Essential Drugs

Guidelines for antimicrobial susceptibility testing *(intermediate laboratories)*

Introduction

Although many communicable diseases have been effectively contained, bacterial infections remain a major cause of morbidity and mortality particularly in developing countries.

It has been stressed repeatedly that the increasing prevalence of strains of common pathogenic bac-teria resistant to widely-available, relatively-cheap antimicrobials such as those included in WHO's Model List of Essential Drugs (1) is dangerously eroding their effectiveness not only for the treatment of individual patients but also for the community at large.

Whenever one such drug or class of drugs is used excessively, genetic changes favouring the emer-gence of antimicrobial-resistant bacteria tend to supervene. Although this can occur after only a single exposure in an individual patient, it usually emerges after a sustained period of use. Resistance is generally stable and passed on for several generations of bacteria. The spread of antimicrobial-resistant clones of bacteria within a host population or environment is also important. This is most noticeable in hospitals where certain resistant organisms can become endemic and may infect many patients. The danger is intensified by poor hygienic practices in the hospital and/or lack of adequate training in control and containment of nosocomial and other infections. The spread of resistant bacteria within the community is of even greater importance. This seriously compromises presumptive initial therapy of common bacterial infections and requires revision of routine antimicrobial strategies.

The need for more systematic and coordinated international approaches to laboratory monitoring of antimicrobial sensitivity has become important and urgent. Microbiological laboratories including national reference laboratories need to be established in developing as well as developed countries if the resistance of important bacterial pathogens is to be monitored. In response to this need, the WHONET (2, 3) programme was developed by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance. It is now operating in some 100 hospitals, mostly in the United States, and it is expanding in South America and the western Pacific region. The system will not be fully effective, however, until every country has a national reference laboratory that is actively reporting to this network.

The concept of "reserve antimicrobials" was introduced to WHO's Model List of Essential Drugs in 1989 (4). This resulted from increasing concern about the emergence of important pathogens that have developed resistance to all "essential" antimicrobials. A reserve antimicrobial was defined as "an antimicrobial which is useful for a wide range of infections but, because of the need to reduce the risk of development of resistance and because of its relatively high cost, it would be inappropriate to recommend its unrestricted use". This concept becomes tangible, however, only when means exist of demonstrating prevailing sensitivity patterns. This implies a need for precise laboratory investigation. Without these facilities seriously-ill patients are endangered.

If antimicrobial resistance is to be controlled, several independent measures need to be undertaken:

1. Routine surveillance of antimicrobial-resistant bacteria using standardized, predictive test methods must be introduced in hospital and community settings.

2. Antimicrobial prescribing guidelines must be developed that will assure effective treatment and not encourage the emergence and spread of antimicrobial-resistant bacteria.

3. Comprehensive national programmes must be implemented that respect standards of good laboratory practice, with the objective of assuring quality and availability of antimicrobials.

4. Adequate training of technical personnel must be assured.

5. Systems must be developed within the context of WHONET whereby each country can collate and disseminate information on antimicrobial resistance to hospitals and health workers.

The following guidelines concern routine surveillance of antimicrobial-resistant bacteria using tests which can be undertaken in intermediate level laboratories.

Susceptibility testing

Since the majority of infections which arise in man are not investigated by microbiological methods, antimicrobial treatment is usually administered on the basis of a presumptive etiological diagnosis determined by the clinical history and findings. Microbiological investigations are carried out where the etiology is uncertain, in severe infections when patients fail to respond to empiric therapy or develop a new infection during the course of therapy, or for public health purposes. Additionally, *in vitro* susceptibility tests are performed when an organism is known to have unpredictable sensitivity.

Susceptibility tests are carried out on antimicrobials to which the organism is normally susceptible in order to determine whether resistance has emerged. Demonstration of a prevalence of resistant strains could influence recommendations for presumptive antimicrobial therapy.

Clinically and epidemiologically important bacteria and essential antimicrobials for susceptibility testing

General principles

i) The antimicrobial substances listed in these guidelines are commonly used in presumptive therapy. This list is illustrative rather than comprehensive; other drugs may be important in various countries or regions.

ii) Some of the drugs listed are index compounds representative of a defined group of drugs, e.g.; oxacillin resistance in staphylococci indicates resistance to other beta-lactams.

iii) The most important types of resistance are easy to detect in rapidly-growing organisms. In contrast, detection of resistance in fastidious and anaerobic bacteria requires a high degree of technical expertise. In such circumstances, testing should be carried out in central reference laboratories, wherever possible.

1. Community-acquired infections

These infections are caused by organisms highly prevalent in the community. Some can be treated on an outpatient basis and others are severe enough to require hospital admission for diagnosis and therapy.

Causative organisms	Drugs used as markers of antimicrobial resistance
Staphylococcus aureus	benzylpenicillin, oxacillin erythromycin, gentamicin
Streptococcus pneumoniae	benzylpenicillin as predicted by the oxacillin disc test erythromycin chloramphenicol, sulfamethoxazole trimethoprim
Streptococcus pyogenes	(benzylpenicillin)[2] erythromycin
Haemophilus influenzae	ampicillin, chloramphenicol sulfamethoxazole, trimethoprim by estimating beta-lactamase production
Neisseria gonorrhoeae	benzylpenicillin, tetracycline (ciprofloxacin[1], ceftriaxone[1])[2] by estimating beta-lactamase production
Escherichia coli (UTI)	ampicillin, sulfamethoxazole trimethoprim, nitrofurantoin nalidixic acid, sulfonamide (gentamicin)[2] (fluoroquinolone)[2], (cefalosporin)[3,2]
Salmonella typhi and other invasive *Salmonella*	ampicillin chloramphenicol sulfamethoxazole, trimethoprim (fluoroquinolone)[2], (cefotaxime)[2]
Shigella spp.	ampicillin, sulfamethoxazole trimethoprim, tetracycline (chloramphenicol)[2], nalidixic acid[4] (fluoroquinolone)[2]
Vibrio cholerae	tetracyclines, sulfamethoxazole trimethoprim, nitrofurantoin (erythromycin)[2], (chloramphenicol)[2]

2. Hospital-acquired infections

These infections arise in patients who are either in hospital or who have recently been discharged from hospital. The causative organism is derived either from the patient's endogenous flora or from the flora which are endemic in the hospital.

Causative organisms	Drugs used as markers of antimicrobial resistance
Staphylococcus aureus	benzylpenicillin erythromycin, oxacillin gentamicin, (vancomycin)[2]
Coagulase-negative staphylococci	benzylpenicillin erythromycin, oxacillin gentamicin, (vancomycin)[2]
Enterococci	ampicillin, gentamicin (vancomycin)[2]
Pathogenic Gram-negative bacilli epidemic or endemic in hospitals including *E. coli, Klebsiella* spp., *Salmonella, Enterobacter*	ampicillin chloramphenicol sulfamethoxazole trimethoprim gentamicin, (fluoroquinolone)[2] (cefalosporin[3])
Pseudomonas aeruginosa	gentamicin, piperacillin, (fluoroquinolone)[2] (ceftazidime)[2]

3. Laboratory facilities required

In most developing countries health laboratory services are generally organized at three levels:

1. **Peripheral laboratories** including those attached to health centres and first referral hospitals (district hospitals).

2. **Intermediate level laboratories,** situated in regional or provincial hospitals.

3. **Central reference laboratories** usually located in the capital city or in a university hospital. Facilities for bacteriological culture and susceptibility testing are generally not available at the peripheral level. Laboratory support in microbiology will be limited to microscopy and to some simple and rapid tests for the detection of antibodies (typhoid, syphilis) or antigens (meningitis, *Chlamydia*).

Most intermediate laboratories should have the facilities to culture, identify and establish the susceptibility of common pathogens such as *Shigella, Salmonella* spp., *Vibrio cholerae,* staphylococci, nosocomial Gram-negative bacilli, streptococci, meningococci, gonococci, pneumococci and *Haemophilus influenzae*. Susceptibility testing should also be routinely performed at least for rapidly-growing pathogens. For fastidious bacteria, testing may be limited to rapid, inexpensive methods notably beta-lactamase testing of *Haemophilus* and gonococci and elementary or disc-test screening, using for instance oxacillin as a marker for penicillin resistance in pneumococci.

All isolates of *Shigella, Salmonella* and *Vibrio* spp. and representative isolates of pneumococci, *H. influenzae*, meningococci and gonococci should be forwarded to a central reference laboratory using an appropriate transport medium. Monitoring of *Shigella,* which should be undertaken on the basis of periodic epidemiological studies, necessitates equipment for refrigeration, confirmatory identification, and susceptibility testing.

4. Laboratory requirements at the intermediate level

A special room should be available for bacteriological procedures. It should include a simple safety cabinet for handling cultures of dangerous pathogens (e.g., *S. typhi*), and facilities for safe disposal of specimens and cultures.

The standard equipment should include:

- an autoclave;

1. Ciprofloxacin and ceftriaxone are reserve antimicrobials that are recommended for single-dose therapy of N. gonorrhoeae infections where there is a high prevalence of resistance to first-line antimicrobials.
2. Compounds in parentheses indicate that results need to be reported to the clinician only when resistance is discovered in the listed antimicrobials.
3. Cefalosporin signifies a so-called "first-generation" cefalosporin such as: cefalotin to represent cefalotin, cefalexin and cefadroxil; or cefazolin to represent cefazolin, cefaclor. Use of wide-spectrum cefalosporin (ceftazidime) may be considered to assure recognition of an extended range of beta-lactamase mediated resistance.
4. Nalidixic acid is a reserve antimicrobial recommended for the treatment of shigellosis

- an incubator thermostat regulated at 35 °C;
- Bunsen burners or spirit lamps;
- a refrigerator with freezer-compartment adequate for correct storage of discs, prepared media, and some delicate reagents. Opened discs will retain potency for one month at 4–8 °C, while unopened disc stocks should be stored at below 8 °C.

In addition to the standard routine culture media needed for isolation and identification, the following media and materials are needed for disc diffusion testing:

- Mueller-Hinton agar from a reputable manufacturer;
- antimicrobial discs of the recommended potency;
- barium chloride standard (0.5 McFarland, for preparation of the inoculum);
- sterile swabs for application of the inoculum;
- ruler or calipers for measuring zone diameters of inhibition;
- standard bacterial strains for internal quality control.

The following are additionally needed for testing fastidious organisms:

- a candle-jar;
- blood, and XV or IsoVitaleX supplement for preparation of Mueller-Hinton chocolate agar (for testing *Haemophilus*);
- GC agar, haemoglobin, XV or IsoVitaleX supplement for testing gonococci;
- blood agar (5% blood in Mueller-Hinton agar) for testing pneumococci;
- discs or other reagents for beta-lactamase testing.

5. Recommended testing method

Strict adherence to well-established techniques (Annex I) and regular quality control of media and reagents are necessary if reproducible and reliable results are to be assured. An unusual or unexpected susceptibility result requires confirmation within a central reference laboratory.

The laboratory must be able to identify bacteria correctly and consistently (Annex IV) before undertaking susceptibility testing. This applies to common Gram-positive and Gram-negative bacteria, as well as more technically-demanding species.

Disc diffusion tests, which are both economical and simple to use (5), are widely employed for testing individual isolates of pathogens. Standardized procedures must be carefully followed if tests are to hold comparative value. The refined Kirby-Bauer disc method has been carefully monitored by the United States National Committee for Clinical Laboratory Standards (NCCLS) and is widely used. It is satisfactory provided it is rigorously standardized. Several other methods have been described involving the use of controlled media, standard disc concentrations and strict compliance with agreed guidelines.

6. Methods of testing

Screening methods described in depth in other WHO publications (6), are recommended for susceptibility testing in intermediate laboratories. These involve inoculating standardized media with a lawn culture of a standardized inoculum of bacteria, followed by incubation in the presence of standardized antimicrobial discs, at 35 °C. The zone of inhibition around the antimicrobial disc is inversely proportional to the minimal inhibitory concentrations of the antimicrobial to the organism.

7. Pathogens to be tested

Most rapidly-growing aerobic bacteria of a non-fastidious nature can grow on Mueller-Hinton agar or other recommended susceptibility-test agars. For these organisms disc susceptibility testing is the most practical method. Other pathogens which could be considered for testing include the *Enterobacteriaceae*, *Pseudomonas* spp., enterococci, *Neisseria meningitidis* for penicillin resistance, and *Streptococcus pyogenes* for erythromycin resistance. The choice of other organisms for susceptibility testing is influenced by local factors. In serious invasive infections such as pneumococcal septicaemia or *H. influenzae* meningitis a test to confirm susceptibility to penicillin or ampicillin may avert the need to use expensive broad-spectrum antimicrobials.

Susceptibility testing of the following organisms should be undertaken in either an intermediate or central reference laboratory on the basis of their importance to the individual patient or to the region.

Salmonella typhi: All isolates should be identified and tested for antimicrobial susceptibility.

Salmonella (other than S. typhi): Isolates should be derived from specimens other than faeces. In addition, the susceptibility of faecal isolates should be monitored periodically.

Shigella: Except in epidemic situations in which the causative organism has been confirmed, all isolates should be identified and tested for antimicrobial susceptibility.

Streptococcus pneumoniae: All invasive isolates and a selection of respiratory isolates should be identified and tested. Penicillin resistance may be moderate (0.12 to 1 mg/L) or high (>1 mg/L), and can be detected using 1µg oxacillin disc for screening. Penicillin resistance should be confirmed quantitatively in a central reference laboratory.

Staphylococcus aureus and coagulase-negative staphylococci: All isolates from wounds, pus or exudates should be identified and tested. Routine testing using oxacillin as the index drug is performed by a disc diffusion test on Mueller-Hinton agar. This requires:

(a) full 24-hour incubation;

(b) an ambient temperature not in excess of 35 °C;

(c) careful reading of colonies growing within the inhibition zone;

d) confirmation of methicillin resistance using Mueller-Hinton agar supplemented with 4% NaCl and 6 µg oxacillin.

Enterococci: Both *E. faecalis and E. faecium* can be identified to genus level by bile esculin. Both show some natural low-level resistance to aminoglycosides and a higher level of resistance to several other antimicrobials. Discs containing ≥120 µg gentamicin are required to detect the important strains exhibiting high-level gentamicin resistance (>500 mg/L MICs). Standard discs are available from several sources and the criteria for interpretation vary with the supplier.

Haemophilus influenzae: All invasive strains isolated from CSF or blood and a proportion of respiratory isolates should be tested. Special media are required. At intermediate level laboratories, tests for beta-lactamase can be performed to predict ampicillin resistance using either nitrocefin discs, a starch-iodine procedure or an acidimetric method. Chloramphenicol and sulfamethoxazole/trimethoprim resistance is best detected by disc diffusion tests. These tests are best performed in central reference laboratories. The break point for chloramphenicol resistance is set at ≤ 2 mg chloramphenicol/l which is lower than that applied to other bacteria (≤ 8 mg/L).

N. gonorrhoeae: A sample of isolates should be tested annually, preferably in central reference laboratories. Testing should be conducted under atmospheric conditions using defined enrichment media. Beta-lactamase production can be detected using nitrocefin discs, starch-iodine procedure or an acidimetric method. Non-enzymic penicillin, tetracycline, ceftriaxone and fluoroquinolone-resistance can be detected by the disc-diffusion method.

Mycobacterium tuberculosis: Testing should be performed in a central reference laboratory with technical expertise in the identification and culture of mycobacterial species.

8. Guidelines for scoring bacterial susceptibility and resistance

There is no formal international agreement, as yet, on scoring for bacterial susceptibility or resistance. However, many countries have developed national standards based on a uniform methodology. In particular, NCCLS guidelines are in general use throughout the USA and in individual laboratories in some other countries. Other standards have been developed by Japan, Germany, Sweden and the United Kingdom. These standards demand the precise adoption of a specific method, including adherence to the prescribed medium, the antimicrobial content in the disc, the density of the inoculum, and the guidelines for interpreting the results.

At the present time, NCCLS methodologies, standards and guidelines (7) are the most extensively used and are regularly updated. For countries where standardized methods are not in use, they provide a suitable framework for implementation. In countries where other methods for detecting resistance are already well established and are known to be reliable, the applied disc methods can be supplemented by the tests listed in Annexes I and III. The interpretive criteria for the essential drugs indicated for resistance pattern monitoring are found in Annex II.

9. Quality assurance

Each laboratory performing antimicrobial susceptibility tests should have an internal, self-administered quality control procedure which should be followed, if possible, on a weekly basis. The selection of the strain will depend upon the organisms and antimicrobials tested, e.g., *S. aureus*

ATCC 25923 should be selected when testing Gram-positive clinical isolates and *E. coli* ATCC 25922 should be selected when testing non-fastidious Gram-negative bacilli. The test results should be carefully recorded and possible causes for values outside prescribed limits should be investigated.

Some countries now additionally require laboratories to submit to external proficiency tests involving the identification of potential pathogens as well as an assessment of the accuracy of susceptibility tests. These provide inter-laboratory comparison of organism identification as well as antimicrobial susceptibility-testing accuracy. All laboratories are encouraged to participate in such programmes.

References

1. WHO Technical Report Series No. 825. *The use of essential drugs.* Geneva, 1992.

2. WHO Technical Report Series No. 624. *Surveillance for the prevention and control of health hazards due to antimicrobial-resistant enterobacteria: report of a WHO meeting.* Geneva, 1978.

3. Antimicrobial resistance. WHO Scientific Working Group. *Bulletin of the World Health Organization,* **61**: 383-394 (1983).

4. WHO Technical Report Series No. 796. *The use of essential drugs.* Geneva, 1990.

5. WHO Technical Report Series No. 610. Twenty-eighth Report of the *WHO Expert Committee on Biological Standardization,* Annex 5. Geneva, 1977.

6. Vandepitte J. et al. *Basic laboratory procedures in clinical bacteriology.* WHO, Geneva, 1991.

7. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disc susceptibility tests – fourth edition: Approved Standard.* NCCLS document M2-A5 and M100-S4. Villanova, PA: NCCLS, 1992 and 1993.

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Annex I: Disc diffusion method (brief outline)

I. Background, selective reporting, interpretive category definitions and media selection (see text and appropriate Annex).

A. Refer to references listed below for more detailed description of the procedures.

II. Medium preparation

A. Prepare Mueller-Hinton agar medium from a performance-tested lot according to manufacturer's recommendations.

B. Pour in 9-cm plates (25 to 30 ml) or 14-cm plates (60 to 70 ml).

C. Store at 2 to 8 °C, medium at pH 7.2–7.4.

III. Disc reagents/inoculum turbidity standard

A. Utilize commercially-prepared discs, stored at 8 °C or below.

B. Allow discs to reach room temperature before use and avoid excessive moisture accumulation.

C. Use 0.5 McFarland turbidity standard ($BaCl_2$) stored in the dark and dispensed in 4 to 6 ml tubes which should be shaken before use.

IV. Procedure steps

A. Select 4 or 5 well-isolated colonies and prepare the inoculum by direct colony-to-broth mixing method, to be equal to the $BaSO_4$ standard turbidity *(critical step).*

B. Inoculum should be prepared in saline or a clear broth.

C. Use a sterile cotton swab for inoculum delivery, removing excess fluid by pressing swab against inner side of tube.

D. Swab the plate surface for even distribution (three directions), allow to dry.

E. Place disks on agar surface, no closer than 24 mm apart:
1. Two to 6 discs on a 9-cm plate
2. Five to 11 discs on a 14-cm plate (lower disc numbers for fastidious species).

F. Incubate at 35 °C as follows:
1. Staphylococc:i 24 hours.
2. *Haemophilus* and *Neisseria*: 20 to 24 hours in 3 to 5% CO_2 or in a candle-jar.
3. All other species: 16 to 18 hours.

G. Measure zones of inhibition to nearest mm, and interpret results with the assistance of tables provided (Annex II).

H. Record test and quality control results.

References

1. Antimicrobial susceptibility testing. In: Vandepitte, J. et al. (Eds). *Basic laboratory procedures in clinical bacteriology.* pp. 78-95. World Health Organization, Geneva, 1991.

2. Performance standards for antimicrobial disc susceptibility tests. M2-A5 Approved Standard. NCCLS, Villanova, PA., USA, 1993.

Annex II: Disc diffusion test criteria (NCCLS method)

Antimicrobial agent	Disc content	Zone diameter in mm		
		Susceptible*	Inter-mediate*	Resistant*
Beta-lactams				
Ampicillin				
when testing Gram-negative enteric organisms	10 µg	≥ 17	14–16	≤ 13
when testing enterococci	10 µg	≥ 17	–	≤ 16
when testing haemophilus	10 µg	≥ 22	19–21	≤ 18
Oxacillin				
when testing staphylococci	1 µg	≥ 13	11–12	≤ 10
when testing pneumococci for penicillin susceptibility [a]	1 µg	≥ 20	–	≤ 19
Benzylpenicillin				
when testing staphylococci	10 units	≥ 29	–	≤ 28
when testing streptococci	10 units	≥ 28	20–27	≤ 19
when testing *N. gonorrhoeae* [c,d]	10 units	≥ 47	27–46	≤ 26
Piperacillin				
when testing *P. aeruginosa*	100 µg	≥ 18	–	≤ 17
Cefazolin[e]	30 µg	≥ 18	15–17	≤ 14
Cefotaxime[e]	30 µg	≥ 23	15–22	≤ 14
Ceftazidime[e]	30 µg	≥ 18	15–17	≤ 14
Ceftriaxone[e]				
when testing *N. gonorrhoeae* [b,f]	30 µg	≥ 35	–	–
Cefalotin[e]	30 µg	≥ 18	15–17	≤ 14
Quinolones				
Ciprofloxacin				
when testing Gram-negative enteric bacilli	5 µg	≥ 21	16–20	≤ 15
when testing *N. gonorrhoeae* [b,f]	5 µg	≥ 36	–	–
Nalidixic acid	30 µg	≥ 19	14–18	≤ 13
Other drugs				
Chloramphenicol				
when testing Gram-negative enteric bacilli	30 µg	≥ 18	13–17	≤ 12
when testing haemophilus and pneumococci [b,h]	30 µg	≥ 26	–	≤ 25

* See note 1 on next page.

Annex II: *Disc diffusion test criteria (continued)*

Antimicrobial agent	Disc content	Zone diameter in mm		
		Susceptible*	Inter-mediate*	Resistant*
Erythromycin	15 µg	≥ 23	14–22	≤ 13
Gentamicin[g]	10 µg	≥ 15	13–14	≤ 12
Nitrofurantoin[i]	300 µg	≥ 17	15–16	≤ 14
Tetracycline				
when testing Gram-negative enteric bacilli	30 µg	≥ 19	15–18	≤ 14
when testing *N. gonorrhoeae* [c,d]	30 µg	≥ 38	31–37	≤ 30
Trimethoprim	5 µg	≥ 16	11–15	≤ 10
Sulfonamides	300 µg	≥ 17	13–16	≤ 12
Trimethoprim/sulfamethoxazole[j]	1.25 µg/ 23.75 µg	≥ 16	11–15	≤ 10
Vancomycin				
when testing enterococci	30 µg	≥ 17	15–16	≤ 14
when testing other Gram-positives [b]	30 µg	≥ 12	10–11	≤ 9

* **Note 1:** The three categories of antimicrobial susceptibilty are as follows:

Susceptible: the infection caused by the tested strain would probably respond to appropriate doses of that antimicrobial.

Resistant: the infection caused by the tested strain would not respond to therapy.

Intermediate: the organism's response to therapy is unpredictable.

(a) Oxacillin (representative for methicillin, nafcillin, cloxacillin, dicloxacillin, flucloxacillin) is preferred because of its superior stability to degradation in storage and the application to *S. pneumoniae* testing. Oxacillin resistance among staphylococci implies resistance to all beta-lactams (penicillins, cefalosporins, carbapenems and beta-lactase inhibitor combinations).

(b) Strains yielding zone diameter results suggestive of a non-susceptible category should be submitted to a reference laboratory for further testing.

(c) An intermediate category indicates a lower patient infection cure rate (85-95%) compared to more than 95% cure rates for susceptible strains.

(d) Gonococci with 10 unit penicillin disc zones of ≤19 mm are likely to be beta-lactamase producers. Similarly, tetracycline 30 µg disc zone diameters of ≤19 mm usually indicate a plasmid-mediated PRNG strain (MIC correlate, ≥ 16 mg/L).

(e) Choices for cefalosporin surveillance testing: cefalotin represents cefalotin, cefalexin and cefadroxil; cefazolin represents cefazolin and cefaclor; ceftazidime maximizes recognition of extended spectrum beta-lactamase mediated resistance; ceftriaxone is a reserve antimicrobial used for gonococcal testing only; and cefotaxime should be tested against salmonellae.

(f) For these drugs, the current rarity of well-documented resistant strains precludes defining any category other than susceptible.

(g) Testing for high-level aminoglycoside resistance should be performed by the agar dilution method (BHI medium) with a screening concentration of 500 mg gentamicin/L. Alternative high-content gentamicin discs (>100 µg content) may be available for this purpose in some geographic areas. Local product criteria should be applied.

(h) These criteria were modified from those utilized by the NCCLS for use in laboratories in developing countries, i.e., no intermediate category.

(i) Used to predict susceptibility to furazolidone.

(j) Also designated co-trimoxazole.

Annex III: Quality control guidelines for disc diffusion tests

Antimicrobial agent	Disc content	Zone diameter limits for control strains				
		E. coli ATCC25922	*S. aureus* ATCC25923	*H. influenzae* ATCC49247	*N. gonorrhoeae* ATCC35218	*P. aeruginosa* ATCC35218
Ampicillin	10 µg	16–22	27–35	13–21	–	–
Oxacillin	1 µg	–	18–24	–	–	–
Benzylpenicillin	10 units	–	26–37	–	26–34	–
Piperacillin	100 µg	24–30	–	–	–	25–33
Cefazolin	30 µg	23–29	29–35	–	–	–
Cefotaxime	30 µg	29–35	25–31	31–39	34–38	18–22
Ceftazidime	30 µg	25–32	16–20	27–35	–	22–29
Ceftriaxone	30 µg	29–35	22–28	31–39	39–51	17–23
Cefalotin	30 µg	17–22	29–37	–	–	–
Ciprofloxacin	5 µg	30–40	22–30	34–42	48–58	25–33
Nalidixic acid	30 µg	22–28	–	–	–	–
Chloramphenicol	30 µg	21–27	19–26	31–40	–	–
Erythromycin	15 µg	–	22–30	–	–	–
Gentamicin	10 µg	19–26	19–27	–	–	16–21
Nitrofurantoin	300 µg	20–25	18–22	–	–	–
Sulfonamides	300 µg	18–26	24–34	–	–	–
Tetracycline	30 µg	18–25	19–28	–	30–42	–
Trimethoprim	5 µg	21–28	19–26	–	–	–
Trimethoprim/ sulfamethoxazole	1.25 µg/ 23.75 µg	24–32	24–32	24–32	–	–
Vancomycin	30 µg	–	15–19	–	–	–

Note 1: Quality control tests should be performed at least weekly if organisms are processed on a daily basis. However, if testing is infrequent and/or irregularly performed, quality control strains (one or more) should be processed concurrently with the clinical isolate tests. **Note 2**: These quality control ranges were suggested for use with Mueller-Hinton agar *(E.coli* and *S. aureus* preferred). **Note 3:** Quality control zone guidelines are listed to assist intermediate and central reference laboratories wishing to test *Haemophilus* and gonococci. The zone diameter ranges were derived for tests on *Haemophilus* test medium (HTM) and supplemented GC agar respectively.

Annex IV: Minimum identification features

Principal pathogens which require accurate identification to monitor the resulting antimicrobial susceptibility are listed below. The minimum identification characteristics and tests are also presented. These conform to those listed in WHO's *Basic Laboratory Procedures in Clinical Bacteriology,* and other widely-recognized reference material.

Organism	Minimum identification features
Staphylococcus aureus	Gram-positive cocci in clusters catalase-positive coagulase-positive
Coagulase-negative staphylococcus	Gram-positive cocci in clusters catalase-positive coagulase-negative
S. pneumoniae	Gram-positive oval-shaped cocci in diplo or short chains alpha (green) haemolytic colonies catalase-negative bile solubility or optochin disc susceptibility
S. pyogenes (group A)	Gram-positive cocci in chains beta-haemolytic colonies catalase-negative bacitracin (0.04 µg) disc-susceptibile sulfamethoxazole/trimethoprim (23.75 µg/1.25 µg) disc-resistant
Enterococci	Gram-positive cocci in diplo or short chains catalase-negative[1] bile-esculin hydrolysis growth in 6.5% NaCl broth
H. influenzae	Gram-negative, slender cocco-bacilli "satelliting" colonies around colonies of *Staphylococci* no growth on blood-free media
N. gonorrhoeae	Oval Gram-negative diplococci with concave opposing edges and long axes parallel oxidase-positive growth on selective GC media no growth on nutrient agar maltose not fermented (gamma-glutamyl-aminopeptidase negative)
N. meningitidis	Oval Gram-negative diplococci with concave opposing edges and long axes parallel oxidase-positive growth on selective GC media no growth on nutrient agar maltose fermented (gamma-glutamyl- aminopeptidase positive)
E. coli	Gram-negative, non-motile rods indole-positive acid slant, gas is produced, H_2S-negative (TSI) [beta-glucuronidase-positive (PGUA)]
Klebsiella spp.	Gram-negative, non-motile rods indole-negative (except *K. oxytoca*) lysine-positive (LIA) acid slant, gas is produced, H_2S-negative (TSI)
Salmonella spp.	Gram-negative motile rods acid butt, alkaline or neutral slant, gas is produced except *S typhi* (TSI) lysine-positive except *S. paratyphi* A (LIA slant) H_2S-positive except *S. paratyphi* A (TSI and LIA slant)
Shigella spp.	Gram-negative non-motile rods lysine-negative oxidase-negative acid butt, alkaline or neutral slant, no gas production except some strains in a few serotypes, no H_2S production (TSI)

(Continued on next column)

1. Some strains may produce a pseudocatalase.