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Special issue on
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The burgeoning challenge of antimicrobial resistance

Rajesh Bhatia*

The discovery of penicillin by Fleming revolutionized mankind’s fight against communicable diseases. By 1940s, this drug was available for clinical use. Since then penicillin and many other antimicrobial agents that were discovered subsequently have saved millions of lives all over the world. So huge was the initial impact of antimicrobial agents that many people erroneously started believing that “time has come to close the chapter of communicable diseases”. Unfortunately, they had not comprehended the versatility of microorganisms and the array of survival mechanisms developed by them over several millennia of their existence.

While the successful clinical use of penicillin is widely known and lauded, detection of resistance to penicillin in 1940 itself was ignored by the global community. Facilitated by the continuous and indiscriminate use of antimicrobials in health, veterinary and industrial sectors, the microorganisms slowly and steadily started developing resistance to several antimicrobial agents giving rise to multidrug resistant organisms. Antimicrobial resistance (AMR) is now proclaimed as the most important challenge being faced by humanity in its fight against infectious diseases. The emergence and spread of resistance in several microorganisms have rendered the management of many infectious diseases difficult. Failure to discover new antimicrobial agents has further hampered the war against infectious agents.

Antimicrobial resistance is now no longer a local problem. It has international ramifications. In the modern era of travel and trade, resistant organisms rapidly cross the man-made boundaries through humans or the food chain. The emerging threat of resistance in malaria, tuberculosis (TB) and the human immunodeficiency virus (HIV)/AIDS is a huge impediment to achievement of the Millennium Development Goals (MDGs) by 2015.

This special issue of the Regional Health Forum provides ample evidence to demonstrate that AMR is a burgeoning and hugely neglected problem in the WHO South-East Asia (SEA) Region. The problem is assuming serious proportions in all Member States. The overview of the status of resistance in various microorganisms in Bangladesh, Nepal and Sri Lanka indicates the extent of the problem and its implications for care of patients and public health.

Bhatia et al. have discussed the problem of resistance to first-line antiTB drugs, which has become a concern for national TB control programmes. It is estimated that around 180 000 cases of multidrug-resistant (MDR)-TB occur annually in this Region with more than 80% of these being in Bangladesh, India, Indonesia, Myanmar and Thailand. The drugs needed to treat MDR-TB are over 100 times more expensive than the first-line drugs used to treat non-resistant forms.

The generic antiretroviral (ART) drugs available in the Region are contributing greatly towards improving the survival rate of patients worldwide and in rendering HIV a chronic but a manageable condition. A large number of

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patients from India have been followed up by Balakrishna et al.\textsuperscript{10} to elaborate the intricacies of ART therapy. Although the response to ART drugs is excellent when these are delivered at health facilities, there also are reports of emergence of resistance, which are a serious cause of concern.

Resistant malaria has already become a major issue for the population of 400 million living in areas that expose them to the high risk of contracting it. Artemisinin-based combination therapies (ACT) have recently been introduced in virtually all countries in which malaria is endemic. However, surveillance data from the Thai Ministry of Public Health indicate that clinical failures of artemisinin-based therapies exist on the Thai-Cambodian border, whereas efficacy with artesunate-mefloquine along the western borders of Thailand remains high\textsuperscript{11, 12}.

There has been a substantial change in the antimicrobial susceptibility of \textit{Neisseria gonorrhoeae}. Thirty years back, gonorrhoea used to respond effectively to penicillin. Now the resistance to penicillin, tetracyclines and fluoroquinolones is widespread across the Region\textsuperscript{13}.

Pentavalent antimonials have been successfully used for treatment of kala-azar since the last six decades. Since the 1970s, however, their conventional dosages have failed to achieve the desired results with 60% unresponsiveness being reported with the WHO regimen in Bihar, India. The newer oral drug, miltefosine is a potent antileishmanial drug with a longer half-life, but requires rational use in affected areas\textsuperscript{14}.

Typhoid and paratyphoid fever continue to be important causes of illness and death, particularly among children and adolescents in the SEA Region where this disease is associated with poor sanitation and unsafe food and water. Shortly after the emergence of MDR S. Typhi in this Region, case fatality rates approaching 10% (close to 12.8% recorded in pre-antibiotic era) were reported\textsuperscript{15}. Rational use of some of the recommended drugs for typhoid fever can prolong the life of these drugs, especially chloramphenicol\textsuperscript{16}.

More than 50% isolates of \textit{Staphylococcus aureus} in hospital settings are now methicillin resistant. The resistant strains are widely prevalent in developing as well as developed countries, and are creating major issues in the proper management of seriously ill patients in hospitals\textsuperscript{17}.

Multiresistant klebsiellae, \textit{Pseudomonas} and \textit{Acinetobacter} species have given a new dimension to the problem of hospital-associated infections. \textit{A. baumannii} has become an important pathogen in intensive care units. In a study done in Thailand, the mortality rate for patients admitted due to imipenem-resistant \textit{A. baumannii} was 52% as compared with 19% for those infected with the sensitive variant\textsuperscript{18}. Kumthorn et al.\textsuperscript{19} describe the growing problem of \textit{A. baumanii} group in hospital settings in Thailand. It is likely that similar situations are prevalent in other countries too.

The presence of a drug-resistant gene, \textit{bla}_{NDM-1}, in several members of the family \textit{Enterbacteriaceae} has given rise to organisms that are resistant to a large number of commonly used antimicrobial agents. A reality check on these organisms and their recent emergence has been articulated by Rodrigues\textsuperscript{20}.

Antimicrobial resistance in viruses and fungi is making management of diseases caused by these microorganisms difficult. The recently detected resistance in influenza viruses\textsuperscript{21} and fungi of medical importance\textsuperscript{22} should draw researchers’ special attention to this menace, which is now threatening the hitherto neglected organisms.

Antimicrobial resistance has several severe consequences. The patient remains sick for a longer period thus requiring prolonged treatment usually with expensive and at times toxic drugs. Not only there is greater morbidity and mortality but the burden on health system
also increases. The impact of modern technological and complex surgeries gets negated when the patient after successful intervention gets infected with resistant microorganisms. From the public health perspective, the patient acts as a reservoir of infection for a longer period thus putting at risk more members of the community and healthcare workers. All these factors have a substantial effect on the economy, at both individual and societal levels. In fact, it is difficult to imagine effective newer surgical procedures, transplantations and prolonged chemotherapy for various cancers, care of the critically ill young and the old, or prolonged treatment of HIV-infected persons in the absence of measures aimed at effective containment of AMR4.

The need for new antibiotics to address the emerging resistance in microorganisms cannot be overstated. There has been a near-empty antibiotic pipeline23. However, there is some light at the end of the tunnel with a few significant new global initiatives that are under way. Some antimicrobial agents are being developed and are awaiting approval of the Food and Drug Administration of the United States of America 24. The European Commission recently sought proposals for new antibiotic research and development for multidrug-resistant Gram negative pathogens. This quickly led to the establishment of a joint EU-US Transatlantic Taskforce on Antimicrobial Resistance. The call by the Infectious Diseases Society of America for a 10x20 initiative viz., development of 10 new antibiotics by 202025, should trigger new research and development by the pharma companies.

We need to recognize that the problem of resistance is complex and encompasses biological, behavioural, technical, economic, regulatory and educational dimensions that require a comprehensive response. It requires ownership and active participation by several stakeholders, and a strategic approach with objectives that include establishment of a national alliance for prevention and control of antimicrobial resistance. The WHO Regional Office for South-East Asia has recently developed one such strategy26. It addresses four areas that need attention of national authorities; governance; regulatory mechanisms; building national capacity; and mobilizing active participation of communities.

Recognizing the emerging importance of this subject and to enhance its visibility for an early action, “antimicrobial resistance” is the theme of the World Health Day 2011. Far too long, antimicrobial resistance has been an unrecognized and neglected problem. The time to act is now.

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Antimicrobial resistance: Bangladesh experience

Md. Abul Faiz* and Ariful Basher**

Abstract

Infectious diseases are major health problems in Bangladesh requiring frequent use of antimicrobials. Diagnosis and treatment of most of the bacterial diseases are empirical. Microbial sensitivity patterns of common infections like respiratory tract infection, urinary tract infection, enteric fever, wound infection are not routinely available for decision making in drug selection. Many infectious diseases do not respond to conventional antimicrobial agents. Standard treatment guidelines of different microbes are not sufficient for the purpose, moreover the community awareness programme is imperfect. There is no routine antimicrobial surveillance or quality assurance in place. Multidrug resistant (MDR) TB in primary infection is ~3%, and MDR enteric fever is an emerging threat in Bangladesh. Due to drug resistant *falciparum* malaria, artemisinin-based combination therapy is used; visceral leishmaniasis is treated with oral miltefosine although sodium stibugluconate is still sensitive but found to be at times toxic and difficult to deliver. Available evidence does not support the optimal diagnosis and treatment of bacterial infections in Bangladesh.

Antibiotics are available as non-prescription drugs in medicine shops and irrational use is not uncommon. Adherence to treatment protocol and compliance with treatment course of antimicrobials need to be emphasized at different levels.

Measures for prevention and containment of antimicrobial resistance are necessary in Bangladesh. It should be taken as a national priority and the establishment of a national alliance or regulation governing the use of antimicrobials should be considered.

Introduction

Antimicrobial resistance is a worldwide problem. The selection and spread of resistant organisms in developing countries that can often be traced to complex socioeconomic and behavioural antecedents contribute to the escalating problem of antibiotic resistance. Factors such as unregulated dispensing and manufacture of antimicrobials, truncated antimicrobial therapy, inadequate access to effective drugs and sometimes drugs of questionable quality and overall poverty are likely to be contributing to antimicrobial resistance.¹

Antimicrobials are the most commonly prescribed group of drugs in general practice and in hospitals. Despite the improved trend of health care in Bangladesh, infectious diseases remain priority public health problem, where widespread use of different antimicrobials against bacterial, fungal, viral and parasitic infections is required. Most antimicrobials are prescribed, with the decision to apply based on best-guess empiric therapy. A majority of the prescribers in Bangladesh diagnose infection by clinical assessment and suspect a microbial aetiology.² The important factors associated with resistant bacteria are poor hospital hygiene, overcrowding, lack of

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resources for infection control and lack of personnel trained in controlling infection in hospital.

**Methodology**

This article is based on a review of related literature, newspaper articles and online searches using Pubmed, Google and Banglajol. Combinations of key words related to each of the subject areas were used. Websites of relevant institutions, government and nongovernmental organizations were also searched. The literature thus obtained was categorized and reviewed carefully.

**Drug resistance pattern of common infectious diseases**

Systematic drug sensitivity reports against microorganisms from countries like Bangladesh are sparse. Many studies and interventions have focused on surveillance in hospitals and on the education of physicians. So far very limited data are available on the use of antibiotics. Indeed, Bangladesh is facing dual problems. On the one hand it failed to eradicate age-old infectious diseases due to indiscriminate and irrational use of antimicrobials. On the other hand also it is facing ICU-related drug-resistant microorganisms like those in developed countries.

Antibiotics are most frequently prescribed for acute respiratory tract infections, acute watery diarrhoea, acute trauma and gastrointestinal symptoms. Ceftriaxone (30.19%) followed by cefixime (18.87%), and amoxycillin (16.98%). It was observed that cephalosporins accounted for more than 55% of the total antibiotics used, where the highest uses were by ceftriaxone, cefixime, and cefuroxime. This probably explains why ceftriaxone and cefixime have abnormally high resistance.

A study suggested that Pseudomonas aeruginosa responsible for wound, urine, ear, throat and other infections were more than 50% resistant to commonly-used antibiotics in Bangladesh, including ciprofloxacin, gentamicin, ceftriaxone, cefixime and azithromycin. Azithromycin was 100% ineffective in wound and urine infections, while ceftriaxone and cefixime was 100% ineffective in tracheal infections. Another study also reports that Escherichia coli was resistant in 40% of cases to commonly used antibiotics ceftriaxone, levofloxacin, ciprofloxacin, amoxicillin and ampicillin and 95% resistant to azithromycin. Klebsiella pneumoniae also showed similar patterns. It was observed that 43.2% and 39.5% of isolated E. coli and K. pneumoniae respectively had ESBL phenotypes. This rate is higher than in countries of the Western Pacific Region, North America or Europe and some South American nations.

Cholera germs have acquired resistance to a number of antimicrobials including tetracycline. Over the year, shigellosis have shown great propensity to develop resistance to antibiotics. In 1996, reports from Matlab and Dhaka showed that more then 95% Shigella dysenteriae isolated were resistant to ampicillin, cotrimoxazole and nalidixic acid and 14%-40% were resistant to methicillin. In 1973, S. flexneri isolates were universally susceptible to ampicillin; however, by 1979 susceptibility decreased to 79% in urban Bangladesh. Similarly, the susceptibility of S. flexneri to tetracycline dropped from 79% in 1973 to 15% in 1979. In a recent study, at least 25% of S. flexneri were resistant to three commonly used antibiotics such as ampicillin, co-trimoxazole and nalidixic acid.

Ciprofloxacin has been extensively used in Bangladesh for the treatment of suspected gonorrhoea as it is relatively cheap and effective, and only a single oral dose is required. But as a consequence of the long-time, versatile and large-scale use of this group of antimicrobial agents in areas where over-the-counter availability of drugs without
prescription is common, a substantial increase in resistant strains occurred. Data from one study seem to reflect the consequence of the long standing usage of ciprofloxacin for the treatment of suspected gonorrhoea using syndromic management at sexually transmitted infections (STI) clinics in Bangladesh. The most striking finding of the study is the emergence of isolates resistant to three or more antimicrobial agents. More than half of the isolates are resistant to three drugs, including ciprofloxacin. Of the multidrug-resistant isolates, more than half were both PPNG (penicillinase-producing Neisseria gonorrhoeae) and TRNG (tetracycline resistant Neisseria gonorrhoeae).14

"Wonder drugs" for treating typhoid have now become a challenge to the physicians. A study conducted in an urban hospital of Bangladesh noted that 75% of Salmonella typhi were resistant to nalidixic acid vis-a-vis ciprofloxacin.15 In another study conducted in one referral centre in 2005 a total of 57% of Salmonella typhi strains isolated were MDR (multidrug-resistant) and NAR (nalidaxic acid resistant).16

A study report from Dhaka Shishu Hospital revealed that the principal organisms for neonatal sepsis are Klebsiella, Acinetobactor, E coli, coagulase negative staphylococci and Staphylococcus aureus. Maximum sensitive drugs are imipenem, ciprofloxacin, gentamycin and cotrimoxazole.17 Imipenem is costly and ciprofloxacin has inadequate safety data. Bacterial isolates are becoming resistant to a different generation of cephalosporin.17

Infections caused by opportunistic organisms are difficult to control due to multidrug resistance, which limits therapeutic options in critically ill and debilitated patients, especially from the intensive care units (ICU), where prevalence of the organism is the most noted. Acinetobacter baumannii is now recognized to be the species commonly found.18

The prevalence of MDR-TB was 3% among new and 15.4% in previously treated patients in Bangladesh.4 In a published report, 42 isolates of sputum samples from different areas of Bangladesh were studied in a supranational reference laboratory (SRL) in Antwerp, Belgium. Among 42 strains, 35 (83%) were found resistant to both isoniazid and rifampicin (MDR). Among these MDR strains, 40% were found resistant to any one of the second-line drugs (kanamycin, ofloxacin, ethionamide, and para- amino salicylic acid). However, none of the strains were found to be extensively drug resistant (XDR).19 Another study found drug resistance of Mycobacterium tuberculosis to at least one drug in 53.68% cases which is highly alarming. The highest resistance (40%) was found against isoniazid, which is the most popular drug, followed by rifampicin (32.63%). Resistance to streptomycin was found in 13.68% cases, to ethambutol in 11.58% and to pyrazinamide in 10.53% cases.20 Experts identified inappropriate and irregular treatment beyond programmatic control, lack of diagnostic facility and treatment of existing MDR cases as the contributing factors to the rising number of MDR and XDR-TB patients.21

Multi-drug resistance of Plasmodium falciparum parasites developed in Asia earlier than in other malarious areas around the world. As early as 1957, chloroquine (CQ) resistance appeared, whilst sulfadoxine—pyrimethamine (SP) resistance first emerged in 1967, both at the Thai-Cambodia border. Since then, it has been described in all Asian countries. In Bangladesh, the restrictions, together with clear case definitions and treatment guidelines for malaria, have not been able to block the spread of resistance to the country’s malaria treatment and the current situation of antimalarial drug resistance. Drug resistance to chloroquine and sulphadoxine-pyrimethamine is reported from areas of Chittagong Hill Tract Districts.22 Antimalarial drug efficacy trials documented that chloroquine had been found to be resistant for treatment of Plasmodium falciparum malaria.
to the extent ranging from 40% to 70% in the highly endemic malaria areas.23,24

The Ministry of Health therefore revised the guidelines for malaria treatment with the introduction of artemisinin-based combination therapy (ACT) for the treatment of uncomplicated *falciparum* malaria.

The adequacy of methods used to conduct studies on treatment of visceral leishmaniasis and reports on them varied. Unresponsiveness to antimony has developed steadily in the past to such an extent that antimony is now being replaced, despite attempts to stop its progression by increasing the dose and duration of therapy. The classic second-line treatments are unsuited — pentamidine is toxic and its efficacy has also declined, and amphotericin B deoxycholate is effective but requires hospitalization for long periods and toxicity is common. Liposomal amphotericin B is very effective and safe but currently unaffordable because of its high price.25 Miltefosine — the first oral drug for visceral leishmaniasis — is now registered but its efficacy is controversial. At present, a combination drug trial is going on in Bangladesh to find out the optimal management and to prevent resistance. Meanwhile, the WHO Expert Committee has published a report on the control of leishmaniasis emphasizing the advantages of combination treatment including cost, toxic effects and reducing the probability of selection of drug-resistant parasites.26 In several phase 3 studies in India, three separate combinations showed 98%-99% cure rates.

There are a wide range of prescribers in the private and public sectors. The private health sector is rapidly expanding and is well supervised and regulation of antibiotics is virtually non-existent. There is no central organization entrusted to oversee the use and investigations related to antibiotic use. Many patients use out-of-pocket expenditure for treatment and investigations even in public sector facilities. There is no national antimicrobial policy and few institutes have antimicrobial guidelines which are also not in practice.27 There is no uniformity among the prescribers in commonly prevalent infections although national guidelines are prepared for certain important prevalent infections, for example malaria, kala-azar and tuberculosis. Completion of the course of the prescribed antibiotic is not supervised and is likely to be poor in compliance for various reasons. All these factors facilitate antimicrobial resistance.

### Societal issues outside the medical sphere

Many developing countries including Bangladesh allow the dispensation of antibiotics without a prescription; this can lead to self-medication and dispensation of drugs by untrained people. In one survey from the Rajbari district, 100,000 doses of antibiotics had been dispensed without a prescription in one month.28 In another study, 92% medications dispensed by pharmacies were without a prescription.29 Poverty-stricken patients may forgo the cost of a physician consultation and self-medicate. They demand antibiotic treatment even when not indicated.

Antimicrobials are available over the counter and any antimicrobials can be prescribed by any health care provider. Most of the drugs are prescribed or sold in Bangladesh by non-qualified or relatively less qualified health workers.30 In the national disease control programmes antibiotics are allowed to be given by health workers in certain cases like ARI. Antibiotics are also prescribed unnecessarily for example in viral fevers, and clean post-operative cases.31 Due to the unresponsiveness of the health system with respect to basic amenities, inappropriate client–provider interaction and staff attitudes, patients especially the poor prefer to seek health care from informal providers. These informal providers are deeply embedded in the local community and culture, easily accessible and provide inexpensive services to the villagers with occasional deferred payments and payment in kind instead of cash.
The providers include traditional practitioners and unqualified allopathic practitioners having varying duration of training in diagnosing and treating common ailments mostly from unregulated private institutions of dubious quality. These categories of providers, of greatest importance to the poor and disadvantaged population in rural areas, have largely been ignored by the public sector/government till now, as well as by NGOs.

The widespread and inappropriate use of antibiotics has resulted in the development of a progressively antibiotic-resistant microbial ecosystem in Bangladesh. A study among children from a rural community showed that 50% children had enteric flora resistant to ampicillin, cotrimoxazole and streptomycin throughout the year.32

No information is available from resource-poor settings on the extent of environmental antibiotic usage and its relationship to the prevalence of antibiotic resistant bacteria in animals. Most surprising was the widespread use of animal antibiotics and the anecdotal reports of "resistant" animal infections.33

Overview of the prevailing drugs market

Unethical drug promotion and marketing of substandard and unnecessary drugs in Bangladesh is not uncommon. A medical practitioner can prescribe any drug used for the common cold to cancer. Moreover, polypharmacy is very common among the rural medical practitioners with antibiotics and vitamins prescribed widely.31 The prescription procedure of antibiotics in Bangladesh is less than ideal as prior identification of the pathogens and its sensitivity to the drug is rarely determined before the drug is prescribed.34 Currently, drug companies are the only organizations in Bangladesh to provide information to health personnel and it is often not appropriate information.33

The excessive and inappropriate use of antibiotics adds an unnecessary economic burden to healthcare system and coincides with an increase in drug-resistant organisms, which has resulted in the use of more expensive and toxic drugs. The quality and efficacy of locally manufactured antimicrobial drugs are also largely unregulated. Often multiple brands of the same agent are available, and potency equivalents of the active antibiotic may be a fraction of the appropriate dose. A recent assay involving 15 brands of ciprofloxacin showed that 47% of samples contained less than the specified amounts of the active ingredient.35 Counterfeit drugs like other counterfeit materials are likely to compete favorably in the markets of a developing country like Bangladesh.

Prescription patterns of antimicrobials

Some sporadic studies reported disturbing self-medication behaviours among the general population in Bangladesh.29 Children are mostly affected by inappropriate prescribing of antibiotics. In a study it was shown that 26% of purchased drugs were antibiotics for children aged 0-4 year(s) and 48% of antibiotics were purchased in quantities of less than a single day's dose.36 Pneumonia and diarrhoea are the two most common infectious diseases among children in Bangladesh with annual deaths of about 230 000 children due to diarrhoea.37 But the percentages of appropriate antimicrobial treatment of pneumonia and diarrhoea were 57.1% and 67.8% respectively as shown in one study.38 Misuse of drugs in the treatment of acute diarrhoea among under-five children is highly prevalent and WHO-recommended treatments were seen in only 26.7% of cases and metronidazole was prescribed in all 38.6% cases.39

Multiple and inappropriate antimicrobial drugs is the most common treatment error in dysentery.39 About 50% to 80% of Bangladeshi patients infected with shigellae have a history
of taking at least one antibiotic in the 15 days before a hospital visit.40

Some examples of obsolete treatment used for a long time are: thiacetazone-based antitubercular regimen; use of ineffective chloroquine; S-P or short course quinine Q3F for treatment of falciparum malaria on clinical criteria; over-reliance on sodium antimony gluconate for treatment of kala-azar; over-the-counter purchase of unnecessary antibiotics for viral syndrome; and provision of incomplete course of antibiotic in public hospitals and private practice.

One study in a medical college hospital revealed that the total number of patients who received antimicrobials (69.0%) were prescribed antibiotics for suspected or proven infection and 31.0% and 42.1% of all antimicrobials prescribed were considered inappropriate for prophylaxis. Lack of hospital restrictions on antibiotic use and inappropriate usage for prophylaxis are the main reasons for inappropriate therapy.41

Conclusion

A good, representative database on the current status of antibiotic resistance among common and important pathogens is essential for the proper treatment of infectious diseases in the country. Energetic measures to slow down the emergence and spread of antimicrobial resistance should include programmes on surveillance, education and research on antimicrobial resistance, and regulation of use of antimicrobials in hospitals and in the community.

It is indeed urgently needed to determine antimicrobial practices in the low-income population, determine the duration of compliance to therapy, reasons for non-compliance, sources of medications and prevalence of use of antimicrobials so that we can better apply the regional recommendations for containment of antimicrobial resistance.

References and bibliography


Antimicrobial resistance at different levels of health-care services in Nepal

K K Kafle* and BM Pokhrel**

Abstract
Infectious diseases are major health problems in Nepal. Antimicrobial resistance (AMR) amongst various pathogens have made it difficult to cure these diseases using economical and safe antimicrobial agents. AMR has been seen at various levels of health care delivery in Nepal. This paper briefly articulates the observations made by Alliance for Prudent Use of Antibiotics (APUA) Nepal.

Introduction
In Nepal, the types of institutions under the Department of Health Services comprise eight Central Hospitals, three Regional Hospitals, two Sub-regional Hospitals, 10 zonal hospitals and 65 district hospitals. The regional, subregional and zonal hospitals provide blood, urine and pus culture/sensitivity services. The district hospitals provide only Gram's and AFB staining services and culture/sensitivity services are not available there¹.

Alliance for the Prudent Use of Antibiotics (APUA) is a non-profit organization with its headquarters in Boston (USA). Its mission is to improve infectious disease treatment and control worldwide through promoting appropriate antibiotic access and use and by reducing antibiotics resistance. APUA has affiliated chapters in 63 countries, and Nepal has a country chapter. APUA Nepal publishes its newsletter annually, which includes reports on sensitivity patterns of common isolates in

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This article compiles and evaluates the available data published in the APUA Nepal Newsletter from 2004 to 2010 on the resistance problem in selected common pathogens.

Material and methods
The data published in the APUA Nepal Newsletter on sensitivity patterns of common isolates in urine, blood and pus samples from different levels of hospitals in Nepal²⁻⁸.

Table 1 shows the list of health facilities included for data collection and their testing dates. It included three zonal and one regional hospital and three medical colleges. The common pathogens isolated in urine, blood and pus samples are shown in Table 2.

The sensitivity patterns of common pathogens from the samples are presented in the Results section. The first two issues (2004 and 2005) of the APUA Nepal Newsletter published the sensitivity of pathogens
Table 1: Name of sampled health facilities and testing dates

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<th>Name of health facility</th>
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<td>Western Regional Hospital, Pokhara</td>
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<td>Bheri Zonal Hospital, Nepalgunj</td>
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</tr>
<tr>
<td>Mechi Zonal Hospital, Jhapa</td>
<td>2010</td>
</tr>
</tbody>
</table>

Table 2: Health facility and common pathogens in urine, blood and pus samples

<table>
<thead>
<tr>
<th>Health facility</th>
<th>Urine</th>
<th>Blood</th>
<th>Pus</th>
<th>Testing date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regional Hospital</td>
<td>E. coli, S. aureus, K. pneumoniae, Citrobacter</td>
<td>S. typhi</td>
<td>E. coli, S. aureus, Pseudomonas</td>
<td>2007–2008</td>
</tr>
</tbody>
</table>

mentioning “more than 50%” rather than giving the actual percentage. The results show the range of sensitivity of each pathogen to individual antibiotic at each level of health facility. However, the results do not compare the status of sensitivity between 2004 and 2010.

Results

Common pathogens in urine and their resistance

The common pathogens in urine included E. coli, S. aureus and K. pneumoniae.

E. coli is highly resistant to ampicillin and cotrimoxazole and their sensitivity is poor with cefotaxime as well. The other gram-ve bacilli K. pneumoniae is mostly resistant to cephalixin and nalidixic acid. Similarly, S. aureus is mostly resistant to ampicillin and cephalixin (Table 3-5).

Common pathogens in blood and their resistance

The common pathogens include S. typhi and S. paratyphi and they are mostly sensitive to antibiotics that were tested (Table 6).

Common pathogens in pus and their resistance

E. coli and S. aureus are the commonest pathogens in pus. E. coli is highly resistant to ampicillin and is mostly resistant to cotrimoxazole. Similarly, S. aureus is also highly resistant to ampicillin (Table 7-8).
Table 3: Antibiotic sensitivity in E. coli from urinary tract pathogens

<table>
<thead>
<tr>
<th>Health facility level</th>
<th>Amikacin</th>
<th>Ampicillin</th>
<th>Azithromycin</th>
<th>Cefalotin</th>
<th>Cefoxitin</th>
<th>Ceftriaxone</th>
<th>Gentamicin</th>
<th>Nitrofurantoin</th>
<th>Ofloxacin</th>
<th>Nitrofurazone</th>
<th>Nalidixic Acid</th>
<th>Gentamycin</th>
<th>Metronidazole</th>
<th>Norfloxacin</th>
<th>Ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonal hospitals</td>
<td>&gt; 50.0-83.3</td>
<td>NR</td>
<td>20.8</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>&gt; 50.0</td>
<td>41.7</td>
<td>&gt; 50.0</td>
<td>62.5</td>
<td>68.8</td>
<td>&gt; 50.0</td>
<td>70.8</td>
</tr>
<tr>
<td>Regional Hospital</td>
<td>80.0</td>
<td>NR</td>
<td>29.2</td>
<td>76.2</td>
<td>58.9</td>
<td>NR</td>
<td>NR</td>
<td>100.0</td>
<td>100.0</td>
<td>56.5</td>
<td>8.3</td>
<td>38.5</td>
<td>22.2</td>
<td>87.5</td>
<td>36.4</td>
</tr>
<tr>
<td>Medical colleges</td>
<td>&gt; 50.0-89.4</td>
<td>21.3</td>
<td>65.3</td>
<td>13.3</td>
<td>NR</td>
<td>NR</td>
<td>45.6</td>
<td>&gt; 50.0</td>
<td>&lt; 50.0</td>
<td>&gt; 50.0</td>
<td>&gt; 50.0</td>
<td>&gt; 50.0</td>
<td>29.5</td>
<td>44.7</td>
<td>91.0</td>
</tr>
</tbody>
</table>

NR: Not reported, not all sites reported all drugs.

Testing dates ranged from 2004–2010

* > 50.0 = first two issues (2004 and 2005) of APUA-Nepal Newsletter published sensitivity patterns of pathogens which were 50% or more sensitive without giving actual percentage.
<table>
<thead>
<tr>
<th>Health facility level</th>
<th>Amikacin</th>
<th>Ampicillin</th>
<th>Achromycin</th>
<th>Cefoxitin</th>
<th>Cephalin</th>
<th>Ciprofloxacin</th>
<th>Cotrimoxazole</th>
<th>Cefazolin</th>
<th>Gentamycin</th>
<th>Nitrofurantoin</th>
<th>Norfloxacin</th>
<th>Ofloxacin</th>
<th>Ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonal hospital</td>
<td>100.0</td>
<td>0.0</td>
<td>NR</td>
<td>NR</td>
<td>0.0</td>
<td>NR</td>
<td>NR</td>
<td>50.0</td>
<td>NR</td>
<td>NR</td>
<td>100.0</td>
<td>NR</td>
<td>100.0</td>
</tr>
<tr>
<td>Regional Hospital</td>
<td>NR</td>
<td>50.0</td>
<td>100.0</td>
<td>NR</td>
<td>100.0</td>
<td>NR</td>
<td>NR</td>
<td>50.0</td>
<td>NR</td>
<td>NR</td>
<td>50.0</td>
<td>50.0</td>
<td>NR</td>
</tr>
<tr>
<td>Medical colleges</td>
<td>&gt;50.0-67.3</td>
<td>16.5-40.1</td>
<td>&gt;50.0-100.0</td>
<td>36.3-64.4</td>
<td>&gt;50.0-85.2</td>
<td>&gt;50.0-92.2</td>
<td>44.2-80.3</td>
<td>57.7-84.0</td>
<td>42.6-62.8</td>
<td>95.6-98.6</td>
<td>50.0-100.0</td>
<td>&gt;50.0</td>
<td></td>
</tr>
</tbody>
</table>

NR: Not reported, not all sites reported all drugs.
* >50.0 = first two issues (2004 and 2005) of APUA-Nepal Newsletter published sensitivity patterns of pathogens which were 50% or more sensitive without giving actual percentage.
Table 5: Antibiotic sensitivity in K. pneumoniae from urinary tract pathogens

<table>
<thead>
<tr>
<th>Health facility level</th>
<th>Amikacin</th>
<th>Amoxicillin</th>
<th>Ampicillin</th>
<th>Azithromycin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
<th>Cephalexin</th>
<th>Cotrimoxazole</th>
<th>Gentamycin</th>
<th>Nalidixic Acid</th>
<th>Nitrofurantoin</th>
<th>Norfloxacin</th>
<th>Ofloxacin</th>
<th>Tetracycline</th>
<th>Zonal hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jr. Regional Hospital</td>
<td>100.0</td>
<td>NR</td>
<td>0.0</td>
<td>NR</td>
<td>NR</td>
<td>0.0</td>
<td>NR</td>
<td>33.3</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>83.3</td>
<td>NR</td>
<td>NR</td>
<td>100.0</td>
</tr>
<tr>
<td>Regional Hospital</td>
<td>NR</td>
<td>NR</td>
<td>100.0</td>
<td>25.0</td>
<td>33.3</td>
<td>100.0</td>
<td>50.0</td>
<td>16.7</td>
<td>75.0</td>
<td>75.0</td>
<td>83.3</td>
<td>NR</td>
<td></td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td>Medical colleges</td>
<td>&gt;50.0-64.7</td>
<td>4.6-69.0</td>
<td>NR</td>
<td>NR</td>
<td>&gt;50.0-93.1</td>
<td>&gt;50.0-88.0</td>
<td>7.6-58.4</td>
<td>&gt;50.0-82.0</td>
<td>40.5-72.2</td>
<td>&gt;50.0-93.1</td>
<td>35.7-93.2</td>
<td>46.0-88.3</td>
<td>&gt;50.0-50.0</td>
<td>&gt;50.0</td>
<td></td>
</tr>
</tbody>
</table>

NR: Not reported, not all sites reported all drugs.
* >50.0 = first two issues (2004 and 2005) of APUA-Nepal Newsletter published sensitivity patterns of pathogens which were 50% or more sensitive without giving actual percentage.
### Table 6: Antibiotic sensitivity in S. enterica typhi and Paratyphi A from blood pathogens

<table>
<thead>
<tr>
<th>Health facility level</th>
<th>Amikacin</th>
<th>Amoxicillin</th>
<th>Ampicillin</th>
<th>Cefixaime</th>
<th>Cefotaxime</th>
<th>Ceftriaxone</th>
<th>Cephalexin</th>
<th>Chloramphenicol</th>
<th>Ciprofloxacin</th>
<th>Cotrimoxazole</th>
<th>Gentamicin</th>
<th>Imipenem</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonal hospital</td>
<td>&gt;50.0</td>
<td>NR</td>
<td>50.0</td>
<td>NR</td>
<td>NR</td>
<td>100.0</td>
<td>NR</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>NR</td>
<td>NR</td>
<td>50.0</td>
</tr>
<tr>
<td>Regional Hospital</td>
<td>100.0</td>
<td>NR</td>
<td>43.5</td>
<td>100.0</td>
<td>100.0</td>
<td>NR</td>
<td>94.5</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>61.1</td>
<td>100.0</td>
<td>NR</td>
</tr>
<tr>
<td>Medical colleges</td>
<td>NR</td>
<td>100.0</td>
<td>&gt;50.0-91.8</td>
<td>100.0</td>
<td>NR</td>
<td>&gt;50.0-100.0</td>
<td>&gt;50.0-100.0</td>
<td>&gt;50.0-100.0</td>
<td>&gt;50.0-100.0</td>
<td>&gt;50.0-100.0</td>
<td>NR</td>
<td>100.0</td>
<td>42.0-100.0</td>
</tr>
</tbody>
</table>

NR: Not reported, not all sites reported all drugs.

* >50.0 = first two issues (2004 and 2005) of APUA-Nepal Newsletter published sensitivity patterns of pathogens which were 50% or more sensitive without giving actual percentage.
Table 7: Antibiotic sensitivity in S. aureus from pus pathogens

<table>
<thead>
<tr>
<th>Health facility level</th>
<th>Amikacin</th>
<th>Ampicillin</th>
<th>Azithromycin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
<th>Cephalexin</th>
<th>Gentamycin</th>
<th>Ofloxacin</th>
<th>Procaine Penicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonal hospital</td>
<td>87.5</td>
<td>25.0</td>
<td>NR</td>
<td>NR</td>
<td>12.5</td>
<td>NR</td>
<td>NR</td>
<td>75.0</td>
<td>NR</td>
</tr>
<tr>
<td>Regional Hospital</td>
<td>100.0</td>
<td>50.0</td>
<td>66.7</td>
<td>70.0</td>
<td>71.4</td>
<td>NR</td>
<td>100.0</td>
<td>57.1</td>
<td>57.1</td>
</tr>
<tr>
<td>Medical colleges</td>
<td>&gt;50.0-79.4</td>
<td>22.7-37.0</td>
<td>NR</td>
<td>NR</td>
<td>&gt;50.0-79.4</td>
<td>31.6-83.6</td>
<td>NR</td>
<td>&gt;50-75.3</td>
<td>45.6-70.9</td>
</tr>
</tbody>
</table>

NR: Not reported, not all sites reported all drugs.

* > 50.0 = first two issues (2004 and 2005) of APUA-Nepal Newsletter published sensitivity patterns of pathogens which were 50% or more sensitive without giving actual percentage.
Table 8: Antibiotic sensitivity in *E. coli* from pus pathogens*

<table>
<thead>
<tr>
<th>Health facility level</th>
<th>Antibiotics</th>
<th>Amoxycillin</th>
<th>Ampicillin</th>
<th>Amikacin</th>
<th>Cotrimoxazole</th>
<th>Cefixime</th>
<th>Cefotaxime</th>
<th>Ceftriaxone</th>
<th>Cephalexin</th>
<th>Cefuroxime</th>
<th>Ofloxacin</th>
<th>Polymyxin B</th>
<th>Gentamycin</th>
<th>Nitrofurantoin</th>
<th>Norfloxacin</th>
<th>Ofloxacin</th>
<th>Polymyxin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonal hospital</td>
<td>&gt;50.0-87.5</td>
<td>NR</td>
<td>25.0</td>
<td>NR</td>
<td>NR</td>
<td>12.5</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>NR</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>NR</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>NR</td>
<td>&gt;50.0</td>
</tr>
<tr>
<td>Regional Hospital</td>
<td>70.0</td>
<td>NR</td>
<td>42.8</td>
<td>61.5</td>
<td>44.4</td>
<td>25.0</td>
<td>100.0</td>
<td>60.0</td>
<td>33.3</td>
<td>50.0</td>
<td>NR</td>
<td>NR</td>
<td>61.5</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Medical colleges</td>
<td>&gt;50.0-85.8</td>
<td>49.2</td>
<td>6.6</td>
<td>NR</td>
<td>NR</td>
<td>55.0-89.3</td>
<td>42.8-88.4</td>
<td>8.9-70.2</td>
<td>38.0-67.3</td>
<td>41.7-62.3</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>NR</td>
<td>NR</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
<td>&gt;50.0</td>
</tr>
</tbody>
</table>

NR: Not reported, not all sites reported all drugs.

* >50.0 = first two issues (2004 and 2005) of APUA-Nepal Newsletter published sensitivity patterns of pathogens that were 50% or more sensitive without giving the actual percentage.
Discussion

Infectious disease is a common ailment in Nepal. In Nepal, antimicrobial resistance is a major problem, contributing to increased treatment costs, hospital stay, morbidity and mortality. The resistance is more common among Gram-negative than Gram-positive organisms, the precise extent of the problem is not known since the majority of the published reports derive from individual units or hospitals9. Resistance to commonly available and affordable antimicrobials poses a major concern in the management of bacterial infection. Improper practices in the use of antimicrobial agents in human medicine and for prophylaxis in animal husbandry may contribute significantly to the emergence of multidrug-resistant (MDR) strains10.

Transmission of highly resistant bacteria from patient to patient within the hospital environment (nosocomial transmission) amplifies the problem of antimicrobial resistance and may result in the infection of patients who are not receiving antimicrobials.

Despite its proven efficacy in containing and preventing additional infection and curbing the associated antibiotics use, infection control is among the slowest disciplines to gain widespread implementation. Failure to carry out simple infection control practices such as changing gloves and washing hands is both dangerous and all too common. It often stems from the failure to recognize its importance, understaffing, and/or forgetfulness. Controlling the spread and emergence of drug resistance in the hospital is best administered by an active, coordinated infection control programme, which may include targeted cohorting of infected patients, enhanced surveillance, isolation or rigorous barrier precautions, early discharge, and alterations in antimicrobial usage11.

References and bibliography

(10) APUA. Multidrug-resistant escherichia coli from apparently healthy children in Kenya. APUA Newsletter. 2007; 25(1).
Antimicrobial resistance in resource-poor settings – Sri Lankan experience

C.G.U.A Patabendige*, N.S. Chandrasiri**, L.I. Karunanayake***, G.K.D. Karunaratne+, P. Somaratne***, J.P Elwitigala++ and P. Chandrasiri+++,

Abstract
An increasing trend in antimicrobial resistance in healthcare settings has been observed and demonstrated in Sri Lanka with the improvement of microbiological services in the country. Some good studies have been carried out in different localities, giving the level of resistance. There is an observation made by health professionals about upward trend in development of antimicrobial resistance in pathogenic microorganisms in the community but data to demonstrate it is scarce.

Though there is no national antimicrobial policy, local policies are in operation in some hospitals. Data from the ongoing antimicrobial resistance surveillance will be used for the formulation of the national antimicrobial policy in the future. The Sri Lanka College of Microbiologists, the Sri Lanka Medical Association and the Task Force in Microbiology are some of the professional organizations who are working hard on this subject. The increasing trend in antimicrobial resistance is likely to be due to multiple reasons. Strengthening rational use, legal provisions for prescribing, registration policies, infection control activities, training of health-care workers and the community, further strengthening of microbiology laboratories by providing continuous supply of consumables and necessary equipment to perform standardized methodology would help in combating this problem. Furthermore, a situational analysis of irrational use of antimicrobials in agriculture and fishery and quick action to minimize it and assuring continuous supply of good quality antibiotics to be used according to the antimicrobial policies are essential in controlling this menace. Otherwise an uncontrollable situation will emerge causing morbidity and mortality due to infections with resistant micro-organisms with no antimicrobials effective for their management.

Introduction
Sri Lanka is a developing country with a state-sponsored free health system with co-existing private health care. The country has achieved good life expectancy as well as low maternal and neonatal mortality rates compared to most other Asian countries during the past few decades. Currently, the country faces a problem of an increasing trend in antimicrobial resistance in microorganisms in both health care and community settings probably due to multiple reasons. Some of these organisms are extended spectrum \( \beta \) lactamase (ESBL) producing coliforms, multidrug-resistant *Pseudomonas* and *Acinetobacter* spp, methicillin resistant *Staphylococcus aureus* (MRSA), penicillin resistant *Streptococcus pneumoniae* and quinolone resistant *Salmonella typhi* and
Salmonella paratyphi A. Appropriate and timely action needs to be taken by the authorities in order to minimize the morbidity and mortality due to antimicrobial resistant infections and also to preserve the effectiveness of antimicrobial agents which are used in management of microbial infections.

**Situation in health-care settings**

For the detection of antimicrobial resistance in different microorganisms which are of medical importance and observing its trends, it is extremely important to identify the microorganisms accurately and carry out antimicrobial sensitivity according to standardized methodology. Till the turn of century, microbiology facilities were not widespread in the country. Currently almost all teaching hospitals and some general hospitals in different provinces are served by consultant microbiologists. With the appointment of these consultants, microbiology services improved countrywide thus helping to implement standardized, universally accepted methodology for antimicrobial sensitivity testing and also proper identification of the infectious agents to the species level. In addition to the onsite consultant microbiologists, they serve as offsite consultants to the hospitals where onsite consultants have not been appointed yet, by giving advice on antimicrobial prescribing and microbiological testing needs. Qualified medical laboratory technologists and laboratory assistants actively work in all microbiology laboratories in the government sector.

At present, there is an ongoing antimicrobial resistance surveillance project in the country involving 10 surveillance centres namely six from the Western and one each from the Sabaragamuwa, Southern, Central and Uva provinces for monitoring of Gram negative microorganisms. The project will be extended for the identification of the gram positive organisms 2011. Data obtained from the surveillance centres during the past year will be analysed early next year and data will be used for the formulation of the national antibiotic policy. The surveillance data will be published in the near future. The Task Force in Microbiology which has been set up under the chairmanship of the Director General of Health Services and with the participation of consultant microbiologists from different healthcare settings in different provinces has recognized the monitoring of antimicrobial resistance as a national priority.

Though there is no national antimicrobial policy, the consultant microbiologists who are working in the hospital settings have prepared local policies for their institutions taking into account the prevalence rates of the microbial pathogens and their sensitivity pattern. But compliance is variable from satisfactory to poor in different settings.

Though limited training programmes have been held locally at hospital settings, training imparted to doctors on rational use of antimicrobials is not satisfactory. Public awareness about rational use of antimicrobials is poor. Drug review committees and drug therapeutic committees have been set up in hospitals as a requirement of the Ministry of Health but their active operation to reach the goals in prevention and control of antimicrobial resistance is questionable at present.

Though there are no regulations directly related to antimicrobial use at present, there are indirect controls such as at the time of registration, it is granted as schedule 11 – B drug for all antimicrobials, leaving them to be prescribed by a registered medical practitioner but prescribing by unqualified personnel is occurring indicating the need for strengthening the legal provisions for prescribing of antimicrobials. Use of substandard antimicrobials is a problem together with the lack of continuous supply of standard antimicrobials. Remedial actions are being taken by the relevant authorities to address this issue. The level of irrational use of antimicrobials in agriculture and fishery in the country is unknown. Through personal

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communications, we have been made aware that some of the antibiotics are being used as growth promoters in agriculture and fishery in the country.

In resource-poor settings, implementation of good infection control practices to prevent cross-infections due to multidrug-resistant organisms is questionable. Though health-care workers are aware about the practices, adequate resources are not available for effective implementation.

Data on antimicrobial resistance in health-care settings

Though the resources available are not adequate, some good studies have been carried out giving the level of resistance in certain localities in the country. They have been published in the Bulletin of the Sri Lanka College of Microbiologists which is released annually by the Sri Lanka College of Microbiologists. The Sri Lanka Medical Association also publishes data on antimicrobial resistance in its publications.

The following are some of the local antimicrobial resistance data published during the past few years:

Kandy Teaching Hospital

Data from a retrospective study carried out to determine the prevalence of multidrug-resistant organisms (MDRO) in 2008.

A total of 158 and 513 wound swabs / pus specimens were received from Intensive Care Units (ICUs) and wards respectively. Out of the Staphylococcus aureus isolated, 63.6% and 48.5% were MRSA in the ICUs and wards respectively.

Out of 441 endotracheal secretions received from ICUs, 346 yielded gram negative bacilli. Out of coliforms, Pseudomonas spp, Acinetobacter spp isolated, 80.5%, 40% and 57.6% respectively were multidrug resistant giving the total percentage of 60.1%.

Table 1: Prevalence of MRSA in ICU and wards

<table>
<thead>
<tr>
<th>Organism</th>
<th>ICU</th>
<th>Wards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus (total)</td>
<td>44</td>
<td>132</td>
</tr>
<tr>
<td>MRSA</td>
<td>28(63.6%)</td>
<td>64(48.5%)</td>
</tr>
<tr>
<td>MSSA</td>
<td>16(36.3%)</td>
<td>68(51.5%)</td>
</tr>
</tbody>
</table>

MSSA=Methicillin Sensitive S. aureus,   MRSA=Methicillin Resistant S. aureus

National Cancer Institute of Sri Lanka

This is the final referral centre for malignancies so the study population is immunocompromised.

(1) A prospective study done on analysis of BACTEC blood cultures in 2009 showed that 89 (52.35%) isolates were gram negative bacilli (GNB) which included 59(66.29%) coliforms of which 20 (33.9%) were ESBL producers, 25(28.08%) Pseudomonas spp and 05(5.62%) Acinetobacter spp. Sixty-five (38.28%) isolates were gram positive cocci which included

Table 2: Prevalence of multi-drug resistant Gram Negative Bacilli

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Gram Negative Bacilli</th>
<th>Multidrug-resistant organisms (including ESBL-GNB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Califors</td>
<td>149</td>
<td>43.19</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>145</td>
<td>41.90</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>52</td>
<td>15.02</td>
</tr>
<tr>
<td>Total</td>
<td>346</td>
<td>208</td>
</tr>
</tbody>
</table>

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Staphylococcus aureus isolates of which 31 (79.49%) were MRSA. Twelve (7.06%) Candida spp and one Cryptococcus neoformans were isolated.

A prospective descriptive study was conducted on antibiotic sensitivity and Minimum Inhibitory Concentration (MIC) of vancomycin in MRSA isolates in 2008. Antibiotic sensitivity testing (AST) was performed according to the 2008 CLSI guideline.

The highest resistance rates were detected for erythromycin and clindamycin, and resistance to clindamycin was mainly due to inducible resistance making these drugs less important. While 97.84% of isolates were sensitive to rifampicin, no resistance was observed for linezolid.

A prospective study was carried out to determine the distribution of MIC of antibiotics vancomycin and teicoplanin in MRSA in 2009 using Etest strips (AB Biodisc, Sweden) method and the results were interpreted according to the 2008 CLSI guideline.

MICs of vancomycin were ranged from 0.25 μg/ml to 2.0 μg/ml with MIC<sub>50</sub> and MIC<sub>90</sub> of 1.0 μg/ml and 1.5 μg/ml respectively. Teicoplanin MICs were ranged from 0.25 μg/ml to 4.0 μg/ml with MIC<sub>50</sub> of 2.0 μg/ml and MIC<sub>90</sub> of 3.0 μg/ml. Higher MIC<sub>50</sub> and MIC<sub>90</sub> obtained for vancomycin implies the need for further clinical studies in detecting the efficacy of glycopeptides in our population as efficacy decreases when MIC is

### Table 3: Antimicrobial sensitivity of GNB

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cefazidime</th>
<th>Gentamicin</th>
<th>Amikacin</th>
<th>Netilmicin</th>
<th>Ciprofloxacin</th>
<th>Meropenem</th>
<th>Imipenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>GN B</td>
<td>57.3%</td>
<td>48.31%</td>
<td>66.29%</td>
<td>51.69%</td>
<td>71.91%</td>
<td>95.51%</td>
<td>94.38%</td>
</tr>
<tr>
<td>Coliforms</td>
<td>50.85%</td>
<td>49.15%</td>
<td>72.88%</td>
<td>52.54%</td>
<td>62.71%</td>
<td>93.3%</td>
<td>98.3%</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>72%</td>
<td>44%</td>
<td>52%</td>
<td>48%</td>
<td>88%</td>
<td>96%</td>
<td>92%</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>60%</td>
<td>60%</td>
<td>60%</td>
<td>60%</td>
<td>100%</td>
<td>60%</td>
<td>60%</td>
</tr>
</tbody>
</table>

### Table 4: Antibiotic sensitivity pattern

<table>
<thead>
<tr>
<th></th>
<th>Vancomycin</th>
<th>Teicoplanin</th>
<th>Clindamycin</th>
<th>Erythromycin</th>
<th>Ciprofloxacin</th>
<th>Cotrimoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>93(100%)</td>
<td>93(100%)</td>
<td>19(20.43%)</td>
<td>5(5.38%)</td>
<td>33(35.48%)</td>
<td>29(31.18%)</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>1(1.08%)</td>
<td>0</td>
<td>6(6.45%)</td>
<td>5(5.38%)</td>
</tr>
<tr>
<td>R</td>
<td>0</td>
<td>0</td>
<td>73(78.49%)</td>
<td>88(94.62%)</td>
<td>54(58.07%)</td>
<td>59(63.44%)</td>
</tr>
</tbody>
</table>

S: Sensitive, I: Intermediates, R: Resistant

<table>
<thead>
<tr>
<th></th>
<th>Gentamicin</th>
<th>Amikacin</th>
<th>Netilmicin</th>
<th>Rifampicin</th>
<th>Fusidic acid</th>
<th>Linezolid</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>35(37.63%)</td>
<td>55(59.14%)</td>
<td>58(62.37%)</td>
<td>91(97.84%)</td>
<td>72(77.42%)</td>
<td>93(100%)</td>
</tr>
<tr>
<td>I</td>
<td>2(2.15%)</td>
<td>16(17.2%)</td>
<td>17(18.28%)</td>
<td>1(1.08%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R</td>
<td>56(60.22%)</td>
<td>22(23.66%)</td>
<td>18(19.35%)</td>
<td>1(1.08%)</td>
<td>21(22.58%)</td>
<td>0</td>
</tr>
</tbody>
</table>

S: Sensitive, I: Intermediates, R: Resistant
increasing. No glycopeptide intermediate or glycopeptide resistant isolates were found. This needs further evaluation by broth or agar dilution methods.

(4) A prospective study on nosocomial candidaemia was carried out from January 2007 to December 2009\(^4\). During the study period, Candida tropicalis 19/515 (3.68%), 31/828 (3.74%) and 32/825 (3.88%), Candida glabrata 20/515 (3.88%), 21/828 (2.53%) and 20/825 (2.42%), Candida albicans 01/515 (0.19%), 03/828 (0.36%) and 05/825 (0.60%), Candida parapsilosis 01/515 (0.19%), 03/828 (0.36%) and 02/825 (0.24%) were isolated and one isolate of Candida guilliermondii in 2008 which is the first blood culture isolation of this Candida spp. in Sri Lanka. The most prevalent species was Candida tropicalis followed by Candida glabrata and Candida albicans. A majority of isolates were sensitive to fluconazole and amphotericin B.
Colombo district study on Haemophilus influenzae b (Hib)

A study was carried out in 2004 to determine the burden of Hib in the paediatric population in Colombo district. There were 26 isolates in the study including 11 cerebro spinal fluid (CSF), 19 blood and 1 joint fluid.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>54%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>38%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>04%</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>15%</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>15%</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>61%</td>
</tr>
</tbody>
</table>

Fifty-seven per cent of CSF isolates are either resistant to ampicillin or chloramphenicol or to both first-line antibiotics for meningitis.

National Tuberculosis Reference Laboratory

An analysis of drug susceptibility of mycobacterium species isolated for 3 years from 2005 was done. Data from 3425 mycobacterium isolates (14.7%) of 23,311 cultures performed in the country excluding Mullative and Killinochchi were analysed retrospectively for sensitivity to rifampicin, INAH, ethambutol and streptomycin. Mycobacterium tuberculosis and atypical mycobacteria accounted for 96.73% and 3.27% of isolates respectively. Multidrug resistance (MDRTB) was observed in 2.48% of Mycobacterium tuberculosis culture isolates.

Table 6: Resistance to individual drugs, 2005–2007

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Streptomycin</th>
<th>Ethambutol</th>
<th>INAH</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>9.45</td>
<td>6.49%</td>
<td>4.47%</td>
<td>3.40%</td>
</tr>
<tr>
<td>Atypical mycobacteria</td>
<td>89.29%</td>
<td>92.86%</td>
<td>89.29%</td>
<td>96.43%</td>
</tr>
</tbody>
</table>

Out of the total isolates 82.81% of cultures were sensitive to all four drugs and 17.19% showed resistance to single or a combination of drugs. Out of Mycobacterium tuberculosis 1.4% and of atypical mycobacteria 81.25% were resistant to all four drugs.

Colombo South Teaching Hospital

(1) A descriptive study was carried out from Jan- Dec 2008 to collect epidemiological data on invasive Streptococcus pneumoniae isolates (ISP) in relation to age, underlying risk factors and clinical outcome in paediatric and adult population. Clinical outcome was correlated in relation to diagnosis and penicillin MIC with 21 isolates from 17 patients with 9 paediatric and 8 adult patients. MIC was performed using penicillin Estrips (AB Biodisk, Sweden) and interpreted according to the new CLSI cutoffs for meningeal and nonmeningeal isolates.

All meningeal isolates were penicillin resistant but all non-meningeal isolates were penicillin sensitive.

(2) A study carried out on the prevalence of pathogenic microorganisms in urine cultures from patients at the outpatient department showed 14.28% ESBL producing coliforms (unpublished data, personal communication DMBT Dissanayake).
Table 7: Epidemiological data and penicillin susceptibility of isolates

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Number</th>
<th>MIC interpretation</th>
<th>Risk factors</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis</td>
<td>4</td>
<td>All penicillin resistant</td>
<td>None</td>
<td>1 death</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5</td>
<td>All penicillin sensitive</td>
<td>None</td>
<td>All recovered</td>
</tr>
<tr>
<td>Pelvic abscess</td>
<td>3</td>
<td>All penicillin sensitive</td>
<td>1 nephrotic</td>
<td>All recovered</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>2</td>
<td>All penicillin sensitive</td>
<td>None</td>
<td>All recovered</td>
</tr>
<tr>
<td>Peritonitis/appendix</td>
<td>2</td>
<td>All penicillin sensitive</td>
<td>1 cirrhosis</td>
<td>1 death</td>
</tr>
<tr>
<td>No diagnosis</td>
<td>1</td>
<td>-</td>
<td>Congenital heart disease</td>
<td>1 death</td>
</tr>
</tbody>
</table>

(3) A study carried out on changing serotype pattern and antimicrobial resistance in blood culture isolates of Salmonella from 2006-2009 has shown that from 2007 ciprofloxacin resistant Salmonella paratyphi A has become the prevalent serotype. This trend is continuing in 2010 making available typhoid vaccines ineffective. In this study ciprofloxacin sensitivity was determined using nalidixic acid disc. Chloramphenicol resistance is seen only in Salmonella typhi. Ciprofloxacin resistance is almost 100% in Salmonella paratyphi isolates in 2008 and 2009. Ciprofloxacin resistance is gradually increasing Salmonella typhi and had reached 50% by 2009. Two isolates showed intermediate resistance to ceftriaxone.

Lady Ridgeway Hospital for Children

This is the final referral centre for children less than 12 years old.

(1) Surveillance of invasive pneumococcal disease was carried out from 2004–2009. Part of this study has been published.

Considerating the current CLSI penicillin break points for meningitic isolates of pneumococci, 92.5% are penicillin resistant. However, with the revision of CLSI break points for non-meningitic isolates, 95.0% isolates are penicillin sensitive. A high resistance was noted to erythromycin (67.5%) and co-trimoxazole (72.5%) compared to certain South Asian countries.

Table 8: Antibiotic sensitivity pattern of isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% Sensitive</th>
<th>Intermediate</th>
<th>% Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>70.0</td>
<td>0</td>
<td>30.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>32.5</td>
<td>0</td>
<td>67.5</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>27.5</td>
<td>12.5</td>
<td>60.0</td>
</tr>
</tbody>
</table>

(2) A study carried out in 2008 on bacterial aetiological agents in children with gastroenteritis has shown 13.9% isolation rate with 58.2% Shigella spp. out of which Shigella flexneri was the most common. Of the Campylobacter spp. 88.5% were Campylobacter jejuni. 78.8% of the bacterial isolates were sensitive to furazolidone while 58.6% were sensitive to mecillinam and 54.1% to nalidixic acid.
Table 9: LRH - Stool cultures, 2008

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella spp.</td>
<td>103 (58.2%)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>32 (18.1%)</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>26 (14.7%)</td>
</tr>
<tr>
<td>Enteropathogenic E. coli</td>
<td>16 (9%)</td>
</tr>
<tr>
<td>Total</td>
<td>177</td>
</tr>
</tbody>
</table>

(3) A study done for two years from February 2008 on the epidemiology of *Haemophilus influenzae* infections and β lactamase production in isolates has shown that there were 19 β lactamase positive isolates out of the total of 45 giving a percentage of 44.4%. There were 4.4% of β lactamase negative ampicillin resistant (BLNAR) isolates. 8.89% and 15.56% strains were resistant to cefotaxime/ceftriaxone and chloramphenicol respectively. ¹¹

NHSL

A retrospective analysis of intravascular catheter tip culture results with the simultaneously drawn peripheral blood culture results was done from January 2008 to April 2010. Standardized methods were used to process 357 catheter tips (325 neckline tips and 32 femoral tips). The most commonly isolated organism was *Staphylococcus aureus* of which 73% were MRSA and 52% positive tips had Gram negative bacilli. *Candida* spp. accounted for 7%¹².

Situation at community level

Data in relation to antimicrobial resistance in the community is scarce.

There were very few community awareness programmes in relation to antimicrobial resistance.

Probable reasons for the increasing trend of antimicrobial resistance

As most problems in health this is also multifactorial. Inappropriate and unrestricted use of antibiotics, prescription by unqualified personnel, nonavailability of well established methodology for monitoring of prescription policies, over-the-counter antibiotic sale, insufficient awareness among the general public about rational use of antimicrobials, insufficient resources and facilities for laboratory testing and information dissemination countrywide, lack of continuous supply of good quality antibiotics, poor post–marketing surveillance, problems related to drug registration and ordering policies, unnecessary and indiscriminate use of antibiotics in situations such as animal husbandry and fishery together with inadequate infection control practices make this a quagmire.

Future plans

- Expanding the ongoing antimicrobial resistance surveillance to the national level and using data generated by it for the formulation of the national antibiotic policy.
- Strengthening legal provisions to minimize the prescribing by unqualified personnel.
- Formation of a national alliance on antimicrobial resistance.
- Introduction of comprehensive community and healthcare personnel education on rational use of antibiotics with national coverage.
- Strengthening the inspection of pharmacies by authorized officers to prevent over-the-counter sales of antimicrobials.
- Assuring continuous supply of good quality antimicrobials.
• Strengthening antimicrobial Registration policies.
• Minimizing the irrational use of antimicrobials in agriculture and fishery by collaboration with the respective ministries.

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HIV-1 drug resistance among drug-naïve and HAART-treated patients in India: Current status

Pachamuthu Balakrishnan*, Shanmugam Saravanan*, Vidya Madhavan*, Greer Waldrop*, Syed H.Iqbal*, Sunil S.Solomon* and Suniti Solomon*

Abstract
Highly active antiretroviral therapy (HAART) has dramatically improved survival and quality of life among people living with HIV/AIDS globally. However, drug resistant mutations of HIV are seriously challenging the benefits of HAART, especially considering the heterogeneity of the epidemic in India. With the introduction of generic HAART, there has been a steep increase in patients initiating HAART in India. Drug resistant mutations easily evolve in the presence of sub-optimal adherence. It should also be noted that since most patients pay for medications out-of-pocket, interruptions in therapy due to monetary constraints are not uncommon. There is limited information on HIV drug resistance in resource constrained settings, like India, where the predominant circulating HIV-1 sub-type is C. The transmissibility of drug-resistant forms of the virus is also a major public health concern especially when formulating treatment guidelines. This article reviews published data available on the patterns of HIV-1 drug resistance among treatment-naïve and drug-exposed patients in India.

Key words: HIV drug resistance, non-B subtypes, antiretroviral drugs, primary drug resistance, HIV monitoring, India.

Introduction
India, with an estimated 2.3 million HIV-infected individuals (adult HIV prevalence: 0.29%) at the end of 2009, remains home to one of the largest populations of HIV infected persons globally and accounts for about half of Asia’s HIV-infected population.1,2 The prevalence of HIV infection is not uniformly distributed across India: Manipur in the Northeast (primarily an epidemic driven by injection drugs use) has the highest adult HIV prevalence of 1.4%, followed by Andhra Pradesh in the south (0.9%) where the epidemic is predominantly driven by heterosexual transmission - other states with high HIV burden include Mizoram (0.81%), Nagaland (0.78%), Karnataka (0.63%) and Maharashtra (0.55%). It is evident from the sentinel surveillance data that HIV in India is both geographically diverse and simultaneously driven by different modes of transmission in different regions.1 While there is data that clearly demonstrate a decline in the HIV incidence and prevalence among heterosexual populations, HIV prevalence appears to be increasing, or at best stable, amongst injecting drug users (IDUs) and men who have sex with men (MSM) in India.

The introduction of generic antiretrovirals (ARVs) in 2000 resulted in a steep increase in the number of persons initiating highly active antiretroviral therapy (HAART) primarily due to the reduction in cost of ART.6,7 The introduction of the government’s ART rollout...
programme has also improved access to HAART in India – it is currently estimated that over 400,000 persons have initiated HAART in India and 357,808 are currently alive and on therapy across the private and public sector. The impact of the improved access is clearly witnessed by improved survival among PLHIV in India.³

HAART prolongs survival and improves quality of life of people living with HIV and AIDS primarily by suppressing viral replication.⁸ In fact in the developed world, survival among people with HIV in the HAART era is comparable to HIV uninfected people.⁹ But the success of ART is dependent on optimal adherence to therapy, the failure of which results in the emergence of HIV drug resistance.¹⁰,¹¹,¹² Importantly, drug resistant viruses can be transmitted to newly infected individuals.¹³,¹⁴ Transmission of drug resistant HIV is a major public health concern, as it could lead to a scenario wherein persons newly infected with HIV might be resistant to commonly used first-line agents. This is particularly worrisome in the developing world where there is limited access to second-line drugs. This review summarizes the common factors contributing to HIV drug-resistance and the extent of HIV-1 drug resistance among treatment naïve and treatment-experienced patients in India.

HIV-1 pol gene diversity in India

India is the first Asian country outside Africa where HIV-2 and HIV-1/HIV-2 mixed infections have been reported.¹⁵ However, there is limited evidence of HIV-2 in the Indian sub-continent – with more than 99% of infections in India attributed to HIV-1. The most common subtype of HIV-1 in India is subtype C.¹⁶,¹⁷,¹⁸ However, investigators have reported other subtypes as well. A study from Mumbai demonstrated the presence of A-C inter-subtype recombinants with 2% and, subtype A and CRF01_AE were also observed.¹⁶ Strains belonging to subtype A1 and A1C recombinants were also observed in Pune, India (Figure 1).³⁹

Evolution of HIV-1 drug resistance

There are numerous factors that result in the development of drug resistant strains of the virus. The high replication capacity of HIV and its error-prone transcription is a major factor contributing to the development of resistance. It has been shown that retroviral replication is a highly error-prone process with varying estimates of roughly 7x10⁻⁶ to 1.4x10⁻⁴ base-pair substitutions occurring per nucleotide per replication cycle.¹⁹,²⁰,²¹ Another significant source of genetic variation is recombination. Recombination between HIV-1 genomes has been demonstrated and probably occurs in vivo as a result of simultaneous infection of an individual by two different HIV-1 strains.²²,²³,²⁴ As the first therapeutic regimen is probably the most important for virologic suppression, drug resistant variants of HIV challenge the efficacy of HAART to suppress viral replication adequately.⁸ In settings of incomplete viral suppression, drug resistant mutations evolve resulting in the development of drug resistant strains.²⁵ Also, impaired drug absorption (e.g., taking proton pump inhibitors can impair absorption of atazanavir), drug interactions (e.g. co-administration of rifampin and nevirapine) and drug metabolism can influence the development of HIV drug resistance. The observed degree of HIV-1 genetic diversity may also be influenced by selective pressure, such as the host’s immune response, cell tropism of the virus and the genetic makeup of the host.²⁶

Adherence to ART remains, perhaps, the most important and certainly the most preventable factor contributing to the development of resistance. Patients in India commonly interrupt ART as most patients pay for ART out-of-pocket.²⁷ Patients also tend to combine their ART with drugs from alternate systems of medicine– interactions that are yet to be quantified.²⁸ As many patients access care in the private sector, prescriptions of sub-
Transmission of HIV-1 drug resistance strains/primary drug resistance in India

Transmission of drug-resistant HIV-1 has been observed in most countries where antiretroviral treatment is available and it jeopardizes the success of antiretroviral therapy. Indeed, transmitted drug resistance generally leads to a delay in virologic suppression and to an increased risk of earlier virologic failure. Although the transmission of drug-resistant strains of HIV has been well documented in developed nations, there are concerns with...
testing chronically infected patients, after years of dormant HIV, since drug-resistant mutations will disappear over time in the absence of drug selection pressures and would hence be undetectable by standard resistance assays. Drug-resistant mutations also become undetectable if the infecting strains revert to wild type or become overgrown by fitter wild-type viruses; the resistant strains then persist as archived viruses or as minority species and that may not be detectable by current assays until selective drug pressure is present.33

Despite widespread ARV use, limited information is available on the prevalence of HIV-1 drug resistance in India, with surveillance and monitoring reports coming only from a handful of major cities.16, 17, 18, 37 As treatment programmes are expanded, the prevalence of primary drug resistance might be expected to increase as has been observed in the developed world. The prevalence of HIV-1 drug resistance among treatment-naïve patients (primary drug resistance) is of paramount importance in informing national policy of appropriate first-line agents. Table 1 presents a review of the literature of primary drug resistance in India.

**HIV-1 primary resistance to nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs**

Between 2004 and 2010 approximately 16 studies have attempted to quantify the prevalence of primary drug resistance across seven sites. The sample size of these studies ranged from 12 – 107; the studies were predominantly conducted among heterosexual populations with the exception of the study by Iqbal and colleagues that was conducted among IDUs in Chennai. The prevalence of primary drug resistance to reverse transcriptase drugs ranged from 0.0% – 78.3%; resistance to lamivudine (3TC) – presence of M184V – was the most common mutation observed.

A 2004 study by Deshpande et. al; from Mumbai has shown that two isolates out of 128 (1.6%) had the M184V mutation, indicating primary drug resistance to 3TC.16 This primary resistance was detected in the early stages of HIV disease (CD4+ T- cell count more than 400/ul) among patients recruited to the study in 2003. A 2004 phenotypic study by Hira et. al; conducted in Mumbai has shown a higher prevalence (6.7%) of primary drug resistance to reverse transcriptase inhibitors.37 The study patients (14/208) were recruited between 1997-2003 and were referred from different clinics in and around Mumbai. The resistance profiles were as follows: 2 (4%) isolates were resistant to AZT, 8 (3%) isolates were resistant to 3TC, 3(11 %) isolates were resistant to NVP and one isolate was resistant to d4T. The Hira et. al 2004 results were not reported in the tables as the results, unlike the other reports, were based upon isolates percentages rather than samples. Rajesh et al of Chennai also reported low rates of resistance in 2010 with 2.8% of 107 co-infected HIV and TB drug-naive patients showing resistance to NRTIs.38

The 2005 study by Sachdeva et. al. from Chandigarh, India, demonstrated the presence of coexisting strains of wild type and drug-resistant mutants among drug-naive patients, attending voluntary counseling and testing center (VCTC) between 2000–2002.39 These patients were recruited between 1999–2001. Similarly, genotypic studies by Balakrishnan et. al from Chennai, India, in 2005 and by Chatterbhuj et. al in Mumbai (2010), India, among treatment-naïve failed to detect any primary drug resistance.17,40 Yet these three studies, despite observing 0% drug resistance, revealed amino acid substitutions at drug resistance positions. Similarly, Arora et. al from Chandigarh (2008), India, Lall et. al from Pune (2008), India, and Neogi and colleagues from Bangalore (2010), India, reported low levels of primary NNRTI resistance 2%, 5% and 4.8% respectively while also observing polymorphisms at known drug resistance positions.41,18,42
The 2005 study by Sachdeva et al. from Chandigarh, India, has demonstrated the presence of coexisting strains of wild type and drug resistant mutants among drug-naïve patients, attending voluntary counseling and testing centres (VCTC) between 2000 – 2002.43 A highly sensitive nested amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for the detection of the minority population of zidovudine- and lamivudine-resistant variants of HIV-1 using pro-viral DNA from Peripheral Blood Mononuclear Cells (PBMC). For the detection of zidovudine resistance they used the two most common resistance-associated codons 70 and 215 primer, while for lamivudine they used codon 184 specific primers.44,45,46 Of 60 patients studied, they found a very high prevalence (32%) of M184V/I point mutations. Of these patients, 16 (84%) showed the presence of both Valine (Val) and Isoleucine (Ile) variants (26.67% of the total patients) and three (16%) showed Val (5.0% of the total patients) coexisting with the wild type. Interestingly, they also observed a high level (78.33%) of K70R thymidine analogue mutation, which along with another TAMs could potentially confer class-wide resistance to NRTIs. Sen et al, from Pune, India, also analysed proviral DNA of PBMCs as well as plasma isolates in two separate publications in 2007.47,48 Both publications revealed no drug resistant mutations in drug-naive patients, however, this approach of testing both the proviral DNA and the circulating virus in plasma represents an important methodological trend which continues to be important for identifying archived mutations which have become undetectable in the circulating virus.

Fleury et. al 2006 found high rates of genotypic and phenotypic resistance in isolate from Mumbai, however the total sample size was less than 20 samples.49

HIV-1 primary resistance to protease inhibitors drugs

Hira et. al (2004), initially showed 2.5% (2/79) prevalence of SQV primary drug resistance.37 Since then, other reports have also shown low levels of primary PI resistance. Balakrishnan et. al (2005) reported 20% of the population had mutations at codons conferring PI resistance,17 followed in 2008 by Arora et. al and Lall et. al, who reported 2% and 2.5 %, respectively.41,18 Furthermore, Iqbal et. al (2009) reported 2.5% IDUs with primary resistance to PIs in Chennai, India.50 Yet, in 2008 Kandathil et. al focused only on the prevalence of PI mutations in 35 patients and found no major resistance mutations.51 The limited sample sizes of these reports quantifying primary resistance to PIs, requires one to interpret these findings with a certain degree of caution.

Given that the majority of patients in India are initiated on a combination of NRTIs and an NNRTI and the use of PIs is limited, it would be expected that primary drug resistance to PIs will be limited. However, with the availability of PIs via the government’s is free rollout programme, primary drug resistance to PIs might be expected to increase in the coming years.

HIV-1 secondary drug resistance in patients exposed to ARVs

HIV-1 secondary resistance to nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs

Between 2004 and 2010 approximately 11 studies have attempted to quantify the prevalence of secondary drug resistance across only five sites (Table 2). The sample size of these studies ranged from 3 – 200; the studies were predominantly conducted among heterosexual populations. The prevalence of secondary drug resistance to reverse
transcriptase drugs ranged from 17% – 100%. As in the naïve populations, resistance to lamivudine (3TC) – presence of M184V – was the most commonly observed mutation.

At virological failure the NRTI and NNRTI resistant mutations are reported for NRTI at 78.79%, 95%, 92.2%, 93% and for NNRTI at 80.65, 95%, 62.7%, 93% by Sen et al, 2007, Kandathil et. al, 2009, Gupta et. al; 2010, and Rajesh et. al; 2009 respectively. At immunological/clinical failure, based on WHO guidelines, Despande et. al, 2010 and Madhavan et. al, 2009, have reported 92%, 94% resistance to NRTIs, respectively. Furthermore, resistance to NNRTIs were reported from the same authors as 100%, 94% and 65%, respectively. The study by Madhavan et. al, 2009 is especially significant as the sample size was the largest in the published literature at 200.

The methodology of comparing drug resistance in the PBMC proviral DNA and circulating virological genetic material (plasma) demonstrated interesting results among treatment-experienced patients. Sen et. al reported 87.5% disagreement between paired PBMC proviral and plasma data, suggesting a significant compartmental component to HIV drug resistant mutation analysis.48

HIV-1 secondary resistance to protease inhibitors drugs

Given the low use of PIs (primarily due to cost) in the management of HIV disease in India, there are limited studies of resistance to PIs among treatment-experienced patients in India. However, four studies reported resistance to PIs ranging from 8% to 50%; the sample size for these studies ranged from 3 to 51.47,51,52,53

Recently, a cross-sectional study was conducted in Chennai among 45 individuals undergoing second-line antiretroviral treatment (ART). The patients were subjected to RT & PR sequencing. Of those 45 individuals, 33 (73%) had any one of the PI mutations, 41 (91%) had NRTI mutations, 33 (73%) had NNRTI mutation and 30 (66.7%) had both NRTI and NNRTI mutations. The mutations observed suggests that darunavir (sensitivity 100%) was the least affected PI followed by tipranavir (sensitivity 98%), lopinavir (sensitivity 53%), atazanavir (sensitivity 42%) and <40% sensitivity to other PIs.56

HIV-1 drug resistance in pediatric patients

Knowledge on HIV-1 drug resistance among children is scarce, especially in India. Soundararajan et. al reported no significant drug resistant mutations among 28 treatment-naive children from south India; all samples demonstrated polymorphisms.57 Contrastingly, in a study from north India, Sehgal et. al reported a high rate of K103N (33%) mutation among naive children using highly sensitive ARMS PCR. Kurle et. al (2007) examined 19 and 13 samples collected at 48 h and 2 months postpartum, respectively, from infants that were given single-dose nevirapine (sd-NVP). They observed high levels of NNRTI mutation (10.5% in 48 hrs and 46.15% at 2 months).58

HIV-2 drug resistance in India

Though many reports indicate HIV-2 infection in India, Maniar et. al, 2006, remains the only one to report HIV-2 drug resistance in a single HIV-1 and HIV-2 co-infected individual. This case presented with undetectable HIV-1 circulating RNA while simultaneously reporting mutations of M184V, Q151M, V71I and L90M in HIV-2.59

The role of laboratory monitoring assays in HIV drug resistance

High-income countries view ART monitoring with viral load and genotypic resistance testing as mandatory elements of patient care. But a
limited availability of assays for routine determination of plasma HIV-1 RNA levels and for detecting drug resistance, acquired and transmitted drug resistance in resource-limited settings present formidable challenges.

In order to limit the emergence of resistance to antiretroviral drugs, HIV treatment should ideally be accompanied by periodic virological and genotypic monitoring. However, in resource-limited countries, viral load monitoring and drug resistance tests are not yet available at an affordable cost and capital for infrastructure requirements limits the scale-up of access to these tests.60 Treatment initiation and modifications are thus guided, when available, by indirect clinical markers of disease progression and CD4 cell counts.61 As a consequence, many patients with virological failure remain on ineffective drug regimens for long time periods, resulting in a scenario ideal for accumulating drug-resistant mutations. Rapid or uncontrolled emergence of HIV drug resistance is thus feared as a potential consequence of the ART scale-up without proper monitoring in resource-limited countries.62 In lieu of expanded access to ARV amongst the Indian HIV-infected population, the context for ART management and monitoring must also be expanded to or adapted to fit the constraints of the resource-limited setting.

Furthermore, there are stringent requirements for storage and transport of plasma, making the option of shipping samples to nearby laboratories difficult. However, use of dried blood spots (DBS) rather than plasma as a source of viral genetic material provides a hopeful alternative for the research-limited setting. Recently, a number of studies have demonstrated the feasibility and reliability of using DBS to monitor viral load and genotypic resistance.63-65 Although studies report good agreement between plasma and DBS genotypes, cell-associated proviral DNA in DBS can also contribute to the amplified PCR products, which adds to variability.66,65

In addition to the use of DBS, implementing in-house assays for molecular diagnostics can help improve the possibility of increased genotypic testing in resource-limited settings. Many in-house genotyping assays with low running cost have been designed and were found to have a high success rate (>&85.3%) of amplifying and sequencing subtype C and non-C HIV-1 samples.67,68 These assays operate at a cost substantially lower than commercial assays ($100 vs. $230). Recently, Saravanan et. al, 2009, has validated the use of an in-house assay in India.69 The implementation of these kinds of in-house assays should substantially increase the use in resource-limited settings and reduce the risk of perpetuating drug resistance-related mutations.

Conclusion and future directions

The available literature on primary drug resistance in India is limited. But data that is available demonstrate a low prevalence of primary drug resistance, with certain studies reporting prevalence higher than 5%, the alert cut-off as defined by the ad hoc working group of the World Health Organization (WHO).

Antiretroviral drug resistance is present wherever antiretroviral drugs are widely used, and as treatment rollout continues in developing countries, the range of resistance will expand. In India, especially, there are limited studies, and moreover no reports from the government sector. The worldwide effort to improve treatment outcomes and reduce transmission of HIV through optimal delivery of ART and HIV prevention programmes must be coordinated with and enlightened by ongoing national, regional, and global evaluations of HIV drug resistance and this should inform treatment guidelines and provide feedback on the success of HIV-1 treatment and prevention programmes. Although it is clear from the currently available reports that among the untreated HIV-1 patients, the prevalence of known drug resistance mutations is low in
India, it is important to routinely implement the threshold survey and based on the experience of the survey, an expanded HIV drug resistance surveillance system can be implemented to support the ART programme as it continues to scale up. Adherence interventions and controlling prescriptions (continuity between doctors and pharmacies) will continue to be important components to decrease rates of drug resistance.

The following should be considered for improving the monitoring system:

1. Survey with the inclusion of recently-infected individuals as reversion of transmitted drug resistance will be minimized.

2. As the majority of epidemiological studies have used population-based genotypic testing, this fails to detect and quantify minorities of drug-resistant quasispecies below 25%. Hence, studies with the highly sensitive assay such as ARMS-PCR and Pyrosequencing would reveal the actual size of the problem of transmitted resistance.

3. Inclusion of the polymorphic substitutions could overestimate the size of the problem as a majority of the earlier studies had interpreted with the subtype B database. Hence, the subtype specific polymorphisms should be established and documented.
<table>
<thead>
<tr>
<th>Site</th>
<th>Author</th>
<th>Year</th>
<th>Sample Size</th>
<th>Resistance Profile</th>
<th>Resistance Mutations</th>
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<td>Year</td>
<td>Sample Size</td>
<td>Resistance Profile</td>
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<td>Vellore</td>
<td>Kandathil et al</td>
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<td>Iqbal et al</td>
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<td>37*</td>
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<td>Chennai</td>
<td>Rajesh et al</td>
<td>2009</td>
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# Genotyping with PBMC specimens.
## Samples from Punjab, Haryana, Himachal Pradesh, Jammu & Kashmir and Chandigarh.
* IDUs.
** TB and HIV Co-infected patients.
Table 2: Summary of Drug Resistant Mutations and its frequency Among ARV-exposed HIV Patients in India

<table>
<thead>
<tr>
<th>Site</th>
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<th>Year</th>
<th>Sample Size</th>
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<th>Resistance Profile</th>
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<td>TAMs</td>
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<td>Delhi</td>
<td>Chaudhary et al</td>
<td>2010</td>
<td>6</td>
<td>17%</td>
<td>17%</td>
<td>M184V (17%)</td>
<td>Y181C (17%)</td>
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<td>D67N (18%), M41L (16%), T215Y (14%), T215F (4%), T215I (2%), L210W (2%),</td>
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<td>K103N (16%), K101E (8%), K103S (2%), V108I (8%), Y188C/L (4%), K238T (2%)</td>
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<td>M184V (40%), K70R (12%), K219E (8%), K219Q (6%), E44D (4%), T69D/A/N (2%), L74V (2%), V75M (2%), F116Y (2%), Q151M (2%)</td>
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<td>K103N (16%), G190A (14%), A98G (12%), K101E (8%), K103S (2%), V108I (8%), Y181C/L (4%), K238T (2%)</td>
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<td>Pune</td>
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<td>72.22%</td>
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<td>D67N (32%), M41L (23%), T215Y (14.7%), D67G (5.8%), T215I (2.9%)</td>
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<tr>
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<td>80.65%</td>
<td>50% (of those on PI) (2/4)</td>
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<td>M184V (7.6%), K219E (8.8%), V18I (8.8%), L74V (5.8%), F116Y (5.8%), Q151M (5.8%), M230I (2.9%), F227L (2.9%), K219E (2.9%), I210S (2.9%), F77L (2.9%), L75M (2.9%), L74I (2.9%), K70Q (2.9%), T69D/A/N (2.9%), A28V (2.9%)</td>
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Note: TAMs = NRTI, PI
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<tr>
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<td>200</td>
<td>94%</td>
<td>D67N (40%), M41L (38%), K70R (24%), T69T/N (13%), L74I (5%), 151Q-complex (8.5%), K65R (7%), L74V (53%), K219E/N (13%), T215F (12%)</td>
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<td>Y181C (39%), K103N (34%), G190A (27%), A98G (14%), K101E/P (11%), V106M (10%), M108I (10%), Y188L/H/C (16%), P225H (2.5%), E138K (2.5%), F227L (2%), G190E/S (1%), V179D (1%), M230L (1%), M184V (76.5%), T215Y (24.5%), E44D/V118I (21%), K219E/Q (16%), T215F (12%), L210W (5.5%), D67N (40%), M41L (38%), K70R (26%), T69T/N (13.5%), L74V (9.5%), Q151M (8.5%), K65R (8%), T69D (1%), A98G (34%), G190A (27%), E44D/V118I (21%), K219E/Q (16%), T215F (12%), L210W (5.5%), D67N (40%), M41L (38%), K70R (26%), T69T/N (13.5%), L74V (9.5%), Q151M (8.5%), K65R (8%), T69D (1%)</td>
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<td></td>
<td>Rajesh et al</td>
<td>2009</td>
<td>18-failing ART</td>
<td>95%</td>
<td>M184V (90%), N46I (20%), K65R (9%), G151M (5%), F116Y (5%), Y115F (5%), F77L (5%), V75I (5%), D67N (40%), N46I (20%)</td>
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<td>K103N/Q (45%), L90M (10%), D240N (10%), P229H (5%), V106M (5%), V106A (5%), K103E/Q (12%), K103N/Q (45%), L90M (10%), D240N (10%), P229H (5%), V106M (5%), V106A (5%)</td>
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<tr>
<td>Chennai</td>
<td>Rajesh et al</td>
<td>2009</td>
<td>15-failing ART</td>
<td>93%</td>
<td>M184V (67%), K65R (7%), L74I (5%), K219E/N (1.3%), V106M (27%), V108I (20%), Y181C (47%), G190A (27%)</td>
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<td>K103E/Q (12%), K103N/Q (45%), L90M (10%), D240N (10%)</td>
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</tbody>
</table>

**Southern Region**

**TAMs**

- Vellore
- Chennai

**Non-TAMs**

- Vellore
- Chennai

**Sample Size**

- 3
- 18-failing ART
- 15-failing ART

**Resistance Profile**

- 33%
- 95%
- 93%

**Resistance Mutations**

- D67N (40%), M41L (38%), K70R (24%), T69T/N (13%), L74I (5%), 151Q-complex (8.5%), K65R (7%), L74V (53%), K219E/N (1.3%), V106M (27%), V108I (20%), Y181C (47%), G190A (27%)
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clade C HIV-1 infected individuals from India.

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from Pune, India.

mutations in antiretroviral treatment-experienced

patients from Pune, India.

mutations in antiretroviral treatment-experienced

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Drug resistance in tuberculosis in South-East Asia

Vineet Bhatia*, Md Khurshid Alam Hyder* and Nani Nair*

Abstract

The South-East Asia Region (SEAR) of WHO bears around one third of the global burden of multidrug-resistant (MDR-TB). Extensively drug-resistant TB (XDR-TB) has also been reported from five countries in the Region. Evidence suggests that drug resistance is essentially a man-made phenomenon because of inadequate or poorly administered treatment. Current treatment regimens recommended under DOTS cure TB patients and prevent emergence of resistance. Even though countries in the Region have 100% geographical coverage, access to DOTS services for marginalized and vulnerable populations remains an issue. International Standards of TB Care is not yet adopted by all providers. For existing resistant cases there is limited capacity and experience in diagnosing and managing MDR-TB cases. Limited laboratory capacity for diagnosis of drug resistant cases and for surveillance, difficulties in procuring quality second-line drugs and long lead times for procurement are some of the constraints. Substantial additional resources are required to scale up programmatic management of drug resistant TB.

Several steps are required to simultaneously scale up diagnosis, treatment and surveillance of MDR and XDR-TB. These include technical and financial support to countries by WHO, technical partners and funding agencies; programme efforts to ensure implementation of all elements of the Stop-TB strategy including mobilization of sufficient resources; regulatory measures to ensure rational use of drugs; an infection control policy to prevent spread and community mobilization to create support structures for TB, MDR-TB and TB-HIV co-infected individuals.

Global and Regional situation of MDR-TB

Multidrug-resistant TB (MDR-TB) is caused by bacteria that are resistant to at least isoniazid and rifampicin, the most effective anti-TB drugs. MDR-TB results from either primary infection with resistant bacteria or may develop in the course of a patient’s treatment.

WHO estimates that globally, 440 000 MDR-TB cases emerged and 150 000 deaths were caused by MDR-TB in 2008. Well-functioning national TB control programmes in

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the South-East Asia (SEA) Region achieving high cure rates has resulted in maintaining the slow but steady decline in TB incidence rates during the past decade. This has also led to low levels (Range: 1.7%- 4.2%) of multidrug-resistance among newly detected cases. Among previously treated cases in the Region, MDR-TB rates range from 10.0% - 34.7%. However, given the large numbers of TB cases, this translates to 130 000 cases, (110 000–170 000) accounting for more than one third of the world’s MDR-TB cases in the SEA Region, with India estimated to have the second highest number globally (Figure 1). The country-wise estimated burden of MDR-TB is presented in Table 1.
Figure 1: Distribution of MDR cases as per WHO regions

Table 1: Estimated MDR-TB cases and rates in SEAR Member States, 2010

<table>
<thead>
<tr>
<th>Country</th>
<th>Source of estimates</th>
<th>% MDR among new TB cases (95% CI)</th>
<th>% MDR among previously treated TB cases (95% CI)</th>
<th>Number of MDR-TB among incident total TB cases (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>model</td>
<td>2.2 (0.0–5.6)</td>
<td>14.7 (0.0–39.6)</td>
<td>9 800 (1 000–19 000)</td>
</tr>
<tr>
<td>Bhutan</td>
<td>model</td>
<td>2.2 (0.0–5.6)</td>
<td>14.7 (0.0–39.6)</td>
<td>33 (4–61)</td>
</tr>
<tr>
<td>DPR Korea</td>
<td>model</td>
<td>2.2 (0.0–5.6)</td>
<td>14.7 (0.0–39.6)</td>
<td>3 900 (658–7 200)</td>
</tr>
<tr>
<td>India</td>
<td>DRS, a 2005</td>
<td>2.3 (1.8–2.8)</td>
<td>17.2 (14.9–19.5)</td>
<td>99 000 (79 000–120 000)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>DRS, b 2004</td>
<td>2.0 (0.5–6.9)</td>
<td>14.7 (0.0–39.6)</td>
<td>9 300 (0–21 000)</td>
</tr>
<tr>
<td>Maldives</td>
<td>model</td>
<td>2.2 (0.0–5.6)</td>
<td>14.7 (0.0–39.6)</td>
<td>3 (0–6)</td>
</tr>
<tr>
<td>Myanmar</td>
<td>DRS, 2007</td>
<td>4.2 (3.2–5.6)</td>
<td>10.0 (7.1–14.0)</td>
<td>9 300 (6 400–12 000)</td>
</tr>
<tr>
<td>Nepal</td>
<td>DRS, 2007</td>
<td>2.9 (1.9–4.3)</td>
<td>11.7 (7.6–17.6)</td>
<td>1 700 (990–2 300)</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>DRS, 2006</td>
<td>0.2 (0.0–1.0)</td>
<td>0.0 (0.0–1.0)</td>
<td>63 (0–130)</td>
</tr>
<tr>
<td>Thailand</td>
<td>DRS, 2006</td>
<td>1.7 (1.1–2.6)</td>
<td>34.5 (28.2–41.5)</td>
<td>2 900 (2 100–3 800)</td>
</tr>
<tr>
<td>Timor-Leste</td>
<td>model</td>
<td>2.2 (0.0–5.6)</td>
<td>14.7 (0.0–39.6)</td>
<td>130 (6–260)</td>
</tr>
</tbody>
</table>

a DRS Survey in Indonesia was completed for Mimika District (2004) and Central Java province (2006).
b Mimika district: MDR-TB in newly diagnosed TB cases: 2.0 %.

Central Java province: preliminary result; MDR-TB in newly diagnosed TB cases was: 1.8 % and among previously treated TB cases was: 16.7 %.

DRS = drug resistance surveillance or survey data; CI = confidence interval; MDR-TB = multidrug-resistant TB
Extensively drug-resistant TB (XDR-TB) has also been reported from five countries in the Region. MDR-TB could potentially replace drug-susceptible TB, and constitutes a threat to global public health security. In areas of high HIV prevalence, the potential for increased transmission of MDR-TB is high.

Considerable efforts are required to expand capacity for quality assured drug susceptibility testing in the Region in order to more accurately estimate the extent of MDR- and XDR-TB. Given the widespread availability and use of second-line drugs, and as laboratory capacity to conduct second-line drugs susceptibility testing increases, additional numbers of patients with XDR-TB are likely to be identified.

### Factors influencing emergence of resistance to TB drugs

Evidence suggests that drug resistance is essentially a man-made phenomenon though multiple factors may be involved. Generally it is an inadequate or poorly administered treatment that causes resistance although MDR-TB can then spread from person to person. Some of the many potential causes of resistance are:

- Sub-standard regimen administration particularly when there are a large number of health care providers outside the national programme.
- Failure to directly observe treatment and specifically situations leading to non-adherence and default of TB patients.
- Poorly organized or funded TB control programmes.
- Poor quality of drugs and/or interrupted drug supply.
- Socio-economic or cultural barriers to access diagnosis/ treatment.
- Inadequate infection control measures at health facilities/hospitals.

Though it has not been possible to establish a direct association between MDR-TB and the HIV epidemic because of missing data from several countries, in areas of high HIV prevalence and MDR-TB, the likelihood for increased transmission of MDR-TB is high.

Most of these factors are playing an important role in the Region. This is evident from the fact that:

- With a case detection rate of 65% of all cases, more than one third of estimated new cases are not registered by NTPs in the Region.
- While the geographical coverage for DOTS in all Member States has reached 100%, there are challenges to access for several pockets of populations due to various reasons.
- The private sector is the first contact for 65% of TB patients in India and 73% in Myanmar as per studies in the Region. A study in Indonesia also reveals that the majority of people in rural areas preferred private practitioners for treatment of TB. Despite significant progress (Figure 2) the involvement of the private and other health sectors in TB control in the Region is far from being optimal.
- Evidence also suggests that treatment success rates in the private sector (unless a part of public-private PPM initiatives) are usually below 50%.
- Less than 5% of the estimated MDR-TB cases are registered for treatment by NTPs. This means that a huge proportion of cases are either not getting treatment or being treated under unknown conditions with high chances of a non-standardized regimen.
Figure 2: Anti Microbial resistance in SEA Region

Tea estates, factories, railways, Ministries of Shipping, Mines, Petroleum and Oil, Railways, Defence, Religious affairs, Labour, Education and Home Affairs

Medical associations in Bangladesh, India, Indonesia, Myanmar, Thailand

National TB Programmes in the SEA Region

> 2500 NGOs

> 25 000 private providers

> 360 medical colleges, 1500 private and public hospitals

Several thousand community initiatives

Over 200 corporate houses

Figure 3: Market of anti-TB drugs in some countries

Ref: Global alliance for TB drug development, May 2007. Pathway to patients: Charting the dynamics of the global TB Drug Market
Poor drug regulation-TB drugs (both first-and second-line) are available over the counter in several countries in the Region. (ref study report Figure 3)

The health infrastructure is overburdened, especially overcrowded hospitals with no infection control policy.

Several countries in the Region face poor housing conditions and specifically overcrowding in urban areas that facilitate spread of infections6.

Preventing emergence of resistance

Steps to prevent the emergence of resistance include:

(1) Strengthening DOTS – All countries in the Region have 100% coverage under DOTS and are thus providing uniform use of standard regimens, free diagnosis and treatment, DOT, strict monitoring of treatment, defaults, outcomes and use of fixed drugs combinations (FDCs). Providing standardized treatment under the DOTS strategy is one of the foremost measures that needs to be adopted to prevent emergence of resistance. The programmes should now strive to provide universal access.

(2) Involvement of all care providers and provision of services as per International Standards of TB Care (ISTC). Treatment success rates amongst TB patients in private–public mix have been found to be comparable to public health settings7. Thus, multi-sector involvement is essential in prevention of drug resistance.

(3) Promoting rational use of drugs and pharmacovigilance. This would be another key area in preventing emergence of resistance. Countries would need to undertake situational analysis that involves - evaluation of prescription policies in health-care settings in public and private sectors and utilization of antimicrobial agents at various levels; assessing therapeutic and non-therapeutic use in animals and appraise impact of pharmaceuticals promotion8. Pharmacovigilance is defined by the World Health Organization (WHO) as “the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems” and involves strengthening of technical and regulatory requirements along with bringing about a change in the behaviour of prescribers and users. Countries need to promote optimal prescription; develop standard national/local treatment guidelines (STG) advocating evidence-based therapy; train professionals in the use of these STGs and assure use of STGs through hospital committees. Preventing over-the-counter availability of TB drugs is specifically important for promoting rational drug use.

Preventing the spread of drug resistance

(1) Infection control measures: Infection control policies and plans are being pursued in six countries in the Region. All countries need to adapt the international guidelines in the local context.

(2) Early diagnosis of drug resistant cases: This would mean strengthening the lab infrastructure and corresponding human resource.
capacity to undertake drug resistance surveys and susceptibility testing.

All Member States (with the exception of Maldives and Timor-Leste) have capacity for mycobacterial culture. However, capacity is quite limited even in these countries. The national reference laboratories in Bangladesh, Indonesia, and Myanmar have recently been accredited for quality assurance for culture and drug susceptibility testing, while Sri Lanka is in the process of upgrading the national reference laboratory for TB.

The national reference laboratories at the Tuberculosis Research Centre, Chennai, India, and at the Bureau of TB at Bangkok, Thailand, are the two designated supranational TB reference laboratories in this Region. These labs are also undertaking DST for second-line anti-TB drugs to determine the extent of XDR-TB. Reference laboratories in Bangladesh, Indonesia, Myanmar and Nepal are also engaged in rapid surveys for XDR-TB among mycobacterial isolates from patients who have failed re-treatment regimens, through linking with the SNRLs in the global network.

(3) Adequate management of MDR-TB cases: During the past two years, steady progress has been made in the Region in initiating MDR-TB cases on treatment. The Green Light Committee had approved the case management of patients with MDR-TB under national programmes in nine countries. Bangladesh, India, Indonesia and Myanmar are in the process of expanding these services, while Nepal has already established ambulatory case management services for MDR-TB throughout the country. Maldives continues to treat the few cases that occur on a case-by-case basis. Bhutan, Sri Lanka and Thailand will begin enrolling cases later in 2010, while DPR Korea will apply to the Greenlight Committee (GLC) to establish MDR-TB case management under their respective national programmes in 2010. Countries would need to choose an appropriate model for ongoing MDR-TB care where a selection and balance between need for hospitalization and community-based treatment has to be met.

(4) TB-HIV programme collaboration – TB and HIV programmes need to further strengthen cross-referrals and adequate management of co-infected cases. This will now assume more importance with the emerging challenge of drug resistance.

Challenges

There are unique challenges to tackling the spread of TB drug resistance in the Region as highlighted below:

(1) **Gaps in basic TB control**: DOTS has been recognized and recommended as standard for TB treatment globally and key for preventing emergence of resistance. Though countries in the Region have 100% geographical coverage through programmatic structures, access to services for the entire population remains an issue, particularly for marginalized and vulnerable populations. There is also sub-optimal access to quality first-line drugs through several
sectors and providers outside the programmes. International Standards of TB Care (ISTC) is not yet widely used by all providers.

(2) **Diagnosing and managing MDR-TB**: In the absence of substantive evidence from national drug resistance surveys (DRS) in many countries, the understanding of the burden of MDR/XDR-TB is based on “best estimates”. Most countries in the Region have limited laboratory capacity for diagnosis of drug-resistant cases and for DRS. Even countries with culture and drug susceptibility testing facilities do not have not enough quality assured laboratories. There is limited capacity and experience in managing MDR-TB cases. Further, many countries face difficulties in procuring quality second-line drugs and long lead times for procurement. Involvement of private and other labs is not easy. Costs for paying for private services are exorbitant (e.g. India) estimated a cost of US$ 20 million for 5% of cultures required for 30 000 MDR-TB cases!) Overall, substantial additional resources need to be mobilized to manage a relatively small number of patients (including training, drugs and service delivery, etc.) which, in turn, inflates the programme budget and countries have to look for additional external funding.

**Regional priorities**

The first priority in dealing with MDR-TB remains prevention of acquired drug resistance through continuing to ensure higher case detection and cure rates using high quality of DOTS services. Secondly, attention needs to be paid to developing comprehensive national plans for the urgent scale-up of diagnostic and case management capacity for MDR-TB, conforming to internationally recommended protocols, including good infection control measures.

In the context of both of the above, the priorities in the Region are:

- Securing adequate external as well as domestic funds, (including from local governments under decentralized systems) for all aspects of TB control;
- Urgent attention to building health systems capacity: skilled personnel and quality infrastructure, focusing on laboratory capacity for diagnosis, and surveillance;
- Supporting countries to develop updated guidelines for MDR-TB diagnosis and case management and treatment regimen in line with international standards for TB care (ISTC) in place in all sectors;
- Encouraging countries to strengthen legislative measures to ensure rational use of drugs;
- Securing adequate quantities of quality assured first- and second-line anti-TB drugs for uninterrupted treatment of the planned number of MDR-TB cases; and
- Increasing the number of manufacturers in the Region meeting WHO pre-qualification or national drug regulatory standards, equivalent to international standards.
References and bibliography


Antimalarial drug resistance is of great concern in the WHO South-East Asia (SEA) Region. A high degree of resistance of *Plasmodium falciparum* to chloroquine and sulfadoxine-pyrimethamine is prevalent in this Region. Multidrug resistance is prevalent in some parts of the Greater Mekong Sub-region. Artemisinin and its derivatives in combination with other effective partner drugs, which showed fastest parasite and fever clearance time, have been introduced in all the countries in the Region. Emergence of artemisinin resistance at the Thai-Cambodia border has been reported recently. *Plasmodium vivax* is sensitive to chloroquine in all the countries except in Indonesia. WHO supports and coordinates global management of drug resistance. It provides technical and financial assistance for therapeutic efficacy studies that serve as the basis for updating malaria treatment policy. It supports national malaria control programmes and other partners in implementing strategies to contain and prevent further spread of artemisinin combination therapy (ACT)-resistant parasites.

Introduction
Malaria is endemic in all countries of the SEA Region except Maldives. Of the 1745 million total population in this Region, 76% are at risk of malaria. Antimalarial drug resistance is a major public health problem and is of great concern as it hinders the effective control of malaria. National malaria control programmes and research institutes in the Region, in collaboration with WHO, have intensified the monitoring of therapeutic efficacy of antimalarial drugs and provided evidence for the updating of national malaria treatment policy.

*Plasmodium falciparum* resistance to chloroquine and sulfadoxine-pyrimethamine is widespread in the Region. Multidrug resistance (resistance to three or more antimalarial compounds of different chemical classes) has been reported from the Greater Mekong Sub-region. *Plasmodium vivax*, the second most common parasite species in the Region, is still sensitive to chloroquine except in Indonesia where resistance is widespread. Chloroquine-resistant *vivax*, was also documented in India and Myanmar but not considered as a serious threat.

The most effective antimalarial drugs introduced for malaria control in the early 2000s are artemisinin and its derivatives. These drugs have the fastest parasite and fever clearance time compared to other antimalarial drugs. In order to prolong its effective life, WHO strongly recommended that artemisinin should be deployed in combination with other effective partner drugs (so called artemisinin based combination therapy – ACT). However, the use of ACTs is seriously threatened by the emergence of artemisinin resistance at the Thai-Cambodia border reported recently. The Thai-Myanmar border, Myanmar, Bangladesh and some north-eastern states of India are potentially vulnerable for the spreading of artemisinin-resistant strains through population movement across the international borders.
WHO's role in the global management of drug resistance is to develop, update and provide support for implementing standardized methods for assessing antimalarial drug efficacy on the basis of expert consensus and feedback from the field. For this, WHO has prepared guidelines for conducting drug efficacy trials at the country level and updating the same as and when required. All countries in the Region are following these guidelines for the routine drug surveillance system. It is found that the failure rate of the currently used ACTs is increasing on both sides of the Thai-Cambodian border, due mainly to local emergence of resistance to artemisinin derivatives. WHO is further investigating this problem and supports national malaria control programmes and partners in implementing strategies to contain and prevent the further spread of resistant parasites to neighbouring countries.

In 1977 the WHO Regional Office for South-East Asia (SEARO) initiated a special project “the Regional Collaborative Studies on Drug Resistant Malaria” to support Member States in conducting drug efficacy trials (both in vivo and in vitro tests). However following the termination of the project the work was meant to be carried out as routine activities by Member States. It was observed that drug resistance monitoring could not be sustained due to several operational factors. WHO developed standard protocols and template for therapeutic efficacy studies (TES) and supported countries to use the protocols. Three out of the five methods (in vitro test, in vivo test and genotyping) are being regularly applied by countries. Myanmar and Thailand that are participating countries of the Mekong Malaria Programme are doing well in terms of drug resistance monitoring. Following the
meeting organized by WHO in Bali, Indonesia in September 2010, other Member States in the SEA Region established a network of sentinel sites for TES that is now functioning. The network is important for long-term monitoring and understanding of the epidemiological pattern of drug resistance.

The map shows the levels of efficacy (Adequate Clinical and Parasitological Response – ACPR) of ACTs in the SEA Region from 2002-2009.

Status of antimalarial drug resistance, by country

Bangladesh

Emergence of drug resistance is posing a serious problem in Bangladesh. The degree of resistance of *P. falciparum* to chloroquine (CQ) has increased tremendously. Both in vitro and in vivo studies were carried out. Studies carried out from 1979 to 1995 showed an increasing trend of high degree of resistance from 10% to more than 70%. Chloroquine and sulfadoxine-pyrimethamine combination (CQ+SP) also showed more than 25% resistance against *P. falciparum*. Oral quinine plus SP combination showed variable degree of resistance from 13% to 30% against *P. falciparum*. Intravenous quinine showed acceptable level of resistance, which was only 6%. Mefloquine was not used routinely in Bangladesh but resistance of *P. falciparum* was found in in vivo test (treatment failure rate of 27%) and in vitro test (61% of total isolates studied). The efficacy of artemether-lumefantrine (an ACT) against *P. falciparum* was very high (ACPR about 93%) in 2005. The 42-day TES of ACT in 2007 revealed 99%-100% efficacy. The Malaria Control Programme switched from CQ to artemether-lumefantrine in 2004 but the widescale implementation of this combination started in 2007. This combination was tested again in a 28-day study during 2009 and results showed 98%-100% ACPR. The TES on *P. vivax* was not carried out as there were very few vivax cases, and no evidence of chloroquine-resistant *P. vivax* had been found in the country.

Bhutan

Therapeutic efficacy studies were conducted on both *P. falciparum* and *P. vivax*. There are five sentinel sites (two hospitals and three basic health units) at the southern district bordering India. The WHO study protocol was followed. All patients were followed up for 28 days and all *falciparum* cases are hospitalized for three days. Artemether-lumefantrine was introduced as the first-line treatment of uncomplicated *falciparum* malaria cases in 2004. The combination was tested annually from 2005 to 2009. Chloroquine for treatment of *P. vivax* cases was also tested annually from 2004 to 2009. Artemether-lumefantrine against *P. falciparum* and chloroquine against *P. vivax* provided very high (100%) ACPR but total cases studied were low especially during the last two years due to reduction of malaria incidence. An in vitro study was not conducted.

India

Chloroquine-resistant *falciparum* was reported throughout the country especially the northeastern states where high degree resistance was documented. Sulfadoxine-pyrimethamine-resistant *falciparum* was also reported but was found to be focal in distribution. The country switched the first-line treatment for uncomplicated *falciparum* malaria to ACT (artesunate + sulfadoxine-pyrimethamine) in 2008 in areas with high drug resistance and the regimen was implemented countrywide in 2009.

Drug resistance monitoring is being carried out regularly by the malaria control programme in collaboration with the National Institute of Malaria Research. Therapeutic efficacy studies were conducted on both *P. falciparum* and *P. vivax*. Eleven and two sentinel sites were assigned for *falciparum* and *vivax* cases, respectively. The most recent study conducted in 2009-2010 revealed that the
efficacy of currently used ACT (artesunate + sulfadoxine-pyrimethamine) on *falciparum* malaria was high (94%-100%) and the efficacy of chloroquine for treatment of vivax was 100%. Parasite clearance time and gametocytaemia pattern were studied. At some sentinel sites less than 80% of *falciparum* cases had the parasite cleared after 48 hours of initial treatment. Genotyping (MSP2) was done to differentiate between recrudescence and new infections. Molecular markers (Pfcrtk76t and dhfr) were studied at all sentinel sites.

**Indonesia**

The country has a long history of drug resistance since 1978. Widespread drug resistance of both *P. falciparum* and *P. vivax* to chloroquine has been reported throughout the country. This is the most important barrier to malaria control in the country. Drug resistance monitoring is being conducted on a regular basis on both parasites on currently used treatment regimen. Moreover, alternative drugs and potential drug combinations are also being studied in order to provide information for subsequent drug policy revision. There are six sentinel sites for drug efficacy studies. During 2000-2004, the ACPR of chloroquine for treatment of vivax malaria was 80%-90% except in S. Lampung district where the ACPR was only 33% in 2002 indicating high chloroquine resistance. Amodiaquine, as an alternative drug for treatment of vivax malaria was studied in Bangka district; the ACPR was 97%. Artesunate + amodiaquine (as an ACT) was deployed as the first-line treatment for uncomplicated *falciparum* malaria in 2004. During 2003-2005, the combination with dosage of amodiaquine at 30mg/kg BW was relatively more efficacious than that of 25mg/kg BW (ACPR higher than 90% and 80%-90%, respectively). In 2005, a comparative study between artesunate + amodiaquine and dihydroartemisinin-piperaquine for *falciparum* malaria was carried out in Timika, Papua district. The ACPRs were 52% and 84% respectively. A high degree of resistance of both vivax and *falciparum* malaria in Timika, Papua district was found. In two studies conducted in 2007 high relapse rates were observed in vivax cases treated with artemether-lumefantrine and artesunate + amodiaquine when compared to dihydroartemisinin-piperaquine. Based on these findings, the national drug policy was changed in 2006 and dihydroartemisinin-piperaquine became the first-line treatment for any uncomplicated cases of all four parasite species and for treatment of malaria in the second and third trimesters of pregnancy.

**Nepal**

Malaria is endemic along the southern districts bordering India. *P. vivax* is the predominant species (80% of the total malaria cases). Drug resistance monitoring started in 1978 and both in vivo and in vitro studies were conducted with the primary focus on *falciparum* resistance. Chloroquine-resistant *falciparum* was first reported in 1984 and subsequently resistance was reported from several districts. In 1996-1997, sulfadoxine-pyrimethamine (SP) that replaced chloroquine lost its efficacy. In 2000, the late treatment failure rate of *falciparum* cases treated with sulfadoxine-pyrimethamine was found to be 57%. In 2003, the efficacy of SP was found to be very low in Jhapa district. This led to revision of national treatment guidelines and adoption of ACT as the first-line treatment of uncomplicated *falciparum* malaria in 2007. Artemether-lumefantrine that was chosen as an ACT was studied in Jhapa district in 2007 and in Dhanusha district in 2008: high efficacy (ACPR 100%) was reported. Chloroquine for treatment of vivax malaria was studied intermittently, i.e. in Kanchanpur district in 2003 and Dhanusha and Dadeldhura districts in 2008, and Kanchanpur district in 2009: results showed a high cure rate (ACPR 100%). The fixed sentinel sites have not been fully established. The country is considering increasing the number of sentinel sites and engaging more partners and setting up a region-wide drug resistance monitoring network.
Sri Lanka

The country reported high proportion of P. vivax (95%) cases. However, the country monitored the efficacy of both P. falciparum and P. vivax against antimalarials. Chloroquine-resistant falciparum was reported in 1984 following two focal falciparum outbreaks. There were several reports of chloroquine resistance in 1996, 2003 and 2004. The first case of sulfadoxine-pyrimethamine-resistant falciparum was reported in 1992. The national malaria treatment guidelines were revised in 2008 and artemether-lumefantrine was adopted for treatment of all falciparum cases as a strategy for malaria elimination. No case of chloroquine-resistant vivax has been reported so far. There are difficulties in enrolling eligible cases for therapeutic efficacy studies due to low malaria incidence. All enrolled falciparum cases are hospitalized for 3 days and followed up for 28 days. All enrolled vivax cases are followed up for 14 days. Due to low malaria incidence all malaria cases are followed up post-treatment by regional medical officers. Large-scale epidemiological studies of drug resistance and molecular studies are not possible due to the low number of malaria patients.

Timor-Leste

Being a newly established country that lacks national capacity, drug resistance monitoring is not conducted. Based on information from the nearby areas of Indonesia where high degree and widespread drug resistance to both species were reported, the National Malaria Control Programme adopted artemether-lumefantrine as the first-line treatment for uncomplicated falciparum malaria in 2007. National capacity in drug resistance monitoring is yet to be established.

Countries in the Greater Mekong Subregion

There are six countries in the Greater Mekong Subregion, namely Cambodia, Lao PDR, Myanmar, People’s Republic of China (Yunnan Province), Thailand and Viet Nam. Resistance to several antimalarial drugs of different chemical classes was reported in the region. Consequently, in order to strategically respond to the problem a network of drug resistance monitoring was established as a part of the Mekong Roll Back Malaria Initiative. All six countries are actively participating in drug resistance monitoring and networking. There are currently 34 fixed sentinel sites that are functioning well. The key factors that have contributed to success include: regular funding; having fixed sentinel sites; good data management; technical support; and regular and effective information-sharing. Countries have been maintaining sentinel sites for several years now, and have contributed significantly in providing evidence for updating the malaria treatment policy.

References and bibliography

A study of oseltamivir-resistant influenza viruses
in Thailand, 2008-2010

Malinee Chittaganpitch*, Sunthareeya Waicharoen*, Jiranant Warachit De silva*,
Passakorn Akrasewi** and Pathom Sawanpanyalert*

Abstract

On 25 January 2008, WHO was notified by Norway of a high prevalence of oseltamivir (Tamiflu©) resistance in seasonal influenza A(H1N1) viruses detected through routine surveillance and testing. Information about drug resistance is now an important piece of information guiding patient treatment recommendations. The Regional Influenza Reference Laboratory of SEA Region (RIRL), Thailand established the capacity to run the fluorescence-based NA enzyme inhibition assay. Throat swabs from patients with influenza-like illness or pneumonia were collected at 11 sentinel sites across the country. All swab specimens were transported to the RIRL. Specimens were identified using the standard protocol for real time reverse transcription polymerase chain reaction (rRT-PCR) from the WHO and US-CDC to detect influenza A/B virus and then A viruses were subtyped with specific primers from US-CDC. All specimens from sentinel sites which demonstrated influenza positive by rRT-PCR during 2008-2010 were selected for virus isolation in MDCK cells. A total of 1,211 representative influenza isolates were tested for susceptibility to oseltamivir by fluorometric neuraminidase inhibition Assay (phenotypic assay). All positive results or resistant isolates and some negative results obtained from phenotypic assay were subsequently performed partial NA gene sequencing which carried the oseltamivir resistance mutation at H274Y (N2 numbering). The study results demonstrated that in 2008-2009, a steady increase in proportion of seasonal A(H1N1) oseltamivir resistance was observed, reaching 95.6% in 2009. In 2009-2010, the H274Y mutation was found in pandemic A(H1N1) viruses and the prevalence of resistance was 1.31%. Oseltamivir resistance was not found with influenza type B or H3 viruses during 2008-2010. Continued monitoring of antiviral resistance in influenza viruses is essential for guiding patient treatment recommendations.

Influenza is an infectious disease caused by influenza viruses which are in the Orthomyxoviridae family. Influenza viruses are single-strand segmented RNA viruses. There are two genuses that commonly cause influenza in humans, Classification of influenza viruses into subtypes is labeled according to the H number (H1 to H16) and the N number (N1 to N9) which represent the type of hemagglutinin and neuraminidase respectively. Although influenza spreads around the world every year as seasonal epidemics, resulting in the death of approximately 250 000 to 500 000 people every year, an influenza pandemic can occur after the appearance of the new strain of a virus in humans. Often, new strains appear when an existing influenza virus transfers from animals to humans. An example of a new
strain is influenza A (H5N1), an avian strain, which raised concern of a new influenza pandemic since it emerged in the 1990s. Fortunately, although its symptoms are severe, it has not evolved to spread easily between people. Another example of a novel influenza strain is the 2009 influenza A (H1N1) virus that evolved by combining genes from human, swine and avian influenza viruses. This virus emerged in North America in April 2009 and spread widely and rapidly to cause a pandemic. In Thailand, the avian influenza A/H5N1 epidemic and recent emergence of the novel strain of influenza virus A(H1N1) in April 2009 led to the usage and stockpiling of influenza antiviral drugs, particularly neuraminidase inhibitors. The neuraminidase inhibitors (NAI) zanamivir (Relenza<sup>TM</sup>) and oseltamivir (Tamilflu<sup>TM</sup>) are representatives of the most effective class of antiviral drugs for the treatment and prevention of influenza A and B infections since the neuraminidase (NA) enzyme plays an essential role in releasing and spreading of progeny virion by the cleaving of sialic acid residues on newly formed virions and from cellular receptors at the site of the virus budding. Neuraminidase antiviral drugs such as oseltamivir (Tamiflu<sup>TM</sup>) and zanamivir (Relenza<sup>TM</sup>) exploit the NA protein function which is required for virus releasing. After being exposed to the neuraminidase inhibitor, the influenza virion aggregates on the surface of the host cell thereby limiting infection. During clinical testing of oseltamivir in 2001, viruses with point mutation in the NA gene which led to an amino acid change from histidine to tyrosine at position 274, commonly referred as H274Y in N2 numbering (H275Y in N1 numbering), were found in some individuals. Although this amino acid change led to decreased binding of the drug, these viruses had a decreased replicate rate and infectivity. It was thought that oseltamivir-resistant influenza viruses would not easily spread in the population. However, during 2007-2008, there was an increasing number of oseltamivir-resistant influenza A (H1N1) viruses with the H274Y mutation in Europe and North America. Similarly, oseltamivir-resistant viruses were also found in the Southern hemisphere. There are various methods to genotypically identify influenza resistance to the neuraminidase inhibitor on NA gene such as conventional sequencing, pyrosequencing, restriction fragment length polymorphism (RFLP), and real time reverse transcription polymerase chain reaction (RT-PCR). Presently, there are four important resistant markers which have been reported, E119V and R292K were noted among subtype A(H3N2), H274Y in subtype A(H1N1), and R152K in type B. The phenotypic analysis of drug resistance, such as chemiluminescence or fluorescence neuraminidase inhibition assay, is very important for confirmation of genotypic assay and investigation of newly emergent markers.

Recently, the neuraminidase inhibitor oseltamivir has been widely used for treatment of influenza illness in Thailand. This antiviral medicine was introduced after the outbreak of avian influenza in 2004, nevertheless it was not commonly used at that time and was not used widely until the 2009 A(H1N1) pandemic. Since the pandemic, oseltamivir has been commonly prescribed by Thai physicians. WHO and the Ministry of Public Health, Thailand, expressed concern about possible increasing influenza virus drug resistance triggered by the extensive usage of oseltamivir. The previous experience with increasing amantadine resistance among influenza A (H3N2) virus strains was an example of what could happen. In 2004, after the emergence of avian influenza H5N1, intensive surveillance of influenza viruses circulating in Thailand was established through the RIRL, National Institutes of Health, Department of Medical Sciences, Thailand in collaboration with the Center for Disease Control and Prevention, Atlanta, United States of America (US-CDC). The seven new sentinel sites set up across the country, in addition to the four existing public health centers, brought up the number of sentinel sites to a total of 11. The surveillance system established the laboratory protocols to identify circulating
strains and also the frequency of oseltamivir resistance in influenza isolates. In this manuscript, we present data on circulating influenza strains and the frequency of oseltamivir resistance in influenza isolates from 2008 to 2010, collected from 11 sentinel sites. Within this time period, the 2009 pandemic influenza A (H1N1) virus emerged.

Methods
A convenience sample of throat swabs from patients with influenza-like illness or pneumonia were collected at 11 sentinel sites across the country. All swab specimens were put into viral transport medium and transported to the National Influenza Centre. The specimens were tested for influenza A and B viruses using the standard protocol for real time reverse transcription polymerase chain reaction (rRT-PCR) from WHO and US-CDC.9, 10. Influenza A viruses were then subtyped with specific primers from CDC. All specimens from sentinel sites which demonstrated influenza positive by rRT-PCR were selected for virus isolation in MDCK cells. A representative number of influenza isolates was tested for susceptibility to oseltamivir, by fluorometric neuraminidase inhibition assay (phenotypic assay). All positive results or resistant isolates and some negative results obtained from phenotypic assay were subsequently put through partial NA gene sequencing which carried the oseltamivir resistance mutation at H274Y (N2 numbering) were performed at the National Influenza Centre, Thai. Some specimens were sent to the WHO Influenza Collaborating Centres at Melbourne, Australia.12 The NA inhibitor (oseltamivir) susceptibility of influenza virus isolates was expressed as the concentration of NA inhibitor needed to inhibit the NA enzyme activity by 50% (IC50). The active form of the prodrug oseltamivir phosphate, oseltamivir carboxylate, was provided by F. Hoffmann-La Roche Ltd, Switzerland.

Results
A total of 1,211 influenza isolates were tested for susceptibility to oseltamivir during 2008-2010 (Table 2). In 2008, 106 isolates were tested and the prevalence of oseltamivir resistance among seasonal A(H1N1) by trimester was 0%, 12.5%, 55.5% and 75.0%, respectively (Table 2). In 2009, the prevalence increased to 95.6%. From 15 September to December 2009, influenza isolates from 11 sentinel sites were predominately pandemic A(H1N1) (Table 1). A higher proportion of pandemic A(H1N1)
were sampled and tested for oseltamivir resistance. A total of 685 pandemic A(H1N1) isolates from May 2009 to November 2010 were tested for oseltamivir resistance. At present, the accumulated number of this mutation was 9 viruses and the prevalence of resistance strains was still low at 1.31% when compared with seasonal A(H1N1) (Table 2). Until now this mutation was not found with influenza type B and A(H3N2). All mutant viruses showed a large reduction in the susceptibility to oseltamivir when compared with the nonresistant viruses and also had H274Y mutation by partial NA gene sequencing. The IC\textsubscript{50} range of H274Y seasonal A(H1N1) was 165.76-840.77 nM and 195.40-806.30 for pandemic A(H1N1) (Table 3). These results were higher than those for nonresistant viruses (N1 subtype) which were around 130-600 fold of the IC\textsubscript{50} (Table 4) which was relevant to Hurt AC study\textsuperscript{12}.

**Conclusion**

The study results in 2008 indicate that oseltamivir-resistant influenza A/H1N1 viruses had already emerged in Thailand where oseltamivir is not so widely used and then rapidly spread, becoming the predominant strain circulating in 2009. It had been suggested that oseltamivir-resistant influenza viruses would not easily spread among populations\textsuperscript{4}, but our findings do not support this hypothesis. Therefore, the origin and spread of this resistant virus needs to be further studied. Fortunately, seasonal A/H1N1 viruses remain sensitive to zanamivir and also to amantadine. These data were supported by the confirmation results from the WHO Collaborating Centres in Melbourne, Australia and CDC, Atlanta, USA. In contrast, the prevalence of oseltamivir-resistant pandemic A(H1N1) viruses is still low as 1.31% but continued monitoring of pandemic A(H1N1) viruses is essential for monitoring the effectiveness of oseltamivir treatment. Physicians should follow updated information on drug resistance prevalence of current circulating influenza strains. Surveillance data should inform policy makers so that they may revise antiviral treatment guidelines accordingly or pandemic stockpiles as warranted.\textsuperscript{18}

**Table 1:** Laboratory diagnosis of influenza positive isolates by type and subtype during 2008-2010 from 11 sentinel sites, Thailand

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Specimens</th>
<th>No. of Positives</th>
<th>Type</th>
<th>Subtype of influenza A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Influenza A</td>
<td>Influenza B</td>
</tr>
<tr>
<td>2008</td>
<td>3736</td>
<td>906</td>
<td>517</td>
<td>389</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(24.25%)</td>
<td>(57.06%)</td>
<td>(42.94%)</td>
</tr>
<tr>
<td>2009</td>
<td>3072</td>
<td>645</td>
<td>562</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20.99%)</td>
<td>(87.13%)</td>
<td>(12.87%)</td>
</tr>
<tr>
<td>2010</td>
<td>3249</td>
<td>857</td>
<td>581</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18.07%)</td>
<td>(67.79%)</td>
<td>(32.21%)</td>
</tr>
</tbody>
</table>

*pdm H1N1 is pandemic influenza A(H1N1) 2009*
Table 2: Distribution of type and subtype of influenza virus resistance to oseltamivir during 2008-2010 Thailand

<table>
<thead>
<tr>
<th>Influenza virus</th>
<th>Result of oseltamivir resistance (NAI assay)</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Resistant per total tested</td>
<td>% Resistant isolates</td>
<td>No. Resistant per total tested</td>
<td>% Resistant isolates</td>
</tr>
<tr>
<td>Flu A(H1N1)</td>
<td>42/48</td>
<td>87.5</td>
<td>43/45</td>
<td>95.55</td>
</tr>
<tr>
<td>Flu A(H3N2)</td>
<td>0/37</td>
<td>0.00</td>
<td>0/83</td>
<td>0.00</td>
</tr>
<tr>
<td>Flu B</td>
<td>0/21</td>
<td>0.00</td>
<td>0/29</td>
<td>0.00</td>
</tr>
<tr>
<td>pdm A(H1N1)</td>
<td>-</td>
<td>-</td>
<td>4/211</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Note: All influenza viruses that showed resistance in fluorometric neuraminidase inhibition assay (NAI assay), also had H274Y mutation by partial NA gene sequencing

Table 3: Range of IC50 values in the fluorometric neuraminidase inhibition assay for oseltamivir susceptible and resistant influenza viruses during 2008-2010, Thailand

<table>
<thead>
<tr>
<th>Type/Subtype</th>
<th>Range of IC50 Value (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Seasonal A(H1N1)</td>
<td>0.24-1.58</td>
</tr>
<tr>
<td>pdmA(H1N1)</td>
<td>0.03-4.13</td>
</tr>
<tr>
<td>A(H3N2)</td>
<td>0.06-2.35</td>
</tr>
<tr>
<td>B</td>
<td>3.09-53.74</td>
</tr>
</tbody>
</table>

Table 4: Mean of IC50 values ± standard derivation in the fluorometric neuraminidase inhibition assay for oseltamivir susceptible influenza viruses during 2008-2010, Thailand

<table>
<thead>
<tr>
<th>Type/Subtype</th>
<th>N</th>
<th>Mean of IC50 values ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>523</td>
<td>1.26 ± 0.76</td>
</tr>
<tr>
<td>N2</td>
<td>137</td>
<td>0.39 ± 0.36</td>
</tr>
<tr>
<td>B</td>
<td>132</td>
<td>24.71 ± 12.84</td>
</tr>
</tbody>
</table>

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hospital, Bangkok Hospital Samui, Hat Yai hospital and the Bamrasnaradura Infectious Diseases Institute (BIDI). We are grateful to the WHO Collaborating Centre, Melbourne, Australia and CDC, Atlanta, USA, for support for the reagents kit and their technical assistance; Dr Rajesh Bhatia, Regional Adviser, Blood Safety and Laboratory Technology, WHO Regional Office for South-East Asia for his valuable advice; Hoffmann-La Roche Ltd (Basel, Switzerland) for the provision of oseltamivir carboxylate.

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Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the funding agency.

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Systematic antimicrobial resistance (AMR) surveillance of Neisseria gonorrhoeae at the local, regional, and global levels is a necessary tool for informing and modifying empirical antimicrobial prescription and for designing and monitoring interventions to control resistance. The accumulation and dissemination of surveillance data require functional and quality-assured laboratories for the pathogen isolation and susceptibility testing, demographic data, databases, and information dissemination channels. Many developing countries in WHO’s South-East Asia (SEA) Region lack more than one of these essential requirements, and thus surveillance data are lacking for N. gonorrhoeae. Comparing resistance trends across different countries is challenged by the lack of continuous surveillance data from many SEAR countries. Establishment of the WHO global Gonococcal Antimicrobial Surveillance Programme (GASP) across all regions assists in generating AMR data, as well as in compiling and disseminating the information. Participation in EQAS programmes and appropriate use of the WHO reference panel by GASP participants is a necessary requirement for the validation of gonococcal AMR data. The currently recommended treatment for gonorrhoea includes the use either of third generation cephalosporins or of spectinomycin. To ensure that the limited resources are used in the best possible ways, gonococcal resistance against these drugs should continuously be surveyed. High dosages and incorrect use of the first-line antibiotics for the treatment of gonorrhoea should be discouraged to delay the emergence of cephalosporin resistance.

Keywords: Antimicrobial resistance, surveillance, Neisseria gonorrhoeae, South-East Asia Region, Gonococcal Antimicrobial Surveillance Programme

Introduction

Antimicrobial resistance (AMR) has become a major public health problem and it contributes to health and economic losses worldwide. AMR to commonly prescribed antibiotics is increasing both in developing as well as developed countries. Resistance has emerged even to newer, more potent antimicrobial agents and some micro-organisms may develop resistance to a single antimicrobial agent (or related class of agent), while others develop resistance to several antimicrobial agents or classes. These organisms are often referred to as multidrug-resistant or MDR strains. In some cases, the microorganisms have become so resistant that no available antibiotics are effective against them.

The growing threat from resistant organisms calls for concerted action to prevent the emergence of new resistant strains and the spread of existing ones. The problem of AMR requires a multi-pronged research approach. There have been several initiatives by the World Health Organization (WHO) and the US Centre for Disease Control (CDC) directed at addressing the problem of antimicrobial resistance such as:

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WHO Global Strategy for Containment of Antimicrobial Resistance (http://www.who.int/emc/amr.html accessed 30 September 2010);

U.S. Public Health Action Plan to Combat Antimicrobial Resistance (draft at: http://www.cdc.gov/drugresistance/actionplan/ accessed 30 September 2010);


The WHO Global Strategy for Containment of Antimicrobial Resistance, was launched in September 20012. The strategy recognizes that AMR is a global problem that must be addressed in all countries. WHO’s Global Strategy urges national governments to strengthen health systems and AMR surveillance strategies, improve and enforce licensing and regulatory policies, increase access to appropriate antimicrobials, promote appropriate drug use, and encourage the development of new drugs and vaccines. Implementation of WHO’s strategy for containment of antimicrobial resistance is more advanced in some countries, but no country has so far implemented all the suggested interventions3. Surveillance of AMR, an important component of WHO’s Global Strategy, is of great importance in monitoring the emergence and spread of resistance and in planning appropriate treatment regimens.

The most deadly infectious diseases in the world today are also the ones for which AMR has emerged. AMR has rendered care for and treatment of such serious illnesses as diarrhoeal diseases, respiratory tract infections, sexually transmitted infections, meningitis, pneumonia, and hospital-acquired infections more difficult and expensive than ever imagined.

Gonorrhoea, a sexually transmitted infection, remains a significant disease globally and gonococcal AMR severely compromises control of gonococcal disease, preventing effective treatment of individuals, increasing the rate of morbidity and complications and enhancing the transmission of HIV. Effective treatment of gonorrhoea depends on having available data on AMR patterns in Neisseria gonorrhoeae. Over the last decade, N. gonorrhoeae strains have developed a high level of resistance against several antimicrobial agents such as sulfonamides, penicillin, tetracycline and quinolones in different countries, posing an increasing problem in the management of gonorrhoea. The emergence of strains resistant to extended-spectrum cephalosporins, the antibiotics used as the first-line treatment for uncomplicated gonococcal infections, is now a serious concern worldwide as it may lead to untreatable gonorrhoea. This review is aimed at analyzing the problem of antimicrobial resistance in N. gonorrhoeae, particularly in countries of the WHO SEA Region.

Methods

A Medline search was conducted using PubMed, for articles published since 1990 under the major headings of “antimicrobial resistance in N. gonorrhoeae in SEA Region countries”, “Surveillance of antimicrobial/drug resistance in N. gonorrhoeae” and “gonorrhoea treatment/therapy.”. Data were compiled from relevant articles.
Sources of data on AMR in *N. gonorrhoeae*

The need to identify and characterize the resistance profile of *N. gonorrhoeae* has been recognized as a public health priority. This has been addressed by a number of bodies while establishing surveillance programmes for AMR in gonococci so as to address the need for quality control (internal) and quality assurance (external) programmes, increasing difficulties accessing isolates as the use of non-culture based testing increases, and the need to define sample populations and sample sizes for valid data generation. These surveillance programmes include the WHO Gonococcal Antimicrobial Surveillance Programme (GASP); the United States Gonococcal Isolate Surveillance Programme (GISP); the Australian Gonococcal Surveillance Programme (AGSP) and the UK Gonococcal Resistance to Antibiotics Surveillance Programme (GRASP). Some of these systems use intermittent and others use continuous surveillance.

The WHO GASP was established in different regions of the world in 1990. GASP is important in assisting health providers in making recommendations regarding effective antibiotics for treatment. The progress of GASP has been slow in some regions due to a delay in establishing laboratories, networks and infrastructure for activities such as quality assurance. However, programmes have been established in Latin America and the Caribbean, and in the WHO Regions of the Western Pacific (WPR) and South-East Asia (SEAR)\(^5\). Within these regions, a number of national networks are at various stages of development. WHO recently reviewed the standards required for surveillance of antimicrobial resistance in *N. gonorrhoeae*\(^6\).

The GASP in WHO-WPR and the GISP in the United States are continuous surveillance programmes with programme-specific quality assurance (QA) and quality control (QC) using published standardised methodology; Data are published annually\(^7\)\(^\text{-}^\text{11}\).

Most of the studies on surveillance have been conducted within hospitals and other closed environments, in more developed countries where gonorrhoea rates are often a fraction of those in less developed countries. Almost all developing countries have been insufficiently studied, but there are some areas from which continuous data and from some countries few or no data are available. Surveillance data from several more developed countries suggest that AMR in gonococci in most developed countries is not as great a problem and often AMR which does exist is primarily imported rather than endogenous.

**Status of AMR of *N. gonorrhoeae* in WHO-SEAR countries**

South-East Asia is most likely an origin of drug resistance\(^12\)\(^,\)\(^13\). Penicillinase-producing strains were first isolated in 1976 in South-East Asia, and resistance to spectinomycin and tetracycline of gonococci emerged in the 1980s. Fluoroquinolone-resistant gonococci were found in several Asian countries during the early 1990s.

GASP became functional in the SEA Region in 1997. Two regional reference laboratories (RRL), one each in India and Thailand, were identified to provide technical and material support to five countries each. The Inter-Regional Reference Laboratory, located at the WHO Collaborating Centre for Sexually Transmitted Diseases, Sydney, Australia, provides technical support, reference panels of gonococci for use in internal quality control and organizes the external quality assurance system (EQAS) for these laboratories. The WHO EQAS programme includes dispatching a set of gonococci each year as unknowns to each participating laboratory. The participating laboratories in this network conduct susceptibility testing and incorporate quality control (e.g. control panels) and participate in the EQAS programme. Participating laboratories send AMR data to
the WHO Collaborating Centre in Sydney where data are analysed. Focal point laboratories from India, Thailand, and Sri Lanka have participated in the programme since its inception. Bangladesh and Nepal participated initially and discontinued later. However, laboratories in Myanmar and Bhutan have started participating for the last few years. Effective surveillance of AMR in these countries is expected to monitor trends in established types of resistance and promptly identify new types of resistance.

India

β-lactamase producing N. gonorrhoeae was observed for the first time in Madras (Chennai), followed by detection from Bombay (Mumbai), Trivandrum (Thiruvananthapuram), Vishakhapatnam and Chandigarh. The regular monitoring of antimicrobial susceptibility is being carried out at the Regional STD Teaching, Training and Research Centre in New Delhi since 1995 and this centre is functioning as a WHO-SEAR GASP Regional Reference Laboratory (RRL) since 2000. Surveillance data available from other centres in India (except from Pune where continuous surveillance since 1995 and AIIMS, Delhi since 2007 - unpublished) are intermittent. The National AIDS Control Organization along with RRL are trying to establish antimicrobial susceptibility testing for N. gonorrhoeae in all Regional STI Centres and State Reference Centres for STIs so as to monitor the trends in different regions of the country.

RRL has been conducting a country-based GASP EQAS programme in India since 2000 to evaluate the quality of the AMR testing data and assess the network capability to detect newly emerging AMR. AMR surveillance data from RRL is based on N. gonorrhoeae isolates obtained from patients attending male and female Sexually Transmitted Diseases (STD) clinics. Data of the first 14 years (Fig. 1), have documented the pattern of AMR in N. gonorrhoeae, highlighting the alarming increase in ciprofloxacin and penicillin resistance from 1995 to 2008. Ciprofloxacin resistance increased from 3.4% in 1996 to 83.3% in 2008. In 2004, it was 97.2%. Penicillinase-producing N. gonorrhoeae (PPNG) strains varied from 3.4% to 35.1% between 1996 to 2008. A rising trend was observed in the isolation of tetracycline-resistant N. gonorrhoeae (TRNG), from 1.7% in 1996 to 19.3% in 2008. Ceftriaxone less-susceptible strains were detected for the first time in 2001 from RRL. Only nine strains (2.4%) were found to be less sensitive to ceftriaxone from 2002 to 2006. Ceftriaxone

![Fig. 1: Trend of Antimicrobial Resistance from 1996-2008 at New Delhi, India](image-url)
less-sensitive strains varied from 1.3% to 1.7% between 2002 to 2004 but an insignificant rise to 5.5% was observed in 2006. These strains almost always exhibited resistance to quinolones or quinolones and penicillin. All the strains were found to be sensitive to spectinomycin except one strain in 2002. The frequency of multiresistant isolates found (23.3%) in RRL was quite high.

The continued high prevalence of penicillin resistance up to 2003 followed by the decrease up to 2006 may reflect the loss of selective pressure from the disuse of penicillin as treatment for gonorrhoea. From 2002 to 2006, 21.2% of isolates were found to be PPNG and the results compared well with another study from north India. In contrast, Bhalla et al. and Khaki et al. in Delhi from the same STD clinic reported 8%, 11.1% and 17.3% of isolates to be PPNG in 1998, 2002 and 2007, respectively.

The increasing trend of TRNG observed in RRL may reflect ongoing selective pressure produced by the use of tetracyclines to treat other infections and its use as adjunct therapy in the syndromic management of STDs. Tetracycline resistance was observed to be 51% in another Indian city without any mention of TRNG. In contrast, Bhalla et al. and Khaki et al. from Delhi reported 28%, 2.8% and 20% of isolates as TRNG in 1998, 2002 and 2007, respectively.

The use of the quinolone group of antibiotics for the treatment of gonorrhoea has been discontinued in India for quite some time because of reported high levels of resistance. However, there were considerable differences in rates of quinolone resistance in different studies in India. All isolates were found to be susceptible to ceftriaxone, spectinomycin, cefixime and azithromycin in a study from Delhi. It is fortunate that, except for one strain, spectinomycin resistance has not been reported from RRL and other STD clinics as it is an alternative drug of choice for cases having hypersensitivity to cephalosporins. Spectinomycin is not easily available in India and this may explain the retention of efficacy of this antibiotic.

Reduced susceptibility towards ceftriaxone was also reported by some laboratories in India. However, two strains isolated from a focal point laboratory in India, showing reduced susceptibility towards ceftriaxone, could not be confirmed at the RRL, New Delhi. Ceftriaxone less-susceptible strains (unconfirmed by MIC) from other focal point laboratories were not received at the RRL for confirmation. In all, the ten cases from RRL, having strains less susceptible to ceftriaxone, responded to treatment with ceftriaxone or cefixime. Treatment failures are documented with oral third-generation cephalosporins such as cefixime, cefdinir and cefetibuten from some countries but not as yet with ceftriaxone.

**Sri Lanka**

PPNG strain was first detected in Sri Lanka in 1980 and routine testing of gonococcal isolates obtained from patients attending the Central STD Clinic, Colombo for PPNG was started the following year. In 1997, a systematic monitoring system for other antibiotics was established with the introduction of GASP. PPNG isolates increased from 3.4% in 1981 to 26% in 1989. However, since 1992, there was a sharp decline in PPNG with none detected in 1995. PPNG isolates were reported to be 61.5% and 52.9% in 2007 and 2008 respectively. A highly significant rise in the percentage of chromosomally mediated resistance to penicillin from 37% in 1996 to 96.8% was observed in 2000. The rest of the strains were less sensitive. Penicillin was withdrawn from use as first-line therapy for gonorrhoea and single dose quinolone therapy introduced in 1993. Occasional clinical resistance to quinolones was first detected in late 1994. Antibiotic susceptibility testing facilities for quinolones were not available routinely in the Central Laboratory of the STD/AIDS Control Programme at that time. As increasing clinical resistance began to
surface during the second quarter of 1995, antibiotic susceptibility testing for quinolones was started in June the same year. The quinolone resistance varied between 14%-50% in the third and fourth quarters of 199535. Resistance to ciprofloxacin varied from 10.3% to 13.7% from 1996 to 1998 and showed a declining trend to 8.2% in 2000 followed by a rise to 76.5% in 20089,29. This rapid emergence of quinolone resistance indicated that 4-fluroquinolones were no longer useful as first-line therapy for gonorrhoea in Sri Lanka. Cefuroxime axetil 1 gm orally is the recommended treatment since 1996. No ceftriaxone less-susceptible and spectinomycin-resistant strains were isolated till 20089,29. TRNG isolates were 13.5% in 1997, which decreased to 0% in 200039.

Bangladesh

In Bangladesh, there was no established systematic antimicrobial susceptibility surveillance for N. gonorrhoeae. The national STI management guidelines recommended the use of ciprofloxacin as the first-line therapy for the treatment of uncomplicated gonococcal infection during 1997-2006. The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) had initiated antimicrobial susceptibility monitoring for N. gonorrhoeae in Dhaka, the capital of Bangladesh, since 1997, and it was subsequently (1999-2003) extended to three major cities (Chittagong, Jessore, and Sylhet), in the southeast, southwest and northeast parts of Bangladesh respectively and later to Faridpur, Barisal and Mymensingh in 200536-39. The programme was part of the STI service-delivery programme established by ICDDR,B. In total, 1,767 N. gonorrhoeae isolates were tested from males and females (population with high-risk behaviour i.e. street-based, brothel-based, hotel-based female sex workers, male having sex with male (MSM) population, male truckers, STI patients and the general population) during 1997-2006.

Approximately 9% of the isolates in 1997 were observed to be resistant to ciprofloxacin compared to 87% in 2006 with the highest (92%) resistance in 2003. All the isolates were susceptible to ceftriaxone except that 1.0% strains in 1997 and 1.5% in 2000 were having reduced susceptibility to ceftriaxone9,29. All the isolates were also susceptible to azithromycin (MIC of ≤1 μg/mL), and spectinomycin, except that one isolate (0.2%) in 2002 and one each isolate in 2003, 2005, and 2006 were resistant to azithromycin and spectinomycin respectively9. Although most isolates were susceptible to azithromycin, a gradual increase in MIC of azithromycin was observed during 2003-2006. While none of the isolates had an MIC of ≥0.25 μg/mL in 1997, approximately 25% of the isolates from 2003 had an MIC of ≥0.25 μg/mL for azithromycin. No significant difference in resistance was observed among isolates collected from different populations and cities in a given year. Approximately 14% of the isolates were PPNG in 1997 compared to 44% in 2006. Of the isolates from 1997, 20% were TRNG compared to 86% in 2006. None of the isolates was both PPNG and TRNG in 1997 and 1998 compared to 42% in 2006. Multidrug-resistant N. gonorrhoeae emerged in 1997, and 44% of the strains isolated during 2006 were multidrug-resistant. Of the multidrug-resistant isolates, none was both PPNG and TRNG in 1997 and 1998, and 83% of the isolates were both PPNG and TRNG in 200639.

Based on the above surveillance data, the National AIDS and STD Programme, Ministry of Health and Family Welfare, Government of Bangladesh, revised the national guidelines for the management of STIs in 2007 and recommended cefixime as the first-line therapy for gonorrhoea39.

Thailand

Spectinomycin became the primary therapeutic drug for the treatment of gonorrhoea in Thailand in 1983 following a
series of studies which documented PPNG rates of up to 71%⁴⁰. Five years later (1987 and 1988), in a survey of patients with STDs in Bangkok, Cholburi, Chiangmai, and Songkhla, none of 3,200 N. gonorrhoeae isolates was spectinomycin resistant⁴¹. However, in a study on the 333 isolates from STD patients attending public health clinics in Bangkok and Cholburi in 1990, 8.9% were reported to be spectinomycin resistant⁴². A total of 70% of isolates were resistant to tetracycline and 28.2% were PPNG. Fewer than 1.5% of isolates were resistant to the extended-spectrum cephalosporins tested. Some 0.3% or fewer isolates were resistant to broad-spectrum cephalosporins, fluoroquinolones, or the monobactam aztreonam. Therefore, norfloxacin, ciprofloxacin, ofloxacin, spectinomycin, ceftriaxone, and ceftaxime were recommended for the treatment of uncomplicated gonorrhoea in 1994⁴³.

In 1994, out of 101 isolates from patients attending the Bangrak STD clinic in Bangkok⁴⁴, 89.1% were resistant to penicillin or tetracycline. A total of 7.9%, 17.8%, and 7.9% were reported to be TRNG, PPNG and PPNG/TRNG respectively. More than one half (52.3%) of strains were chloramphenical resistant N. gonorrhoeae (CMRNG). All strains were susceptible to spectinomycin. Approximately, one fifth (21.8%) of all strains exhibited decreased susceptibility to fluoroquinolones and resistance to norfloxacin; these strains included strains exhibiting chromosomally mediated resistance to tetracycline (TetR), CMRNG, and PPNG strains. More than 75% of strains exhibited decreased susceptibility to kanamycin and tiamphenicol; 21% were resistant to kanamycin.

The prevalence of ciprofloxacin-resistant (CipR) strains in Bangkok increased substantially in the 1990s. Trees et al⁴⁵ reported in 1998 and 1999, that CipR strains increased from 13.8% in 1998 to 25.4% in 1999. Because of the high level of CipR isolates at Bangrak Hospital, in 2000, the Thai Ministry of Public Health issued recommendations against the use of fluoroquinolones for the treatment of gonococcal infection in Thailand. Third generation cephalosporins and spectinomycin are recommended for treatment since then.

Lawung et. al⁴⁶ reported the elevated trend of antimicrobial resistance from Bangrak hospital, Bangkok (National Centre for Sexually Transmitted Infections) during 2000-2002. The PPNG isolates increased significantly from 50.7% in 2000 to 87.9% in 2002. The ciprofloxacin resistance also increased from 28% during 2000 to 56% during 2002. In addition, there was a positive correlation between the susceptibility to ciprofloxacin and ofloxacin in all strains tested. All isolates were susceptible to ceftriaxone. The incidence of double resistance determinants, penicillin and quinolone resistance, were significantly increased from 34.3% in 2000 up to 77.3% in 2002. In addition, a PPNG and norfloxacin resistant isolate obtained in 2002 was resistant to spectinomycin with a high MIC (>1.024 g/L).

In a study on 122 gonococcal isolates from HIV-positive male and female STD patients (including CSWs 22.1%), during June 2005 to May 2007, none of the isolates was susceptible to penicillin or tetracycline⁴⁷. Among the 122 isolates, 83.6% were PPNG, and most (79.5%) of these 122 isolates were further identified as PPNG plus TRNG, with only 4.1% being PPNG alone. With respect to fluoroquinolones, 90.2% and 91% of the isolates were resistant to ciprofloxacin and ofloxacin respectively, much higher than previously reported⁴⁴,⁴⁵. No gonococcal isolate with resistance to ceftriaxone and cefotaxime was detected. Recently, 66.9% and 75.6% of strains were reported to be quinolone resistant and 86% and 80.8% as PPNG in 2007 and 2008 respectively⁹.
Nepal

Specific data on the incidence of gonorrhoea and AMR of N. gonorrhoeae in Nepal is lacking. A report based only on nine isolates in 2001 showed four as PPNGs, four TRNGs, and only one isolate resistant to ciprofloxacin. No resistance was reported to ceftriaxone and spectinomycin.29

In 2001, National STI case management guidelines in Nepal recommended a single oral dose of 500 mg ciprofloxacin as first-line therapy for the management of uncomplicated gonococcal infection.48 A pilot study was conducted to assess the effectiveness of this recommendation49. In this pilot study, a total of 16 gonococcal isolates isolated from symptomatic and asymptomatic males and females attending an STI service delivery clinic in Eastern Nepal were tested for antimicrobial susceptibility testing between May and September 2003. Among the isolates, two (12.5%) were resistant to penicillin, including one PPNG isolate; eight (50%) were resistant to tetracycline, including one TRNG; 14 (87.5%) were resistant to ciprofloxacin; and three (19%) exhibited reduced susceptibility to azithromycin. All isolates were susceptible to ceftriaxone, cefixime, and spectinomycin. Six ciprofloxacin-resistant isolates had chromosomally mediated resistance to tetracycline and one had chromosomally mediated resistance to penicillin.

Limited data from these two studies demonstrate the need for continuous monitoring of antimicrobial susceptibility of N. gonorrhoeae in Nepal to revise the national STI case management guidelines for treatment of N. gonorrhoeae.

Bhutan and Myanmar

Antimicrobial resistance data from Myanmar are available from one recent report on antibiotic surveillance in WHO-WPR and SEAR countries9, 50. Data for Myanmar are based on only 12 isolates in 2008. Out of 12 isolates, 10 were reported to be penicillin resistant, including two PPNG strains. Four and six strains were observed to be resistant and less susceptible to quinolones respectively.

In Bhutan, JDW National Referral Hospital started surveillance of AMR in 2008 in a systematic way. All the 161 isolates were found to be resistant to penicillin and 95% resistant to quinolones in 200850. Susceptibility testing for spectinomycin and tetracycline is also being carried out at this centre. However, data are not mentioned in the above report.

Conclusion

The present review indicates that high rates of penicillin, tetracycline and quinolone resistance have been detected in all countries of the SEA Region. Reduced susceptibility to third-generation cephalosporins used at present as first-line therapy, although rare, has been reported from some SEAR countries. Therefore, AMR surveillance should be continuous in order to reveal the emergence of new resistant strains, to monitor the changing patterns of resistance, and to be able to update treatment recommendations so as to assist in disease control. The factors such as unregulated drug availability, antimicrobial misuse, inadequate antimicrobial drug quality assurance and inadequate surveillance must be addressed to permit a holistic strategy for resistance control. WHO has already started making elaborate plans for a response to the threat of untreatable gonococcal infections.
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Methicillin-resistant *Staphylococcus aureus* (MRSA) in developing and developed countries: implications and solutions

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**Abstract**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global public health problem, associated with considerable morbidity and mortality. The MRSA infections can be hospital- or community-acquired. Hospital-associated MRSA (HA-MRSA) characteristically colonizes or infects hospitalized individuals with predisposing risk factors, usually harbours SCCmec type I, II or III, and is multi-drug resistant (MDR). In contrast, community-associated MRSA (HA-MRSA) infects healthy individuals without any previous health-care contact, often harbours smaller and more mobile SCCmec types, is usually Panton-Valentine leucocidin (PVL) positive, susceptible to non-β-lactam antimicrobial drugs, and frequently manifests as skin and soft-tissue infections. However, this distinction between CA- and HA-MRSA is gradually fading owing to the emergence of pvl negative and/or MDR CA-MRSA clones, and its invasion into hospitals. The incidence of HA- and CA-MRSA infections, as well as the relative abundance of different MRSA clones varies considerably among countries. The HA-MRSA is endemic in many hospitals worldwide. The CA-MRSA has a smaller fitness burden, higher transmissibility and virulence compared to HA-MRSA, and is epidemic in many geographical locations. In addition, some MRSA clonal lineages exhibit superior survival and transmissibility, and are more frequently isolated than others. Limited options are available for the therapeutic management of MRSA infections. The CA-MRSA-associated skin and soft-tissue infections are treated with oral antibiotics including doxycycline, minocycline, clindamycin, trimethoprim-sulfamethoxazole, rifampicin and fusidic acid. Severe CA-MRSA infections and HA-MRSA necessitate intravenous vancomycin therapy. Asymptomatic carriers represent an important MRSA reservoir. The transmission of MRSA infections may be limited by universal infection-control measures, patient education, screening and decolonization of asymptomatic MRSA carriers in both health-care and community settings.

**Introduction**

*Staphylococcus aureus* (s. aureus) is a frequent cause of bacterial infections in both developed and developing countries. It is a highly versatile and adaptable pathogen, causing a range of infections of varying severity affecting the skin, soft tissue, respiratory system, bone, joints and endovascular tissues. The organism also exists as a commensal, colonizing the anterior nares of about one third of the healthy human population. Asymptomatic nasal carriers are at a high risk of subsequent *S. aureus* infection and are presumed to be an important source of strains that spread and cause infection in contacts. In addition, *S. aureus* represents a prototype for drug resistance, especially to β-lactam antibiotics. Although this bug has been naturally susceptible to almost every antibiotic developed so far, it frequently gains resistance by gene mutations and horizontal gene transfer, that protect the bug under antibiotic selection pressure, and has been implicated...
in episodes of epidemic and pandemic proportions.1,4 The effectiveness of penicillin, introduced in the early 1940s, was annulled within a decade owing to the rapid spread of plasmid-encoded *S. aureus* β-lactamase.1,5 Resistance to meticillin, a penicillinase-stable β-lactam, was reported within two years of introduction in 1959, and rapidly spread worldwide.1,5 Methicillin-resistant *S. aureus* (MRSA) first emerged in hospitals in the 1960s, re-surfaced as a community-based infection in the 1990s, and is currently a frequently encountered antibiotic-resistant pathogen.1,2,4,6

**Emergence and resurgence of MRSA**

Unlike penicillin resistance that results from a plasmid-encoded penicillin-degrading enzyme (β-lactamase), methicillin resistance is genetically mediated by staphylococcal-cassette-chromosome (*SCCmec*), a mobile genetic element encoding for an altered penicillin-binding protein (PBP2a, *mecA*) with a decreased affinity to β-lactams.5-7 *SCCmec* probably evolved in penicillinase-negative *Staphylococcus sciuri*, a frequent colonizer in animals, under selective pressure of penicillin and was subsequently acquired by *S. aureus* following inter-species horizontal gene transfer.5-7 Eight different *SCCmec* types are known on the basis of *mecA* class and new types continue to emerge.5-7 Although nearly all clinically significant MRSA produce PBP2a, infrequent isolates with alterations to existing PBPs, named moderately-resistant *S. aureus* (MODSA) and isolates exhibiting low-level methicillin resistance due to penicillinase overproduction, named borderline oxacillin-resistant *S. aureus* (BORSA), have been rarely described.8 The clinical significance of such strains remains doubtful.8

The emergence of MRSA typically coincides with that of penicillin-resistant *S. aureus* (PRSA).7 It was first described in the 1960s, and has been traditionally regarded as a nosocomial pathogen, endemic in hospitals and health-care facilities of most countries.1,2,4,6 Hospital-associated MRSA (HA-MRSA) characteristically colonizes or infects hospitalized individuals with predisposing risk factors such as surgery, presence of indwelling medical devices (IMDs), an immunocompromised state or prior antibiotic exposure.1,2,4,6 It is often isolated from wound infections, line-associated bacteremia and ventilator associated pneumonia.5-7 The strains usually harbour *SCCmec* type I, II and III, and are multidrug-resistant (MDR).5-7

About three decades after the emergence of HA-MRSA, the organism spilled over to the community increasing the staphylococcal disease burden.1 The community-acquired strains evolved either from the hospital strains and underwent genetic changes or were the result of mec gene transfer to formerly susceptible subsets in the community.9 True community-associated (CA) MRSA, infecting healthy individuals without any previous health-care contact, was initially reported (1990s) in Australia, followed by reports from the United States of America (USA), and is now highly prevalent worldwide.1,2,4,6 The CA-MRSA infects healthy individuals without any health-care contact, harbours smaller and more mobile *SCCmec* types (IV and V), is susceptible to non-β-lactam antimicrobial drugs and typically manifests as skin and soft tissue infections. Life-threatening conditions, including osteomyelitis, severe necrotizing pneumonia, and fatal sepsis have also been reported.1,2,4-7 It has a superior epidemicity than HA-MRSA, courtesy the presence of more mobile *SCCmec* types, faster growth and relatively smaller fitness burden.1,5-7 Consequently, the CA-MRSA strains possess a high attack rate in outbreak settings, are more virulent than HA-MRSA, and have rapidly disseminated among countries.1 Panton-Valentine leucocidin (PVL), a prophage-encoded two-component cytotoxin (*lukS-PV* and *lukF-PV*) presents itself in only 2-3 % of methicillin-sensitive *S. aureus* (MSSA), is frequently associated with CA-MRSA and may play a role in skin and soft-tissue infections and severe necrotizing pneumonia caused by
these bacteria. Moreover, these strains exhibit increased expression of chromosomally-encoded \( \alpha \) hemolysin, a pore-forming toxin thatlyses many types of eukaryotic cells, as well as \( \alpha \)-type phenol-soluble modulins (PSMs), amphipathic peptides that recruit, activate and destroy leucocytes. Both \( \alpha \) hemolysin and PSMs are over-expressed in CA-MRSA compared to HA-MRSA and are major determinants of its pathogenesis and virulence.

The CA-MRSA is evolving rapidly, and lineages differing in classical CA-MRSA characteristics, including pvl negative and/or MDR clones, have also been reported.

**Molecular epidemiology**

The MRSA is usually transmitted by direct skin-to-skin contact with a colonized or infected individual and occasionally via fomites. Five factors or “Cs” have been implicated in MRSA outbreaks — contact; lack of cleanliness; compromised skin integrity; contaminated objects; and crowded living conditions.

Methicillin resistance in *S. aureus* isolates (mostly health-care-associated MRSA) varies from less than 1 % in Norway, Sweden and Denmark, less than 5 % in the Netherlands, 5 - 10 % in Canada, 40 % in Greece and the United Kingdom of Great Britain and Northern Ireland, 25 - 50 % in the USA, 37.5 % in India, to more than 50 % in China, Hong Kong Special Administrative Region (Hong Kong SAR) and Singapore. About 51.6 % of *S. aureus* isolates among patients admitted to burns and orthopaedic units in India were reported to be MRSA.

The CA-MRSA is highly prevalent in the USA, accounting for about 59% infections in emergency departments. However, Kallen et al. have recently reported a decrease in health-care-associated community (17%) as well as hospital (28%) MRSA infections in nine geographically diverse metropolitan areas in the USA. An upsurge of MRSA has been reported in India. The MRSA isolation rates have increased from 9.83 % (1992) to 45.44 % (1998), with the strains being more common in south than in west or north India. In Japan, the percentage of MRSA isolated from skin infections has been shown to vary from 10 to 20%. By comparison, CA-MRSA has not reached the same proportions in Europe. Although its incidence is steadily increasing in many European countries including France, the Netherlands and the United Kingdom, the overall prevalence of PVL-positive CA-MRSA in Europe is about 1-3%. It was reported to constitute 6.4% *S. aureus* isolates, 10% MRSA and 1.8% MRSA in Italy, Austria and Ireland respectively. A very low incidence of CA-MRSA has also been described in Switzerland (0.09%), the United Kingdom (0.005%), Spain and Portugal. However, the prevalence of CA-MRSA in Greece is very high, accounting for about 55% MRSA infections.

Despite the increased global presence of MRSA, its carriage among healthy population remains low in most countries. Different studies have reported MRSA nasal carriage rates ranging from 0.26 to 9.2 % in USA. Similarly, 1% *S. aureus* asymptomatic carriers harbour MRSA in the United Kingdom. About 3.89% and 5.3% healthy children and adults respectively, have been reported to be colonized with CA-MRSA in India. The carrier rate is generally higher in AIDS and diabetic patients, those who are frequently hospitalized or are on dialysis or are of advancing age. The HA-MRSA carriage rate is around 15.6 % in inpatients and 1.8 - 2 % among health-care workers in India.

The MRSA isolates are genetically characterized by multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), SCCmec typing (I-VIII), accessory gene regulator (agr) typing and staphylococcal protein A (spa) typing. The MLST classifies *S. aureus* isolates on the basis of allelic variation in seven housekeeping genes — clones consist of isolates with identical
sequences at all the seven loci and are assigned a unique sequence type (ST); clonal complexes (CCs) comprise closely-related STs differing by single nucleotide polymorphisms (SNPs) at lesser than three loci. It indexes slowly accumulated variation and can be used to measure long periods of evolution among strains. In contrast, PFGE involves the analysis of Smal-digested genomic DNA, and is more discriminatory but appropriate for evaluation of the recent evolution.

All MRSA clones have evolved from five groups of related genotypes (clonal complexes), each arising from a distinct ancestral genotype. The prevalence of different lineages varies with the geographical location probably due to socioeconomic factors and antimicrobial policies. Some clonal lineages have a superior ability to survive and transmit, and are consequently more frequently isolated than others. Five major MLST clonal complexes (CCs) have been implicated in HA-MRSA infections globally, with the archaic clone being the first HA-MRSA clone to be identified. Although most HA-MRSA clones harbour SCCmec I-III, type IV has been reported in many lineages. The major clonal complexes include:

(1) CC5: ST5-MRSA-I, ST5-MRSA-II (New York/Japan clone; PFGE type USA100), ST5-MRSA-IV (paediatric clone; PFGE type USA 800) and ST22-MRSA-I (southern German clone);
(2) CC8: ST250-MRSA-I (archaic clone), ST247-MRSA-I (iberian clone), ST239-MRSA-III (Brazilian/Hungarian/Portuguese clone), ST8-MRSA-II (Irish 1 clone), ST8-MRSA-IV (PFGE type USA500);
(3) CC22: ST22-MRSA-IV;
(4) CC30: ST36-MRSA-II (PFGE type USA 200); and
(5) CC45: ST45-MRSA-IV (Berlin clone), ST45-MRSA-II (PFGE type USA600).

Many of these multidrug-resistant clones have disseminated globally and account for the majority of HA-MRSA infections in several regions. The ST5-MRSA-II (USA100) and ST22-MRSA-IV are the prominent HA-MRSA clones in the USA, Europe and Australia respectively. The ST36-MRSA-II (USA200) is the single most abundant clone in the United Kingdom hospitals. The Indian HA-MRSA strains are related to the Brazilian/Hungarian clone and belong to ST239-MRSA-III/IIIA. The same clone is also prevalent in China, along with ST5-MRSA-II that has a low prevalence. In addition, a novel variant of SCCmec III has been described among Indian ST239 HA-MRSA isolates.

Among CA-MRSA, the ST1-MRSA-IVa (CC1; USA 400) was the predominant CA-MRSA clone in the USA till 2001. It was subsequently replaced by ST8-MRSA-IVa (CC8; USA 300) in most communities and is currently the leading cause of CA bacterial infections in the USA. It was isolated from more than 50% infections in the United States emergency departments. The USA 300 is more virulent than the USA 400 owing to over-expression of α-toxin, PSMs and many secreted proteases. It also harbours arginine catabolite response element (ACRE), a putative pathogenicity island encoding for arginine deaminase pathway. The ACRE promotes bacterial survival on acidic human skin, proliferation under low oxygen conditions such as in abscesses and evasion of host defences. Consequently, ACRE-positive lineages exhibit superior colonization and transmissibility in comparison to many other clones with similar virulence, such as ST8-MRSA-IVh (CC8; USA 500). However, USA 400 still remains the major cause of CA-MRSA-associated infections in some regions of North America and Canada. About 45 distinct CA-MRSA clones have been described in Australia; ST30-MRSA-Iva (CC30; USA 1100) and USA 400 are the leading cause of infections. The ST80-MRSA-IV is the
A predominant CA-MRSA clone in Europe. The ST22-MRSA-IV and ST772-MRSA-V, ST59-MRSA-Iv, and ST30-MRSA-Iv have been reported from India, China (Province of Taiwan) and Singapore. Many recent CA-MRSA clones have been shown to have transmitted between animals and humans. The ST398-MRSA-V, a PVL-negative strain, was first identified in pigs and pig farmers in Europe and has been subsequently reported from the USA and Canada. Considering the universal use of multiple antibiotics such as tetracycline in livestock, the antibiotic resistance of these strains and their subsequent transmission to humans is a major concern. Furthermore, CA-MRSA is now invading the hospitals. The CA-MRSA-infected individuals transmit these strains in the hospital setting and result in nosocomial infections. The USA 300 and USA 500 are currently a more notable cause of hospital-acquired MRSA infections than the USA 100. The USA 300 accounted for about 20% nosocomial cases of MRSA blood stream infections in Atlanta, USA. This epidemiological transition may pose a significant therapeutic challenge. The CA-MRSA clones have a relatively smaller fitness burden and enhanced virulence, and may therefore increase the seriousness of hospital-acquired drug-resistant S. aureus infections.

Therapeutic and prophylactic management

Methicillin resistance in S. aureus is usually detected by the cefoxitin disk and oxacillin-salt-agar screen test according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), (USA). Many other methods including PCR-based meca detection, latex agglutination test (PBP2a detection), E test, agar and broth dilution, quenching fluorescence assay, as well as chromogenic media such as Spectra MRSA are also available for MRSA identification. The MRSA has markedly influenced the empirical therapy for suspected staphylococcal infections. Most β-lactam antibiotics are ineffective against both HA- and CA-MRSA. The HA-MRSA isolates are usually MDR. The CA-MRSA strains are often susceptible to non-β-lactam drugs although MDR clones are also emerging. Unlike methicillin resistance, the prevalence of MRSA resistance to non-β-lactam agents varies geographically and may change over time. Local susceptibility patterns of community S. aureus isolates should therefore be regularly monitored to frame policies for MRSA management. Cutaneous abscesses need surgical incision and drainage irrespective of the antibiotic susceptibility pattern of the causative organism. Antibiotics provide little or no benefit in most cases, and are not routinely recommended except for patients of advanced age or those with severe disease symptoms of systemic illness, immunosuppression or abscess in an area that is difficult to drain. The CA-MRSA-associated skin and soft-tissue infections are often treated using oral antibiotics including doxycycline and minocycline, clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), rifampicin, and fusidic acid. Clindamycin is active against CA-MRSA strains as well as against the Group A streptococci, and is therefore an appealing therapeutic choice. However, clindamycin resistance seems to be increasing. In India, about 15.65% and 7.23% CA-MRSA strains were shown to exhibit inducible and constitutive clindamycin resistance respectively. A D-zone test is therefore recommended for identification of inducible clindamycin resistance in erythromycin-resistant, clindamycin-susceptible S. aureus isolates. Long-acting tetracyclines (doxycycline and minocycline) and TMP-SMX are also efficacious against CA-MRSA. The prevalence of tetracycline resistance remains low among MRSA isolates in the community and the resistance described so far (tetK mediated) has been specifically associated with tetracycline, not doxycycline and minocycline. However, their activity against...
Group A streptococci is variable and these antibiotics are contraindicated in children younger than eight years (tetracyclines) or pregnant women (tetracyclines and cotrimoxazole). Rifampicin or fusidic acid may be used as adjuncts to another active drug but never singly — monotherapy frequently results in emergence of resistance. As rifampin achieves high concentrations in mucosal surfaces, it may promote eradication of MRSA carriage theoretically. In India, use of rifampicin as an anti-MRSA drug is discouraged owing to the high prevalence of tuberculosis. Fluoroquinolones are usually not recommended for MRSA treatment. Therapy with these agents frequently results in selection of resistant mutants, and consequent relapse and treatment failure.

Vancomycin remains the first-line intravenous drug for severe CA-MRSA and HA-MRSA infections. However, high rates of microbiological and clinical failure, nephrotoxicity and emergence of non-susceptible strains have limited the effectiveness of this drug. Fortunately, the number of VRSA isolates has remained limited worldwide and availability of highly (chromatographically purified vancomycin has shown a falling incidence of nephrotoxicity. Linezolid exhibits an excellent anti-staphylococcal activity, comparable to that of vancomycin, and can also be administered orally. Resistance to this drug has been rarely reported. Resistance to this drug has been rarely reported. Nonetheless, owing to the expense and potential toxicity, linezolid has been approved by the Food and Drug Administrator (FDA), United States of America, for the treatment of serious MRSA infections only. In addition, daptomycin and tigecycline have been approved by the FDA for MRSA management. Many glycopeptide derivatives including telavancin, dalbavancin and oritavancin, and two cephalosporins (ceftobiprole and cefaroline) are also effective against MRSA, both in vitro and in animal models. Telavancin has now been approved by the FDA for treatment of complicated skin and soft-tissue infections. Cefotibiprole has been approved for clinical use in Canada and Switzerland. However, they are broad-spectrum drugs, and require parenteral administration. Orally-bioavailable alternatives, such as an oxazolidinone with an eight-fold higher activity than linezolid, are in early stages of development. Iclaprim and quinupristin/dalfopristin have also been reported to be effective against Gram-positive pathogens including MRSA.

Patient education is a critical aspect of MRSA management. Patients, their care-takers and household members should take appropriate precautions, such as good hygiene practices, and proper cleaning, coverage and management of draining wounds for limiting the spread of infection in their household and close contacts.

Asymptomatic carriers, both patients and health-care workers, constitute important MRSA reservoirs. The use of hand hygiene, environmental cleaning, patient isolation as well as barrier precautions such as gloves, gowns and masks plays an important role in preventing MRSA transmission in hospital settings. Routine screening of health-care workers and patients is also effective in preventing the MRSA spread and is cost-effective in the long run. Inclusion of swabs from colonization sites other than the anterior nares has been shown to increase the sensitivity of MRSA screening from 80 to 92%. In addition, prophylactic approaches based on the use of vaccines, and/or passive immunization with antistaphylococcal antibodies directed against PVL/PSMs/α-toxin are also being studied.

Decolonization presents an effective strategy to prevent infection in MRSA carriers as well as its transmission to non-carrier population. Many topical and systemic antimicrobial agents, and antiseptic bodywashes have been employed in decolonization regimens to prevent MRSA outbreaks in health-care settings and community. Mupirocin is the best topical antimicrobial currently available, and is a very
effective MSSA and MRSA decolonizing agent.\textsuperscript{36-38} Completion of nasal mupirocin treatment (2\%) can successfully decolonize 81.5 to 100\% patients.\textsuperscript{36} However, poor compliance in some community settings, recolonization following regimen completion, and development of resistance to this drug may limit the widespread use of such interventions.\textsuperscript{30} In India, 2-5\% and 1\% of MRSA strains have been reported to exhibit high-level (plasmid-mediated) and low-level (chromosomal mutational) mupirocin resistance respectively.\textsuperscript{39,40} A plasmid encoding for mupirocin resistance has been detected in the genome of CA-MRSA USA300. Apart from mupirocin, chlorhexidine washing can also reduce the risk of MRSA infection and colonization.\textsuperscript{19}

\textbf{Conclusion}

\emph{S. aureus} has a remarkable ability to develop antibiotic resistance, leading to four distinct resistance waves that have occurred in the past sixty years. The advent of PRSA, then MRSA and now vancomycin resistance has resulted in a steady decline in the efficacy of these valuable antibiotics. The MRSA first emerged as a nosocomial pathogen (HA-MRSA; in the 1960s), then further surfaced as a community-based infection (CA-MRSA; in the 1990s) and has subsequently increased the staphylococcal disease burden. It is a global public health problem and represents the most commonly identified antibiotic-resistant pathogen. The incidence of HA- and CA-MRSA infections as well as the prevalence of different MRSA clones varies considerably among countries. Some MRSA clonal lineages are more frequently isolated than others owing to their superior survival and transmissibility. The HA-MRSA is endemic in many hospitals worldwide. The CA-MRSA has a smaller fitness burden, higher transmissibility and virulence compared to HA-MRSA and is epidemic in many countries. In addition, the distinction between CA- and HA-MRSA is gradually fading, owing to the emergence of \textit{pvl} negative and/or MDR CA-MRSA clones and its invasion into hospitals as well. The MRSA has markedly influenced the empirical therapy for staphylococcal infections. Limited therapeutic options are available for the management of these infections. Most \textbeta-lactam antibiotics are ineffective against both HA- and CA-MRSA. The HA-MRSA is usually MDR but CA-MRSA is often susceptible to non-\textbeta-lactams. Resistance to non-\textbeta-lactam drugs varies geographically and may change over time. The CA-MRSA-associated skin and soft-tissue infections are treated with oral antibiotics including doxycycline, minocycline, clindamycin, trimethoprim-sulfamethoxazole, rifampicin, and fusidic acid. Severe CA-MRSA infections and HA-MRSA demand intravenous vancomycin therapy. Transmission may be prevented by following universal infection-control strategies and decolonization therapy.

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Carbapenem-resistant enterobacteriaceae: a reality check

Camilla Rodrigues*

Abstract

Resistance to antimicrobial agents in several bacteria is on the increase because of the irrational and rampant use of antimicrobial drugs. This is destroying the premise of modern medicine that whenever required, adequate antibiotic cover will be available to save the patient’s life. Such premise is no longer true. Several multidrug-resistant bacteria are now detected with great frequency. Carbapenem-resistant enterobacteriaceae belong to this category of resistant bacteria that were recently labelled as superbugs too, simply because the commonly available antibiotics are ineffective against them.

It was destined to happen. Call it the breach of the last bastion or the burst of the antibiotic bubble, the emergence of antimicrobial resistance to carbapenem with little or no back-up drugs is hardly unsurprising. In fact it is the naïve response of the worldwide scientific community over the last fifty years that has been unexpected and perhaps disappointing. Bacterial resistance to antimicrobials has been increasing at a dizzying pace — a remarkable testimony to the ability of bacteria to collect and exchange resistant genes with unimaginable efficiency and complete lack of species specificity. The ultimate success in the practice of modern day medicine is based on the premise that whenever required, adequate antibiotic cover will always be available. However, if the efficacy of the top-of-the-line antibiotics is going to be reigned in by the ubiquitous bacteria as E. coli and K. pneumoniae, then in the near future, treatment options especially in critical care, oncology and transplant medicine are likely be in serious jeopardy. Carbapenems (currently ertapenem, imipenem, meropenem and doripenem, etc.) are the most effective and potent β lactam antibiotics, with the broadest spectrum and the least resistance. They are reliably active against multidrug-resistant Gram-negative bacteria and form the mainstay in the treatment of serious infections in most hospitals across the world today. Resistance to these top-of-the-line antimicrobials in the traditional, established nosocomial pathogens namely Pseudomonas aeruginosa and Acinetobacter baumannii has been extensively described over the last eight years.1 But when bacteria residing in the gut as enterobacteriaceae find ingenious ways of survival, despite treatment with carbapenems, is time to admit that the war against the microbes is taking an ominous turn.2 With the Gram-negative antibiotic pipeline having practically dried up for the next 10 -15 years, it is also time to dispel our misplaced optimism that some new drug is bound to arrive. The common mechanisms that are responsible for carbapenem resistance include changes in outer membrane proteins, overexpression of drug efflux pumps and carbapenem hydrolyzing enzymes. Carbapenemases potentially herald the end of treatment of Gram-negative infections because of all the major mechanisms conferring resistance the

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most menacing are these hydrolyzing enzymes, as they also compromise efficacy to other β-lactams. Additionally, there is the inevitable co-resistance to the other main classes of commonly used antibiotics, namely the fluoroquinolones and the aminoglycosides.

The high-level resistance to carbapenems by such carbapenemases is essentially of three types – *Klebsiella pneumoniae* carbapenemase (KPC); metallo beta lactamasases (MBLs); and oxacillinases. Carbapenemases are beta lactamasases and by tradition the nomenclature of beta lactamasases is based on their substrates, biochemical properties, location of their discovery, location of the gene on the chromosome, strains of bacteria, patients providing the sample or even the investigator who described them! Various MBLs in the recent past have been named after the cities associated with them, such as VIM (Verona Integron-encoded Metallo β-lactamase); GIM (German IMipenemase); SIM (Seoul IMipenemase). The infamous NDM-1 (New Delhi Metallo-1) was ostensibly named as it was first isolated from a Swedish patient who was admitted to a hospital in New Delhi. However, the fact that the gene encoding for this resistance actually originated in India cannot be proven. The NDM-1 gene shares little identity with the other MBLs and is apparently mobile on a plasmid that is readily transferable.

The Catch 22 situation in countries that have high rates of extended spectrum beta lactamasases (ESBLs) in enterobacteriaceae compels them to place a higher reliance on carbapenems. The inevitable use of carbapenems is consequently bound to exert greater selective pressure. A recent pilot study from our tertiary care centre in central Mumbai that receives referral samples from the city and the State found a steady increase in carbapenem resistance in enterobacteriaceae from 0% in 2006 to 8% in 2009. In our cohort of cases where NDM-1 was detected, the main risk factor was the prior use of carbapenems, usually up to a month.

Multicentric studies across major cities are required to validate whether the NDM-1 gene is the predominant resistance mechanism responsible in carbapenem-resistant enterobacteriaceae (CRE), and whether transmission can occur in the community; more importantly, whether asymptomatic carriage can occur and if so for how long. The dissemination of transposons and integrons have given rise to gene epidemics and new genetic and biochemical mechanisms continue to be described such as plasmid addiction and the phenomenon of hypermutability (especially with fluoroquinolones). It is simplistically assumed that resistance comes at a fitness cost and reduction in prescribing will directly translate to a reduction in the prevalence of resistance. Unfortunately, this is not necessarily entirely true. Evolution ultimately selects bacteria with the least fitness burden.

Gastrointestinal carriage of resistant commensals in the absence of direct selective pressure with carbapenems is what we now need to worry about. A disturbing example is that of the ESBLs produced by enteric pathogens that have spread worldwide since their first description in 1983. As a result of mutations, more than 300 types of ESBLs are described today in various species of the Enterobacteriaceae family and even in other non-enteric organisms. The TEM and SHV type β-lactamases, mainly produced by *Klebsiella pneumoniae*, have spread throughout hospital settings, and CTX-M enzymes, mainly produced by *Escherichia coli*, have become predominant in the community. The alarming precedence set by the spread of these ESBL-producing organisms in the community is now proving a force to contend with, especially in community-acquired infections of the urinary tract. Also, in some regions, fecal carriage of ESBLs has become a fairly frequent feature (up to 20% patients). As a corollary to this, the possibility of a similar phenomenon occurring with gut microbiorganisms acquiring resistance to carbapenems at no apparent fitness cost and
in the absence of any selective pressure is very real.

How does this happen? We must not forget at the same time that essentially, it is the antibiotic usage that is the main driver of resistance, and that resistance is clearly a function of the volume consumed. If resistance to antibiotics is unavoidable, then the simple truth about antibiotics is — the more you use them, the more you will lose them. Increased prescription of antibiotics, paradoxically whether appropriate or indiscriminate, is responsible. Prescribing an antibiotic to a single patient also amounts to prescribing it at the same time to billions of bacteria who have certainly not been taking it “lying down” for the last five decades. It is not the appropriate use but the inappropriate use of antibiotics not only in medical practice but also as growth promoters in veterinary practice and their use in agriculture, etc. that we need to address and curb.

From the perspective of medical health, there is an urgent call to take stock of the situation and salvage what we can. National antibiotic policies should form the framework on which we can tailor local guidelines. These guidelines will help in maximizing the outcome for an individual patient while minimizing the collateral damage to our microbial ecology. But writing and formulating guidelines for the rational use of antimicrobials is easy — the caveat lies in their strict audit and implementation. Mandatory institutional mechanisms to regulate antibiotic prescription must be in place. Evidence-based medicine dictates that in seriously ill patients, inappropriate treatment is the most important predictor of mortality, and this is probably the basis for hitting hard and early with carbapenems. Commitment to rapid de-escalation to narrower spectrum drugs is where we now need to focus. To enable such a streamlining of therapy to actually take place, “cultures” have to be sent before any antimicrobial is initiated, so that the etiologic pathogens are isolated and their susceptibility is available. Additionally, development and evaluation of advanced, improved and rapid diagnostic methods is a vital need.

Needless to add, good infection prevention policies in hospitals that prevent cross-transmission of resistant bacteria from patient to patient is certainly warranted. However, good infection control is less likely to be that effective when resistance arises readily by mutation or when the ultimate pathogen has long been a colonizer in the bowel.

The sad truth is that no single strategy to combat this burgeoning problem of antibiotic resistance seems to be working effectively worldwide. The “10 x 20” initiative promises a global commitment to develop ten new antibacterials by 2020. Whether this will come to pass is a matter of conjecture. If we, as responsible doctors, have to hand over our antibiotic legacy to the next generation, there is an urgent need for introspection. After all, resistance in bacteria is not a matter of “if” but of “when”.

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Growing problem of multidrug-resistant Acinetobacter baumannii in Thailand

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Abstract

Acinetobacter species have become important pathogens in health care settings and are now responsible for substantial mortality and morbidity. The resistance among Acinetobacter spp. has also been continuously rising since 2000. Multidrug-resistance (MDR), is defined as simultaneous resistance to fluoroquinolones, aminoglycoside, and third-generation cephalosporins. Data reviewed from Thailand’s national surveillance mechanism shows that in 2009, at least 62% of isolates of this bacterium were carbapenem and multidrug-resistant. This paper provides a brief summary of our experience.

Introduction

Antimicrobial resistance has become a major health care challenge over the past few decades. Various bacterial species have developed an ability to survive in hostile environments of heavy antimicrobial pressure. For example, methicillin-resistant Staphylococcus aureus (MRSA) has been problematic since 1980s, initially among hospitalized patients, and is now becoming more common in communities in some countries. Extended-spectrum β-lactamase (ESBL) producing organisms were first reported from Germany and have now spread around the globe such that community-acquired strains were also found in many areas. However, the most worrisome trait is the simultaneous resistance to multiple antibiotics among Pseudomonas aeruginosa and Acinetobacter baumannii. These organisms are frequently resistant to carbapenems, the broadest antibiotic available to date, as well as to broad-spectrum cephalosporins, aminoglycosides, and fluoroquinolones.

The National Antimicrobial Resistance Surveillance Centre, Thailand (NARST), which is the national antimicrobial surveillance system under the administration of the Ministry of Public Health with support from the World Health Organization (WHO), has been monitoring the situation of antibiotic resistance among common bacterial pathogens since 1998. In the beginning, bacterial species that showed high resistance rates were P. aeruginosa and MRSA. However, it was noted that resistance among Acinetobacter spp., has been continuously rising since 2000. Multidrug-resistance (MDR), defined as simultaneous resistance to fluoroquinolones, aminoglycoside, and third-generation cephalosporins in the NARST report published in 2009, was estimated to be at least 62% of carbapenem-resistant A. baumannii. This led to training of microbiology laboratory personnel in the surveillance network in 60
hospitals around the country to be aware of this particular bacterial species. Because MDR is defined differently in various literatures, this article will focus mainly on carbapenem resistance.

Prevalence of carbapenem- and multidrug-resistant A. baumannii in Thailand

During 2000-2005, 28 hospitals in the surveillance network submitted their results of susceptibility tests to NARST. In 2000, over 5000 isolates of A. baumannii were identified from various types of clinical specimens. This number almost doubled in 2005, when nearly 100,000 isolates were identified. Approximately 60% were found in respiratory tract specimens. Resistance to carbapenem was the most dramatic in that the rate of resistance was only 2.1% in 2000 and sharply increased to 46.7% in the last year. The next prominent resistant phenotype was the resistant to cefoperazone/sulbactam, which used to be the drug of choice for the treatment of infections caused by A. baumannii. In 2000, 97% of isolates were susceptible to this β-lactam/β-lactamase inhibitor combination due to dual antimicrobial activity of the two compounds. In contrast, only 88% remained susceptible to this agent in the last year of that study period. The highest carbapenem-resistance rates were found in the northeastern region, northern region, and Bangkok, respectively. Furthermore, over 50% of A. baumannii were not only resistant to carbapenems, but also to many other broad-spectrum agents such as ceftazidime, cefoperazone/sulbactam, ciprofloxacin, and amikacin. In accordance with reports from around the world, a high rate of resistance was seen in intensive care units (ICU). For example, the overall rates of imipenem-resistance in ICUs were 64% and 48% in non-ICU populations in 2005.1

Continuing surveillance performed after the above data were published revealed an even more difficult situation. Overall, carbapenem-resistance rates increased to 59.1% in 2009. A high rate of resistance was seen in all hospitals in the network. These hospitals are mainly medium-sized, secondary to tertiary care hospitals. A few hospitals were large (>500-1000 beds) and were also university-affiliated. One tertiary-care, university-affiliated hospital in the eastern region reported up to 76.1% resistance. This figure was similar to what we found in Bangkok where at least three university hospitals which are not in NARST reported a similar rate of resistance. However, if one explores further into each individual area of the hospital, the highest rate of resistance will be found in the ICU. In these university hospitals, only 10% to 15% of A. baumannii in ICUs are still susceptible to carbapenems, and most of these isolates are resistant to almost all available antimicrobial agents in the hospitals. Another unpleasant fact is that nearly all these ICU isolates are now almost completely resistant to major classes of antibiotics including carbapenems, third- and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones. In addition, resistance to cefoperazone/sulbactam is also increasing, which further limits treatment options for these problematic organisms. The only two antibiotics that might still be used with little hope are tigecycline and colistin. Tigecycline is a minocycline derivative that has bacteriostatic activity against several bacterial species including carbapenems, third- and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones. It has some limitations. Firstly, there is no standardized susceptibility breakpoint for...
A. baumannii and the susceptibility test should be performed using the broth dilution technique, which is not routinely done in microbiology laboratories in developing countries. Therefore, if one wants to prescribe this agent for patients, close monitoring of clinical response is critical. Secondly, resistance can emerge easily during therapy. Lastly, colistin can lead to impaired renal function and/or neurotoxicity in a large number of patients. These factors are therefore limitations for clinical use of colistin.

Factors influencing epidemiology of carbapenem- and multidrug-resistant A. baumannii

It has been well documented that the emergence and increasing rate of resistance to an antibiotic correlates with the use of that particular agent. In the case of carbapenem-resistance, studies regarding this correlation have been published as well. Our observation found that carbapenems have been used increasingly in the past two decades. From 1994 to 2001, the rate of carbapenem use in a university hospital was lower than 10 defined daily doses (DDD) per 1000 patient-days. During that period, the carbapenem-resistant A. baumannii ranged between 2% and 10%. From 2001 onwards, carbapenems were used increasingly every year until in 2009 when the rate of use was about 54 DDD/1000 patient-days. Along with this increased use, the rate of carbapenem-resistant A. baumannii increased dramatically until 2009 when it reached about 76% as mentioned earlier. The driving force leading to increased use of carbapenem in healthcare facilities is the emergence and spread of extended-spectrum β-lactamase producing Gram-negative bacilli, mainly enterobacteriaceae, in hospitals nationwide. Polwichai P et al. pointed out that the prevalence of ESBL-producing E. coli and K. pneumoniae increased gradually from around 20% in 2000 to 40% in 2005. In the early years of the epidemic, ESBL-producers were mainly hospital isolates. However, community-onset infections caused by these organisms have been increasingly reported. The production of this type of enzyme has been associated with poor outcome when a third-generation cephalosporin was used and many cases needed to change the antibiotic to one of carbapenems. Furthermore, a higher proportion of community strains of E. coli have become resistant to fluoroquinolones and gentamicin, other alternatives to β-lactam agents. Because of widespread concern regarding multidrug-resistance, particularly ESBL producers, many practicing physicians now choose a carbapenem as empirical therapy while waiting for the results of microbiologic studies. Once the results of the cultures have been reported, we would expect physicians to adjust the regimen accordingly, but this was not true in all circumstances. Other forms of inappropriate use of carbapenems included use as routine surgical prophylaxis or prescribed to patients whose fever did not respond to other antibiotics even though the cause of fever might not be an infectious process. Frequently, patients came to medical care because of high fever and physicians tend to prescribe a carbapenem because of the misconception that an antibiotic that has the broadest spectrum of coverage would be “the best” for their patients.

In addition to inappropriate use of carbapenems and other broad-spectrum agents, inadequate infection control measures is another important aspect of the endemicity. Hospitals in Thailand were mainly built so that many patients stay in a common large room consisting of 4 to 30 beds. Spaces between beds are usually small. In many circumstances, these “common wards” were not meant to function as intensive care units but they were modified to accommodate critically-ill patients due to limited space in the hospitals. Old buildings may not have an adequate number of sinks for hand washing. Medications are prepared in a designated area in each patient care unit, where it might be near a sink, which
creates a moist environment, not at the pharmacy department. Along with limited space and suboptimal design, the numbers of healthcare workers are also limited. The nurse: patient ratio had hardly ever been at 1:1, but mostly is around 1:2-8. With this working environment, it is easy for any bacterial pathogens to spread from person to person via the hands of health-care personnel and/or contaminated inanimate objects.

Resources for isolation in hospitals are also limited. In order to isolate patients who carry MDR pathogen effectively, an adequate number of single-bed isolation rooms equipped with bathroom and necessary space for keeping patient care items in place is needed. Hand hygiene facilities including sink, soap, and paper towels for hand washing and alcohol-based hand rub solution should also be easily accessible in the room. Water-proof isolation gowns, gloves, and surgical masks are essential items as well as medical instruments and devices such as sphygmomanometer, blood pressure cuffs, and stethoscopes. However, all these resources are limited in most hospitals.

The higher the prevalence, the more resources and efforts are needed to bring the epidemic under control. Since the prevalence of carbapenem-resistance and other epidemiologically important type of resistance have reached high levels in many hospitals, it is a true challenge for health-care providers to work tirelessly to reduce the magnitude of this problem.

Examples of efforts to reduce the prevalence of MDR A. baumannii

Controlling MDR A. baumannii is not an easy task because the epidemiology of MDR A. baumannii is complicated. In brief, its emergence and spread in healthcare facilities involved both inappropriate uses of broad-spectrum antimicrobial agents and inadequate infection control. Therefore, multifaceted intervention is the only possible means to control the epidemic. These measures include an antimicrobial stewardship programme, education for health-care workers of all disciplines, standard and contact precautions, an effective hand hygiene campaign, and/or active surveillance culture for early detection. These efforts have been tried in many hospitals in Thailand. Some of these have been published in medical journals. For example, a medical school in central Bangkok has been trying to establish an antimicrobial stewardship programme focusing on carbapenems via the use of an antibiotic order form. This programme resulted in a stable rate of carbapenem use in the institution despite increasing prevalence of ESBL producers. However, it has not affected the prevalence of MDR A. baumannii since many other broad-spectrum antibiotics were not included in the order form because of the manpower limitation to closely monitor the use of these agents. In addition, isolation precautions have not been fully implemented. This particular university hospital is now using a computer-assisted antimicrobial prescription programme with direct linkage to microbiology data so that more antibiotics can be included; the prescriptions are easier for monitoring and feedback; and educational messages can be placed for prescribing physicians. The initial phase of the use of such a programme resulted in a significantly decreased use of carbapenems. Another university hospital has implemented multifaceted interventions in the ICU including an educational programme, introduction of an antibiogram, use of antibiotic prescription forms, and prescribing controls. After the intervention, the inappropriate use of broad-spectrum antibiotics was significantly reduced. The incidence of infections due to several MDR pathogens such as MRSA, ESBL-producing E. coli and Klebsiella pneumoniae, and third-generation cephalosporin-resistant A. baumannii was significantly decreased. The total cost-saving value of these interventions was estimated to be US$ 32,231.5 The authors further expanded the intervention to regular wards that had 30 beds and where the
nurse-patient ratio was 1:8. They created a cohort area in addition to the isolation measures. The intervention brought about successful reduction of MDR pathogens as well.6 According to our personal conversation with colleagues in many hospitals, we are aware that, in fact, efforts to control MDR A. baumannii are under way in their areas.

In summary, antimicrobial resistance in A. baumanii, particularly resistance to carbapenems and other broad-spectrum antibiotics, is a challenge for healthcare services worldwide. Its emergence and spread are tied closely with inappropriate use of antibiotics, not only carbapenem itself, but also other broad-spectrum agents such as third- and fourth-generation cephalosporins. Inadequate infection control efforts further aggravate the problems. Interventions that encompass these two major aspects have been demonstrated to work well even in areas with limited resources like Thailand. We therefore encourage all health-care sectors to create such programmes taking into account normal practice, culture, and resources available for practicality of the intervention. With determined efforts, we should be successful, at least in retarding the accelerated increase in the incidence of these pathogens before we are left in a world that seems to have no effective antibiotics to fight against serious bacterial infections.

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Preserving efficacy of chloramphenicol against typhoid fever in a tertiary care hospital, India

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Abstract
A decrease in the incidence of multidrug resistant Salmonella Typhi was observed in a tertiary care hospital along with an increase in non-multidrug resistant isolates of same organism. This is most likely due to reduced use of traditional antimicrobial agents (ampicillin, chloramphenicol, co-trimoxazole) and increasing reliance on ciprofloxacin as the first line of treatment of patients with typhoid fever.

Background
Typhoid fever, caused by Salmonella enterica serovar Typhi, is a major health problem in developing countries, particularly in the Indian subcontinent and in South-East Asia. Typhoid fever is the most serious form of enteric fever and in 2000 it was estimated that the global number of typhoid cases exceeded 21 000 000, with more than 200 000 deaths1. In cases of enteric fever, it is often necessary to commence treatment before the results of laboratory sensitivity testing become available, and in this respect, ciprofloxacin has become the first-line drug for treatment, especially since the widespread emergence of S. Typhi isolates that are multidrug resistant (MDRST) to the more traditional antimicrobial agents comprising chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole [cotrimoxazole]2. However, this switch to ciprofloxacin and selective pressures exerted by the irrational use of ciprofloxacin in human and veterinary therapeutics in a population endemic with nalidixic acid resistant S. Typhi (NARST) strains has led to a subsequent increase in the occurrence of S.Typhi isolates resistant to this antimicrobial agent and decline in MDRST, including in India3,4. In endemic countries uncomplicated enteric fever is treated on an out-patient basis with oral antibiotics. WHO guidelines for treatment of uncomplicated enteric fever exist but are non-specific and are becoming out-of-date5.

In India, the incidence of MDRST isolates has been reported to be as high as 60% in Pune, in 19996, but then declining to 22% in Nagpur (2001)7. The resurgence of resistant isolates in Ludhiana, India, in 2002 has, however, been a cause for concern8. A US-based study of imported strains9 noted an increase in the number of MDR and NARST globally, although all isolates remained sensitive to ciprofloxacin and ceftriaxone. In Bangladesh10 there has been a reported decrease in MDR isolates with no corresponding increase in sensitive strains. For ciprofloxacin there has been an increase in minimum inhibitory concentration in strains imported into the UK11, in Bangladesh12, as well as in India3.
There have been several reports of therapeutic failure of ciprofloxacin in patients with enteric fever and in 2009 we have reported a strain of S. Typhi showing high-level resistance to ciprofloxacin (at a MIC value of 64 μg/mL). Reports of typhoidal salmonellae with increasing MIC and resistance to newer quinolones raise the fear of potential treatment failures and necessitate the need for new, alternative antimicrobials. Extended-spectrum cephalosporins and azithromycin are the options available for treatment of enteric fever. The emergence of broad spectrum β-lactamases in typhoidal salmonellae constitutes a new challenge. Worldwide, there are sporadic reports of high level resistance to beta-lactam antibiotics including ceftriaxone in typhoidal salmonellae, as well as the presence of CTX-M-15 and SHV-12 extended spectrum β-lactamases (ESBLs), and resistance to third generation cephalosporins. Further, we recently reported on an ACC-1 AmpC β-lactamase producing S. Typhi that had been isolated from the blood of a girl aged 14 years.

In developing countries such as India, ciprofloxacin continues to be the mainstay in the treatment of enteric fever as it is orally effective and economical. The emergence of S. Typhi highly resistant to ciprofloxacin is a cause for worry for both clinicians and microbiologists as well as for patients. Though fluoroquinolone resistance is chromosomally mediated, selective pressures exerted by the overuse of these drugs may see such isolates becoming more common in the future. Of interest, though, is the possibility of turning to an older drug such as co-trimoxazole for treatment, in case of susceptible isolates. Therefore, a study was undertaken to characterize trends in antimicrobial resistance in clinically relevant S. Typhi isolates originating from Pondicherry, India, to help guide clinicians on successful treatment therapies.

**Study**

Blood cultures from a total of 3744 patients presenting with fever at the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) Pondicherry and Government General Hospital, Pondicherry during the period January 2005 to December 2009, as part of prospective surveillance for typhoid fever were taken for microbial diagnosis. The patients ranged in age from 2 years to 74 years (median, 43 years). Standard blood culture protocols were followed. Colonies were identified as S. Typhi using standard biochemical methods, and confirmed using Salmonella polyvalent O, O9 and H:d antisera (Murex Biotech, England). Isolates were tested for susceptibility to antimicrobials using the Kirby Bauer disk diffusion method and antibiotic MIC was determined by both agar dilution and Etest (AB Biodisk, Solna, Sweden) against the antibiotics ciprofloxacin, ampicillin, chloramphenicol and ceftriaxone. MICs against gatifloxacin and ofloxacin were determined by Etest only. Genotyping was performed on a representative sample of isolates using pulsed field gel electrophoresis (PFGE).

A total of 338 S. Typhi isolates from two hospitals recovered during the period 2005 to 2009 were included in this study representing 100% of the S. Typhi isolates recovered during this period. Of these isolates, 222 (66%) were fully susceptible to ampicillin, chloramphenicol and cotrimoxazole; 74 (22%) were MDRST; 264 (78%) were nalidixic acid resistant S. Typhi (NARST); and 27 (8%) were both MDRST and NARST. The following resistance pattern of S. Typhi was observed: chloramphenicol, 22%; ampicillin, 24%; cotrimoxazole, 30%; ciprofloxacin, 8% and ceftriaxone, 0.3%. We compared our present observations with those from previous years (Table 1). There was a steady decline in the number of MDRST isolates over the study period, as well as a parallel increase in NARST.
A remarkable decrease over the years in resistance to chloramphenicol, ampicillin, and cotrimoxazole was noticed.

**Table 1**: Antimicrobial resistance pattern of Salmonella enterica serotype Typhi, Pondicherry, India

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>No. of resistant isolates/ total no. tested (%)</th>
<th>January 2002 to November 2003&lt;sup&gt;a&lt;/sup&gt;</th>
<th>January 2005 to December 2009&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>84/157 (53)</td>
<td>82/338 (24)</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>82/157 (52)</td>
<td>76/338 (22)</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>102/157 (65)</td>
<td>103/338 (30)</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>131/157 (83)</td>
<td>264/338 (78)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0/157 (0)</td>
<td>27/338 (8)</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0/157 (0)</td>
<td>1/338 (0.3)</td>
<td></td>
</tr>
</tbody>
</table>

Note:  
<sup>a</sup> see reference 4.
<sup>b</sup> observed in the present study.

**Figure 1**: Proportion of NAR and MDR among the isolates of S. Typhi.

(Number in brackets indicate total number of blood culture positive S. Typhi isolates cultured in each year at JIPMER and Government General Hospital, Pondicherry, India)

NAR - nalidixic acid resistant S. Typhi
MDR – multidrug resistant S. Typhi

**Discussion**

Typhoid fever remains a public health concern in developing countries such as India, largely due to socioeconomic problems involving poor sanitation and poverty. In this study, we investigated 338 blood culture-positive S. Typhi isolates and similar to previous research, the study indicated a higher number of culture-positive cases occurring in persons aged 6–20 years [17]. Our findings indicate a remarkable decline in the number of MDRST isolates over the study period. This was accompanied by an increase in non-MDR isolates, though the majority of these (78%) were NARST and showed reduced sensitivity to ciprofloxacin. These findings are most likely due to decreased prescribing of traditional antimicrobial agents, and an increasing reliance on ciprofloxacin as the first-line treatment for S. Typhi in Pondicherry<sup>4</sup>.

Antimicrobial resistance in our study was not associated with a particular clonal genotype of S. Typhi and the MDRST isolates belonged to different PFGE genotypes, similar to previous research<sup>18</sup>. Further, during the study period, there was a gradual increase in MICs against ceftriaxone (a third-generation cephalosporin), from a MIC<sub>90</sub> value of 0.064 μg/mL in 2005 to 0.25 μg/mL in 2009, though still well within the susceptible range. Resistance to quinolones and, more recently, increases in MIC levels to third- and fourth-generation cephalosporins, re-emphasize the importance of continued surveillance in the revision of enteric fever treatment protocols<sup>16</sup>.

A recent meta-analysis of treatment trials for typhoid suggests that among sensitive cases of typhoid, cure rates with oral first-line agents may be comparable with fluoroquinolones<sup>19</sup>. The current situation also offers an opportunity to evaluate alternative regimens and combination therapies for treatment of drug-resistant typhoid<sup>20</sup>. For example, treatment with antimicrobials such as third-generation cephalosporins and even macrolides (azithromycin) has resulted in favourable outcomes, although the cost of therapy with these agents remains prohibitive for use in developing countries<sup>21</sup>.

In view of the current antimicrobial susceptibility trend (overall decline in
MDRST) in Pondicherry, of the first-line antimicrobials, ampicillin, chloramphenicol and cotrimoxazole, especially chloramphenicol may be of use again in endemic areas with control over antibiotic use (antibiotic stewardship). Decline in MDRST, probably due to the loss of a high-molecular-weight self-transferable plasmid encoding chloramphenicol, ampicillin, and cotrimoxazole resistance should lead to the cautious reuse of classical first-line antibiotics, such as chloramphenicol. Also, a high relapse rate, a high rate of continued and chronic carriage, and bone marrow toxicity are other concerns with reuse of chloramphenicol for the treatment of typhoid fever. The spread of fluoroquinolone resistant S. Typhi may necessitate a change towards 'evidence-based' treatment for typhoid fever. In order to better manage and prevent the spread of antimicrobial resistance, both clinicians and governments require accurate information.

References and bibliography

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Drug resistance in fungi — an emerging problem

Arunaloke Chakrabarti*

Abstract
Over the past quarter of a century, invasive fungal infections have emerged as an important cause of morbidity and mortality in immunocompromised patients. Although several new antifungal drugs have been licensed in recent years, antifungal drug resistance is becoming a major concern during treatment of such patients. The resistance may be intrinsic, acquired or clinical. The understanding of the mechanism of resistance and clinical impact is important while planning treatment strategies. Four altered gene expression pathways have been identified in azole resistance. The mechanism of resistance in polyene and echinocandins is still not clearly understood. Recent studies have revealed that molecular chaperone heat shock protein (Hsp90) can alter the relationship between genotype and phenotype leading to a profound impact on antifungal drug resistance. Though definite progress has been made to correlate standardized in vitro antifungal susceptibility testing with prediction of treatment outcome, limitations still exist due to time required for testing and understanding the factors leading to clinical resistance. Overall, the level of resistance to antifungal agents is still relatively low, but there is a possibility of antifungal resistance becoming a crucial determinant of outcome following antifungal therapy in future.

Introduction
Medical progress has led to an expanding population of susceptible hosts with impaired immunological defenses against infection in the community and hospitals. These populations are at heightened risk for many opportunistic fungal diseases including candidiasis, aspergillosis, mucormycosis (zygomycosis), cryptococcosis, pneumocystosis. Traditionally Candida and Aspergillus species accounted for the majority of infections. Candidemia is the fourth leading cause of blood-stream infections and carries 35-55% mortality1. The incidence of mould infections has also increased in the recent past, especially infectious caused by Aspergillus spp. where the mortality rate crosses 50% in such patients2. Mucormycosis is a threat in uncontrolled diabetes in developing countries like India3. In this scenario, contemporary epidemiological trends also indicate a certain shift of the fungal pathogen towards resistant species among those common two genera, Candida and Aspergillus, and emergence of the previously uncommon fungi that are particularly difficult to manage4,5. These include C. glabrata and C. krusei in yeast with their reduced drug susceptibility, and among the mould fungi, these include the non-fumigatus Aspergillus spp. like Aspergillus terreus, zygomycetes and Fusarium spp. Selective pressure due to increased use of antifungal prophylaxis in high-risk patients has been suggested as a contributory factor for this shift and emergence of uncommon mould6.

Antifungal drugs and the problem of resistance
For a long time amphotericin B deoxycholate and 5 fluorocytosine were the only therapeutic options for invasive fungal infections. The first
therapeutic alternatives began to emerge with the introduction of fluconazole and itraconazole in the late 1980s. Expansion in antifungal research in the last two decades has led to the development of lipid formulations of amphotericin B (amphotericin B colloidal dispersion, amphotericin B lipid complex, and liposomal amphotericin B), a second-generation broad spectrum triazoles (voriconazole, posaconazole) and an entirely new class of antifungal agents, the echinocandins (caspofungin, anidulafungin and micafungin). A few of the new antifungal agents are under clinical trial (Table 1). Despite the increase in the spectrum of antifungal agents now available, the choice of suitable antifungal agents remains relatively limited due to the emergence of comparatively more resistant fungal species, slow mycological diagnosis, variable drug bioavailability in immunocompromised patients, toxicity of antifungal agents, lack of either oral or intravenous preparations, drug interaction, and most importantly due to development of resistance and breakthrough infections\(^7,8\).

Unfortunately, the increased use of triazoles in prophylactic and empiric antifungal therapy in high-risk patients has led to selective pressure towards drug-resistant Candida and Aspergillus species\(^9\). It has resulted in infection either through the inherently resistant fungi (primary resistance) or through the resistant subpopulation of the normally susceptible fungi (secondary resistance). Fortunately, development of acquired resistance in fungi is not a “fast-track” event as in bacteria or viruses, except in the event of nearly one third patients with advanced AIDS harbouring fluconazole-resistant C. albicans in their oral cavity\(^9\). As no known mechanism of horizontal resistance gene transfer was known in fungi, it was believed that exceptionally large number of viable fungi when exposed to high levels of antifungals in the oropharyngeal candidiasis might become resistant to antifungal agents\(^10\). The episode of rapid emergence of antifungal resistance ended with the advent of effective antiretroviral therapy in patients with AIDS. However, there is no scope for complacency as recently in a genome-wide analysis of three Fusarium species, it was shown experimentally that complete chromosomes could be transferred between different fungal strains\(^12\). Prior to this it was believed that fungi were generally confined to vertical gene transfer, a slower type of genetic change based on mutation, recombination and the effect of

| Table 1: Currently available and “under trial” antifungal agents |
|---------------------|-------------------------------|---------------------|---------------------|
| **Compound** | **Currently available** | **Under clinical trial** | **Target site** |
| Polyenes | Amphotericin B deoxycholate and lipid formulations (amphotericin B lipid complex, amphotericin B colloidal dispersion, liposomal amphotericin B) | Liposomal nystatin | Ergosterol in cell membrane |
| Fluorinated pyrimidine | 5 fluorocytosine | DNA, RNA synthesis |
| Triazoles | Fluconazole, itraconazole, voriconazole, posaconazole | Isavuconazole, ravuconazole, albiconazole | Ergosterol biosynthesis - 14\(\alpha\) demethylase |
| Echinocandins | Caspofungin, anidulafungin, micafungin | Aminocandins | 1,3-β-d glucan synthesis in cell wall |
| Allylamines | Terbinafine | Ergosterol biosynthesis – squalene epoxidase |
This new understanding of fungal genetics would help researchers understand the types of fungi that are most likely to develop resistance to antifungal agents.

The overall resistance in Candida spp. to fluconazole and voriconazole is considered to be around 3-6% and level of resistance has remained constant over a decade. However, a recent report from India revealed panazole resistance in ~10% of Candida species. Triazole resistance in A. fumigatus is increasingly being recognized and up to 6% of clinical isolates were found to be resistant to triazole in the United Kingdom and the Netherlands. In contrast to azoles, echinocandin resistance does not seem to be the major cause of concern, as global surveillance studies indicate that there has not been any significant epidemiological shift in the susceptibility of Candida spp. isolates to echinocandins. However, since 2005 there have been multiple case reports of breakthrough infections after echinocandin therapy in patients with AIDS or acute myeloid leukaemia. The prevalence of flucytosine resistance in yeast remains low (<2%). But the speed at which yeast can develop resistance to flucytosine has prompted clinicians to use the compound in combination with mainly amphotericin B. Overall, though the incidence of antifungal resistance is low, it remains a serious problem in the management of high-risk patients. Recently, concern has been expressed on the possibility of induction of resistance in opportunistic fungi in the environment as azole fungicides are used in agriculture.

**Mechanism of antifungal resistance**

The mechanism of drug resistance in microorganisms traditionally takes the path of either identifying a cellular determinant that prevents entry of the drug or removes the drug from the cell or inactivates the drug or prevents the drug from inhibiting the target of various combinations of the above-mentioned pathways. In fungi, mutation in gene encoding target proteins, up-regulations of expression of multidrug efflux pumps and drug target themselves, altering the stoichiometry of the inhibitor target ratio in favour of fungus are possible mechanisms. However, no fungus has yet been shown to have the ability to degrade an antifungal agent like beta-lactamase in bacteria. Multidrug resistance, called pleiotrophic drug resistance (POR) in Saccharomyces cerevisiae is possibly an ancient model for multidrug resistance that operates in pathogenic fungi through the efflux pump. Therefore, most studies on the antifungal drug resistance mechanism have targeted the efflux pump mechanism. Inhibition of the pump over-expression or drug pump activity may transform a fungistatic drug like azole into a fungicidal drug. In C. albicans a unique mechanism of gene amplification leading to azole resistance has been identified. The mechanism involves formation of aneuploidy or isochromosome, in which the chromosome arm bearing both transcription factor (regulating ABC transporter) and target of the azoles Erg11 is duplicated. The different genetic alterations and mechanism of resistance in Candida spp. and Aspergillus spp. are summarized in Table 2.

**Table 2: Genetic mechanism of resistance in Candida and Aspergillus (modified from reference 9)**

<table>
<thead>
<tr>
<th>Antifungal Azoles</th>
<th>Candida</th>
<th>Aspergillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased drug concentration (efflux pumps)</td>
<td>↑ CDR gene of ATP binding cassette (for all azoles)</td>
<td>Mdr1, Mdr3, Mdr4</td>
</tr>
<tr>
<td></td>
<td>↑ MDR gene of major facilitator class (for fluconazole)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. albicansCDR1, CDR2, MDR1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. glabrataCDR1, PDH1, Snq2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. dubliniensiscdCDR1, CDMDR1</td>
<td></td>
</tr>
</tbody>
</table>
### Antifungal Azoles

<table>
<thead>
<tr>
<th>Candida</th>
<th>Aspergillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Target cell alteration</td>
<td></td>
</tr>
<tr>
<td>- Mutation of ERG11</td>
<td></td>
</tr>
<tr>
<td>- Decrease affinity ERG11p (intrinsic resistance to fluconazole in <em>C. krusei</em> isolates)</td>
<td></td>
</tr>
<tr>
<td>- ↑ ERG11p</td>
<td></td>
</tr>
<tr>
<td>- Cyp51A (mutation at codon 220 develops resistance to all azoles, mutation at codon 54 develops cross-resistance to itraconazole and fluconazole)</td>
<td></td>
</tr>
<tr>
<td>- ↑ Cyp51A</td>
<td></td>
</tr>
<tr>
<td>- Bypass pathway</td>
<td></td>
</tr>
<tr>
<td>- Mutation of ERG3 (prevent formation of toxic products from 14-α methyl fectosterol)</td>
<td></td>
</tr>
<tr>
<td>- Chromosomal aneuploidy or isochromosome</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em> – chromosome 5</td>
<td></td>
</tr>
</tbody>
</table>

### Polyenes

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Target site alteration</td>
<td></td>
</tr>
<tr>
<td>- Mutation of ERG3 (accumulation of other sterols)</td>
<td></td>
</tr>
<tr>
<td>- Alteration of drug : target ration by any mechanism</td>
<td></td>
</tr>
</tbody>
</table>

### Echinocandins

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Target site alteration</td>
<td></td>
</tr>
<tr>
<td>- Point mutation mostly at Ser 645 of Fks1</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em>, Fks1</td>
<td></td>
</tr>
<tr>
<td><em>C. glabrata</em>, Fks1, Fks2</td>
<td></td>
</tr>
<tr>
<td>- Activation of salvage or compensatory pathway for chitin synthesis (PKC cell integrity pathway)</td>
<td></td>
</tr>
</tbody>
</table>

From the evolutionary perspective, none of the mechanisms acts alone. Phenotypic resistance depends on the genetic variation occurring in a particular genome. However, the development of resistance is often accompanied by a deleterious effect of mutation on the fitness of fungi in the absence of the drug. Compensatory mutation may mitigate this effect and enhance fitness. Hsp90 is known to play an important role in remodelling the relationship between phenotype and genotype in distant species. In antifungal drug resistance its role has been emphasized recently. Hsp 90 acts as a capacitor for accumulation of genetic variation. When its function is compromised by genetic alteration, pharmacological inhibitors or environmental stress, genetic variations are revealed, which lead to alteration of the relationship between genotype and phenotype. Hsp90 acts through calcineurin. Any inhibitor of Hsp90 or calcineurin would possibly act synergistically with the antifungal agent.

### Antifungal drug susceptibility testing

Both the Clinical Laboratory Standard Institute (CLSI), United States of America, and the
European Committee on Antimicrobial Susceptibility Testing (EUCAST) have published approved protocol antifungal susceptibility testing either by broth microdilution or disc diffusion assay. Drug threshold levels for in vitro growth inhibition yield a minimum inhibitory concentration (MIC). The CLSI has recommended antifungal MIC breakpoints to separate susceptible and resistant population for azoles and echinocandins by analysing the in vitro susceptibility data, in vitro outcome and pharmacokinetics/pharmacodynamic studies6, 9, 25. However, EUCAST defined the breakpoint derived from MIC as the Epidemiological Cut-off Value (ECV) to avoid confusion with clinical breakpoints. The EUCAST uses ECV “as the most sensitive measure of resistance development — for measuring resistance development in hospitals and the community, for measuring the effect of interventions and for developing strategies to counteract further resistance development”6, 26. The breakpoint derived through MIC tends to be lower than the clinical breakpoint, as this procedure is independent of dosage regimens. In contrast, the clinical breakpoint is based on distribution of MIC, pharmacokinetics of the antimicrobial agent, and the clinical outcome. Therefore, “the clinical breakpoint should be used in every day clinical laboratory work to provide evidence for rational therapy in the patient”26. While correlating the therapeutic outcome in multiple studies with in vitro antifungal susceptibility testing data, especially the combination of Candida species andazole antifungal agents, a pattern of “90-60” rule emerged like in bacteria: infections due to susceptible isolates respond to therapy ~90% of the time, whereas infections due to resistant isolate respond ~60% of the time27. However, in spite of all these studies and recommended standards, antifungal susceptibility testing rarely influences management protocol in an individual patient, as it takes 48-72 hours after isolation of the fungus. Therefore, there is a need for a more rapid test procedure or “real time” antifungal susceptibility testing for clinicians.

Cross-resistance among antifungal agents

Cross-resistance among azoles is expected as the target of action on fungi is similar. In HIV-positive patients a high level of cross-resistance to itraconazole was observed in fluconazole-resistant C. glabrata and C. tropicalis compared with C. albicans and C. krusei isolates28. Cross-resistance in C. glabrata strains was due to increased expression of CgCDR1, CgCDR2 genes and CDR efflux pumps. Though voriconazole also has cross-resistance with other azoles, the rate is low, and after performing in vitro susceptibility testing it may be used in patients who have previously been exposed to fluconazole or itraconazole6. However, in a study conducted in India, high cross-resistance to fluconazole, itraconazole and voriconazole was reported in C. albicans and C. tropicalis blood isolates, though the mechanism of resistance was not studied.14 Cross-resistance has been observed among the three echinocandins.17, 18 Cross-resistance should not be expected between the echinocandin class of drugs and either the polyene or azoles, as the sites of action are different.

Clinical antifungal resistance

Non-specific symptoms and signs of invasive fungal infections present difficulties in early diagnosis; delay in diagnosis is the major cause of treatment failure28. Even in empiric and targeted therapy the success rate ranged from 32% to 74%19. The major causes of treatment failure have been summarized by Kanafani and Perfect19: (i) incorrect diagnosis of specific fungal disease including immune reconstitution inflammatory syndrome (IRIS) in patients with AIDS after antiretroviral therapy; (ii) failure of antifungal agents to overcome the state of severe immune deficiency in such patients; (iii) more virulent infection such as Cryptococcus gattii infections; (iv) toxicities of antifungal agents (nephrotoxicity in polyene and hepatitis in azoles); (v) poor penetration of antifungal agents at certain sites of fungal
infections such as the central nervous system or the necrotic tissue with poor blood supply; (vi) reduced blood concentration of the antifungal agent due to drug interaction, especially during voriconazole therapy; (vii) suboptimal duration of the antifungal therapy; and (viii) the underlying disease as the main barometer of clinical success and failure in antifungal therapy.

Like bacteria, fungi also produce a biofilm in vitro. It is well known that the biofilm is an important obstruction in antibacterial therapy. In fungal infections similar studies have been conducted. Enhanced extracellular matrix especially beta glucan synthesis during biofilm growth has been shown to prevent penetration of antifungal agents such as azole and polyene29. It is believed that the echinocandins and lipid formulations of amphotericin B can penetrate biofilm better than amphotericin B deoxycholate and azoles19. The clinical trials also indicate the importance of the biofilm. Numerous clinical trials on candidemia have demonstrated that the treatment failure and mortality are high in patients who are on catheters for long periods.30

Conclusion

With increase in the incidence and spectrum of invasive fungal infections, antifungal drug resistance has become an important consideration in the management of patients. Though unlike bacteria, the level of resistance to antifungal agents is relatively low due to the possible absence of drug-resistant plasmid or transposons in fungi, the recently conducted experiment of horizontal gene transfer in pathogenic Fusarium species shows that there is no scope of complacency. The emergence of intrinsically resistant fungal species as a human pathogen is compounding the challenge of planning treatment strategies. Beyond these confounding factors, the conditions leading to clinical resistance should be kept in mind while managing invasive fungal infections in immunocompromised patients.

Acknowledgement

The author acknowledges the help of M. Manpreet Dhaliwal for organizing the references.

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Role of nurses in prevention of antimicrobial resistance

Wanchai Moongtui *, Wilawan Picheansathian** and Wilawan Senaratana**

Abstract

Antimicrobial resistance (AMR) is an important issue in many countries especially at tertiary health care facilities. Improvements in quality in the area of AMR are urgently needed in many countries including Thailand to provide effective and efficient services with the ultimate goal of improving quality of care and patients’ quality of life. Nurses play a major role in controlling AMR problems because patients are in their "hands" and infections with resistant organisms are primarily transmitted through direct contact. Thailand has initiated several steps to build capacity of nurses in this area. These include a Master’s degree programme and short training courses in the area of infection control with the focus on AMR. The nursing and midwifery workforce can play an important role in prevention of nosocomial infection (NI) including AMR prevention and control if they have been properly educated and trained. This paper focuses on the situation of AMR in Thailand and the initiatives of the Faculty of Nursing, Chiang Mai University in building capacity of nurse-midwives in general and infection control nurses in NI and AMR prevention and control and in other areas.

Antimicrobial resistance in Thailand

The primary mission of the health care facility is to respond to the needs of the client or patient, family and community by providing effective and efficient services with equal access to quality of care. Inadequate quality of care could cause nosocomial infection (NI), an infection that can be acquired during hospitalization, and antimicrobial resistance (AMR). AMR has become one of the serious health problems in many countries including Thailand. Its consequences have impacts on the patient and on the family, health personnel and hospitals. In most hospitals, infection prevention and control activities have been integrated into the system of care. NI is one of the key indicators for hospital accreditation in Thailand.

For the hospital to be certified as an accredited hospital, the hospital must incorporate the following components of mission1: (a) has a written policy, strategy and protocol of NI prevention and control; (b) has effective and efficient NI prevention and control programme; (c) has assigned a person responsible for NI prevention and control programme; (d) provides opportunities for personnel to gain knowledge and skills in the NI prevention and control programme with updated information; (e) focuses on the process of NI prevention and control programme activities; and (f) has continuous quality improvements and activities on NI prevention and control.

Incidence of antimicrobial resistant infection

Antimicrobial resistance has been a serious problem especially at the tertiary health care level. The high incidence and impact of AMR
In Thailand has been reported in many studies. Inchai et al. (2003) examined the incidences and distribution of methicillin-resistant Staphylococcus aureus (MRSA) infection in one tertiary care university affiliated hospital during a three-month period. The results showed that the incidence of MRSA infection was 8.2 infections per 1000 admitted patients. At the department level, the majority of the incidences occurred in the medical department (10.8 incidences/1000 admitted patients) followed by the orthopaedic and surgical departments with an incidence of 9.9 and 7.4 per 1000 admitted patients, respectively. The highest incidence of MRSA infection was found in the paediatric intensive care ward with the incidence of 103.4 incidences per 1000 admitted patients followed by the burn unit and surgical intensive care unit with the incidence of 90.9 and 68.2 incidences per 1000 admitted patients, respectively.

Further, Sritippayawan et al. (2009) determined the incidence and risk factors of multi-drug resistance (MDR) NI in a paediatric intensive care unit (PICU) of a university hospital in Thailand during an eight-month period in 2005 among children aged 15 years or less who developed a PICU-related NI. The results showed that 44 patients developed 58 episodes of PICU-related NI with the rate of 28.3 per 1000 patient-days. Thirty episodes (52%) were MDR NI. One of the major risk factors was the history of previous use of a broad-spectrum antibiotic (OR = 9.7, p < .01).

It is clear that the AMR rate is increasing and has evidently an impact on the patients. Apisarnthanarak and Mundy (2009) utilized a matched case control study of mortality associated with MDR Acinetobacter baumannii infections at a Thai tertiary health care centre from July 1, 2007, to June 30, 2008. A case was defined as a patient who was infected with MDR Acinetobacter baumannii and met the definition of a NI. A matched control patient had non-MDR Acinetobacter baumannii infection at a similar infection site, hospital unit, and date of admission. Fifty-six patients with MDR Acinetobacter baumannii infection had higher mortality (80% VS 14%; p < .001) and length of hospitalization was higher (9 days VS 5.4 days, p < .001).

Factors associated with AMR infection in Thailand

Many factors influence the occurrence of AMR problems in Thailand including availability of antimicrobial drugs without prescription, inappropriate physician prescription, and inappropriate practice of AMR prevention and control among health care workers.

1. Availability of antimicrobial drugs without prescription

Antimicrobial drugs are available for purchase in local pharmacies, or in private shops as over-the-counter preparations. Some are without prescription. The use is often inappropriate because of inadequacy in the law enforcement for the regulations regarding antimicrobial purchases. There is also evidence that antimicrobial agents are often sold inappropriately in indications and in quantities in many developing countries.

2. Inappropriate prescription

The use of antimicrobial drugs should be based on appropriate indications. However, a survey of antimicrobial drug use among 42 university hospitals, regional, and provincial hospitals in Thailand in 1999 showed only 0.8% of appropriate use of antimicrobial drugs. Studies are needed to update the situation.

3. Inappropriate practice of AMR prevention and control

The Healthcare Infection Control Practices Advisory Committee and the Society for Healthcare Epidemiology of America has recommended approaches to control or
eradicate multidrug-resistant organisms in health care settings. These include administrative support, judicious use of antimicrobials, surveillance, standard and contact precautions, and decolonization. There is evidence that these approaches were not followed by health care workers regularly. For example, Duangmoragot (1995) found that only half of nurses reported the MDR suspected cases to an infection control nurse (ICN). Similarly, hand hygiene was only practiced approximately 80% of the time, after providing direct care to MDR-infected patients. Gown, mask, and glove were used only 50% of the time when cleaning the bed and accessories of the MDR-infected patients. Inappropriate practice of AMR prevention and control plays a major role in the spread of AMR infections from cross-infections. Therefore, improving infection control practices of MDR is crucial. Without initiatives, AMR problems could be even worse. Evidence shows that one trained ICN per 100 beds is assigned to do full-time work on infection control in the health care facilities and one additional ICN per 250 beds yields effective infection control outcomes. The majority of ICNs in Thailand had experiences providing nursing care for patients. However, if they are assigned to do full-time infection control work, they need additional training to acquire knowledge and skills in the area of infectious diseases, epidemiology, microbiology, disease surveillance, environmental sanitation, quality management, data analysis, leadership skills, and teaching. Unfortunately, one of the major findings showed that ICNs had insufficient knowledge and skills in infection control and they suggested the schools to provide infection control training courses for them. Studies of the roles of ICNs found an insufficient number of full-time ICNs per hospital bed due to the shortage of ICN posts in most hospitals.

The Faculty of Nursing, Chiang Mai University, recognized the problems of NI and AMR, the need to produce competent ICNs for the health-care system. The school decided to meet the challenges by building capacity of nurse-midwives and initiated some related work.

**AMR infection control initiatives in capacity building**

Since 1992, the Faculty of Nursing, Chiang Mai University has initiated and implemented many programmes to reduce AMR and NI problems including a Master’s programme, the only one in the country and in the Region, and a series of short training courses. All of these programmes have been approved by the Thailand Nursing and Midwifery Council. At the beginning of these initiatives, the programmes were financially supported by the China Medical Board of New York, a USA foundation. Details of some of the programmes are as follows:

1. **Master’s programme in infection control nursing**

The two-year Master of Nursing Science Programme in Infection Control Nursing was started in 1992. The primary objective of the programme was to educate and prepare nurses to have an advanced role in infection control nursing. The curriculum composed of a total of 42 credit hours. The main topics include concepts and main theories in nursing, research design in nursing and data analysis, systematic review and research utilization, nursing leadership in health system, competency development of advanced practice nurses, advanced health assessment, basic medical microbiology, advanced infection control nursing, advanced practicum in infection control nursing, epidemiology of infectious diseases, issues and problems in infectious diseases, and a thesis. AMR is included in the courses and theses.

This programme has been conducted successfully. Recently the programme has been
approved and certified by the Thailand Nursing and Midwifery Council as one of the advanced nursing practice programmes at the Master’s level. The programme admits 15 students annually. As of October 2010, the school has produced approximately 225 nurses who are now functioning as ICNs in health-care settings throughout the country.

Studies on AMR by graduates can be broadly categorized into the following aspects: epidemiology of and risk factors of AMR, and intervention for the prevention and control of AMR. Selected examples are presented as follows: Judang (2002)12 studied the incidence, risk factors, and impact of pneumonia caused by AMR among patients (n = 223) admitted at a medical intensive care unit during the year 2000-2001. Results showed that incidences of ventilator-associated pneumonia were 18.8 infections per 1000 ventilator-days. Risk factors included underlying diseases such as chronic obstructive pulmonary diseases and duration of ventilator uses (p < .001). All causative agents were AMR Staphylococcus aureus and AMR Acinetobacter baumannii. The costs of the AMR treatment were twice as high compared with those who did not develop the infection. This study provided empirical data that AMR is a major problem in Thailand especially at a tertiary health care setting.

Judang el al. (2000)13 examined risk factors for AMR infections in a tertiary university hospital among cases of infection (n=100) and control (n = 100) in 2000. It was found that long duration of admission (>4 weeks), a history of receiving antibiotics, and invasive instruments were significant factors for AMR infections. The results of the study guided the policy regarding the appropriate use of antibiotics as well as provided education to medical students and physicians to realize the importance of antibiotic usage and understand the indications for antibiotic use. The results of the study also guided nursing personnel to strictly adhere to the practices of prevention and control of AMR infection especially among patients who had those risk factors.

Duangmoragot et al. (1995) studied the effect of participatory learning on practices among nurses for the prevention and control of AMR infection in one provincial hospital. This quasi-experimental research aimed to compare the practice of prevention and control of AMR infection among nurses before and after participatory learning. The sample consisted of 63 nurses working in the surgical department. Data collection was carried out from October 2004 to January 2005. The research tools consisted of the participatory learning plans, the practice observation form and the practice questionnaire. The results of the study illustrated that, after completing the participatory learning, the nursing practices for the prevention and control of AMR infection was significantly different (p < .05). Activities that nurses improved their behaviour significantly after joining the programme included notifying infection control nurses when there is a case of MDR bacterial infection, patient isolation, hand hygiene, glove wearing, mask and gown wearing, management of patient’s belongings, and linen management. This study shows that participatory learning can change nursing practice according to the guidelines for prevention and control of AMR infection. Therefore, it is recommended that participatory learning should be used to promote nursing practice for prevention and control of AMR infection.

These studies are presented as examples. There have been many studies focusing on the interventions that could reduce not only inappropriate infection control practices but also the incidence of infection.

2. Short training courses

Although the Master’s degree programme is being offered, the annual intake is only 15 students, which is not sufficient. Furthermore, there have been requests to offer the tailor-
made programmes to meet the needs of each individual. Many ICNs could not leave the full-time job to attend the two-year Master’s degree programme. Thus, many short training courses have been offered in series.

2.1 The first infection control training course

This course was initiated, implemented, and tested in 2003. The course consists of 80 hours of theoretical knowledge and a practicum. The theoretical course includes an update in epidemiology of NI, investigation of outbreaks, appraisal of data for nosocomial infection control, data analysis and management, evidence-based practice in infection control, project development and implementation in infection control, information technologies in infection control, practice guidelines development and implementation, leadership in infection control, and health economics in infection control. As for the practicum, each participant developed and conducted the infection control project in their own setting with the supervision of the faculty members responsible for the course using a variety of methods including an infection control newsletter, internet-based web board, email, and telephone calls.

Picheansathian et al. (2003)\textsuperscript{14} conducted an evaluation of this course by asking the participants to share their opinions on the course and the perceived self-efficacy in ICN role ($n = 46$), while asking the supervisors to provide their opinions on the quality of the role of the participants comparing before and after the training course ($n = 46$). A majority of participants strongly believed agreed that all topics in the course were highly applicable to their work. They also stated that networking among participants was good and that they could share their experiences and knowledge. When comparing their self-efficacy on the infection control roles before and after attending the course, it was found that their perception of the self-efficacy increased significantly ($p < .05$) in the following roles; infections disease surveillance, routine quality control monitoring, consultation, serving as a committee member, monitoring of and support to the infection control activities, education, and research utilization. All supervisors agreed that the training course was beneficial to the hospital and on the role development of ICNs. A majority of them (93.2\%) stated that ICNs improved their roles as an ICN after attending the course. The differences in perception regarding the ICN’s roles as compared with the baseline, were rated higher for each role but the differences were not significant. The results of this study provided critical inputs for course revision.

2.2 The certificate programme of nursing speciality in infection control and nursing care for infectious patients

This four-month programme was initiated and has been implemented since 2006. The primary goal of the programme was to improve knowledge and skills of ICNs not only in the area of infection control but in the area of health system, health policy, and infection control at the national level, infectious diseases epidemiology, infectious diseases surveillance, evidence-based utilization for infection control, research process and utilization, information technologies, and strategies to promote the work of infection control.

This programme has an intake of 45 students annually. All graduates who complete the programme work as ICNs in many health-care settings throughout the country.

Other related initiatives

In addition to capacity building, a best practice model to reduce AMR problems, guidelines, and networking have been developed.

1. Best practice model

The best practice model to reduce AMR problems consists of six steps.
Step 1. AMR problems identification and prioritization

In this step, the AMR problem should be quantified. Data and information from infectious disease epidemiological studies should be conducted and reviewed. Consultations with experts in this area or hospital epidemiologists could provide fruitful information. Other important aspects to discuss in this step included impact of the problems. The morbidity and mortality impacts should be highlighted in the discussion to prioritize the problem. Then, the meeting to establish a committee to solve the problem should be conducted. The infection control committee should be appointed with members recruited from all parties involved. One of the members should work full-time and be responsible only in the area of infection control. Policies related to AMR and quality improvement should be reviewed and updated. The AMR quality improvement plan should be initiated. Importantly, collaboration with affiliated hospitals for the practicum is essential. A win-win approach has been used for the practices by helping the unit in the hospital in prevention and control of infection and, in turn, participants could learn from the actual infection control practicum.

Step 2. Development of AMR prevention and control programme

The AMR prevention and control programme can be implemented using a variety of strategies and methods under the quality improvement programme. Under these programmes, many projects of AMR prevention and control could be initiated. Meetings among the infection control committee, hospital administrators and stakeholders should be conducted to initiate and conduct AMR quality improvement through evidence-based practices. Based on our experiences, many projects failed to finish on time or were delayed if the project was started without adequate preparation. Therefore, workshops to prepare the committee and the involved staff are crucial.

Experts in the area of infection control and in the area of evidence-based practices should be invited to provide the basic principles and knowledge in these areas. Time should be allowed to discuss, identify, and prioritize the AMR problem. Possibility of an AMR project for each unit should be encouraged, discussed and initiated with support from the hospital administrator. During this step, experts should be available for consultation when needed. The results from the discussion regarding the projects should be summarized and classified into categories.

Step 3. Project planning and development

In this step, the project should be started. It is the time to assign a responsible person, set up a timeline, and initiate a process for activities and their evaluation. The duration of each project could be six months or longer for all processes depending on the situation. Experts should be involved in project planning and development to facilitate the progress and to identify and solve the problems or barriers that might exist. The projects should be submitted for approval from the hospital administrator.

Step 4. Project implementation

In this step, project staff identify guidelines or best available evidence from a variety of sources. It could be the results from primary research, related guidelines, or expert opinions. This step is critical when identifying guidelines based on evidence. Experts must be available to help identify the source of guidelines, evaluate the quality of the guidelines and develop guidelines applicable to the local context. Since some of the evidence is not in the native language, experts play an important role to help in translation during this period. Then, guidelines based on the evidence should be drafted and refined. A meeting among those who will utilize the guidelines such as nursing staff working in the unit, patients, and other involved health-care workers should be conducted to get a
consensus on the guidelines and an agreement to implement them.

Step 5. AMR practice, promotion and support
This step is important and could determine the success or failure of the projects. The responsible staff must identify strategies and use them to promote the practices of the guidelines. A variety of methods could be used including group discussion, morning conference, lecture, training workshop, or rewarding activities. Experts and responsible staff should provide advice, support, and consultation when needed.

Step 6. Project evaluation and conclusion
In this step, staff responsible for each project should conduct the evaluation and conclusion of the project as well as writing the report. Experts should provide training for responsible staff on the project evaluation, and writing the report. Responsible staff should conduct the workshop or the meeting to discuss, evaluate, and conclude the project. Sharing of knowledge and experiences should be the main objectives so that each individual can learn from each other. Then, the schedule to have a project presentation should be developed with the participants and the hospital administrators.

2. Development of guidelines
A few guidelines were developed, for example, the guidelines related to eight important infection control aspects with financial support from the WHO Country Office, Thailand. The guidelines were utilized in many infection control projects. Also, guidelines on management of avian influenza for nurses were developed, tested, and utilized. However, there is a need to develop AMR-specific guidelines applicable to the local context.

3. Development of networking
Two networking exercises were initiated and conducted actively by members.

- The infection control nurse group was formed with the primary purpose of sharing information on infection control practices among its members.
- The Nursing Association for Prevention and Control of Infection was formally established with the primary purpose of providing education training activities for its members as well as other related health care workers.

The above-mentioned initiatives have started yielding results but yet a lot more needs to be done so that nurses can play a critical role in reducing NI as well as AMR in any health care setting.

References and bibliography


Response to antimicrobial resistance in a globalized world

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Abstract

Antimicrobial resistance is a major challenge for public health in the current globalized trade-based environment. Antimicrobial drugs are susceptible to obsolescence due to inappropriate use. This relates to use of these drugs in humans and use of antimicrobials in developing food products: plant, animal and aquaculture, etc. Antimicrobial resistance-related issues are becoming important in the work of a range of multilateral bodies such as World Health Organization’s International Health Regulations, World Trade Organization’s Trade Related Aspects of Intellectual Property Rights and Sanitary and Phytosanitary Measures Agreements, the Food and Agriculture Organization, Office International des Epizooties, and the Codex Alimentarius Commission, etc. Norm-setting at a global level is being undertaken by these multilateral organizations in their own capacity and through joint collaboration. This paper argues that regulatory and policy measures distinguished from drugs in general are imperative to preserve the efficacy of antimicrobials. It also argues that multilateral acceptance needs to develop synergy with norm-setting measures at regional/national levels in order to achieve an effective response to antimicrobial resistance.

Background

In the twentieth century, antimicrobial drugs, antibiotics and antivirals provided a critical breakthrough in man’s successful fight against disease. Penicillin, the first truly safe antibiotic agent, was discovered by Sir Alexander Fleming in 1928. In 1942, penicillin was made available to some members of the public and in 1944 to the general public. Currently, though many antibiotics are available, there are only about twelve classes of antibiotics. The most recently approved new class of antibiotics, the Oxazolidinones, was approved in 2000. It is the only new antibiotic class to be introduced into a clinical setting in thirty years. By 2001, clinical resistance to this drug was observed.

Antimicrobial drugs are susceptible to obsolescence by overuse. This happens when the target of the antimicrobial drug develops resistance, a process that can occur with alarming rapidity. According to Dawkins, “life results from the non-random survival of randomly varying replicators. Resistance to antimicrobials is a result of such evolution. Given a sufficient amount of time and a large enough population, a particular life-form will find some way to adapt to a particular environmental stimulus.” Unnecessary use of an antimicrobial occurs when the compound is administered to treat a condition for which the compound is wholly unsuited or is genuinely irrelevant. The tendency of patients to consume sub-therapeutic doses, partial courses, or to under-use the antimicrobials is problematic.
The adaptive potential of the microbial world is such that for each new antibiotic that is introduced, several escape mechanisms are soon devised. According to a forecast by the University of California at San Francisco, the United States of America, this dynamic will lead to the exhaustion of all antimicrobial drug options by 2070. Many developing countries have inadequate or non-existent regulatory controls over antimicrobial use for human and animal health purposes. Regulatory and policy measures are imperative to preserve the efficacy of these “wonder drugs” for coming generations in the current fast-paced, globalized and trade-centred national and international environment.

**Legal developments**

The advent of antibiotics has had a profound impact on the societal response to communicable diseases. These drugs changed the hitherto prevalent quarantine-based response. A leading case in quarantine law occurred in 1902 when a public health resolution was upheld prohibiting the entry of any person in any city or town in quarantine, regardless of whether the person was healthy or infected with disease. Antibiotics made coercive personal control mechanisms unnecessary and legal decisions shifted the focus to the due process in law. It was held that a person alleged to have tuberculosis (TB) must be accorded procedural due-process rights. However, in the 1980s, the courts upheld the substantive authority of public health authorities in responding on behalf of a community. They also reaffirmed the historical public health legal concepts.

The delicate balance between individual liberty of action and community public health interests is at the forefront in discussion on antimicrobial resistance (AMR). On the one hand is the necessity for promotion of use of antibiotics for containment of disease and on the other, containment of use of antimicrobials to make certain that they do not lose efficacy through indiscriminate use. This has engaged national and international entities and resulted in certain directives governing such use as is the case in the European Union (EU). One of the only antimicrobial agents available for treatment of multidrug-resistant enterococci and methicillin-resistant Staphylococcus aureus (MRSA) infections was vancomycin. The Danish Minister of Agriculture and Fisheries banned the use of avoparcin nationally because scientific evidence showed that avoparcin used as a growth promoter in food animals constituted a potential threat to human health. In July 1995, the ban became effective in all countries in the EU. The avoparcin ban called attention to the wide array of antimicrobial substances being used in food animals for growth promotion or disease control and to the risk for transfer of other resistant bacteria or resistance genes from animals to humans through the food chain. It was decided that all use of antimicrobial growth promoters should be terminated within the EU starting 1 January 2006. This EC Regulation 2821/98 came up for adjudication and President of the Court of First Instance of the European Court of Justice (CFI) held that the documents before him confirmed that bacteria resistant to virginiamycin in animals were transmissible to humans.

The importance of preserving these drugs has led courts in the EU to exercise the precautionary principle in law. The mere existence of this potential risk was enough in

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2 Antibiotics and antimicrobials are used interchangeably in this text.
itself to exercise the precautionary principle and justify taking the protection of human health into account in the balancing of interests. Thus it has been reiterated at national and international forums that the “protection” of the efficacy of antimicrobial drugs is of vital importance.

**Trade and intellectual property issues**

Trade and globalization have contributed to international and national movement in medicines, food products and persons. The globalization of the food industry is having enormous health (and regulatory) effects. The term “globalization of public health” has emerged in policy discourses to express the transnational or globalized nature of public health threats (including the spread of communicable diseases) in an interdependent world and de-emphasizes the “territorialization” or “nationalization” of diseases. This process has fostered AMR. It has been stated that easy access to cheap antibiotics under the globalised trade regime in the third world has furthered antimicrobial resistance. This can occur both by human consumption of antimicrobial drugs and in animal and plant use. Overuse of antimicrobials in livestock and poultry production can lead to the rapid development of resistant strains of food-borne pathogens like *Campylobacter jejuni*.4

Antimicrobial resistance is relevant in international trade and public health in issues related to intellectual property rights (patents), compulsory licensing, parallel trade, counterfeit drugs and internet (web-based) procurement of different food and drug products.

Of all intellectual property rights, the pharmaceutical industry relies primarily on patents. The pharmaceutical industry’s dependence on the patent system as a panacea of all problems has resulted in the approach to find a “patent” solution for all problems. The intellectual property law including on patents, that endows drug makers with a temporary monopoly on new drugs, can make effective preventive medications inaccessible to poor countries.

The Infectious Diseases Society of America (IDSA) correctly identifies the need for effective antibiotic therapies, but has mistakenly called for significant changes in patent law to remedy the problem, including patent extensions and wildcard patent extensions for antibiotics.5 It is assumed that apart from the direct stimulation of resistance by increasing consumption, AMR has resulted in retardation of innovation and research efforts in this genre of drugs. According to surveys conducted in 1999 and 2003 by the Pharmaceutical Research and Manufacturers Association, the trade organization for the pharmaceutical industry in the United States, the number of drugs in development for AIDS and related conditions declined from 102 to 83, respectively. Similarly, the supply of antibiotics is threatened by high levels of antibiotic resistance, an uneven supply of novel classes of antibiotics over the last few decades, and a dramatic reduction in the number of pharmaceutical companies engaged in research and development (R&D) in the area of antibiotics. Despite the critical need for new antimicrobial agents, the development of these agents is declining. Steps are therefore needed to encourage and facilitate the development of new antimicrobial agents.

Although knowledge is a classic public good, in practice the enormous cost of R&D means that patents are used to transform it into a private good thus providing the incentive for private sector investment.

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4 C. jejuni became resistant to fluoroquinolone once that antimicrobial was approved for use in poultry

5 A wildcard patent extension grants additional years of patent life on any drug of a company’s choice if the company achieves some socially desirable goal, in this case, development of a novel antibiotic. Wildcard patent extensions have generated sharp academic exchanges in recent years.
However, market-based pricing is an important element of the patent system and its absence in pharmaceuticals is intriguing. For dealing with AMR the most effective and immediate solutions might be based on conservation rather than production, and on reimbursement rather than patent law.

Several methods have been suggested to avoid or remedy this problem, including transferring the responsibility for research funding to the public sector, requiring all drug manufacturers to pay into a research and development fund, and increasing the drug patent term. Perhaps a total rethinking of how antibiotic funding is handled is in order.

Collective action on a number of fronts is therefore necessary, including the reform of international patent laws and the coordination of licensing and regulatory requirements. Conservation and issues of reimbursement in the current globalized trade-dependent milieu thus call for concerted national and international intervention tailored specifically to prevent AMR.

The debate on compulsory licensing and AMR illustrates the dichotomy of accessibility of drugs on the one hand and their overuse on the other. Compulsory licensing is one of the flexibilities permitted under the Trade Related Aspects of Intellectual Property Rights Agreement (TRIPS) under the World Trade Organization (WTO). A compulsory licence is granted by a government to allow the use of a patented product without the permission of the patent holder and is one of the major safeguards for access to medicine in a public emergency. Opposition to compulsory licensing is based on concerns about the potential misuse and abuse of antimicrobial drugs in developing countries. It has been stated that lack of control over the prescription and distribution of antimicrobial drugs has contributed to the irrational use of such drugs and the resulting development of AMR. Antimicrobial resistance in diseases such as TB and malaria has resulted from the undisciplined use and misuse of pharmaceutical drugs in developing countries. With some strains of the human immunodeficiency virus (HIV) already developing resistance to HIV/acquired immunodeficiency syndrome (AIDS) therapies, concern exists that widespread compulsory licensing of HIV/AIDS therapies in developing countries could increase the potential for the development of resistant strains of HIV. While some developing countries may have the public health infrastructure to administer HIV/AIDS drugs properly on a large scale, the historical problems encountered in the developing world with the irrational use of antimicrobials suggests that compulsory licensing of HIV/AIDS therapies might have a significant long-term downside. Therefore, opponents of compulsory licensing advocate restricted access to antimicrobial drugs to developing countries.

Instead of preventing access, it is important to address the constraints of effective drug use in developing countries such as the lack of infrastructure etc. Disease knows no boundaries as has been observed in the H1N1 and H5N1 epidemics. Hence, rather than restricting accessibility to antimicrobial drugs, availability of drugs in countries with high disease burdens should be ensured combined with appropriate national regulatory mechanisms especially designed to address AMR.

Parallel imports, or grey-market goods, are imported copies of a product protected by some class of intellectual property, which are legitimately and legally purchased in a foreign jurisdiction. Parallel imports are likely to increase the improper use of antimicrobials by increasing both unnecessary consumption and underconsumption. Underconsumption may increase as a secondary effect of price, and is likely a result of patient capriciousness. On the other hand, patients may encourage overconsumption themselves merely on the ground of information that an antimicrobial
may help. Both parallel imports and drug subsidies function by lowering the cost of drugs to consumers. Rather than restricting parallel imports that ensure accessibility to much needed drugs, both patients and doctors need to be sensitized to AMR. This calls for enhanced communication to the public on AMR and its implications. This process may be given a stimulus through appropriate national regulation on communications measures specifically designed for AMR.

Antimicrobial resistance is a matter of serious concern in counterfeit medicines. Counterfeit medicines are more commonly found “in countries with weak drug regulation control and enforcement, scarcity [in the] . . . supply of basic medicines, unregulated markets, and unaffordable prices. Counterfeit drugs pose a serious risk to the safety of patients who take them as they are unsafe and ineffective. Counterfeit medicines also affect the health of the public at large by increasing the risk of producing more antimicrobial-resistant organisms. In view of their importance, counterfeit antimicrobials need to be addressed differently from other counterfeit products or drugs. Hence, it is necessary to have national and international regulations on counterfeits that distinguish this class of drugs from the others. This would help focus attention on AMR and thereby preserve the efficacy of this genre of drugs.

Internet and web-based transactions (and parallel imports through the web) enhance the ability of patients to order medications from pharmacies and suppliers from all over the world. More complicated, however, is the purchase of drugs from overseas pharmacies, when the exporting jurisdiction does not require a prescription for export, or where the pharmacies can easily avoid the regulations that exist. It has been suggested that in these cases, parallel importing jurisdictions should require that all imported medication be screened by the respective border control agency, and every prescription drug matched with a valid prescription. Without curbing the freedom of communication and information in internet transactions, suitable mechanisms to address AMR are the need of the hour. Hence, it may be advisable to encourage the development/use of nationally/internationally recognized antimicrobial provider indicators e.g. trademark/ logos/ certification marks on web sites. These indications on the source of drugs would encourage discerning use through widespread dissemination and communication among doctors and patients and assist in containing AMR.

International developments

Issues related to AMR have assumed importance in a range of multilateral organizations such as WHO’s International Health Regulations (IHR), WTO’s Trade Related Aspects of Intellectual Property Rights (TRIPS) and Sanitary and Phytosanitary Measures (SPS) Agreements, the Food and Agriculture Organization (FAO), Office International des Epizooties (OIE), and the Codex Alimentarius Commission Standards on Food Safety, etc.

The role of WHO: The genesis of WHO’s role in containment of communicable diseases is the nineteenth-century international sanitary

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6 CEO of the Centre for Mental Health in the United Kingdom, stated that, “potent substances are freely available on the Internet and can be ordered easily without any prescription and any authentication of sources, making the public vulnerable to health hazards and public health vulnerable to growing antimicrobial and drug resistance.”
conferences initiated by France in 1851 and attended by eleven European states. From 1851 to the end of the nineteenth century, ten such international sanitary conferences were convened. Surveillance was an important part of the mandate of the Pan American Sanitary Bureau, Office International d’Hygiène Publique, Health Organization of the League of Nations, and the OIE. In 1951, WHO adopted the International Sanitary Regulations, the product of the nineteenth-century international sanitary conferences, which were re-named the International Health Regulations (IHR) in 1969, and modified in 1973, 1981 and 2005. The IHR are a set of regulations for the control and sharing of epidemiological information on the transboundary spread of cholera, plague and yellow fever; to ensure “maximum security against the international spread of diseases with a minimum interference with world traffic”. The IHR (2005) are legally binding on virtually all (i.e. 194) States worldwide, and impact governmental functions and responsibilities across many ministries, sectors and governmental levels.

The role of WTO: The role of WTO and TRIPS in communicable diseases was brought into focus by the situation presented by HIV/AIDS in developing countries, especially South Africa. The complexity of striking a balance between intellectual property rights and access to essential medicines by vulnerable populations has placed TRIPS at the centre of global public health policy in recent years. The exceptions under TRIPS (commercial licensing and parallel importation of generic drugs) could be exploited to protect public health. Despite these exceptions, thirty-nine multinational pharmaceutical companies initiated litigation against the Government of South Africa in 1998. In another case, the United States filed a complaint against Brazil at the WTO in 2001. These interventions challenged the aspects of South African and Brazilian legislations as infringing the TRIPS obligations. This revealed the complexity of balancing intellectual property rights with access to drugs. As a result in November 2001, a WTO Ministerial Conference adopted the Doha Declaration and stated that “the TRIPS Agreement does not and should not prevent Members from taking measures to protect public health”.

Apart from TRIPS, the Agreement on the Application of Sanitary and Phytosanitary Measures (“the SPS Agreement”) of the WTO sets out the basic rules for food safety and animal and plant health requirements while allowing countries to set their own standards. However, SPS also specifies that regulations must be based on scientific findings and should be applied only to the extent that they are necessary to protect human, animal or plant life or health; they should not unjustifiably discriminate between countries where similar conditions exist.

The SPS Agreement was the subject of legal interpretation in the WTO Dispute Settlement mechanism in a major international dispute relating to AMR. In EC-Hormones, the challenged measures were EC regulations prohibiting the sale and importation of meat and meat products that had been treated with growth hormones. The WTO dispute Appellate Board (AB) found that the different levels of sanitary protection set by the EC for meat (beef) treated with growth hormones as compared with meat (pork) treated with antimicrobial agents were unjustifiable. Although they all involve the same health risk (carcinogenicity), growth hormones were banned while antimicrobial agents were allowed. Notably, the EC allowed carbadox and olaquindox to be used as antimicrobial feed additives that promoted the growth of pigs; yet the EC banned the use of hormones as growth promoters in cows although the hormones resulted in similar (or lower) risks to humans. However, the AB observed that the

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EC regulations had legitimate purposes and were genuinely designed to protect its population from the risk of cancer. Thus the normative role of Member states to set up their own standards and regulations under SPS is relevant for AMR issues.

Role of the Office International des Epizooties (OIE), Food and Agriculture Organization (FAO) and the Codex Alimentarius Commission: The Office International des Epizooties (OIE) established in 1924 is the international organization designated to fight animal diseases at the global level. The OIE’s standard-setting activities in this field focus on eliminating potential hazards existing prior to the slaughter of animals or the primary processing of their products (meat, milk and eggs, etc.) that could be a source of risk for consumers. FAO has been concerned with antimicrobial resistance, related to the use of antimicrobials in agriculture (including aquaculture) and veterinary medicine.

The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice. The Joint FAO/WHO Food Standards Programme aims to protect health of consumers and ensure fair trade practices in the food trade. Use of antimicrobial agents in animals in the food chain has long-term implications. As a result many noncommunicable diseases may have their base in communicable diseases. Codex, WHO and FAO are currently engaged in risk analysis of food-borne antimicrobial resistance-specific interventions and implementation strategies to control and contain antimicrobial resistance in various areas, including animal husbandry.

Hence, a number of international organizations are currently engaged in AMR prevention under their mandates and their activities augment norm-setting at international level.

Conclusion

International and national movement of persons coupled with global trade in food items mandates examination of crossborder effects of policies and regulations for rational use of antimicrobials. Due to the challenges posed by movement of pathogens in this globalized trade environment and paucity of new drug development through research, the need to preserve antibiotics is more important than ever before. Since the sum of actions by individual nations does not equal the optimal global response, some element of collective action is required. Collective action to address AMR at international level is seen in the collaboration among WHO, FAO, OIE and Codex, etc. The AMR issues are engaging international courts and WTO is increasingly relying on standards set up under such collaborations in settlement of trade disputes.

Certain work has been carried out to develop international standard treatment guidelines, which are similar, for example, to current WHO AMR surveillance standards, WHO guidelines for the treatment of MDR-TB, and WHO protocols for the detection of drug-resistant malaria. WHO has begun development of a global strategy to contain AMR.

This strategy follows the historical preference of WHO and other international bodies for operating through recommendations and guidelines, relying largely on ad hoc harmonization of individual national mechanisms, legislation and strategies, rather than on formal international legislation. Unfortunately, evidence suggests that this may be inadequate to contain AMR in the long run. In view of the importance of this genre of drugs, developing sustained mechanisms to contain AMR through formal norm-setting is imperative. Formal norm-setting to contain AMR that is binding both nationally and internationally should be encouraged at national and regional levels.
WHO, FAO, OIE and Codex, etc. are undertaking norm-setting at the global level. Multilateral acceptance at this level needs to develop synergy with norm-setting measures at regional/national level. For these responses to yield tangible results, it is necessary to extend the global collective action at regional and national forums in order to have a comprehensive approach to AMR. Such collective action must be initiated not only in single-line WHO programmes at national and regional levels but also be sustained in horizontal formal collaboration with organizations responsible for plant and animal health. A dialogue-exchange mechanism should be established at global, regional and national levels. These forums could then develop national, regional and international normative action that encourages optimal use of antimicrobials. This would lead to acceptance of measures to contain antimicrobials and development of national and international norms in synergy with practical requirements for local and global response. The regional and national forums may also take up normative action on enforcing strategies covering, for example, intellectual property rights, the requirement for AMR data in the pre-approval evaluation of drugs, the use of subtherapeutic doses as growth promoters in animals, the labelling of drugs, and prescription requirements.

There is an additional benefit of collective norm-setting as envisaged above. The collective forum norm-setting at regional and national levels would also enable dissemination of information and appropriate communication aimed at national and regional settings. This would give the process an advantage of being “owned” by the constituents, resulting in better compliance mechanisms for AMR containment. The collective forums at national and regional levels may also develop management mechanisms for antimicrobials, as distinct from drugs used for other purposes. The creation of a separate set of mechanisms at all levels dealing with antibiotics: research, production, marketing, communication and advertisement, and health systems handling, etc. would enable an effective response to AMR.

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Promoting the rational use of antibiotics

Kathleen Anne Holloway*

Abstract

Irrational use of antibiotics is a worldwide problem that contributes to dramatically increasing resistance and causes significant mortality, morbidity and increased health-care costs. This article reviews the available evidence on how we are promoting the rational use of antibiotics in the WHO South-East Asia (SEA) Region, using various WHO sources, and makes some suggestions on how to go forward.

Evidence shows that there is very serious antibiotic misuse, for example: (1) serious overuse of antibiotics in viral upper respiratory tract infection but underuse of appropriate antibiotics for pneumonia; and (2) serious overuse of antibiotics in acute cases of diarrhoea but underuse of oral rehydration solution. The few interventions conducted and adequately evaluated in the Region show that targeted multi-component interventions involving educational and managerial interventions are effective and can improve antibiotic use by 20%-30%.

A country’s policy framework greatly influences antibiotic use. In South-East Asia, antibiotics are available over the counter without prescription in all countries and very few countries are monitoring antibiotic use. Many countries still do not have important policies in place to encourage appropriate antibiotic use and most policies are aimed at the public sector, whereas most people get their medicines from the private and informal sectors. Thus, it is not surprising that irrational use of medicines continues.

There are now global and regional recommendations to have a government structure dedicated to monitoring and improving medicines use and to undertake a national situation analysis in order to develop a roadmap for action. The WHO Regional Office for South-East Asia is now undertaking national situational analyses, at country requests, in order to help them develop coordinated plans of action.

Introduction

Irrational use of medicines is a global problem. It has been estimated that less than half of all medicines are prescribed, dispensed or sold inappropriately1 and that less than half of all patients take their medicines as prescribed or dispensed2. Irrational use of medicines can harm patients in terms of poor patient outcome, unnecessary adverse reactions and wastage of resources, often out-of-pocket payments by patients. Irrational use of antibiotics is particularly serious because it is contributing to antimicrobial resistance that is increasing rapidly worldwide and is causing significant morbidity and mortality4,5,6 and millions of dollars worth of extra health-care costs annually7,8. The situation is so serious that WHO published a global strategy for containment9, a regional strategy for South-East Asia Region10 and Member States of WHO have adopted three World Health Assembly resolutions to contain antimicrobial resistance – the last one in 2005 (WHA58.27)11 – and another resolution in 2007 on promoting rational use of medicines (WHA60.16)12. In South-East Asia, the

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Regional Committee adopted a resolution in 2010 (SEA/RC63/R4) on the prevention and containment of antimicrobials. Despite global concern, few countries, globally or in South-East Asia, have taken serious measures to contain resistance or promote rational use of antibiotics. Unless serious action is taken we risk a future without effective antibiotics.

Use of Antibiotics

Although medicines are one of our most cost-effective health-care interventions and antibiotics are one of our most effective therapeutic classes of medicine, few low- and middle-income countries are monitoring how they are used. Data on medicines use is conspicuously absent in many health management information systems. By contrast, developed rich regions, such as Europe, are now monitoring antibiotic use and taking action to combat irrational antibiotic use.

In order to monitor the progress in developing countries, WHO headquarters developed a database of quantitative information on medicines use in primary care in developing countries that has been systematically extracted from studies published between 1990 and 2007. Studies numbering 679 from 97 countries were identified of which 151 studies came from the SEA Region. Data show that in low- and middle-income countries less than 40% patients in the public sector and less than 30% in the private sector are treated in compliance with clinical guidelines, and that the situation has not improved significantly over the last 20 years. With regard to use of antibiotics in the Region, it was found that:

- 50% of viral upper respiratory tract infection cases are treated unnecessarily with antibiotics, yet only 53% of pneumonia cases receive an appropriate antibiotic;
- 54% of acute diarrhoea cases are treated unnecessarily with antibiotics, yet only 55% receive oral rehydration solution as recommended in the guidelines; and
- 40% of prescribed antibiotics are prescribed in under-dose.

It was further found that the average patient-dispenser interaction time was less than 1 minute, that only about 40% patients were given dosage instructions, very few drugs were adequately labelled, and that only about 60% patients knew how to take their medicines immediately on leaving the facility.

In many countries of the SEA Region, antibiotics are freely available over the counter without prescription even if this is contrary to regulations. In one study, in three Indian cities it was found that the most commonly prescribed antibiotic class in the community was fluoroquinolones, often for coughs and colds, which is entirely inappropriate. In the same study, high levels of resistance of E.Coli to fluoroquinolones were found in the urine of pregnant women (who were not taking any antibiotics) thus showing how fluoroquinolone use in some community members was contributing to the carriage of resistant organisms in other members of the same community.

Determinants of irrational use

In order to further promote rational use of antibiotics, it is important to understand the reasons why providers and consumers behave the way they do. Box 1 summarizes some of the reasons why people use antibiotics unnecessarily.
Box 1: Determinants of irrational antibiotic use

- Lack of provider knowledge, particularly with regard to prescribers who are insufficiently qualified, supervised or supported;
- Prescriber habit (it takes time to look up guidelines so prescribing by habit is faster);
- Poor availability of independent medicines information such as clinical guidelines and drug bulletins;
- Lack of unbiased, independent, government-funded continuing medical education and supervision that includes prescribing;
- Excessive pharmaceutical promotion - which often constitutes the only information prescribers receive and may be biased, emphasising use of the medicines and underplaying the negative consequences such as side-effects, antimicrobial resistance and cost to the patient;
- Very short consultation time (one minute) that does not allow sufficient time to make a proper diagnosis;
- Very short patient-dispenser interaction time (seconds) that does not allow sufficient time to explain to patients how to take their medicines;
- Peer pressure, for example, where doctors fear to be seen to be prescribing differently to their colleagues particularly if those colleagues are senior consultants who may set inappropriate prescribing norms;
- Patient demand in reality and as it is perceived by prescribers (who may perceive a greater demand than the real demand);
- Lack of diagnostic support services such as laboratory services;
- Poor infrastructure such as the inability to undertake observation or follow-up of patients;
- Economic incentives where prescribers gain income from dispensing or selling the medicines they prescribe; and
- Inappropriate medicines supply, for example, where inappropriate antibiotics are supplied while available and appropriate ones are not.

Sources: 1, 20, 21, 22

If an intervention is to be successful it must address any of the above factors that are found to be influencing provider and consumer behaviour. For example, in Indonesia investigation into high injection use found that doctors felt that patients asked for injections while patients did not like injections but felt that doctors liked giving them and were too afraid to refuse. The investigators organized a moderated interactional group discussion between doctors and patients, demonstrating their different viewpoints, after which the injection use decreased by 30%. In India, profit motive and fear of losing patients have been found to influence prescribers.

Targeted interventions to improve antibiotic use

The vast majority of evidence concerning what kinds of intervention are effective in improving the use of medicines comes from developed countries, relatively few studies having been conducted in developing countries. The WHO database on medicines use identified 386 interventions (evaluated in...
313 studies), but only 121 of these interventions (in 81 studies) had been evaluated using adequate study design (randomized controlled trial, pre-post study with control group or time series). It was found that many of the interventions targeted antibiotic use and that most were educational in nature. Provider education alone or the distribution or printing of materials were found to have little impact on medicines use (<10%), whereas multi-component interventions involving education of prescribers and consumers, together with supervision (i.e. a combination of educational and managerial interventions targeting both providers and consumers) could improve the use by more than 20%-30%.

About half of the intervention studies indentified in the WHO database come from Asia. A brief description of the studies that came from the SEA Region can be found in a regional publication on the role of education in the rational use of medicines18, which also highlights particular strategies that have been used in the Region. Most interventions targeted antibiotic or injection use or the treatment of acute respiratory infection or diarrhoea. The effectiveness of interventions carried out in the SEA Region is similar to that in other regions. Interventions that aim to increase antibiotic use, for example, training community members to diagnose and ensure the treatment of childhood pneumonia cases with antibiotics, generally result in large improvements of 15%-25%25,26 and can reduce mortality27,28. However, interventions that aim to decrease antibiotic use, for example, training private pharmacies not to sell antibiotics for mild viral upper respiratory tract infection or acute diarrhoea, tend to have more modest effects of less than 15%29,30. Such modest effects have also been found in training health professionals31,32,33,34. However, where education is accompanied by peer review, self-monitoring and feedback, then it is possible to achieve large reductions of more than 30% in antibiotic use35,36,37.

The monitoring, training and planning (MTP) intervention developed in Indonesia38 is an innovative problem-solving approach. This involves providers identifying a problem, measuring it, discussing the underlying factors and how to improve the situation, setting the improvement target and monitoring to see if they attain this target. This method has resulted in large decreases in inappropriate antibiotic use in hospitals38 and has been successfully used in Laos and Cambodia18,38. A similar cyclical quality improvement intervention is also being field tested with success in communities in Indonesia39.

**National policies to improve use of antibiotics**

National policies greatly influence how medicines are used. Without a favourable policy framework, it will be very difficult to achieve and maintain improved antibiotic use. The Second International Conference on Improving the Use of Medicines40 noted that irrational use of medicines continued, that there was a relative lack of implementation of interventions, almost all of which were small scale, and that the problem was multifactorial in nature, involving many stakeholders. Therefore, they recommended that countries implement national programmes to monitor medicines use and to coordinate implementation of interventions, targeting multiple levels of the health care system in both public and private sectors, to improve use. They also recommended that successful small-scale interventions be scaled up and that more interventions be implemented targeting the community, particularly with regard to informal and private sectors and private pharmacy shops, all of which are particularly relevant in the SEA Region. WHO has developed a database on pharmaceutical policy based on a questionnaire that is sent out to countries once every four years for ministries of health (MoH) to fill in14,41. The last surveys were done in 2003 and 2007.
Table 1: Medicines policies to encourage rational use of medicines globally and in the SEA Region

<table>
<thead>
<tr>
<th>National policies implemented*</th>
<th>Globally</th>
<th>SEA Region N=10 (overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size of countries responding to questions in the policy questionnaire</td>
<td>2003 n&gt;90</td>
<td>2003 n=9</td>
</tr>
<tr>
<td>Prescription audit in the last two years</td>
<td>28%</td>
<td>40%</td>
</tr>
<tr>
<td>National strategy to contain AMR</td>
<td>36%</td>
<td>43%</td>
</tr>
<tr>
<td>Antibiotic non-availability over the counter (OTC)</td>
<td>30%</td>
<td>25%</td>
</tr>
<tr>
<td>Public education on antibiotics undertaken</td>
<td>45%</td>
<td>51%</td>
</tr>
<tr>
<td>Drug and Therapeutic Committees (DTCs) in more than half of general hospitals</td>
<td>53%</td>
<td>58%</td>
</tr>
<tr>
<td>National Drug Information Centre for prescribers</td>
<td>40%</td>
<td>52%</td>
</tr>
<tr>
<td>Obligatory continuing medical education for doctors</td>
<td>49%</td>
<td>56%</td>
</tr>
<tr>
<td>Training for medical students on essential medicines list (EML) and standard treatment guidelines (STG)</td>
<td>67%</td>
<td>68%</td>
</tr>
<tr>
<td>National EML used in public sector procurement</td>
<td>56%</td>
<td>84%</td>
</tr>
<tr>
<td>National EML updated in the last two years</td>
<td>46%</td>
<td>58%</td>
</tr>
<tr>
<td>National STGs updated in the last two years</td>
<td>23%</td>
<td>34%</td>
</tr>
</tbody>
</table>

*If a country did not respond to a particular question, it was assumed that the policy did not exist in that country.

and Table 1 shows the percentage of countries stating that they had various policies in place – both globally and for the Region. Since different countries contributed to the different surveys, results for both surveys (2003 and 2007) are given.

It can be seen that many countries are not implementing many basic policies that WHO recommends to encourage appropriate use of medicines. Although it may appear that the number of countries implementing policies has increased between 2003 and 2007, caution must be used when interpreting the figures, particularly for the SEA Region, since the sample sizes are very small and different countries responded in different years. The situation is probably worse than it appears here since many countries are not implementing fully the policies that are supposedly in place. Globally and in the SEA Region, many countries are using an updated Essential Medicines List for public sector procurement, but few have updated national clinical guidelines or have a drug and therapeutic committee in most health facilities to undertake monitoring and education of staff. Furthermore, it should be noted that most of these policies are aimed at the public and not the private sector, which provides the major chunk of health care in the Region. It is of particular concern that in all countries antibiotics are available over the counter and no regular monitoring of drug use is being undertaken.

**Future challenges**

There is now ample evidence of rampant irrational use of antibiotics together with under-implementation of effective interventions and policies to promote rational use of antibiotics. This is contributing to antimicrobial resistance and it is now urgent that countries and the international community take action. While we may not know all the ways to tackle irrational use of antibiotics, we have enough evidence to
know what our first steps should be. However, we are not taking these steps and investment in this area remains low. Why? There are a number of possible reasons for this.

Firstly, promoting rational use of medicines and containing antimicrobial resistance are not institutionalized within the health care systems of many countries. If there is no department in the MoH dedicated to ensuring appropriate use, who will do the necessary monitoring of antibiotic use and coordination of policy and actors? By contrast the industrial rich nations have invested in national monitoring of antibiotic use and nationwide campaigns to promote rational use of antibiotics\textsuperscript{15,16,17,42,43}.

Secondly, a great deal of extra investment will be needed to restructure the health care systems to undertake the necessary activities. While this may result in significant future savings from reduced misuse of medicines, governments may be reluctant to invest initially, particularly in those countries, as in South-East Asia, where most drugs are paid for out of pocket by patients. By contrast, in rich nations, where there is a substantial health-care infrastructure, most medicines are paid for through insurance reimbursement that can be organized to encourage more rational use e.g. reimbursement of antibiotics when they are used in compliance with clinical guidelines.

Thirdly, health systems have become increasingly fragmented due, among other reasons, to increased verticalization and donor demands. Some countries, for example, have more than 50 drug supply systems from various donors and global drug facilities\textsuperscript{44} – making coordination very difficult – despite global initiatives to improve donor coordination such as the Paris Declaration 2005\textsuperscript{45} and the International Health Partnership\textsuperscript{46}.

Fourthly, there is a huge imbalance of information, with the pharmaceutical industry spending huge amounts of money to promote their products to prescribers and dispensers while governments spend virtually nothing on continuing medical education. Most prescribers, globally and especially in the SEA Region, are receiving most of their information about medicines from the pharmaceutical industry and this information is often biased in favour of more use and less caution\textsuperscript{47,48}.

Fifthly, while quite a lot is known about how to improve the use of medicines in a targeted way in the public sector, not much is known about how to promote rational use at a national level, incorporating the private and informal sectors, particularly in countries lacking in resources to fund huge government bureaucracies. Further research is needed into what kind of coordinating structures will be cost-effective in resource-constrained settings. Furthermore, the basic skills needed to monitor and promote rational use of antibiotics are often lacking and not taught in schools of public health, clinical pharmacology and pharmacy. Such courses should include pharmaco-epidemiology for monitoring use, drug evaluation for selection and management of formularies and how to manage drug and therapeutic committees and antibiotic subcommittees.

The way forward

The causes of irrational use of medicines, and in particular antibiotics, are multiple and small-scale interventions will not change behaviour. Rather a system change is needed and this will be very context specific as different countries have widely differing health-care systems. Multiple global recommendations have been made to have national programmes to promote rational use of antibiotics and other medicines\textsuperscript{9,40} including two recent World Health Assembly resolutions\textsuperscript{11,12} and a regional one\textsuperscript{13}. In July 2010, WHO held a regional meeting for South-East Asia on promoting rational use of medicines\textsuperscript{39}, where delegates from nine
countries recommended, among other things, that:

- All countries have a fully resourced unit or department within their ministry of health dedicated to promoting rational use of medicines and supported by a broad-based steering committee involving all stakeholders; and
- All countries undertake a national situational analysis of the health-care system and pharmaceutical sector with the focus on medicines use in order to identify and prioritize the major problems and develop a coordinated roadmap for action.

WHO is now undertaking such a situational analysis in countries of the SEA Region at their request and developing recommendations for them to use in future planning. In addition, WHO is developing a tool for national stakeholders to use to undertake such an analysis and to monitor progress. However, in order to make progress it will be very important that the recommendations made in a national situational analysis are acted upon. This will require resources. Who will pay? If 5% of all the funds spent on medicines were spent on promoting rational use of medicines, much progress could be made and probably the costs would be recovered through reduced misuse and overuse of antibiotics and other medicines. Governments, donors and global drug facilities can contribute to this process by ensuring that a proportion of all donated funds for medicines goes towards building capacity to monitor and coordinate policies to promote rational use of medicines and antibiotics. Professional bodies and academia can build the necessary skills. Political will is crucial. Will we act now to promote rational use of antibiotics and preserve their effectiveness for future generations or will we sit back and wait for a new non-antibiotic era where future generations will die of infections that are easily treatable today?

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Antimalarial and artemisinin resistance in the Greater Mekong subregion

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Antimalarial resistance is a global concern. Over the past few years, considerable effort and resources have been invested to monitor, detect and understand the basis for the different facets of emerging and increasing resistance of these antimalarial drugs. The development of new antimalarial agents has always been triggered by the development of resistance by the parasites.

The Greater Mekong Subregion (GMS) is known as the epicentre of *P. falciparum* resistance to antimalarial drugs in South-East Asia. The *in vivo* therapeutic efficacy studies (TES) of the WHO Mekong Malaria Programme (MMP) network have been monitoring the therapeutic efficacy of the first-line therapies to *P. falciparum* and *P. vivax* malaria with the use of a single standardized WHO in vivo protocol across the six Mekong countries, and accurately checking the quality of data generated by studies carried out in strategically located sentinel sites. During the last decade, all six countries in the Mekong subregion, namely Cambodia, China, Lao PDR, Myanmar, Thailand and Viet Nam, have officially shifted to the use of artemisinin-based combination treatment (ACT), with the exception of Thailand that started using the artesunate-mefloquine (A+M) combination since 1995. China and Viet Nam, meanwhile, have also been using the five-day or seven-day artemisinin/artsunate monotherapy since the 1980s, as well as Myanmar and Cambodia in the private sector.

A WHO MMP informal consultation in January 2007 in Cambodia acknowledged the decreased sensitivity of ACTs for the treatment of *P. falciparum* on the Cambodia-Thailand border triggering immediate in-depth research studies to confirm this worrisome situation. In addition to increased treatment failure rate, delayed parasite clearance was reported leading to the fear of reduced efficacy of the artemsinin component of the ACT. The Mekong Malaria Programme has been intensifying its support to 35 sentinel sites in the six countries since September 2007. The efficacy of antimalarial drugs was studied in *P. falciparum* and *P. vivax* patients against clinical symptoms and parasitemia with 28- or 42-day follow-ups, with adherence to standardized entry criteria, quality microscopy, data entry management and molecular genotyping techniques to differentiate true failures from re-infections. TES training workshops for field staff and country...
monitoring visits to assess TES performance are being conducted in all countries to ensure proper implementation.

Results from the six GMS countries showed decreasing adequate clinical and parasitological response (ACPR) of ACTs especially in the border areas: in Kawthaung on the south eastern part of Myanmar bordering Thailand, the province of Ranong, where the cure rate to AS+M has been slowly declining since 2006. The Tak province bordering eastern Myanmar reported <90% ACPRs from 2004-2009. In the 2009 studies, the proportion of patients with longer parasite clearance (on Day 3 and beyond after 72 hours of treatment) to A+M was observed in sentinel sites along the western border of Thailand: with >15% Day 3 parasitemia in Tak, Kanchanaburi and in Ranong, whereas the neighbouring Kawthaung in Myanmar had 19% Day 3 parasitemia to dihydroartemisinin-piperaquine (DHA-PIP). On the other side of the eastern border of Thailand with Cambodia, results showed 90% cure rates to the A+M combination in Pailin and Pursat, and 34% Day 3 parasitemia to DHA-PIP in Pailin. Results also showed a longer parasite clearance time (25% Day 3 parasitemia) to 7-day artesunate (AS7) monotherapy in Yingjiang, Dehong county, Yunnan province of China bordering Myanmar, and in Binh Phuoc province in southern Viet Nam bordering Cambodia, province of Snoul. Such worrying but yet preliminary results are currently being validated with in-depth studies on AS7 monotherapy with pharmacokinetic assays. In the absence (yet) of molecular markers for artemisinin resistance, a top research and development priority, and an established in vitro threshold, measuring the parasite clearance time is now considered as an early warning signal to monitor failing ACTs or AS7. However, despite the early stage of developing resistance, there is yet no correlation between the prolonged parasite clearance time and the proportion of therapeutic failure of *P. falciparum* to ACTs. The current working definition of *P. falciparum* resistance to artemisinin is: “an increase in parasite clearance time, as evidenced by ≥ 10% of cases with parasites detectable on day 3 after treatment with an ACT (suspected resistance)” or “treatment failure after treatment with an oral artemisinin-based monotherapy with adequate antimalarial blood concentration, as evidenced by the persistence of parasites for seven days, or the presence of parasites at day 3 and recrudescence within 28/42 days (confirmed resistance)”. There were several possible reasons raised for these ACT failures: failure of the partner drug mefloquine since pre-existing high level mefloquine *in vivo* resistance was documented in the past and is still being observed *in vitro*; the short half-life of artesunate relative to that of mefloquine, the latter drug being no longer very effective; under-dosing and poor compliance by both the patients and the public/private health practitioner despite treatment guidelines, the widespread uncontrolled use of substandard or counterfeit artemisinin derivatives or ACTs that created drug pressure against the artemisinins, and population movement across borders for socioeconomic and political reasons.

The varying topographies and drug policies of countries in the region, systematic findings and reports of substandard and

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counterfeit drugs, injudicious use of medicines in the private sector, as well as the high degree of population mobility, justifies the need for periodic assessment of therapeutic efficacy of antimalarial medicines, sharing of efficacy surveillance data and more intensive intercountry crossborder collaboration. All countries need to be pro-active, as the TES provided early warning information on the emergence or spread of artemisinin resistance/tolerance from its initial foci on the Cambodia-Thailand border and now possibly spreading to other sites or emerging de novo in the Mekong region. This calls for harmonization and a careful implementation and review of country drug policies and case management in general in the GMS and beyond.
Antimicrobial resistance in Leishmania donovani in Bihar, India

P.K. Sinha* and S.K. Bhattacharya**

Leishmaniasis is a member of the neglected tropical diseases that are a major public health problem in Bangladesh, India and Nepal and is targeted for elimination by 2015. It is a vectorborne disease caused by a protozoan parasite belonging to the genus Leishmania donovani in the Indian subcontinent. Fever with chill, hepatosplenomegaly, pancytopenia, weight loss and pallour are the striking clinical features of kala azar (visceral leishmaniasis- VL); if left untreated the disease is almost always fatal.

Drug resistance to kala azar (VL) has been a public health problem in India since long, particularly in Bihar, where the unresponsiveness of Leishmania donovani to sodium antimony gluconate (SAG) is in excess of 50%.¹ Treatment with antimonials dates back to the early twentieth century when Noble laureate Paul Ehrlich first used it for the treatment of trypanosomiasis and syphilis.² Sodium antimony gluconate (SAG) has been used as a drug of choice for the treatment of kala-azar for more than six decades. In the early twentieth century the trivalent antimony was used in the treatment of kala azar with 100% cure rate following treatment for 10-15 days, but due to its alarming toxicity it was replaced with Urea Stibamine, which also had more than 90% efficacy but was toxic. Thereafter, WHO recommended³ a dose of 20mg/kg bodyweight for 28 days to be given intramuscularly or through the intravenous route. However, the current unresponsiveness to SAG is around 60%.¹ Hence, SAG is no longer recommended as the first-line drug for the treatment of VL in north Bihar.

Miltefosine, the first-ever antileishmanial oral drug, had 94% efficacy for the treatment of VL⁴. The drug is now being used as a first-line drug in the kala azar elimination programme in Bihar. A major concern is the possible emergence of resistance due to incomplete treatment and its long half-life (approximately 150 hours).

In view of the emerging problem of antimicrobial resistance of leishmania donovani particularly in Bihar, it is essential to promote rational use of antimicrobials, and to establish an antimicrobial resistance surveillance mechanism to support regional efforts for elimination of kala-azar.

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Antimicrobial resistance results in increased morbidity, mortality and costs of health care. Prevention of the emergence of resistance and dissemination of resistant microorganisms will reduce the adverse effects and their attendant cost.\(^1\)

Antimicrobial stewardship aims to promote the appropriate use of antimicrobials—the right selection, duration, dose and route of administration. Promoting the appropriate use of antimicrobials is intended to improve clinical outcomes by reducing the emergence of resistance, limiting drug-related adverse events, and minimizing the risk of unintentional consequences associated with antimicrobial use. The clinical microbiology laboratory plays a critical role in antimicrobial stewardship by providing patient-specific culture and susceptibility data to optimize individual antimicrobial management and by assisting infection control efforts in the surveillance of resistant organisms.\(^2\)

The antibiotic sensitivity method of disk diffusion by Kirby and Bauer has been standardized and is a viable alternative to broth dilution methods for laboratories without the resources to utilize the newer automated methods for broth microdilution testing.\(^3\)

The current interpretation standards of disk diffusion testing are found in the Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standards (Ninth Edition).\(^4\)

Besides qualitative determination of antimicrobial resistance or susceptibility, periodic review of zone diameters in disk-diffusion techniques can detect early trends of emerging resistance, even within the “susceptibility” cut-offs.\(^5\)

The disk diffusion method involves application of a different battery of antibiotics for individual organisms. The procedure is time consuming if there are a large number of antibiotic sensitivity tests for large number of organisms. We have devised a template for application and interpretation of antimicrobial sensitivity test. The following is the method of preparing the template (Figure 1)\(^6\).

(1) The panel of antibiotic to be tested is designated for each organism as per recommendations and guidelines of the Clinical and Laboratory Standards Institute (USA) and the local antibiotic usage.

(2) The template is prepared using a computer.

(3) A black circle of the size of the petri plate (e.g. 90 cm) used for sensitivity testing is drawn (Petri Circle)

(4) Seven smaller disk circles of 6 mm diameter are drawn with their centres 15 mm away from the margin of the outer circle. The name of the code as per WHONET is written on these circles. Antibiotic disks are placed on these circles after inoculation of the plate.
The distance between the two disks is 24 mm — from centre to centre.

Two circles are drawn from outside the disk circles with the same centre.

The zone diameter inside the inner circle indicates resistance.

The zone diameter outside the outer circle indicates sensitivity.

The zone diameter between the two circles indicates intermediate resistance.

This template can simplify the application of the disks and make it easy for interpretation of the zone diameter. The use of calipers for measuring the zone diameters is minimized. The time for application and reading of plates is reduced by half. Similar templates can be prepared for the quality control strains and all the bacteria. This template acts as a good substitute for automated systems in resource constraint settings. The exact zone diameters can also be measured by drawing more circles at 2mm distance. The zone diameters are read to the nearest millimeter. The convenience of the procedure helps in maintaining the quality of the test.

The zone diameter data gained from disc sensitivity testing have been used for many purposes including determination of the normal distribution of “sensitive” populations. Such data are necessary to identify strains with possible mechanisms of resistance. They are also useful for surveillance of resistance, daily monitoring of performance of the method with reference to control strains and interpretation of sensitivity by comparing the test zones with zone diameter breakpoints. All these procedures rely on a standardized method of testing and the accurate measurement of zones of inhibition. This simple tool supplements the role played by the microbiology laboratory in providing antimicrobial stewardship.3

References and bibliography


Classic publications in Antimicrobial Agents and Resistance

The discovery of antibiotics, their clinical use and detection of antibiotic inactivating enzymes by bacteria was heralded by some publications as providing a new paradigm for the medical management of infectious diseases. Though these papers were published more than seven decades ago, they continue to be relevant in contemporary times. Three such landmark papers are brought together in this special issue of the Regional Health Forum, and are reprinted with permission from the respective journals.

1929
On the antibacterial action of cultures of a Penicillium, with special reference to their use in isolation of B. influenzae.
A. Fleming
British Journal of Experimental Pathology 1929; 10: 226-236
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The discovery in 1929 of in vitro action of a product of Penicillium on several bacteria of medical importance is believed to be the first published report of the antibacterial action of a fungal product. This was published in 1929 by A. Fleming (who subsequently received the Nobel Prize for this discovery) in the British Journal of Experimental Pathology (now the International Journal of Experimental Pathology).

1940
Penicillin as a chemotherapeutic agent.
E Chain, HW Florey, AD Gardner, NG Heatley, MA Jennings, J Orr-Ewing and AG Sanders

Lancet 1940; ii: 226-228
Copyright ©1940. The Lancet Ltd./Elsevier

The discovery of antibacterial action of a fungal product by Fleming (1929) was expanded into a product fit for use in human beings by Chain, Florey et al., who demonstrated its clinical efficacy for the first time in 1940. This heralded an era in which new and potent weapons against infectious diseases were developed, and substantially reduced mortality and morbidity from these diseases.

1940
An enzyme from bacteria able to destroy penicillin
EP Abraham and E Chain
Nature 1940; 146: 837
Copyright ©1940. Nature Publishing Group.

The world has always celebrated the discovery and clinical use of penicillin—however, the fact that in the same year a study was published showing that an enzyme could be produced by bacteria that inactivated penicillin has largely been ignored. This publication should have forewarned us of the ingenuity of microorganisms against antibiotics, which is now manifesting itself as the major problem of antimicrobial resistance in several microorganisms.
ON THE ANTIBACTERIAL ACTION OF CULTURES OF A PENICILLIUM, WITH SPECIAL REFERENCE TO THEIR USE IN THE ISOLATION OF B. INFLUENZÆ.

ALEXANDER FLEMING, F.R.C.S.

From the Laboratories of the Inoculation Department, St. Mary's Hospital, London.

Received for publication May 10th, 1929.

While working with staphylococcus variants a number of culture-plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various micro-organisms. It was noticed that around a large colony of a contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis (see Fig. 1).

Subcultures of this mould were made and experiments conducted with a view to ascertaining something of the properties of the bacteriolytic substance which had evidently been formed in the mould culture and which had diffused into the surrounding medium. It was found that broth in which the mould had been grown at room temperature for one or two weeks had acquired marked inhibitory, bactericidal and bacteriolytic properties to many of the more common pathogenic bacteria.

CHARACTERS OF THE MOULD.

The colony appears as a white fluffy mass which rapidly increases in size and after a few days sporulates, the centre becoming dark green and later in old cultures darkens to almost black. In four or five days a bright yellow colour is produced which diffuses into the medium. In certain conditions a reddish colour can be observed in the growth.

In broth the mould grows on the surface as a white fluffy growth changing in a few days to a dark green felted mass. The broth becomes bright yellow and this yellow pigment is not extracted by CHCl₃. The reaction of the broth becomes markedly alkaline, the pH varying from 8·5 to 9. Acid is produced in three or four days in glucose and saccharose broth. There is no acid production in 7 days in lactose, mannite or dulcite broth.

Growth is slow at 37°C, and is most rapid about 20°C. No growth is observed under anaerobic conditions.

In its morphology this organism is a penicillium and in all its characters it most closely resembles P. rubrum. Biourge (1923) states that he has never found P. rubrum in nature and that it is an "animal de laboratoire." This penicillium is not uncommon in the air of the laboratory.
IS THE ANTIBACTERIAL BODY ELABORATED IN CULTURE BY ALL MOULDS?

A number of other moulds were grown in broth at room temperature and the culture fluids were tested for antibacterial substances at various intervals up to one month. The species examined were: *Eidemia viridescens*, *Botrytis cineria*, *Aspergillus fumigatus*, *Sporotrichum*, *Cladosporium*, *Penicillium*, 8 strains. Of these it was found that only one strain of penicillium produced any inhibitory substance, and that one had exactly the same cultural characters as the original one from the contaminated plate.

It is clear, therefore, that the production of this antibacterial substance is not common to all moulds or to all types of penicillium.

In the rest of this article, allusion will constantly be made to experiments with filtrates of a broth culture of this mould, so for convenience and to avoid the repetition of the rather cumbersome phrase "Mould broth filtrate," the name "penicillin" will be used. This will denote the filtrate of a broth culture of the particular penicillium with which we are concerned.

METHODS OF EXAMINING CULTURES FOR ANTIBACTERIAL SUBSTANCE.

The simplest method of examining for inhibitory power is to cut a furrow in an agar plate (or a plate of other suitable culture material), and fill this in with a mixture of equal parts of agar and the broth in which the mould has grown. When this has solidified, cultures of various microbes can be streaked at right angles from the furrow to the edge of the plate. The inhibitory substance diffuses very rapidly in the agar, so that in a few hours before the microbes show visible growth it has spread out for a centimetre or more in sufficient concentration to inhibit growth of a sensitive microbe. On further incubation it will be seen that the proximal portion of the culture for perhaps one centimetre becomes transparent, and on examination of this portion of the culture it is found that practically all the microbes are dissolved, indicating that the anti-bacterial substance has continued to diffuse into the agar in sufficient concentration to induce dissolution of the bacteria. This simple method therefore suffices to demonstrate the bacterio-inhibitory and bacteriolytic properties of the mould culture, and also by the extent of the area of inhibition gives some measure of the sensitiveness of the particular microbe tested. Fig. 2 shows the degree of inhibition obtained with various microbes tested in this way.

The inhibitory power can be accurately titrated by making serial dilutions of penicillin in fresh nutrient broth, and then implanting all the tubes with the same volume of a bacterial suspension and incubating them. The inhibition can then readily be seen by noting the opacity of the broth.

For the estimation of the antibacterial power of a mould culture it is unnecessary to filter as the mould grows only slowly at 37°C., and in 24 hours, when the results are read, no growth of mould is perceptible. Staphylococcus is a very suitable microbe on which to test the broth as it is hardy, lives well in culture, grows rapidly, and is very sensitive to penicillin.

The bactericidal power can be tested in the same way except that at intervals measured quantities are explanted so that the number of surviving microbes can be estimated.
Fig. 1.—Photograph of a culture-plate showing the dissolution of staphylococcal colonies in the neighbourhood of a penicillium colony.

Fig. 2. Fleming.
Fig. 3.—Photograph of a culture-plate (Filces medium) which had been evenly planted with a mixture of staphylococci and B. influenzae. Six drops of penicillin were then spread over the lower half of the plate. Note complete inhibition of staphylococci in the penicillin treated area with resultant pure culture of B. influenzae.

Fig. 4.—Photograph of a culture-plate (Filces medium) which had been evenly planted with nasal mucus from an individual suffering from a "cold." Six drops of penicillin were spread over the lower half of the plate before incubation. Note profuse growth of staphylococci and diphtheroid bacilli in untreated half, whereas in treated half only some three colonies of B. influenzae are seen.
A. FLEMING.

PROPERTIES OF THE ANTIBACTERIAL SUBSTANCE.

Effect of heat.—Heating for 1 hour at 56° or 80° C. has no effect on the antibacterial power of penicillin. Boiling for a few minutes hardly affects it (see Table II). Boiling for 1 hour reduces it to less than one quarter its previous strength if the fluid is alkaline, but if it is neutral or very slightly acid then the reduction is much less. Autoclaving for 20 minutes at 115° C. practically destroys it.

Effect of filtration.—Passage through a Seitz filter does not diminish the antibacterial power. This is the best method of obtaining sterile active mould broth.

Solubility.—It is freely soluble in water and weak saline solutions. My colleague, Mr. Ridley, has found that if penicillin is evaporated at a low temperature to a sticky mass the active principle can be completely extracted by absolute alcohol. It is insoluble in ether or chloroform.

Rate of development of inhibitory substance in culture.—A 500 c.c. Erlenmeyer flask containing 200 c.c. of broth was planted with mould spores and incubated at room temperature (10° to 20° C.). The inhibitory power of the broth to staphylococcus was tested at intervals.

After 5 days complete inhibition in 1 in 20 dilution.

| ... | 6 .. | .. | 1 in 40 .. |
| ... | 7 .. | .. | 1 in 200 .. |
| ... | 8 .. | .. | 1 in 500 .. |

Grown at 20° C. the development of the active principle is more rapid and a good sample will completely inhibit staphylococci in a 1 in 500 or 1 in 800 dilution in 6 or 7 days. As the culture ages the antibacterial power falls and may in 14 days at 20° C. have almost disappeared.

The antibacterial power of penicillin falls when it is kept at room temperature. The rate of this fall can be seen from Table I.

<table>
<thead>
<tr>
<th>TABLE I.—Effect of Keeping at Room Temperature on the Anti-Staphylococcal Power of Penicillin.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth of staphylococci in dilutions of penicillin as under</td>
</tr>
<tr>
<td>At time of filtration</td>
</tr>
<tr>
<td>After 4 days</td>
</tr>
<tr>
<td>..</td>
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<td>..</td>
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<td>..</td>
</tr>
</tbody>
</table>

If the reaction of penicillin is altered from its original pH of 9 to a pH of 6-8 it is much more stable.

The small drops of bright yellow fluid which collect on the surface of the mould may have a high antibacterial titre. One specimen of such fluid completely inhibited the growth of staphylococci in a dilution of 1 in 20,000 while the broth in which the mould was growing, tested at the same time, inhibited staphylococcal growth in 1 in 800.

If the mould is grown on solid medium and the felted mass picked off and
PENICILLIN.

**Table II.** — Inhibitory Power of Penicillin (Heated and Unheated) on Various Microbes (Agar Plate Method).

<table>
<thead>
<tr>
<th>Type of microbe</th>
<th>Extent of inhibition in mm. from penicillin embedded in agar, serum agar, or blood agar plates.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unheated</td>
</tr>
<tr>
<td>Experiment 1:</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus pyogenes</em></td>
<td>23</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>17</td>
</tr>
<tr>
<td>&quot;viridans (mouth)&quot;</td>
<td>17</td>
</tr>
<tr>
<td>Diphtheroid bacillus</td>
<td>27</td>
</tr>
<tr>
<td>Sarcina</td>
<td>10</td>
</tr>
<tr>
<td><em>Micrococcus lysisodeikticus</em></td>
<td>6</td>
</tr>
<tr>
<td>&quot;from air (1)&quot;</td>
<td>20</td>
</tr>
<tr>
<td>&quot;(2)&quot;</td>
<td>4</td>
</tr>
<tr>
<td><em>B. anthracis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>B. typhosus</em></td>
<td>0</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>0</td>
</tr>
<tr>
<td>Experiment 2:</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus pyogenes</em></td>
<td>24</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>30</td>
</tr>
<tr>
<td>&quot;viridans (mouth)&quot;</td>
<td>25</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>30</td>
</tr>
<tr>
<td>Diphtheroid bacillus</td>
<td>35</td>
</tr>
<tr>
<td><em>B. pyocyaneus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>B. pneumonia</em> (Friedlander)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. coli</em></td>
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</tr>
<tr>
<td><em>B. paratyphosus A</em></td>
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</tr>
<tr>
<td>Experiment 3:</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus pyogenes</em></td>
<td>16</td>
</tr>
<tr>
<td>Gonococcus</td>
<td>16</td>
</tr>
<tr>
<td>Meningococcus</td>
<td>17</td>
</tr>
<tr>
<td>Experiment 4:</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus pyogenes</em> epidermidis</td>
<td>17</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> viridans (feces)</td>
<td>15</td>
</tr>
<tr>
<td><em>B. diphtheriae</em> (2 strains)</td>
<td>14</td>
</tr>
<tr>
<td>Diphtheroid bacillus</td>
<td>10</td>
</tr>
<tr>
<td>Gram-negative coccus from the mouth (1)</td>
<td>12</td>
</tr>
<tr>
<td>&quot;(2)&quot;</td>
<td>0</td>
</tr>
<tr>
<td><em>B. coli</em></td>
<td>0</td>
</tr>
<tr>
<td><em>B. influenza</em> (Pfeiffer) 6 strains</td>
<td>0</td>
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**Table III. Inhibitory Power of Penicillin on Different Bacteria.**

<table>
<thead>
<tr>
<th></th>
<th>1/5</th>
<th>1/10</th>
<th>1/20</th>
<th>1/40</th>
<th>1/80</th>
<th>1/160</th>
<th>1/320</th>
<th>Control</th>
</tr>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>±</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td></td>
<td>epidermidis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>±</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td><em>Streptococcus ( hämolytic )</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>viridans (mouth)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>fecalis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. anthracis</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. pseudo-tuberculosis rodentium</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. pullorum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. dysenteriae</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. coli</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. typhosus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. pyocyaneus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>K. proteus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>V. cholera</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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*B. diptheriae* (3 strains)  
<table>
<thead>
<tr>
<th></th>
<th>1/60</th>
<th>1/120</th>
<th>1/300</th>
<th>1/600</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em> (13 strains)</td>
<td>0</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td></td>
<td>(1 &quot;&quot;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2 strains)</td>
<td>0</td>
<td>0</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td><em>faecalis</em> (11 &quot;&quot;)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td><em>viridans at random from feces</em></td>
<td>(1 strain)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(2 strains)</td>
<td>0</td>
<td>0</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(1 &quot;&quot;)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(1 &quot;&quot;)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>at random from mouth</td>
<td>(1 strain)</td>
<td>0</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(2 strains)</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(1 &quot;&quot;)</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(1 &quot;&quot;)</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

0 = no growth; ± = trace of growth; + = poor growth; + + = normal growth.
extracted in normal salt solution for 24 hours it is found that the extract has bacteriolytic properties.

If this extract is mixed with a thick suspension of staphylococcus suspension and incubated for 2 hours at 45° C. it will be found that the opacity of the suspension has markedly diminished and after 24 hours the previously opaque suspension will have become almost clear.

Influence of the medium on the antibacterial titre of the mould culture. — So far as has been ascertained nutrient broth is the most suitable medium for the production of penicillin. The addition of glucose or saccharose, which are fermented by the mould with the production of acid, delays or prevents the appearance of the antibacterial substance. Dilution of the broth with water delays the formation of the antibacterial substance and diminishes the concentration which is ultimately reached.

INHIBITORY POWER OF PENICILLIN ON THE GROWTH OF BACTERIA.

Tables II and III show the extent to which various microbes, pathogenic and non-pathogenic, are inhibited by penicillin. The first table shows the inhibition by the agar plate method and the second shows the inhibitory power when diluted in nutrient broth.

Certain interesting facts emerge from these Tables. It is clear that penicillin contains bacterio-inhibitory substance which is very active towards some microbes while not affecting others. The members of the coli-typhoid group are unaffected as are other intestinal bacilli such as B. pyocyaneus, B. proteus and V. cholerae. Other bacteria which are insensitive to penicillin are the enterococcus, some of the Gram-negative cocci of the mouth, Friedländer’s pneumobacillus, and B. influenzae (Pfeiffer), while the action on B. dysenteriae (Flexner), and B. pseudo-tuberculosis rodentium is almost negligible. The anthrax bacillus is completely inhibited in a 1 in 10 dilution but in this case the inhibitory influence is trifling when compared with the effect on the pyogenic cocci.

It is on the pyogenic cocci and on bacilli of the diphtheria group that the action is most manifest.

Staphylococci are very sensitive, and the inhibitory effect is practically the same on all strains, whatever the colour or type of the staphylococcus.

Streptococcus pyogenes is also very sensitive. There were small differences in the titre with different strains, but it may be said generally that it is slightly more sensitive than staphylococcus.

Pneumococci are equally sensitive with Streptococcus pyogenes.

The green streptococci vary very considerably, a few strains being almost unaffected while others are as sensitive as S. pyogenes. Gonococci, meningococci, and some of the Gram-negative cocci found in nasal catarrhal conditions are about as sensitive as are staphylococci. Many of the Gram-negative cocci found in the mouth and throat are, however, quite insensitive.

B. diphtheriae is less affected than staphylococcus but is yet completely inhibited by a 1% dilution of a fair sample of penicillin.

It may be noted here that penicillin, which is strongly inhibitory to many bacteria, does not inhibit the growth of the original penicillium which was used in its preparation.
A. FLEMING.

The Rate of Killing of Staphylococci by Penicillin.

Some bactericidal agents like the hypochlorites are extremely rapid in their action, others like flavine or novarsenobillon are slow. Experiments were made to find into which category penicillin fell.

To 1 c.c. volumes of dilutions in broth of penicillin were added 10 c.mm. volumes of a 1 in 1000 dilution of a staphylococcus broth culture. The tubes were then incubated at 37° C. and at intervals 10 c.mm. volumes were removed and plated with the following result:

<table>
<thead>
<tr>
<th>Time after addition</th>
<th>Control</th>
<th>1/80</th>
<th>1/40</th>
<th>1/20</th>
<th>1/10</th>
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<tbody>
<tr>
<td>Before</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>After 2 hours</td>
<td>116</td>
<td>73</td>
<td>51</td>
<td>48</td>
<td>23</td>
</tr>
<tr>
<td>4½ hours</td>
<td>α</td>
<td>13</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>8 hours</td>
<td>α</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 hours</td>
<td>α</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It appears, therefore, that penicillin belongs to the group of slow acting antiseptics, and the staphylococci are only completely killed after an interval of over 4½ hours even in a concentration 30 or 40 times stronger than is necessary to inhibit completely the culture in broth. In the weaker concentrations it will be seen that at first there is growth of the staphylococci and only after some hours are the cocci killed off. The same thing can be seen if a series of dilutions of penicillin in broth is heavily infected with staphylococcus and incubated. If the cultures are examined after four hours it may be seen that growth has taken place apparently equally in all the tubes but when examined after being incubated overnight, the tubes containing penicillin in concentrations greater than 1 in 300 or 1 in 400 are perfectly clear while the control tube shows a heavy growth. This is a clear illustration of the bacteriolytic action of penicillin.

TOXICITY OF PENICILLIN.

The toxicity to animals of powerfully antibacterial mould broth filtrates appears to be very low. Twenty c.c. injected intravenously into a rabbit were not more toxic than the same quantity of broth. Half a c.c. injected intraperitoneally into a mouse weighing about 20 gm. induced no toxic symptoms. Constant irrigation of large infected surfaces in man was not accompanied by any toxic symptoms, while irrigation of the human conjunctiva every hour for a day had no irritant effect.

In vitro penicillin which completely inhibits the growth of staphylococci in a dilution of 1 in 600 does not interfere with leucocytic function to a greater extent than does ordinary broth.

USE OF PENICILLIN TO DEMONSTRATE OTHER BACTERIAL INHIBITIONS.

When materials like saliva or sputum are plated it is not uncommon to see, where the implant is thick, an almost pure culture of streptococci and...
PENICILLIN.

pneumococci, and where the implant is thinner and the streptococcal colonies are more widely separated, other colonies appear, especially those of Gram-negative cocci. These Gram-negative cocci are inhibited by the streptococci (probably by the peroxide they produce in their growth) and it is only when the mass effect of the streptococci is reduced that they appear in the culture.

Penicillin may be used to give a striking demonstration of this inhibition of bacteria by streptococci and pneumococci. Sputum is spread thickly on a culture plate, and then 5 or 6 drops of penicillin is spread over one half of it. After incubation it may be seen that on the half untreated with penicillin there is a confluent growth of streptococci and pneumococci and nothing else, while on the penicillin-treated half many Gram-negative cocci appear which were inhibited by the streptococci and pneumococci, and can only flourish when these are themselves inhibited by the penicillin.

If some active penicillin is embedded in a streak across an agar plate planted with saliva an interesting growth sometimes results. On the portion most distal from the penicillin there are many streptococci, but these are obscured by coarsely growing cocci, so that the resultant growth is a copious confluent rough mass. These coarse growing cocci are extremely penicillin sensitive and stop growing about 25 mm. from the embedded penicillin. Then there is a zone of about 1 cm. wide of pure streptococci, then they are inhibited by the penicillin, and as soon as that happens Gram-negative cocci appear and grow right up to the embedded penicillin. The three zones of growth produced in this way are very striking.

USE OF PENICILLIN IN THE ISOLATION OF B. INFLUENZÆ (PFEIFFER) AND OTHER ORGANISMS.

It sometimes happens that in the human body a pathogenic microbe may be difficult to isolate because it occurs in association with others which grow more profusely and which mask it. If in such a case the first microbe is insensitive to penicillin and the obscuring microbes are sensitive, then by the use of this substance these latter can be inhibited while the former are allowed to develop normally. Such an example occurs in the body, certainly with B. influenzae (Pfeiffer) and probably with Bordet's whooping-cough bacillus and other organisms. Pfeiffer's bacillus, occurring as it does in the respiratory tract, is usually associated with streptococci, pneumococci, staphylococci and Gram-negative cocci. All of these, with the exception of some of the Gram-negative cocci, are highly sensitive to penicillin and by the addition of some of this to the medium they can be completely inhibited while B. influenzae is unaffected. A definite quantity of the penicillin may be incorporated with the molten culture medium before the plates are made, but an easier and very satisfactory method is to spread the infected material, sputum, nasal mucus, etc., on the plate in the usual way and then over one half of the plate spread 2 to 6 drops (according to potency) of the penicillin. This small amount of fluid soaks into the agar and after cultivation for 24 hours it will be found that the half of the plate without the penicillin will show the normal growth while on the penicillin treated half there will be nothing but B. influenzae with Gram-negative cocci and occasionally some other microbe. This makes it infinitely easier to isolate these penicillin-insensitive organisms, and repeatedly
B. influenzae has been isolated in this way when they have not been seen in films of sputum and when it has not been possible to detect them in plates not treated with penicillin. Of course if this method is adopted then a medium favourable for the growth of B. influenzae must be used, e.g. boiled blood agar, as by the repression of the pneumococci and the staphylococci the symbiotic effect of these, so familiar in cultures of sputum on blood agar, is lost and if blood agar alone is used the colonies of B. influenzae may be so minute as to be easily missed.

Figs. 3 and 4 are photographs of culture-plates made after the method described above. On the plate shown in Fig. 3 a mixture of staphylococci and B. influenzae was spread over the whole plate of Fildes medium (Fildes, 1921), then 6 drops of penicillin were spread over the lower half of the plate. The upper half shows the mixed culture while the lower half gives a pure culture of B. influenzae. Fig. 4 represents a culture of nasal mucus from a “cold” made on the same medium. Here, on the upper half (untreated with penicillin) staphylococci and diphtheroid bacilli grow abundantly, while on the treated (lower) half only some three or four colonies of B. influenzae appear.

In conjunction with my colleague, Dr. McLean, a series of cultures were made from the throats of 25 nurses warded for “influenza.” The swabs were planted on boiled blood agar and over half of each plate was spread 3 or 4 drops of penicillin. The results are set forth in Table IV.

<table>
<thead>
<tr>
<th>Without penicillin</th>
<th>With penicillin</th>
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<tr>
<td>Pneumococci</td>
<td>E. influenzae</td>
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<td>1. + + + + +</td>
<td>- + + + +</td>
</tr>
<tr>
<td>4. + + - + -</td>
<td>- + + + +</td>
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<tr>
<td>13. + + + + +</td>
<td>- + + + +</td>
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</table>

In the above Table account has only been taken of the common microbes found in these cultures. In some there were a few diphtheroid bacilli which were always penicillin sensitive, and in others there were Gram-negative bacilli which were penicillin insensitive, although they were inhibited by streptococci or pneumococci. Pneumococci and streptococci were classed together, as complete tests were not made to differentiate one from the other.
(From the appearance of the colonies and the morphological characters pneumococci were evidently present in most cases in much larger numbers than were streptococci.)

The swabs were generally planted thickly and in some cases where the growth on the portion of the plate without penicillin was almost confluent, the cultures were sampled by taking smears from thick portions of the growth. In these cases it is possible that the results given do not give a quite complete picture of the cultures. This, however, does not affect the present argument that by the addition of penicillin to the culture medium, and the consequent inhibition of the pyogenic cocci, the isolation of B. influenzae is very much easier. And in a number of cases it was isolated when it was completely missed in the cultures without penicillin.

It is quite immaterial how many pneumococci and streptococci are present in a specimen—they are completely inhibited—and even a few B. influenzae can be isolated from a mixture with an enormous number of these cocci.

From a number of observations which have been made on sputum, post-nasal and throat swabs it seems likely that by the use of penicillin, organisms of the B. influenzae group will be isolated from a great variety of pathological conditions as well as from individuals who are apparently healthy.

DISCUSSION.

It has been demonstrated that a species of penicillium produces in culture a very powerful antibacterial substance which affects different bacteria in different degrees. Speaking generally it may be said that the least sensitive bacteria are the Gram-negative bacilli, and the most susceptible are the pyogenic cocci. Inhibitory substances have been described in old cultures of many organisms; generally the inhibition is more or less specific to the microbe which has been used for the culture, and the inhibitory substances are seldom strong enough to withstand even slight dilution with fresh nutrient material. Penicillin is not inhibitory to the original penicillium used in its preparation.

Emmerich and other workers have shown that old cultures of B. pyocyaneus acquire a marked bacteriolytic power. The bacteriolytic agent, pyocyanase, possesses properties similar to penicillin in that its heat resistance is the same and it exists in the filtrate of a fluid culture. It resembles penicillin also in that it acts only on certain microbes. It differs however in being relatively extremely weak in its action and in acting on quite different types of bacteria. The bacilli of anthrax, diphtheria, cholera and typhoid are those most sensitive to pyocyanase, while the pyogenic cocci are unaffected, but the percentages of pyocyaneous filtrate necessary for the inhibition of these organisms was 40, 33, 40 and 60 respectively (Bocchia, 1909). This degree of inhibition is hardly comparable with 0.2% or less of penicillin which is necessary to completely inhibit the pyogenic cocci or the 1% necessary for B. diphtheriae.

Penicillin, in regard to infections with sensitive microbes, appears to have some advantages over the well-known chemical antiseptics. A good sample will completely inhibit staphylococci, Streptococcus pyogenes and pneumococcus in a dilution of 1 in 800. It is therefore a more powerful inhibitory agent than
is carabolic acid and it can be applied to an infected surface undiluted as it is non-irritant and non-toxic. If applied, therefore, on a dressing, it will still be effective even when diluted 800 times which is more than can be said of the chemical antiseptics in use. Experiments in connection with its value in the treatment of pyrogenic infections are in progress.

In addition to its possible use in the treatment of bacterial infections penicillin is certainly useful to the bacteriologist for its power of inhibiting unwanted microbes in bacterial cultures so that penicillin insensitive bacteria can readily be isolated. A notable instance of this is the very easy isolation of Pfeiffer’s bacillus of influenza when penicillin is used.

In conclusion my thanks are due to my colleagues, Mr. Ridley and Mr. Craddock, for their help in carrying out some of the experiments described in this paper, and to our mycologist, Mr. la Touche, for his suggestions as to the identity of the penicillium.

SUMMARY.

1. A certain type of penicillium produces in culture a powerful antibacterial substance. The antibacterial power of the culture reaches its maximum in about 7 days at 20° C. and after 10 days diminishes until it has almost disappeared in 4 weeks.

2. The best medium found for the production of the antibacterial substance has been ordinary nutrient broth.

3. The active agent is readily filterable and the name “penicillin” has been given to filtrates of broth cultures of the mould.

4. Penicillin loses most of its power after 10 to 14 days at room temperature but can be preserved longer by neutralization.

5. The active agent is not destroyed by boiling for a few minutes but in alkaline solution boiling for 1 hour markedly reduces the power. Autoclaving for 20 minutes at 115° C. practically destroys it. It is soluble in alcohol but insoluble in ether or chloroform.

6. The action is very marked on the pyogenic cocci and the diphtheria group of bacilli. Many bacteria are quite insensitive, e.g. the coli-typhoid group, the influenza-bacillus group, and the enterococcus.

7. Penicillin is non-toxic to animals in enormous doses and is non-irritant. It does not interfere with leucocytic function to a greater degree than does ordinary broth.

8. It is suggested that it may be an efficient antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes.

9. The use of penicillin on culture plates renders obvious many bacterial inhibitions which are not very evident in ordinary cultures.

10. Its value as an aid to the isolation of B. influenzae has been demonstrated.

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EMMERICH, LOEUW AND KOSCHUN.—(1902) Zbl. Bakt., 30, I.
BCCCHA.—(1909) Ibid., 50, 220.
PENICILLIN AS A CHEMOTHERAPEUTIC AGENT

BY

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A. D. GARDNER, D.M. OXFORD, F.R.C.S.
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(From the Sir William Dunn School of Pathology, Oxford)

In recent years interest in chemotherapeutic effects has been almost exclusively focused on the sulphonamides and their derivatives. There are, however, other possibilities, notably those connected with naturally occurring substances. It has been known for a long time that a number of bacteria and moulds inhibit the growth of pathogenic micro-organisms. Little, however, has been done to purify or to determine the properties of any of these substances. The antibacterial substances produced by Pseudomonas pyocyanea have been investigated in some detail, but without the isolation of any purified product of therapeutic value.

Recently, Dubos and collaborators (1939, 1940) have published interesting studies on the acquired bacterial antagonism of a soil bacterium which have led to the isolation from its culture medium of bactericidal substances active against a number of gram-positive micro-organisms.1 Pneumococcal infections in mice were successfully treated with one of these substances, which, however, proved to be highly toxic to mice (Hotchkiss and Dubos 1940) and dogs (McLeod et al. 1940).

Following the work on lysozyme in this laboratory it occurred to two of us (E. C. and H. W. F.) that it would be profitable to conduct a systematic investigation of the chemical and biological properties of the antibacterial

1. See Lancet, 1940, 1, 1173.
RESULTS OF THERAPEUTIC TESTS ON MICE INFECTED WITH *Strep. pyogenes*, *Staph. aureus* AND *Cl. septicum*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Dose of infecting culture (c.m.)</th>
<th>Interval before starting treatment (hrs.)</th>
<th>Single dose (mg.)</th>
<th>Total dose (mg.)</th>
<th>No. of mice</th>
<th>Survivors at end of—</th>
<th>Days</th>
<th>Hours</th>
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*Staph. aureus* *

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<th>Total dose (mg.)</th>
<th>No. of mice</th>
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*Cl. septicum* *

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<th>Interval before starting treatment (hrs.)</th>
<th>Single dose (mg.)</th>
<th>Total dose (mg.)</th>
<th>No. of mice</th>
<th>Survivors at end of—</th>
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1. A control mouse which was killed by mistake at 24 hrs. is counted. Heart-blood cultures strongly positive.
2. BETWEEN EXPERIMENTS 1 AND 2 THE VIRULENCE OF THE ORGANISM WAS INCREASED BY PASSAGE.

Substances produced by bacteria and moulds. This investigation was begun with a study of a substance with promising antibacterial properties, produced by a mould and described by Fleming (1929). The present preliminary report is the result of a cooperative investigation on the chemical, pharmacological and chemotherapeutic properties of this substance.

Fleming noted that a mould produced a substance which inhibited the growth, in particular, of staphylococci, streptococci, gonococci, meningococci and *Corynebacterium diphtheriae*, but not of *Bacillus coli*, *Homoophilus influenzae*, *Salmomella typhi*, *P. pyogenes*, *Bacillus pseudus* or *Vibrio cholera*. He suggested its use as an inhibitor in the isolation of certain types of bacteria, especially *H. influenzae*. He also noted that the injection into animals of broth containing the substance, which he called "penicillin," was no more toxic than plain broth, and he suggested that the substance might be a useful antiseptic for application to infected wounds. The mould is believed to be closely related to *Penicillum notatum*. Clutterbuck, Lovell and Raistrick (1932) grew the mould in a medium containing inorganic salts only and isolated a pigment—chrysogenous—which had no antibacterial action. Their culture media contained penicillin but this was not isolated. Reid (1933) reported work on the inhibitory substance produced by Fleming's mould. He did not isolate it but noted some of its properties.

During the last year methods have been devised here for obtaining a considerable yield of penicillin, and for rapid assay of its inhibitory power. From the culture medium a brown powder has been obtained which is freely soluble in water. It and its solution are stable for a considerable time and though it is not a pure substance, its anti-bacterial activity is very great. Full details will, it is hoped, be published later.

**EFFECTS ON NORMAL ANIMALS**

Various tests were done on mice, rats and cats. There is some evidence at the site of subcutaneous injection of strong solutions (e.g. 10 mg. in 0.3 c.c.m.). This may well be due to the hypertonicity of the solution. No sloughing of skin or suggestion of serious damage has ever been encountered, even with the strongest solutions or after repeated injections into the same area.

**Intravenous injections** showed that the penicillin preparation was only slightly, if at all, toxic for mice.

An intravenous injection of as much as 10 mg. (dissolved in 0.3 c.c.m. distilled water) of the preparation we have used for the curative experiments did not produce any observable toxic reactions in a 23 g. mouse. It was subsequently found that 10 mg. of a preparation having twice the penicillin content of the above was apparently innocuous to a 20 g. mouse.

Subcutaneous injections of 10 mg. into two rats at 3-hourly intervals for 56 hours did not cause any obvious change in their behaviour. They were perhaps slightly less lively than normal rats but they continued to eat their food. Their blood showed a fall of total leucocytes after 24 hours, but after 48 hours the count had risen again to about the original level. The decrease was, however, a relative decrease in the number of polymorphs, but the normal number was restored 24 hours after stopping the administration of the drug. One of these two rats was killed for histological examination; there was some evidence that the tubule cells of the kidney were damaged. The other has remained perfectly well, and its weight increased from 76 to 110 g. in 28 days. It is to be noted that these rats received, weight for weight, about five times the dose of penicillin used in the curative experiments in mice. No evidence of toxic effects was obtained from the treated mice, which received penicillin for many days.

**Other pharmacological effects.**—On the blood-pressure, heart-beat and respiration of cats no effects have been observed after intravenous injection of 40 mg.—enough to bring up the concentration in the blood just after injection to 1/5000. *Perfusion* of the isolated cat's heart, with Ringer-Locke solution containing 1/5000 penicillin produced progressive slowing during 15 minutes and at the end of that time the heart looked as though it would stop beating; however, it was quickly revived by perfusing with Ringer-Locke solution alone.

The same depressant action was seen at 1 10,000 dilution but the effect was less than at 1 5000. *Solutions* are absorbed from the intestine in the rat without causing any observable damage to the mucous. They are also readily absorbed after subcutaneous injection and the substance can be detected in the blood. It is excreted by the kidneys, the urine becoming bright yellow. At least 40 mg. appears in the urine in a still active form.

*Human leucocytes* remain active in a 1 1000 solution for at least 3 hours.

It must be emphasised that the results of these preliminary tests have been obtained with an impure sample of penicillin.
substance and such slight toxic effects as have been noted may possibly be due, in part at least, to these impurities.

**EFFECTS ON BACTERIA IN VITRO**

In view of this slight evidence of tissue toxicity it is all the more striking that the substance in a dilution of one in several hundred thousand inhibits in vitro the growth of many micro-organisms, including anaerobes. Of those so far tested in this laboratory the following are sensitive to the inhibiting action of our preparation: *Clostridium welchii* (2 strains); *Clostridium septicum* (1 strain, Nat. coll. type cultures No. 458); *Clostridium botulinum* (1 strain, N.C.T.C. No. 277); *C. diphteriae* (1 strain, mils type); *Streptococcus pyogenes* (Lancefield group A); *Staphylococcus aureus* (type 8); *Staphylococcus albus* (type 8); *Staphylococcus hominis*; and *Staphylococcus intermedius*. Penicillin is not immediately bactericidal but seems to interfere with multiplication.

**THERAPEUTIC EFFECTS**

From all the above tests it was clear that this substance possesses properties which made it suitable for trial as a chemotherapeutic agent. Therapeutic tests were therefore done on mice infected with streptococci, staphylococci and *Clostridium septicum*. The results are summarised in the accompanying table, the preliminary trials on small numbers of mice being omitted.

**Pathologic anaerobes**—The therapeutic effects were tried in mice infected with spores of *Clostridium septicum* in the manner described by Eddeson and Over (1940). Attempts to carry out similar tests with *Clostridium welchii* were temporarily abandoned owing to the difficulty of establishing in mice a type of infection which is both certainly fatal and allows adequate time before death for treatment to take effect. A spore suspension of *Clostridium septicum* was made by anaerobic growth at 37° C. for 48 hours, with air passage through the broth cultures injected intraperitoneally. According to opacity measurements with Brown's tubes, doses of 450 and 350 million cocci, living and dead respectively, were given in the two staphylococcal experiments, and doses of 760 and 200 million in the staphylococcal experiments.

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Hutchins, H. D., and Dubos, R. J. (1940) J. Hig. 40, 412.


An Enzyme from Bacteria able to Destroy Penicillin

FLEMING\(^1\) noted that the growth of \textit{B. coli} and a number of other bacteria belonging to the coliform group was not inhibited by penicillin. This observation has been confirmed. Further work has been done to find the cause of the resistance of these organisms to the action of penicillin.

An extract of \textit{B. coli} was made by crushing a suspension of the organisms in the bacterial crushing mill of Booth and Green\(^1\). This extract was found to contain a substance destroying the growth-inhibiting property of penicillin. The destruction took place on incubation with the penicillin solution with the bacterial extract at 37\(^\circ\), or at room temperature for a longer time. The following is a typical experiment showing the penicillin-destroying effect of \textit{B. coli} extract.

A solution of 1 mgm. penicillin in 0.8 c.c. of water was incubated with 0.2 c.c. of centrifuged and dialysed bacterial extract at 37\(^\circ\) for 3 hours. When the penicillin concentration was reduced to 1/400 without enzyme for the same time. (The penicillin used was extracted from cultures of \textit{Penicillium notatum} by a method to be described in detail later. It possessed a degree of purity similar to that of the samples used in the chemotherapeutic experiments recorded in a preliminary report.\(^1\))

1 Fleming, A. \textit{Br. J. Exp. Path.}, 13, 220 (1932).

The growth-inhibiting activity of the solutions was then tested quantitatively on agar plates against \textit{Staphylococcus aureus}. The penicillin solution incubated with the enzyme had entirely lost its growth-inhibiting activity, whereas the control solution had retained its full strength.

The conclusion that the active substance is an enzyme is drawn from the fact that it is destroyed by heating at 80\(^\circ\) for 5 minutes and by incubation with papain activated with potassium cyanide at pH 6, and that it is non-dialysable through 'Cellophane' membranes. It can be precipitated by 2 volumes of alcohol, but much of its activity is lost during this operation. The activity of the enzyme, which we term penicillinase, is slight at pH 5, but increases considerably towards the alkaline range of pH. It is very active at pH 8 and 9. Higher pH's could not be tested as penicillin is unstable above pH 8.

The mechanism of the enzymatic inactivation of penicillin is being studied. No oxygen uptake occurs during the reaction, and the inactivation proceeds with equal velocity under aerobic and anaerobic conditions. No appearance of acid groups could be detected by pH measurement with the hydrogen electrode. Extracts of a number of other microorganisms, made by crushing the bacteria in the bacterial grinding mill, were tested for penicillinase. The enzyme was abSENT from extracts of the penicillin-sensitive \textit{Staphylococcus aureus}, of yeast and of \textit{Penicillium notatum}. It was present in a Gram-negative rod, insensitive to penicillin, found as a contaminant of some Penicillum cultures. Unlike \textit{B. coli}, it was not necessary to crush the organism in the bacterial mill in order to obtain the enzyme from it; the latter appeared in the culture fluid.

The enzyme was also found in \textit{M. luteolitica}, an organism sensitive to the action of penicillin, though less so than \textit{Staphylococcus aureus}. Thus, the presence or absence of the enzyme in a bacterium may not be the sole factor determining its insensitivity or sensitivity to penicillin.

The tissue extracts and tissue autolysates that have been tested were found to be without action on the growth-inhibiting power of penicillin. Prof. A. D. Gardner has found staphylococcal pus to be devoid of inhibiting action, but has demonstrated a slight inhibition by the pus from a case of \textit{B. coli} cystitis. The bacteriostatic action of the sulphonamide drugs is known to be inhibited in the presence of tissue constituents and pus.\(^4\) That the anti-bacterial activity of penicillin is not affected under these conditions gives this substance a definite advantage over the sulphonamide drugs from the chemotherapeutic point of view. The fact that a number of bacteria contain an enzyme acting on penicillin points to the possibility that this substance may have a function in their metabolism.

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E. CHAIN.

Sir William Dunn School of Pathology, Oxford.

Dec. 5.

1 Fleming, A. \textit{Br. J. Exp. Path.}, 13, 220 (1932).
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