

Establishment of national laboratory-based surveillance of antimicrobial resistance



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of antimicrobial resistance**

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Introduction

During the past six decades antimicrobial agents¹ have played a critical role in reducing the burden of communicable diseases all over the world. However, the emergence of resistance and its rapid spread is negating the impact of these drugs, and hindering effective application of modern technologies in mitigating human misery. While the appearance of resistance is a continuous phenomenon in microorganisms, its amplification and spread is through an array of practices conducted by human beings. Improper utilization of antimicrobial agents, especially in high disease-burden settings, results in strong selection pressure that allows the resistant strain to grow and rapidly replace the susceptible isolates.

Diseases due to resistant organisms take longer to heal, and require expensive and at times toxic drugs for longer periods, often making the disease untreatable. The resistant organisms can also move across countries through travel and trade. In that sense, antimicrobial resistance is a global challenge requiring concerted efforts at national and international levels to preserve the available antimicrobial agents. This is possible through treatment policies such as combination therapy, rational prescription, patient adherence, a strong regulatory mechanism coupled with educational activities, along with an efficient surveillance system that monitors the emergence and spread of resistance as well as the utilization of antimicrobial agents.

To facilitate this activity at the country level, WHO has developed a strategy that is simple, practical and easy to scale up. The regional

¹ In the strict sense, all antimicrobials are not antibiotics since some of these are chemically synthesized. However, for ease of reading these two terms will be used synonymously in this document.

strategy aims to accord particular attention to interventions involving the introduction of legislation and policies governing the use of antimicrobial agents, ensuring the rational use of these drugs at all levels of health-care settings and establishing laboratory-based networks for surveillance of resistance.

Scope of the document

This document aims to provide an overview of the steps that can be initiated to establish national laboratory-based surveillance of antimicrobial resistance. The document also provides guidance on key elements of a good surveillance system. The document relies heavily on the experience gained in Thailand where the national antimicrobial resistance surveillance programme has been in operation for several years.

Guideline development process

The WHO Regional Office for South-East Asia commissioned the WHO Collaborating Centre on Antimicrobial Resistance, National Institute of Health, Ministry of Public Health, Thailand to develop the first draft of the guidelines. The objectives were to provide a tool to developing countries in establishing procedures and practices for a national system for a laboratory-based surveillance of antimicrobial resistance which should generate evidence for forming policies and programmes for the rational use of antibiotics. The guidelines were reviewed by several experts at the WHO Collaborating Centre and subsequently by colleagues in the WHO Regional Office for South-East Asia and WHO Country Office for India.

Guidelines development team

WHO wishes to acknowledge the support provided by various experts in drafting, reviewing and finalization of the guidelines (please see list in Annex 1).

Surveillance of antimicrobial resistance

Surveillance is defined as *“the ongoing and systematic collection, analysis and interpretation of health data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know”*.

In simpler terms surveillance is data collection for action.

Surveillance of antimicrobial resistance

Antimicrobial resistance (AMR) surveillance data will help to formulate, monitor and identify the prevailing and emerging problem, which can be contained by effective strategy. Understandably, the majority of surveillance programmes are laboratory -based. Very few clinical data are collected and the data obtained by most surveillance programme are not useful to implement control and/or prevention measures. One strategy to improve collection, collation and dissemination for effective use in the hospital/community is to integrate this function of antimicrobial resistance surveillance activities into the existing disease surveillance activities.

Need for a national antimicrobial resistance surveillance programme

The purpose of surveillance at the national level is to monitor susceptibility patterns of microorganisms to antimicrobial agents which will reveal the antimicrobial resistance status. This will provide reliable data that can be utilized by policy-makers or health administrators to

review and revise the recommendations for empirical treatment for community or hospital-acquired infections. The regular dissemination of antimicrobial resistance information to physicians may improve the empirical selection of antimicrobial agents when treating community or hospital-acquired infections.

Information on antimicrobial resistance derived from the surveillance can be used as an indicator of the quantitative consumption of antibiotics in a particular area covered by the network of hospitals. Data on the prevalence and resistance patterns of different pathogens derived from the surveillance will lead medical authorities to produce prioritized steps and recommendations or guidelines at the national level on the control of community and hospital-acquired infections and reduce the rate of resistance. In addition, information on emerging and the increasing antimicrobial resistance obtained from continuous surveillance will alert both medical personnel and the people in the country, raise awareness and generate a common commitment to systematically combat the antimicrobial resistance crisis.

Advantages of antimicrobial resistance surveillance

Antimicrobial resistance surveillance is necessary to:

- Understand when, where, how and why drug resistance is emerging.
- Reveal antimicrobial efficacy.
- Ensure the better management of patients and infection control in hospital settings.
- Improve management of community infection control.
- Inform policy-makers on evidence-based action in developing drug policy, essential medicines lists, standard treatment guidelines, procurement strategies, resource allocation, health professional curricula and training.
- Improve the empirical selection of antimicrobial agents when treating community or hospital acquired infections.

It is clear that an efficient surveillance system for AMR can play a critical role in improving the efficacy of health services and reduce mortality and morbidity due to infectious diseases.

Establishing a national surveillance system

To establish national antimicrobial resistance surveillance, the following components and mechanisms are essential:

National commitment

The Government or the highest public health authority should make a commitment and provide support to improve laboratory capacity for undertaking surveillance. This should be part of a national agenda for the control and prevention of antimicrobial resistance.

Designate national coordinator

The first critical step to establish national antimicrobial resistance surveillance is to designate a national coordinating centre and give it the mandate to coordinate nationwide activities.

Forge a national network

The national coordinating centre shall set up a national network of selected representative laboratories from hospitals and public health institutes in different regions of the country. For better representation the members of the network may be from the public, private and not-for-profit organizations as well as institutes in different settings where antimicrobial susceptibility patterns are determined. These laboratories should ideally have quality microbiological facilities for the determination of causative organisms, pathogen identification and susceptibility patterns.

These regional laboratories shall provide uniform and validated data to the national coordinating centre to collate antimicrobial resistance patterns, analyse these and disseminate timely surveillance information to potential users on a regular basis.

Ensure uniformity of testing and reporting

The members of the network need to:

- Use standardized methods on collection of clinical specimens, laboratory test and reporting.
- Assure quality of the microbiological testing.
- Be well versed with data input, analysis of data and contribution of information.
- Reach an agreement on the mechanism to develop a national/international centralized database and ensure accurate and timely output as well as feedback.

Quality of surveillance data

The national coordinating centre should be responsible to provide support to participating units in assuring quality of data through the following activities:

- Organization of External Quality Assessment Scheme.
- Supply of essential materials, ie. antimicrobial susceptibility disks, transport media, reference control bacterial strains, testing reagents.
- Follow-up visits by experts/auditors.
- Provision of referral services for the confirmation of results.
- Organization of training courses as per the requirements.

Dissemination of technical information

A mechanism needs to be established for the efficient utilization of data generated by this network. The potential users should be identified and a dialogue initiated with them for ascertaining their needs and fulfilling the same.

Types of surveillance

To set up national resistance surveillance the country should decide the appropriate type of surveillance required to be set up depending on its needs, available infrastructure and resources and the feasibility of meeting the targets.

In a country with limited resources, surveillance may be performed by collecting primary laboratory data of common bacterial pathogens from the routine testing of representative hospitals without seeking additional reports from the primary data collectors (passive surveillance). If sufficient resources are available, passive surveillance can be enhanced by the collection of additional data, which may help in initiating appropriate actions.

Laboratory-based surveillance

Prerequisites for establishing laboratory-based surveillance

- Identify organisms that should not be monitored.
- Identify organisms that may be included in resistance surveillance.
- Finalize the antimicrobial agents to be used for each isolate.
- Develop a protocol for the determination of susceptibility.
- Establish quality system.
- Establish an information sharing mechanism.

The following chapters describe these elements.

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Selection of organisms for surveillance

A large number of microorganisms infect human beings. Not all of these need to be included in the surveillance programme. Based upon local prevalence of diseases and the importance of these in specific health care or community settings, selection criteria should be applied. During the initial phase of the surveillance network, it may be better to have fewer organisms. The spectrum can be gradually expanded to include additional organisms.

Given below is a brief description of some organisms with their characteristics to facilitate decision-making:

Organisms that should not be monitored

These include the followings:

- (a) Organisms for which no standardized disk diffusion method is currently available for important antimicrobial agents, and hence may not be included in routine surveillance activities.

Some of these are:

- *Streptococcus viridans* group versus penicillin.
- *Streptococcus pneumoniae* versus cephalosporins/carbapenems.
- *Staphylococcus* spp versus vancomycin.
- *Neisseria meningitidis* versus penicillin.
- *Burkholderia pseudomallei* versus all antimicrobial agents.

- (b) Unusual organisms which are rarely involved in serious infections. These include:
- *Achromobacter*.
 - *Flavobacterium*.
 - *Bacillus*.
 - *Haemophilus non-influenzae/non-parainfluenzae*.
- (c) Organisms which do not pose serious treatment problems. These include:
- *Bordetella*.
 - *Corynebacterium*.
 - *Listeria*.
- (d) Bacteria which are doubtful enteric pathogens and rarely cause outbreaks. These include
- *Aeromonas*.
 - *Plesiomonas*.
 - *Edwardsiella*.
- (e) Enteric pathogens for which no antibiotic treatment is recommended. These include:
- All categories of diarrhoeagenic *Escherichia coli*.

Organisms that may be included in resistance surveillance

The following groups of organisms may be included in resistance surveillance:

- Organisms which are proven pathogens and not commensal or contaminants.
- Organisms that have high potential for spread in the community and the hospital setting.
- Organisms that are known to acquire resistance against currently used and recommended antibiotics.
- Organisms that have standard interpretation of susceptibility test.
- Organisms that are widespread in the surveillance area and are a frequent cause of disease in the population.

Guidelines for the choice of organisms

Table 1 shows suggested organisms that are to be considered for inclusion in the surveillance system

Table 1: Organisms to be included in the surveillance system

Respiratory pathogens and agents of meningitis	<p><i>Streptococcus pneumoniae</i></p> <ul style="list-style-type: none"> from respiratory isolates (i.e. sputum, ear, sinus) and invasive isolate (i.e. blood, CSF, pleural fluid). <p><i>Haemophilus influenzae</i></p> <ul style="list-style-type: none"> from respiratory isolates (i.e. sputum, ear, sinus) and invasive isolates (i.e. blood, CSF, pleural fluid).
Sexually transmissible diseases	<i>Neisseria gonorrhoeae</i>
Gram-positive cocci	<p><i>Staphylococcus aureus</i>:</p> <ul style="list-style-type: none"> community acquired. nosocomial acquired. <p><i>Staphylococcus saprophyticus</i>:</p> <ul style="list-style-type: none"> novobiocin resistance urinary isolates. <p><i>Coagulase-negative staphylococci</i>:</p> <ul style="list-style-type: none"> only from sterile site. <p><i>Enterococcus faecalis</i>. <i>Enterococcus faecium</i>. <i>Enterococcus species</i>.</p>
Pathogens of diarrhoeal diseases	<p><i>Shigella dysenteriae</i>. <i>Shigella boydii</i>. <i>Shigella flexneri</i>. <i>Shigella sonnei</i>. <i>Salmonella</i> Typhi. <i>Salmonella</i> Paratyphi A. <i>Salmonella</i> (non-typhoid, non-paratyphoid A). <i>Vibrio cholerae</i> O1. <i>Vibrio cholerae</i> O139.</p>

<p>Other members of family Enterobacteriaceae (This group is responsible for community-acquired urinary tract infections and for all type of nosocomial infections)</p>	<p><i>Escherichia coli.</i> <i>Enterobacter:</i></p> <ul style="list-style-type: none"> • Three categories are recommended, <i>E. cloacae</i>, <i>E. aerogenes</i> and <i>Enterobacter</i> spp. for those laboratories that make no specific identification. <p><i>Klebseilla:</i></p> <ul style="list-style-type: none"> • Two categories are suggested <i>K. pneumoniae</i>, (only subspecies <i>pneumoniae</i> should be monitored), and <i>K. oxytoca</i> (indole positive). <p><i>Serratia spp.</i> <i>Proteus:</i></p> <ul style="list-style-type: none"> • <i>Proteus mirabilis</i> (indole negative). • <i>P. penneri</i>, <i>P vulgaris</i> (indole positive). <p><i>Morganella morganii.</i> <i>Providencia rettgeri.</i> <i>Providencia Stuartii.</i> <i>Providencia spp.</i> <i>Citrobacter freundii.</i></p>
<p>Other Gram-negative bacilli</p>	<p><i>Pseudomonas aeruginosa</i> <i>Acinetobacter spp.</i></p> <ul style="list-style-type: none"> • <i>Acinetobacter</i> spp. OF-glucose positive, and non-hemolysis tentatively identified as <i>A. calcoaceticus-baumannii</i> complex, whereas <i>Acinetobacter</i> spp. (OF-glucose negative) presumptively identified as <i>Acinetobacter</i> sp. other than <i>A. baumannii</i>.

The following list can act as a guide for prioritizing the organisms that may be included in the national surveillance system:

Acinetobacter calcoaceticus-baumannii complex

(OF - glucose positive, non hemolysis, growth at 44°C)

Acinetobacter sp.

(OF - glucose negative)

Citrobacter freundii

Enterobacter aerogenes

Enterobacter cloacae

Enterobacter spp.

<i>Enterococcus faecalis</i>	
<i>Enterococcus faecium</i>	
<i>Enterococcus</i> spp.	
<i>Escherichia coli</i>	community-acquired (urine isolates)
<i>Escherichia coli</i>	hospital-acquired (non-urine isolates)
<i>Haemophilus influenzae</i>	invasive infections (encapsulated)
<i>Haemophilus influenzae</i>	respiratory isolates (sputum, otitis, sinusitis)
<i>Klebsiella oxytoca</i>	
<i>Klebsiella pneumoniae</i>	(subspecies <i>pneumoniae</i>)
<i>Neisseria gonorrhoeae</i>	
<i>Proteus mirabilis</i>	(indole negative)
<i>Proteus vulgaris</i>	(indole positive)
<i>Providencia rettgeri</i>	
<i>Providencia stuartii</i>	
<i>Providencia</i> spp.	
<i>Pseudomonas aeruginosa</i>	
<i>Salmonella</i> non-Typhi-Paratyphi A	
<i>Salmonella</i> Paratyphi A	
<i>Salmonella</i> Typhi	
<i>Serratia</i> spp.	
<i>Shigella boydii</i>	
<i>Shigella dysenteriae</i>	
<i>Shigella flexneri</i>	
<i>Shigella sonnei</i>	
<i>Staphylococcus aureus</i>	community-acquired (out - patients)
<i>Staphylococcus aureus</i>	hospital-acquired
<i>Staphylococcus coagulase</i> negative	significant repeated blood isolates
<i>Staphylococcus saprophyticus</i>	(urine)
<i>Streptococcus pneumoniae</i>	invasive isolates (blood, cerebrospinal fluid)
<i>Streptococcus pneumoniae</i>	respiratory isolates (sputum, ear, sinus)
<i>Vibrio cholerae</i> O1	
<i>Vibrio cholerae</i> O139	

Selection of antimicrobial agents

The organisms mentioned in Chapter 4 and the antimicrobial agents for which these need to be tested can also be categorized in a basic set, additional set as well as supplemental set only for urinary pathogens (Table 2)

Table 2: Suggested antimicrobial agents for antimicrobial resistance surveillance

Micro-organisms	Basic set	Additional set*	Supplemental set for urine
<i>Staphylococcus</i> spp.	Oxacillin (for <i>Staph. aureus</i>) Cefoxitin (for <i>Staph. aureus</i> and coagulase negative staphylococci) Penicillin G Erythromycin ¹ Clindamycin ¹ Gentamicin Trimethoprim-sulfamethoxazole	Levofloxacin or ciprofloxacin MIC of vancomycin	Norfloxacin Nitrofurantoin
<i>S. pneumoniae</i>	Oxacillin for penicillin Erythromycin ¹ Trimethoprim-sulfamethoxazole	Levofloxacin MIC of penicillin, MIC of cefotaxime or ceftriaxone or meropenem for CSF and blood isolate	
Beta-haemolytic ^{3,2} <i>Streptococcus</i> group A, B, C, G,	Penicillin Erythromycin ¹ Clindamycin ¹	Levofloxacin Cefotaxime or ceftriaxone MIC of penicillin ⁴	

Micro-organisms	Basic set	Additional set*	Supplemental set for urine
<i>Streptococcus</i> spp. Viridans group ^{3,2}	MIC of Penicillin	Cefotaxime Ceftriaxone	
<i>Enterococcus</i> spp.	Penicillin or Ampicillin Gentamicin (120 µg) β-lactamase test	Vancomycin or teicoplanin Streptomycin (300 µg)	Norfloxacin or ciprofloxacin or levofloxacin Nitrofurantoin
Enterobacteriaceae	Ampicillin Amoxicillin-clavulanic acid or ampicillin-sulbactam Cefazolin Cefoxitin Gentamicin Trimethoprim-sulfamethoxazole Ciprofloxacin	Cefuroxime Cefotaxime or ceftriaxone Cefoperazone Cefepime or ceftipime Amikacin Imipenem Ertapenem Meropenem	Norfloxacin or ofloxacin Nitrofurantoin
<i>Shigella</i> spp. ⁵	Ampicillin Trimethoprim-sulfamethoxazole Ciprofloxacin or Norfloxacin	Levofloxacin	
<i>Salmonella</i> spp. ⁵	Ampicillin Trimethoprim-sulfamethoxazole Nalidixic acid ⁶ Ciprofloxacin or norfloxacin ⁶	Cefotaxime or ceftriaxone ⁵ Levofloxacin ⁶ MIC of ciprofloxacin ⁶	
<i>Vibrio cholerae</i>	Ampicillin Tetracycline Trimethoprim-sulfamethoxazole		
<i>Pseudomonas aeruginosa</i>	Ceftazidime Gentamicin Ciprofloxacin	Piperacillin-tazobactam Cefoperazone Cefepime or ceftipime Imipenem Meropenem Amikacin	Norfloxacin or ofloxacin

Micro-organisms	Basic set	Additional set*	Supplemental set for urine
<i>Acinetobacter</i> spp.	Cefotaxime or ceftriaxone Ceftazidime Gentamicin Ciprofloxacin Ampicillin-sulbactam	Piperacillin-tazobactam Cefoperazon-sulbactam Cefepime or Cefpirome Imipenem Meropenem Amikacin	
<i>Burkholderia cepacia</i>	Trimethoprim-sulfamethoxazole	Ceftazidime Meropenem	
<i>Haemophilus influenzae</i> (CSF isolate)	Ampicillin (β -lactamase test)	Cefotaxime or ceftriaxone Meropenem	
<i>Haemophilus influenzae</i> , <i>H. parainfluenzae</i> (respiratory isolate)	Ampicillin (β -lactamase test) Amoxicillin-clavulanic acid or Ampicillin-sulbactam Trimethoprim-sulfamethoxazole	Azithromycin or clarithromycin Ciprofloxacin	
<i>Neisseria meningitidis</i> ²	Cefotaxime Ceftriaxone Meropenem MIC of penicillin	(Only for prophylaxis: Azithromycin, Trimethoprim-sulfamethoxazole, Rifampin, Ciprofloxacin)	
<i>Burkholderia pseudomallei</i>	MIC of trimethoprim-sulfamethoxazole MIC of amoxicillin-clavulanic acid MIC of ceftazidime MIC of imipenem MIC of tetracycline or doxycycline		
<i>Moraxella catarrhalis</i>	β -lactamase test		

Foot notes

1. Not routinely reported on organisms isolated from the urinary tract.
2. Use Mueller Hinton agar added with 5% sheep blood for susceptibility test.
3. The beta-haemolytic group includes the large colony-forming pyogenic strains of streptococci with Group A (*Strept. pyogenes*), C or G antigens and strains with group B (*Strept. agalactiae*) antigen. Small-colony-forming beta-hemolytic strains with Group A, c, F, or G antigens (*S. anginosus* group)

are considered part of the viridians group, and interpretive criteria for the viridians group should be used.

4. Determine MIC only on isolates from sterile sites.
5. When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone and trimethoprim-sulfamethoxazole should be reported routinely. In addition, chloramphenicol and a third-generation cephalosporin should be tested and reported for extraintestinal isolates of *Salmonella* spp.
6. Fluoroquinolone-susceptible strains of *Salmonella* that test resistant to nalidixic acid may be associated with clinical failure of delayed response in fluoroquinolone-treated patients with extraintestinal salmonellosis. Extraintestinal isolates of *Salmonella* should also be tested for resistance to nalidixic acid. For isolates that test susceptible to fluoroquinolones and resistant to nalidixic acid, the physician should be informed that the isolate may not be eradicated by fluoroquinolone treatment, A consultation with an infectious disease practitioner is recommended.

The selection of antimicrobial agents is a dynamic process and hence the list may need frequent revisions based upon availability of new drugs and data generated through this surveillance mechanism. In resource-limited settings it may be useful to identify antimicrobials from the national essential drugs list. An example of the essential drugs list can be seen at Annex 2.

Protocols for antimicrobial susceptibility testing

Several laboratory techniques are available to determine susceptibility of microorganisms to antimicrobial agents. Some of these are

- Kirby-Bauer disc diffusion method.
- Stokes' disc diffusion method.
- Minimum inhibitory concentration (MIC) determination.
- E-test.

Several variations of these are now in use. The Clinical Laboratory Standards Institute–USA (CLSI) has provided standards for disc diffusion method for determination of antimicrobial susceptibility of most of the pathogens. These standards are also updated regularly. The laboratories need to procure these standards along with interpretation charts from CLSI for use in their facilities.

The protocol should address the following issues for each of which the laboratory must have standard operating procedures:

a. Preparation of agar media

Some of the media used in laboratories are:

- Mueller-Hinton agar (MHA)
- MHA+ 5% sheep blood agar (MHB)
- Gonococcus (GC) agar + 1% defined growth supplement
- *Haemophilus* test medium (HTM)

b. Preparation of reagent

c. Preparation or procurement of antimicrobial discs

Antimicrobial discs should be purchased from reliable companies which are shown by the given certificate of analysis inside the cartridges. These should be stored at -14°C. Do not use the self-defrosting freezer. Before

using, antimicrobial discs should be placed at room temperature to have the same ambient temperature. This minimizes the condensation of warm air to the cold discs. Once a cartridge is unsealed, the cartridge should then be kept at +4°C, in a box containing silica gel to ensure the anhydrous condition. This refrigerated condition is for a small working supply which should not be stored longer than one week. Some discs with labile drugs (e.g. imipenem cefaclor and clavulanic acid combinations) may retain better stability if stored frozen until it is used. Discard discs that reach the expiration date stated on the label.

d. Standard turbidity using 0.5 McFarland Turbidity Standard

A 0.5 McFarland Standard is used to compare the standard turbidity. It is available commercially or can be prepared in the laboratory.

e. Measuring zone of inhibition and interpreting results

The result is obtained by measuring the diameter of the zone of inhibition, including the diameter of a disc in mm. A sliding caliper or ruler should be used and the zone on the back of the inverted agar plate must be measured with the following exceptions:

- The zone margin is the area showing no obvious growth. If the faint growth or tiny single colonies are seen, measure the colony-free inner zone.
- Ignore the swarming growth of *Proteus spp.* and measure the obvious zone of inhibition.
- On MHA containing blood, measure the growth inhibition zone not the zone of inhibition of haemolysis.
- Interpretation is done by comparing the measured diameter to the breakpoint diameter shown in CLSI Tables.

Report the bacteria as susceptible, intermediate or resistant to the antimicrobial agents and record the zone size of the test and control strains in the computer using WHONET programme.

Protocol for rapidly growing aerobic pathogens

Rapidly growing aerobic pathogens include *Staphylococcus spp.*, *Enterococcus spp.*, *Salmonella spp.*, other *Enterobacteriaceae*, *Vibrionaceae*, *Pseudomonas aeruginosa*, *Acinetobacter spp.*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia*.

The direct colony suspension method is recommended for these organisms for the CLSI approved protocol.

Protocol for testing fastidious organisms

Streptococcus pneumoniae and other *Streptococcus spp.*

MHA containing 5% sheep blood is recommended for susceptibility testing of *S. pneumoniae* and other streptococci. The test method is Kirby Bauer and direct colony suspension method, with the following exceptions:

- Prepare inoculum by suspending pure culture grown on sheep blood agar for 18-20 hours in saline or MHB to have the turbidity of 0.5 McFarland standard.
- Take the antimicrobial disc (according to the list in Table 2) from the cartridge by using sterile forceps. Place not more than five antimicrobial discs onto the surface of the agar plate (diameter of 100 mm) or no more than 12 discs on a 150 mm plate.
- Incubate at 35°C in 5% CO₂ atmosphere or in the candle jar for 20-24 hours.

Measure diameter of the zone of inhibition and interpret by comparing with the breakpoints shown in CLSI tables. Record the zone size of the test and control strains in the computer using WHONET programme.

Table 3. Zone diameter interpretative standards for *Streptococcus pneumoniae* (in mm)

Antimicrobial agent	Resistant	Intermediate	Susceptible
Oxacillin 1 µg*	-	-	≥ 20
Erythromycin 15 µg	≤ 15	16-20	≥ 21
Co-trimoxazole 25 µg	≤ 15	16-18	≥ 19
L.evofloxacin 5 µg	≤ 13	14-16	≥ 17

* Isolates of *S. pneumoniae* that show oxacillin zone size ≥20 mm are interpreted as susceptible to penicillin V (MIC ≤ 0.06 µg/ml), ampicillin, amoxicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, ceftriaxone, cefuroxime, imipenem, loracarbef and meropenem. The disc does not however, distinguish penicillin-immediate strains (minimum inhibitory concentration [MIC] = 0.12-1.0 µg/ml) from strains that are resistant (MIC ≥ 2µg/ml). A penicillin MIC should be tested for the isolates that show oxacillin zone size ≤19 mm before reporting it as resistant. This can be done by using a (micro) dilution method or, more simply, by using the E-test.

Susceptibility test of other streptococci to oxacillin is not recommended. For beta-haemolytic streptococci only penicillin or ampicillin disc is used to do the test. In contrast, both penicillin and ampicillin discs are not reliable for viridans group streptococci. A penicillin MIC should be performed on the Viridans group streptococci isolated from sterile sites (e.g., CSF, blood, bone).

Haemophilus influenzae* and *H. parainfluenzae

HTM is recommended for susceptibility testing of *H. influenzae* and *H. parainfluenzae*. Mueller-Hinton chocolate agar is not appropriate for antimicrobial susceptibility testing of *Haemophilus* spp. The test method is Kirby Bauer and direct colony suspension method with the following exceptions:

- Inoculum is prepared by suspending pure culture grown on chocolate agar for 20-24 hours in saline or MHB to have the turbidity of 0.5 McFarland standard.
- Take the antimicrobial disc (according to the list in Table 1) from the cartridge by using sterile forceps. Place not more than five antimicrobial discs onto the surface of the agar plate (diameter of 100 mm) or no more than 12 disks on a 150 mm plate.
- Incubate at 35°C in 5% CO₂ atmosphere or in the candle jar for 16-18 hours.
- Measure diameter of the zone of inhibition and interpret by comparing with the breakpoints shown in CLSI Tables.

Record the zone size of the test and control strains in computer using WHONET programme.

Table 4: Zone diameter interpretative standards for *Haemophilus influenzae* (in mm)

Antimicrobial agent	Resistant	Intermediate	Susceptible
Ampicillin 10 µg*	≤ 18	19-21	≥ 22
Chloramphenicol 30 µg	≤ 25	26-28	≥29
Co-trimoxazole 25 µg	≤ 10	11-15	≥ 16

* Most of the ampicillin resistance among *H. influenzae* is caused by the presence of a β-lactamase. In some settings, less than 1% of the clinical isolates of *H. influenzae* are ampicillin-resistant due to another mechanism (altered penicillin binding proteins). For this reason, a negative β-lactamase test can be reported as susceptible to ampicillin (without performing the disc test). Since many other antimicrobial agents have a predictable activity against *H. influenzae* (3rd generation cephalosporins, quinolones, amoxicillin-clavulanic acid) there rarely exists a clinical need for performing more than a rapid test for β-lactamase. Susceptibility testing, however, remains a useful tool in surveillance programmes.

Neisseria gonorrhoeae

GC agar containing 1% defined growth supplement is recommended for susceptibility testing of *N. gonorrhoeae*. Cysteine-free growth supplement is not required for disc diffusion test and chocolate agar enriched with other supplements is not appropriate for susceptibility testing of *N. gonorrhoeae*.

The test method is Kirby Bauer and direct colony suspension method with the following exceptions:

- Inoculum is prepared by suspending pure culture grown on chocolate agar for 20-24 hours in 5% CO₂ atmosphere, in saline or MHB to have the turbidity of 0.5 McFarland standard.
- Take the antimicrobial disc (according to the list in Table 1) from the cartridge by using sterile forceps. Place not more than five antimicrobial discs onto the surface of the agar plate (diameter of 100 mm) or no more than 12 discs on a 150 mm plate. For antimicrobial disks that produce vary large zone e.g., fluoroquinolones or cephalosporins, only two to three discs may be tested per 100 mm diameter plate.
- Incubate at 35°C (do not exceed 37°C) in 5% CO₂ atmosphere or in the candle jar for 20-24 hours.

Measure diameter of the zone of inhibition and interpret by comparing with the breakpoints shown in CLSI Tables. Record the zone size of the test and control strains in computer using WHONET programme.

Table 5. Zone diameter interpretative standards for *Neisseria gonorrhoeae* (in mm)

Antimicrobial agent	Resistant	Intermediate	Susceptible
Penicillin 10 units*	≤ 26	27-46	≥ 47
Tetracycline 30 µg	≤ 30	31-37	≥ 38
Spectinomycin 100 µg	≤ 14	15-17	≥ 18

* Isolates of *N. gonorrhoeae* that show zone of 10-µg penicillin disk ≤19 mm indicate β-lactamase production. A test for production of β-lactamase is recommended for rapid accurate recognition of plasmid-mediated penicillin resistance. A convenient method to detect β-lactamase is the use of nitrocefin-impregnated paper disc.

7

Quality control of antimicrobial susceptibility testing

Quality control (QC) in the laboratories should cover all diagnostic tests from the collection and evaluation of specimen through the interpretation and accuracy of the test results. In this way, guideline for laboratory practices should be established and revised consistently and concomitant with the establishment of internal quality control of all reagents, media, and equipment used.

The main objectives of quality control of antimicrobial susceptibility by the disc diffusion method are to control the following:

- (1) The precision and accuracy of the test method.
- (2) The quality of reagents, medium, and antimicrobial discs used.
- (3) Laboratory performance, reading, and interpretation of test results.

With the use of reference bacterial strains recommended by CLSI, this process will ensure the highest achievement in the control of precision and accuracy of the test method. The standard bacterial strains should be obtained from reliable sources (e.g., American Type Culture Collection or Culture Collection Center, National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand).

Storing and testing quality control strains should follow the procedure described by CLSI.

Internal quality control for the rapid-growing aerobic pathogens

Quality control of the test method is performed by using the following standard bacterial strains.

: <i>Staphylococcus aureus</i>	ATCC 25923 (DMST 8840)
: <i>Escherichia coli</i>	ATCC 25922 (DMST 4212)
: <i>Pseudomonas aeruginosa</i>	ATCC 27853 (DMST 4739)
: <i>Enterococcus faecalis</i>	ATCC 29212 (DMST 4737)

The *Enterococcus* standard strain is used to test the minimum level of thymidine and thymine in Mueller-Hinton medium for susceptibility testing of bacteria to co-trimoxazole. The high level of thymidine or thymine gives rise to the false negative result. Thus, all batches of this medium should be tested by performing the susceptibility of *E. faecalis* ATCC 29212 or 33186 to co-trimoxazole. Sufficient low level of thymidine and thymine is indicated by an inhibition zone of ≥ 20 mm. This QC should be done once a week or whenever the new batch of medium is prepared or whenever the new disc cartridge is opened.

The acceptable minimum and maximum inhibition zones for a single QC test are shown in corresponding CLSI Tables. If the QC result is out of range only one out of 20 tests, it is acceptable. If the QC result is out-of-control more than one test, corrective action is required immediately.

Internal quality control for fastidious organisms

Streptococcus pneumoniae

Standard bacterial strain used for QC is *S. pneumoniae* ATCC 49619. Perform the same test procedure as described for clinical isolates of *S. pneumoniae* (Mueller-Hinton sheep blood agar, incubated at 35°C in 5% CO₂ atmosphere or in the candle jar for 20-24 hours). The minimum and maximum inhibition zones for a single QC test are shown in CLSI Table.

Neisseria gonorrhoeae

Standard bacterial strain used for QC is *N. gonorrhoeae* ATCC 49226. Perform the same test procedure as described for clinical isolates of *N. gonorrhoeae* (GC agar base plus 1% defined growth supplement, incubated at 35°C (do not exceed 37°C) in 5% CO₂ atmosphere or in

the candle jar for 20-24 hours). The minimum and maximum inhibition zones for a single QC test are shown in CLSI Table.

Haemophilus influenzae

Standard bacterial strain used for QC is *H. influenzae* ATCC 49766. Perform the same test procedure as described for clinical isolates of *H. influenzae* (HTM consisting of 15 µg/ml β-NAD; 15 µg/ml bovine hematin; and 5 g/L yeast extract; adjust pH to 7.2 to 7.4 incubated at 35°C in 5% CO₂ atmosphere or in the candle jar for 16-18 hrs). The minimum and maximum inhibition zones for a single QC test are shown in CLSI Table.

Table 6 shows the list of standard bacterial strains collected at the Culture Collection Center, NIH, Department of Medical Sciences, Nonthaburi, Thailand (DMST-CC). The detail of strains used for QC is also described.

Table 6. List of standard bacterial strains for QC of antimicrobial susceptibility testing

	Species	Strain No.	Primary use/characteristics
1	<i>Enterococcus faecalis</i>	DMST 4736 =ATCC 29212	Sensitive to vancomycin, high level gentamicin (16-23 mm), and high-level streptomycin (14-20 mm) Use to check media that is acceptable for testing sulfonamides, trimethoprim, and their combination, use to test vancomycin screen plate (will not grow) and high-level aminoglycoside disk and MIC tests for enterococci.
2	<i>Enterococcus faecalis</i>	DMST 4737 =ATCC 51299	Resistant to high level gentamicin, high -level streptomycin, and vancomycin. Use to test vancomycin screen plate (will grow) and high-level aminoglycoside disc and MIC tests for enterococci.
3	<i>Enterococcus faecium</i>	DMST 4743 =UCLA 192	An example of vancomycin resistant strain.

4	<i>Escherichia coli</i>	DMST 4212 =ATCC 25922	Sensitive to all gram negative organisms
5	<i>Escherichia coli</i>	DMST 7948 =ATCC 35218	Ampicillin-resistant but sensitive to β -lactamase/ β -lactamase inhibitor combinations. Use to test Amp/Clav (17-22 mm), Ticar/Clav, and other combination drugs.
6	<i>Haemophilus influenzae</i>	DMST 7943 =ATCC 49247	Recommended reference strain for antimicrobial susceptibility tests
7	<i>Haemophilus influenzae</i>	DMST 7944 =ATCC 49766	Ampicillin susceptible, recommended reference strain for antimicrobial susceptibility tests
8	<i>Pseudomonas aeruginosa</i>	DMST 4739 =ATCC 27853	Use to monitor cation content and pH of media. Aminoglycosides are especially sensitive to cations.
9	<i>Staphylococcus aureus</i>	DMST 8840 =ATCC 25923	Oxacillin sensitive (18-24 mm) and penicillin sensitive - β -lactamase negative. Use to test gram-positive drugs with disc diffusion test and as negative control for the oxacillin agar screen plate (will not grow).
10	<i>Streptococcus pneumoniae</i>	DMST7945 =ATCC 49619	Use to test disc diffusion and E-test, MIC test for <i>Streptococcus</i> spp. including <i>S. pneumoniae</i> .

DMST = Department of Medical Sciences , Thailand

ATCC = American Type Culture Collection

UCLA = University of California, Los Angeles

External quality assessment scheme

The hospitals that contribute in the surveillance network of antimicrobial resistance must participate in the external quality assessment scheme (EQAS). This EQAS can be organized by the coordinator of the national network or assigned to some other institute which has adequate expertise and willingness to organize it.

8

Troubleshooting for disc diffusion tests

Various factors that influence the results of the disc diffusion tests are summarized below:

Element	Check possible reason
Standard bacterial strain	<ul style="list-style-type: none">- Use of wrong strain- Improper maintenance, e.g. storage and frequent subculture- Contamination or changes in the genetic characteristics
Testing supplies	<ul style="list-style-type: none">- Improper storage- Contamination or use of the expired materials- Use of damaged or defective agar plates
Testing procedure	<ul style="list-style-type: none">- Use of wrong incubation temperature or length of time- Use of incorrectly prepared inocula (too light or too heavy)- Use of deteriorated or expired turbidity standard- Inoculums prepared from the uncontrolled pH Mueller–Hinton medium or from selective or differential agar medium- Use of the wrong disk or placement of disk provides inadequate contact with agar
Reading errors	<ul style="list-style-type: none">- Inhibition zone is too small or a faint growth appeared (particular with sulfonamide, trimethoprim and their combination). This may due to the inhibitory substances in the medium.
Equipment	<ul style="list-style-type: none">- No calibration or calibration functioning improperly

Quality control by result verification

A clinical bacteriologist should review all test results critically in addition to the regular quality control of test procedures. This is done to verify whether the results are suspect or illogical, which usually results from:

- Reading errors or use of the wrong discs
- Incorrectly identified clinical organisms
- Improper test performance

Some bacteria provide the typical or consistent susceptibility profile, e.g.

- *Proteus* species. are known to be nitrofurantoin and tetracycline-resistant bacteria.
- *Streptococcus pyogenes* usually shows no resistance to penicillin.
- *Serratia*, *Citrobacter*, *Enterobacter*, and *Klebsiella pneumoniae* are usually resistant to ampicillin.
- Most staphylococci and streptococci are susceptible to vancomycin. (If resistant strains are found, recheck the identification of the organisms. *Lactobacillus*, *Leuconostoc*, and *Pediococcus* which have similar cell morphology to staphylococci and streptococci are resistant to vancomycin)
- *Shigella* spp. is usually norfloxacin susceptible.

The clinical isolates that give the suspected or illogical results and are important for epidemiology e.g., norfloxacin-resistant *Shigella*, vancomycin-resistant *Staphylococcus*, should be sent for confirmation at the reference laboratory. A clinical bacteriologist should be informed of the information on typical, atypical, impossible or unusual test results regularly and revise as necessary.

Examples of the suspect test results

- Clinical isolates show resistance to all antimicrobial discs (possible contamination with yeast).
- Clinical isolates express resistance to amikacin but are susceptible

to gentamicin and tobramycin or gram-negative bacilli show resistance to the third generation cephalosporin but are susceptible to the first or second generation cephalosporin.

- *Staphylococcus aureus* that is oxacillin-resistant but susceptible to penicillin.

The important thing in antimicrobial susceptibility testing using disc diffusion test is the precision of test result reporting. Clinical isolates that are susceptible but are reported as drug resistant will result in the administration of higher drug classes to the patients. A very major error is reporting susceptibility instead of resistance which leads to administration of antibiotics to patients which will have no effect on disease causing organisms.

Unusual test results

Table7: Example of phenotypes that have not been reported

Gram-negative Organisms	Antimicrobial agent	Gram-positive organisms	Antimicrobial agent
<i>Citrobacter freundii</i> <i>Enterobacter</i> spp. <i>Serratia marcescens</i>	Ampicillin, cefazolin, or cephalothin-S	<i>E. faecalis</i>	Ampicillin or penicillin-R Daptomycin-NS Linezolid-R Quinupristin-dalfopristin-S
<i>Klebsiella</i> spp. <i>Proteus vulgaris</i> <i>Providencia</i> spp.	Ampicillin-S	<i>E. faecium</i>	Daptomycin-NS Linezolid-R
<i>Stenotrophomonas maltophilia</i>	Carbapenem-S	<i>S. aureus</i>	Daptomycin-NS Linezolid-NS Quinupristin-dalfopristin-I or R Vancomycin-I or R

Gram-negative Organisms	Antimicrobial agent	Gram-positive organisms	Antimicrobial agent
<i>Haemophilua influenzae</i>	Aztreonam-NS Carbapenem-NS 3 rd generation cephalosporin-NS Fluoroquinolone-NS	<i>Staphylococcus</i> , coagulase-negative <i>Streptococcus</i> , viridans group	Daptomycin-NS Linezolid-NS Vancomycin-I or R
<i>Neisseria gonorrhoeae</i>	3 rd generation cephalosporin-NS	<i>S. pneumoniae</i>	Fluoroquinolone-R Linezolid -NS Vancomycin -NS
<i>Neisseria meningitidis</i>	Aztreonam-NS 3 rd generation cephalosporin-NS Meropenem-NS Minocycline-NS Chloramphenicol-I or R Fluoroquinolone-I or R Rifampin-I or R	<i>Streptococcus</i> , beta group	Ampicillin or penicillin ^c -NS 3 rd generation cephalosporin-NS Daptomycin-NS Linezolid-NS Vancomycin-NS
Any organism	Resistant to all agents routinely tested	Any organism	Resistant to all agents routinely tested

Note: R: Resistant; I: Intermediate susceptible; S: Susceptible; NS: nonsusceptible are used for antimicrobial agents for which only susceptible interpretive criteria are provided.

When these phenotypes are observed, they should be verified as follows:

Confirm the identification of the isolate and repeat the susceptibility test to ensure that the unusual results are not due to transcription errors, contamination, or use of a defective agar plate. For the isolates showing antimicrobial susceptibility results as “NS” and staphylococci with vancomycin-intermediate or vancomycin-resistant results, the isolates should also be saved and submitted to a reference laboratory for a CLSI reference dilution method.

Table 8: Example of uncommon phenotypes possibly resulting from technical errors

Gram-negative	organisms	Gram-positive	organisms
<i>Escherichia coli</i>	ESBL - confirmed positive	<i>Enterococcus</i> spp.	Vancomycin-R
<i>Klebsiella</i> spp.	ESBL - confirmed positive	<i>E. faecalis</i>	High-level aminoglycosides-R (particularly if an isolate from sterile body site)
<i>Salmonella</i> spp.	3 rd generation cephalosporin-I or R Fluoroquinolone-I or R or nalidixic-R	<i>E. faecium</i>	High-level aminoglycosides-R (particularly if isolate from a sterile body site), Quinupristin-dalfopristin-R
<i>Pseudomonas aeruginosa</i>	Concurrent gentamicin and tobramycin and amikacin -R	<i>S. aureus</i>	Oxacillin-R
<i>Stenotrophomonas maltophilia</i>	Trimethoprim-sulfamethoxazole-R	<i>S. pneumoniae</i>	Penicillin-R 3 rd generation cephalosporin-R
<i>Neisseria gonorrhoeae</i>	Fluoroquinolone-R	<i>Streptococcus</i> , viridans group	Penicillin-I or R

Note: R: Resistant; I: Intermediate susceptible

When these phenotypes are observed on individual patient isolates, the verification steps as outlined after Table 7 should be considered in a given situation. Reports of *Salmonella* spp. that are intermediate or resistant to 3rd generation cephalosporins and/or intermediate or resistant to fluoroquinolone or resistant to nalidixic acid should also be submitted to a public health laboratory.

Data collection analyses and dissemination

WHONET software which can be freely downloaded from (<http://www.who.int/drugresistance/whonetsoftware/en/>) is recommended to input the data of susceptibility test results. WHONET is a Windows-based database software developed for the management and analysis of microbiology laboratory data with a special focus on the analysis of antimicrobial susceptibility test results.

Essential data set

The recommended minimal data to be inputted by hospital laboratory is as follows:

- Patient identity (hospital number)
- Sex
- Age
- Specimen identity (laboratory number)
- Location (ward where patient admitted)
- Location type (whether the patient is inpatient, outpatient, intensive care, or from community)
- Specimen type (site of infection)
- Name of isolate (genus species, genus only or group of species)
- Quantitative susceptibility data (diameter of inhibition zone or minimum inhibition concentration)

Data from each hospital is inputted preferably on a monthly basis (one file per month of data). After collecting and inputting of data of a certain period (monthly, quarterly, biannually or annually), the data can be collated, analysed and reported locally to the coordinating centre from which the data from hospital network members is collated and analysed and reported nationally. It has to be emphasized that the periodical reports should be prepared in a timely fashion and disseminated to the relevant medical institution in the form of a brochure, book, poster, or digital data on website.

Further reading

- (1) AMR Surveillance: Questionnaire for Assessment of National Networks 2003. WHO/CDS/CSR/RMD/2003.1
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- (11) *WHO Recommended Surveillance Standards*. World Health Organization, 1999. WHO/CDS/CSR/ISR/99.2
- (12) World Health Organization and Centers for Disease Control and Prevention. *Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera*. Centers for Disease Control and Prevention, 1999. WHO/CDS/CSR/EDC/99.8
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Annex 1

Contributors

Ms Surang Dejsalert
Director
WHO Collaborating Centre on
Antimicrobial Resistance
National Health Institute
Department of Medical Sciences
Ministry of Public Health,
Thailand
Nonthaburi

and several scientists working in
WHO CC on AMR

Dr Vikas Manchanda
Head of Microbiology
Chacha Nehru Bal Chikitsalya
Shahdara
Delhi

Dr Anuj Sharma
NPO (Laboratories)
WHO Country Office India
New Delhi

Dr Rajesh Bhatia
Regional Adviser and Focal Point
for Antimicrobial Resistance
WHO Regional Office for South-
East Asia
New Delhi

Annex 2

WHO Model List (March 2010) Essential Medicines (excerpts)

16th edition (updated)

The **core list** presents a list of minimum medicine needed for a basic health-care system, listing the most efficacious, safe and cost-effective medicines for priority conditions. Priority conditions are selected on the basis of current and estimated future public health relevance, and potential for safe and cost-effective treatment.

The **complementary list** presents essential medicines for priority diseases for which specialized diagnostic or monitoring facilities, specialist medical care, or specialist training are needed. In case of doubt, medicines may also be listed as complementary on the basis of consistently higher costs or less attractive cost-effectiveness in a variety of settings.

The **square box symbol (□)** is primarily intended to indicate similar clinical performance within a pharmacological class. The listed medicine should be the example of the class for which there is the best evidence for effectiveness and safety. In some cases, this may be the first medicine that is licensed for marketing; in other instances, subsequently licensed compounds may be safer or more effective. Where there is no difference in terms of efficacy and safety data, the listed medicine should be the one that is generally available at the lowest price, based on international drug price information sources. Not all square boxes are applicable to medicine selection for children. Therapeutic equivalence is only indicated on the basis of reviews of efficacy and safety and when consistent with WHO clinical guidelines. National lists should not use a similar symbol

and should be specific in their final selection, which would depend on local availability and price.

Antibacterials

1. Beta lactam antimicrobial agents

amoxicillin

amoxicillin + clavulanic acid

ampicillin

benzathine benzylpenicillin

benzylpenicillin

cefalexin [c]

cefazolin*

cefixime* * Only listed for single-dose treatment of uncomplicated anogenital gonorrhoea.

ceftriaxone*

cloxacillin

Phenoxymethylpenicillin

Procaine benzylpenicillin is not recommended as first-line treatment for neonatal sepsis except in settings with high neonatal mortality, when given by trained health workers in cases where hospital care is not achievable.

Complementary list

cefotaxime 3rd generation cephalosporin preferred for use in hospitalized neonates.*

ceftazidime

imipenem* + cilastatin* only listed for the treatment of life-threatening hospital-based infection due to suspected or proven multidrug-resistant infection. Meropenem is indicated for the treatment of meningitis and is licensed for use in children over the age of 3 months.

2. Other antibacterials

azithromycin* Only listed for single-dose treatment of genital *Chlamydia trachomatis* and of trachoma.

Chloramphenicol Only for the presumptive treatment of epidemic meningitis in children older than 2 years.

ciprofloxacin

doxycycline (Use in children <8 years only for life-threatening infections when no alternative exists.)

erythromycin

gentamicin

metronidazole

nitrofurantoin

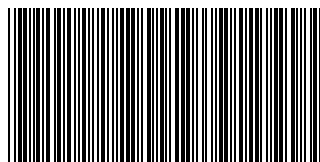
sulfamethoxazole + trimethoprim

Laboratory-based antimicrobial resistance (AMR) surveillance data helps to monitor susceptibility patterns of microorganisms to antimicrobial agents and reveals the antimicrobial resistance status. This can provide reliable data for utilization by policy-makers or health administrators to review and revise the recommendations for empirical treatment for community or hospital-acquired infection. It is clear that an efficient national surveillance system for AMR can play a critical role in improving the efficacy of health services and reduce mortality and morbidity due to infectious diseases. This document provides essential information on key elements of laboratory-based national surveillance programme for AMR and its utilization for prevention and containment of AMR.



**World Health
Organization**

Regional Office for South-East Asia
World Health House
Indraprastha Estate
Mahatma Gandhi Marg
New Delhi-110002, India



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