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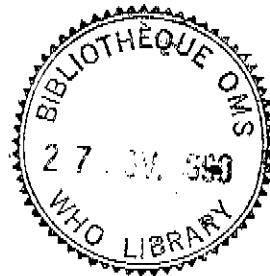


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GUIDELINES FOR SURVEILLANCE AND CONTROL OF  
ANTIMICROBIAL RESISTANCE

(Edited by Dr W J Brinley Morgan)

1990

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1. INTRODUCTION: SCOPE AND OBJECTIVES OF THE GUIDELINES

Modern livestock production and agricultural developments throughout the world rely on the use of antimicrobial substances, not only for the treatment of infection, but also for the promotion of growth and the prevention of disease. As a consequence some bacteria in livestock are becoming increasingly resistant to antimicrobial substances and may also affect the human population, thus creating a public health problem.

The occurrence of antimicrobial-resistant pathogenic bacteria in clinical materials as well as in the normal flora of humans, in food and the environment urgently require elucidation to solve the problems encountered in treating the numerous infections due to such bacteria. In particular, systemic invasion by pathogenic bacteria such as salmonella, which are common to animals and man and/or are transmissible from animals to man, is proving difficult to treat, both in man and animals.

Epidemiological analysis of this situation is being carried out in some countries, but because of inadequate, commonly-applicable monitoring and surveillance schemes, it is difficult to compare data and decide on measures that can be supplied globally.

The joint efforts of the sectors concerned are now needed to eliminate discrepancies and differences in the use of methodologies linked with surveillance and monitoring by the various disciplines involved, through a process of harmonisation. This will result in baseline data that are comparable and make assessment on a wider and more common basis possible. Only at this stage can the establishment of prevention and control measures against antimicrobial-resistant bacteria be envisaged.

Objectives

The aim of the proposed project is to elaborate guidelines for regular and systematic monitoring and surveillance of antimicrobial sensitivity testing worldwide using a common method of testing. The step-by-step activities include:

- i) selection of species of bacteria to be examined as indicators of antimicrobial resistance specific to animals and to humans and/or common to both;
- ii) selection of antimicrobial substances to be used for susceptibility tests;
- iii) to standardize methods for testing for antimicrobial resistance and reporting systems;
- iv) attempt to evaluate the influence of the increase in antimicrobial-resistant bacteria.

The baseline data can be shared and used by both the medical and veterinary public health sectors, food hygienists and environmental and animal health specialists alike in order to develop international cooperation between them.

Immediate objectives

These are:

to collect up-to-date information on antimicrobial resistance in clinical medicine, public health and in agriculture and on the occurrence of public health hazards (this activity has been ongoing since April 1987);

to analyse the current state of the art according to the information so far available and to elaborate a monitoring and surveillance scheme based on standardized antimicrobial susceptibility tests common to the various sectors and to identify the degree of public health risk; and

to assist with the elaboration of measures specifically related to public health and the safe and economical production of livestock.

These guidelines do not deal with issues of the safety of food of animal origin that may contain residues of veterinary drugs as this aspect is being considered by the Codex Committee on Residues on Veterinary Drugs,<sup>1</sup> nor do they cover susceptibility testing of bacterial species such as sexually transmitted pathogens for which special media and techniques are required.<sup>2</sup>

2. APPRAISAL OF THE PRESENT EPIDEMIOLOGICAL SITUATION

Acquired resistance to antimicrobial substances may be mediated by genes carried either on the bacterial chromosome or on smaller pieces of extrachromosomal DNA called R-plasmids. By virtue of the ability of R-plasmids to be transferred to recipient bacteria, both Gram-positive and Gram-negative, they play the major role in the epidemiology of antimicrobial resistance. Plasmids, in general, carry genes coding for a wide range of functional properties which confer an evolutionary advantage on host bacteria. It is likely that a rare occurrence of R-plasmids in bacterial hosts, found in ecosystems where antimicrobial resistance is produced naturally, have given them survival advantage. Within a few years of antimicrobial substances being available, the incidence of bacteria carrying R-plasmids increased dramatically due to the selective pressure imposed by their use for whatever purpose (therapy and prophylaxis in man, animals, fish and plants, and growth promotion in animals).

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<sup>1</sup> Residues of Veterinary Drugs in Foods. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 32, 1985.

<sup>2</sup> Bench level laboratory manual for sexually transmitted diseases. WHO Geneva, 1989, document WHO/CDT/89.443.

The incidence of antimicrobial resistance in any ecosystem is inevitably related to the intensity of antimicrobial usage and, for various reasons, varies from country to country and between different locations within the same country. To reduce this selective pressure legislation to restrict the use of antimicrobial substances has been adopted in some countries.

For instance, in the United Kingdom following the recommendations of the Swann Committee<sup>1</sup> therapeutic antimicrobials are not allowed to be used for growth promotion in animals and their use for therapy and prophylaxis is only permitted if prescribed by a veterinary surgeon for animals under his care. Similar measures have been made in some other countries to restrict the use of therapeutic antimicrobials for growth promotion in animals. A recent publication indicated that in the USA about 50% of their total antibiotic production was used in farm animals and about 50% of all antibiotics used in farm animals was in the form of tetracyclines fed at sub-therapeutic concentrations to meat-producing animals primarily for the purpose of collective prophylaxis. In the European Community legislation has been introduced to control the use of veterinary medicinal products and of feed additives.

Many countries have licensing systems before medicinal products for both human and veterinary use can be marketed as well as on methods of distribution. Distinction is made between medicines that can with reasonable safety be sold directly to the public (the so-called "over the counter products") and those that can only be supplied on the prescription or written direction of a qualified person licensed or authorized to prescribe. In addition, hospitals in certain countries have introduced non-legislative practices to control the pattern of use of particular antimicrobials. But in other countries antimicrobial usage is unrestricted and they can be purchased directly by members of the public. This often leads to the unnecessary use of antimicrobials, encouraging the widespread emergency of resistant organisms. Similarly in animals, therapeutic antimicrobials are often used improperly for alleviating disturbances caused by "stress" or to cover up inadequate animal management and husbandry practices on farms. It follows that if antimicrobials are to continue to be effective in treating bacterial diseases in man and animals, they must be used responsibly.

In any strategy for the control and prevention of the emergency and spread of antimicrobial resistance and resistant organisms, serious consideration must be given to restricting the availability of antimicrobial substances to the general public on the one hand and the encouragement of responsible prescribing by those authorized to do so on the other. This can be achieved by continuously updating knowledge and principles of prescribing drugs, including antimicrobial substances, and encouraging continuing professional development and training by the medical, veterinary and pharmaceutical professions.

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<sup>1</sup> Report 1969 HMSO, London, Cmnd 4190, pp.83.

The withdrawal of antimicrobial substances after they had been used for a period of time has not always resulted in a reduction of antimicrobial resistance. Reports from the Netherlands<sup>1</sup> claim that the withdrawal of tetracycline as a growth promoting agent resulted in a fall in the incidence of tetracycline-resistant coliforms but workers in the USA<sup>2</sup> showed that a total ban on the use of antibiotics over a 10-year period in an isolated pig herd resulted in an initial fall from 80% to 42% resistant coliforms which then persisted at around 42% throughout the monitoring period. This work and that reported from Bristol, UK,<sup>3,4</sup> and France<sup>5</sup> indicates that, once antimicrobial-resistant strains of *Escherichia coli* have been selected and established in an ecosystem, or particular R-plasmids have become established in naturally occurring indigenous strains, the withdrawal of the use of antimicrobial substances reduces but does not eliminate the occurrence of resistant strains.

Surveys of antimicrobial resistance to the older antimicrobial substances in hospitals, carried out in the USA<sup>6</sup> indicate that the incidence of antimicrobial resistance in human pathogens and indigenous bacteria of the normal flora have shown little change over the last 10 years. However, differences between countries occur, and even within countries, and between hospitals in the same country. For instance, a higher incidence of antimicrobial resistant pathogens is reported in the south of Europe compared with the north. Why these differences exist is not known but more information could lead to a better understanding of the epidemiology of antimicrobial resistance and appropriate measures taken to improve the position.

For these and other reasons, it is not possible to predict with any degree of accuracy the incidence of antimicrobial resistance among particular bacterial species in a particular situation. Monitoring for antimicrobial resistant bacteria in animal and human populations is already being carried out but varies in extent between countries and, in most, is very limited. The only rational approach to obtain comprehensive

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<sup>1</sup> Leeuwen van, J.J., Embden van, J., Guinee, P., Kampelmacher, E.H., Manten, A., Schothorst van, M. & Voogd, C.E., 1979, *Antimicrob. Ag. Chemother.* 16: 237-239.

<sup>2</sup> Langlois, B.E., Cromwell, G.L., Stahly, T.S., Dawson, K.A. & Hays, V.W., 1983, *Appl. & Environ. Microbiol.* 46: 1433-1434.

<sup>3</sup> Hinton, M., Hedges, A.J. & Linton, A.H., 1985a, *J. appl. Bact.* 58: 27-35.

<sup>4</sup> Hinton M., Linton A.H. & Hedges A.J., 1985b, *J. appl. Bact.* 58: 131-138.

<sup>5</sup> Chaslus-Dancla, E., Gerbaud, G., Lagorce, M., Lafont, J.P. & Courvalin, P., 1987, *Antimicrob. Ag. Chemother.* 31: 784-788.

<sup>6</sup> Atkinson, B.A. & Lorian, V., 1984, *J. clin. Microbiol.* 20, 792-796.

information is to apply an agreed monitoring programme worldwide. Any monitoring programme must include the identification of isolates into species or biotypes. For instance, it has been shown<sup>1</sup> that Enterococcus faecium which becomes dominant in the gut of the older chick is more resistant to cephalothin, the Macrolide, Lincosamides and Streptogramins (MLS) antibiotics and tetracycline than Enterococcus gallinarum that occurs earlier in life. The apparent change in resistance was due to the displacement of one species by another and not due to acquired plasmids. The importance of phage typing of serotypes of Salmonella, e.g. in S. typhimurium, in the epidemiology of antimicrobial resistance is well established.<sup>2</sup>

Considerable controversy exists on the relative importance for man of the reservoir of antimicrobial resistant strains which arise in animals. Both overt pathogens and indigenous members of the normal bacterial flora must be considered. Apart from Salmonella spp. and Campylobacter jejuni, animal and human pathogens which carry R-plasmids rarely overlap. However, evidence is available that E. coli derived from animal sources can colonise the human gut for some days,<sup>3</sup> but how frequently this occurs or whether their R-plasmids are transferred to indigenous strains in man are not known. R-plasmids found in bacteria of the normal bacterial flora of the body of both animals and man can be transferred to pathogenic species either in vivo or in the environment, thereby enlarging the genetic pool.<sup>4</sup>

The application in recent years of molecular biological techniques for subtyping bacteria and identifying specific genes, has opened up a new chapter in the study of the epidemiology of antimicrobial resistance. This approach takes advantage of the fact that genes coding for antimicrobial resistance in bacteria are commonly carried on plasmids. Plasmids are sufficiently large and subject to enough evolutionary events, to generate discriminating structural variation indicating, on the one hand, a common origin but, at the same time, exhibiting enough diversity to make it possible to distinguish strains that would otherwise be indistinguishable. Using the techniques of plasmid profile analysis, restriction enzyme (endonuclease) cleavage, DNA-DNA hybridization and other more recent molecular techniques it is possible to trace the spread of clones of strains carrying specific plasmids and also the transfer and modification of plasmids, as they are identified in different bacterial hosts.

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<sup>1</sup> Kaukas, A., Hinton, M. & Linton, A.H., 1988, *J. appl. Bact.* 64: 57-64.

<sup>2</sup> Threlfall, E.J. & Rowe, B., 1984, in: *Antimicrobials and Agriculture*, ed. M. Woodbine. Butterworths: 513-524.

<sup>3</sup> Linton, A.H., Howe, K., Bennett, P., Richmond, M. & Whiteside, E.J. 1977, *J. appl. Bact.* 43: 464-469.

<sup>4</sup> Levy, S.B., *Release of genetically modified organisms*, 1987. Regional Conference, Cardiff.

### 3. METHODS AND PROCEDURES FOR SURVEILLANCE AND MONITORING OF ANTIMICROBIAL RESISTANCE.

#### 3.1 Introduction

Clinical and veterinary diagnostic laboratories in countries throughout the world receive clinical materials for diagnostic purposes. As part of the identification process, the isolated organisms are frequently, but not always, subjected to tests for antimicrobial susceptibility.

The range and concentrations of the antimicrobial substances used often vary according to local circumstances since the primary object of such testing is to guide the clinician or veterinary surgeon in selecting the most appropriate agent for treating the individual patient and/or preventing the spread of disease.

Many or all of these isolates may also be sent to a central or reference laboratory for more detailed tests which may include further tests for antimicrobial susceptibility to a different or a wider range of antimicrobial substances, to tests for the production of beta lactamases and other enzymes and to carry out sophisticated molecular techniques including plasmid profile studies.

Whilst medical and veterinary laboratories are mostly concerned with problems pertinent to their own respective spheres, many antimicrobial substances in use are however common to both professions; a range of causal organisms (the zoonoses) is common to both man and animals and there is always the potential for harmless but resistant organisms to pass from animals to man - and vice versa, and to transfer their resistance to host-specific pathogens thereby seriously compromising therapy.

It is therefore essential that there should be harmonization in techniques, interpretation of results and proper use of quality control not only in all laboratories involved in such work within a country but also between countries/regions. Only then will it be possible to establish meaningful measures to combat the development and spread of antimicrobial resistance and resistant organisms and to monitor progress.

#### 3.2 Isolation and identification of the minimal list of bacterial species necessary for monitoring

##### 3.2.1 Salmonellas

Salmonellas being members of the Enterobacteriaceae are most frequently isolated from intestinal contents or faeces. They can be isolated from apparently healthy individuals or from a wide variety of clinical syndromes both in man and in animals. Members of the genus have also been frequently isolated from farm effluent, sewage, animal products and animal feedstuffs.

There are over 2000 different serotypes, some of which may be further sub-divided by means of phage typing. Descriptions of extensive serotyping and phage typing are outside the scope of most diagnostic laboratories and isolates should be referred to a central/national reference laboratory.

### Isolation

There are numerous techniques and methods described which claim to be most successful for isolating different serotypes from various sources<sup>1</sup>. However, we cannot recommend a single method, only general techniques which have been found to be satisfactory.

Salmonellas grow well on simple media but many differential and selective media have been devised. Deoxycholate citrate agar greatly suppresses or inhibits the growth of coliforms and Gram-positive bacteria. Some salmonellas, e.g. *S. abortus ovis* and *S. choleraesuis*, do not grow readily on this medium, especially after attempted enrichment in selenite broth. Brilliant green agar inhibits enteric organisms other than salmonellas, although some formulations have been found to be unsatisfactory for the isolation of *S. dublin*.

Selenite and tetrathionate broths are widely used for selective enrichment of salmonellas although they are not satisfactory for the isolation of *S. choleraesuis* and *S. abortus ovis*. Rappaports broth and variations are now coming into prominence. For isolation of salmonella from feed and water samples, a pre-enrichment stage of buffered peptone water is used.

Non-lactose fermenting colonies on the selective agars are tested first by slide agglutination in polyvalent typing serum. Colonies which react with the serum are then subjected to further biochemical tests, in composite media such as triple sugar iron agar or Kohn's tube medium, and to further serological tests. They can then be sent to a central/reference laboratory for serotyping and phage typing.

#### 3.2.2 Escherichia coli

The aetiological significance of the isolation of *E. coli* has to be assessed in conjunction with the host history and post-mortem examination. Samples are cultured directly on blood agar and MacConkey agar and incubated at 37°C overnight. Colonies of *E. coli* are smooth, large, shiny and pink on MacConkey agar. Most strains isolated from faeces or pathological material produce a positive result in the Eijkman test. Full serotyping involves the determination of the 'O' (somatic), 'K' (capsular) and 'H' (flagellar) antigens and is usually performed at reference laboratories. Some of the animal enteropathogenic strains may be identified serologically by their possession of a fimbrial/adhesin antigen e.g. K88 or K99; or CFAI or CFII in the case of human enterotoxigenic strains.

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<sup>1</sup> Further details can be obtained from standard microbiology textbooks and also from: "Manual for laboratory investigations of acute enteric infections", WHO Geneva, 1987, CDD/83.3, Rev 1; second part of "Bench-level procedure manual on basic bacteriology", WHO, Geneva 1987. WHO document WHO/LAB/87.1.

### 3.2.3 Campylobacters

For many years campylobacters, under their earlier name of *Vibrio*, have been associated with reproductive disease of sheep and cattle. Since the introduction of improved isolation techniques, campylobacters are now recognised as a major cause of enteritis in man. By using similar cultural techniques on animal material, many isolations have been made from apparently healthy animals but, in other cases, clinical disease may be present.

For surveillance purposes, the catalase positive campylobacters constitute most isolates and are the more important. At present, all selective media used for isolation are dependent on incorporating mixtures of antibiotics to inhibit contaminants. These supplements (e.g. Skirrow's, Butzler's & Preston's)<sup>1</sup> consist of mixtures of antibiotics which may be purchased for incorporation into the medium.

To isolate campylobacters, the faeces are inoculated directly, or after suspension in sterile saline, onto the selective media. For isolation of *C.fetus* subsp *jejuni* and *C.coli*, plates are incubated at 42-43°C. *C.fetus* subsp *intestinalis* will not grow at this temperature and, if it is suspected, incubation must be performed at 37°C. The plates should be incubated in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 24-28 hours. If such facilities are unavailable, a candle jar can be used.

Confirmatory tests should be performed on colonies isolated on the selective medium. Campylobacter spp are motile, Gram-negative, spirally curved rods. *C.fetus* subsp *jejuni* is oxidase and catalase positive and nalidixic acid sensitive. In contrast, *C.fetus* subsp *intestinalis* is resistant to nalidixic acid and will grow at 42°C.

### 3.2.4 Staphylococci and Micrococci

Pathogenic staphylococci are commonly found in pyogenic processes in all species of animals - including man, and in birds. They have been frequently isolated from cases of pyaemia and septicaemia as well as from cases of arthritis and mastitis. Pathogenic strains are commonly found on the skin and in the noses of healthy persons and in some species of animals.

They grow readily on nutrient or blood agar and also in the presence of high salt concentrations (10% NaCl). After 24 hours of incubation, colonies on sheep blood agar are 1-2mm diameter, smooth, shiny and convex, and often strongly haemolytic. The major characteristic for differentiating pathogenic *S.aureus* from other micrococci is the production of coagulase.

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<sup>1</sup> Manual for laboratory investigations of acute enteric infections. WHO Geneva, 1987, document CDD/83.3, Rev 1.

### 3.2.5 Streptococci and enterococci

Man and domestic animals suffer from streptococcal infections and the conditions vary from localised suppurative lesions to mastitis, meningitis, arthritis and septicaemia. They can also be isolated from various sites in healthy animals and man.

Samples should be cultured on blood agar and MacConkey agar and incubated aerobically at 37°C. It is also advisable to inoculate a serum broth or Todd-Hewitt broth at the same time. On sheep blood agar, small, dull, semi-translucent colonies are formed after 24 hours of incubation. They may be alpha or beta haemolytic depending on whether the strain produces a soluble haemolysin.

They are catalase and oxidase negative. Sugars are fermented without the formation of gas. Serological classification is carried out using the Lancefield technique for grouping.

Enterococci are being increasingly isolated from blood and urine from humans and, therefore, they would be useful organisms for monitoring for resistance to newer antimicrobial substances.

### 3.2.6 Yersinia

*Yersinia* spp. are widely distributed in nature and may be isolated from the faeces of diseased and healthy animals - including man, as well as from chronic caseous lesions or occasionally acute septicaemic conditions.

Material from suspect lesions is cultured on blood agar and MacConkey agar and incubated at 37°C. In the case of faeces, selective media (e.g. Oxoid CM653) and antibiotic supplement<sup>1</sup> may be used. The typical colonies of *Yersinia enterocolitica* develop as a dark red "bull's eye" surrounded by a transparent border. Biochemical tests and serotyping may be used to differentiate the different *Yersinia* species.

### 3.2.7 Vibrio cholerae

Faeces or a sub-culture from enrichment media, e.g. Alkaline peptone water, are inoculated onto the surface of thiosulphate citrate bile sucrose agar (TCBS) cholerae medium and incubated for 18-24 hours at 37°C. Cultures should be examined immediately after removal from an incubator because the yellow colonies of *Vibrio* may revert to a green colour when left at room temperature.

Pure cultures are tested for fermentation of lactose, sucrose, mannose and arabinose, the VP reaction and haemolysis. A specific O- antiserum is used for agglutination test. Only strains of O1 group are of epidemic importance.

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<sup>1</sup> Schiemann, D.A. , 1979, Can. J. Microbiol. 25: 1298-1304.

### 3.2.8 Shigella

Faeces may be emulsified in saline prior to plating out onto deoxycholate citrate or modified Salmonella-Shigella agar and into selenite broth; the latter is sub-cultured onto a fresh plate after 18-24 hours of incubation. Shigella colonies are pale or colourless after primary incubation and should then be tested either in composite medium or conventional sugars to confirm the group and also serotyped.

### 3.3 Antimicrobial sensitivity testing

#### 3.3.1 Media

In designating a suitable medium for performing antimicrobial susceptibility tests by the agar diffusion technique using antimicrobial susceptibility discs, the following characteristics are considered desirable:

- (1) The composition of the medium should be defined, at least to the point of specific production details, for crude components such as peptone and agar.
- (2) The medium should be capable of producing control zone sizes within published limits.<sup>1</sup>
- (3) The medium, without enrichment, should support good growth of the majority of rapidly growing pathogens for which susceptibility tests are required.
- (4) The medium should not be antagonistic to any of the antimicrobial substances used for susceptibility tests.
- (5) The medium should resist marked pH changes during the growth of common bacterial species.
- (6) The medium should be approximately isotonic to blood and suitable for the addition of blood, which is necessary for the growth of fastidious organisms.
- (7) The medium should be reproducible to minimize batch-to-batch variations.

#### Agar medium

Müller-Hinton agar medium from different manufacturing sources has been found to meet most of these criteria. It is available in a dehydrated form and should be prepared according to the manufacturer's recommendation. Other suitable standardized media can also be used.

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<sup>1</sup> Manual for laboratory investigations of acute enteric infections. WHO Geneva, 1987, document CDD/83.3, Rev. 1.

Liquid medium

Any nutrient broth that is available in the laboratory can be used for growing the inoculum, e.g. trypticase soy; soybean-casein digest; Müller-Hinton; brain heart infusion broths.

3.3.2 Antimicrobial substances

The following 'core' list of antimicrobial substances is recommended for use using concentrations recommended in NCCLS document:<sup>1</sup>

Gram-positive bacteria:

Penicillin G	10 units
Methicillin/oxacillin	5g/lg
Tetracycline	30g
Chloramphenicol	30 "
Erythromycin	15 "
Gentamicin	10 "
Clindamycin	2 "
Vancomycin	30 "
Cefotaxime	30 "

Gram-negative bacteria:

Ampicillin	10g
Cefotaxime	30 "
Tetracycline	30 "
Chloramphenicol	30 "
Gentamicin	10 "
Trimethoprim	5 "
Sulphonamides	250-300g
Nalidixic acid	30g

International standard preparations can be obtained from the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts EN6 3QB, United Kingdom.

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<sup>1</sup> Performance Standards for antimicrobial susceptibility testing. Second information supplement. National Committee for Clinical Laboratory Standards, Doc M100-S2, 7, No 10.

### 3.3.3 Procedures and methods of testing

#### General principle of antimicrobial susceptibility testing

Antimicrobial susceptibility tests measure the ability of an antimicrobial substance to inhibit the *in vitro* bacterial growth. This ability may be estimated by either the dilution or diffusion method.

#### The dilution test

For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or agar medium which is then inoculated with a standardized suspension of the test organism. Multipoint or semi/fully automated systems can also be used. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent, and the lowest concentration that kills the organism is the minimum bactericidal concentration (MBC).

#### The diffusion test

Paper discs, impregnated with the antimicrobial substance, are placed on agar medium uniformly seeded with a standardized inoculum of the test organism. A concentration gradient of the antimicrobial substance forms by diffusion from the disc and the growth of the test organism is inhibited to a distance from the disc that is related, among other factors, to the susceptibility of the organism. There is an approximate linear relation between log MIC as measured by a dilution test and the inhibition zone diameter in the diffusion test. Several technical factors may influence the size of inhibition zones e.g. the inoculum density, timing of disc application, temperature and time of incubation, depth of agar medium, spacing of the antimicrobial discs, storage and potency of antimicrobial discs, composition of the medium etc. Therefore the standardization of the disc diffusion method is highly important. For sulphonamide testing, a dilute inoculum should be used if an ideal medium is not available.

#### Preparation of plates

The medium is poured into dishes on a flat horizontal surface to a uniform depth of 4mm. This corresponds to 60ml of medium for dishes of 14cm internal diameter and 25ml for dishes of 9cm internal diameter.

When the agar has set, the plates for immediate use should be dried for 30 minutes in a 37°C incubator. The unused prepared plates may be stored in a plastic bag which is then sealed and placed in the refrigerator. Plates stored in this way will keep for two weeks.

#### Preparation of inoculum for seeding of plates

Using a pure culture of the test organism, or of the reference strains, a young actively growing culture is obtained and its turbidity is then adjusted against a turbidity standard (see pages 16-17).

Adjustment is made by diluting the broth culture with sterile broth or saline. Proper adjustment of the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent.

The inoculum may also be prepared by transferring sufficient growth from the primary culture plate or from a pure culture into a tube of broth or saline until its turbidity matches the standard. The direct method of adjusting the inoculum without preincubation is acceptable for routine purposes.

#### Seeding of plates

The plates are inoculated with a sterile cotton swab dipped into the inoculum. A bent glass rod may also be used. The swab is streaked all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. Finally encircle around the edge of the agar surface with the swab. The inoculum is left to dry for a few minutes at room temperature with the lid closed.

Plates can also be flooded with the bacterial suspension and the excess removed. A multipoint inoculator may be used on an antibiotic-containing medium.

#### Application of disc

The antimicrobial discs may be placed on the inoculated plates using a pair of sterile forceps, a sterile needle or a disc dispenser. A maximum of 8 discs (depending on their size) may be placed on a 9cm plate. Each disc should be gently pressed down to ensure even contact with the medium. The plates should be placed in an incubator at 35-37°C within 30 minutes of their preparation.

#### Reading zone diameter

After overnight incubation the diameter of each zone (including the diameter of the disc) is measured and recorded in mm. The results should then be interpreted by comparison with an approved standard put up under identical conditions on the same day. The measurements can be made with a ruler, a pair of calipers, or a template.

#### Quality control

The standard reference strains of bacteria are used in parallel with the clinical cultures. These tests should be run every week, or every fifth batch of tests and each time that a new batch of culture medium or a new batch of discs is used. The following reference strains can be used:

- Staphylococcus aureus ATCC 25923 or from another approved culture collection;
- Escherichia coli ATCC 25922 or from another approved culture collection;
- Pseudomonas aeruginosa ATCC 27853 or from another approved culture collection.

#### Turbidity standard

In the absence of a commercially available standard, a turbidity standard can be made by adding 0.5ml of 1.17% (w/v) barium chloride dihydrate ( $\text{BaCl}_2 \cdot \text{H}_2\text{O}$ ) solution to 99.5ml of 1N sulphuric acid. The turbidity standard solution should be placed in a tube identical to the one

used for the broth sample. It can be stored in the dark at room temperature and can be used for up to 6 months, provided it is sealed to prevent evaporation.

For details on quality control see "Second Part of Bench-Level Procedure Manual on Basic Bacteriology".<sup>1</sup>

### 3.3.4 Recording and interpreting the results

The measured zone size in mm should be interpreted according to the approved interpretation standard,<sup>1,2,3</sup> or the guideline of manufacture.<sup>4</sup> National interpretation standard may also be used when the antimicrobial content of the discs differ greatly from those of ref.1. An interpreting template is very useful in reading the results.

The results should be reported using the three-category classification as susceptible, intermediate or resistant.<sup>1,2</sup>

## 3.4 Mechanisms and Methods of Surveillance and Monitoring

### 3.4.1 Information retrieval

Information available in reports and published papers show that bacterial resistance is a problem occurring in all parts of the world. It is just as serious in developing countries where the older and often cheaper, and more readily available antimicrobial substances are becoming progressively less effective and the newer generation are often not readily available and in any case are much more expensive.<sup>5,6</sup> The problem is also increasing amongst the general population and is not confined to hospitals and immune-compromised patients.

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<sup>1</sup> Second Part of Bench-Level Procedure Manual on Basic Bacteriology, WHO/FAO/87.1, 1987: (3. Guideline for antimicrobial susceptibility testing, Table 5, page 35).

<sup>2</sup> WHO Technical Report Series, 673, 1982 (Thirty-second report of the WHO Expert Committee on Biological Standardization). Annex 5: Requirements for susceptibility discs, Appendix 2.

<sup>3</sup> Manual for laboratory investigations of acute enteric infections. WHO Geneva, 1987, document CDD/83.3, Rev. 1.

<sup>4</sup> Antimicrobial Sensitivity Testing using Neo-sensitabs (A/S Rosco-Denmark)

<sup>5</sup> Report of Scientific Group on the Control of Bacterial Resistance. Manila, Philippines, 1984, document LCP/CLR/002.

<sup>6</sup> WHO Technical Report Series, 624, 1978 (Surveillance for the prevention and control of health hazards due to antibiotic resistant enterobacteria).

Much of the information which is available is not co-ordinated on a national, regional or global scale. More information is required on any significant regional differences in the prevalence of resistant bacteria or in the levels of their resistance to various antimicrobial substances and on how rapidly resistance develops, for example, after the introduction of a new antimicrobial substances.

This information is needed in order to assess the extent of over-use or mis-use of antimicrobials in common clinical situations both in clinical and veterinary agricultural practice in order to avoid a situation developing where large numbers of patients are denied effective therapy. Without such information, it is not possible to monitor the effectiveness of any corrective measures that may be introduced, for example the introduction of greater controls on availability of antimicrobial substances or of policies regarding usage. Previous reports issued by the World Health Organization<sup>1,2,3,4,5</sup> have elaborated on how information derived from surveillance programmes can be used.

3.4.2 Local laboratories, national central/reference centres and international coordination

Local laboratories

These will vary from hospital or university laboratories, public health laboratories as well as veterinary diagnostic laboratories at veterinary schools, government service or in the private sector. Their functions will include the following:

- isolating, identifying strains, determining antimicrobial susceptibility according to agreed methods;
- sending results to clinicians, epidemiologists and national reference centre(s);
- sending random or selected strains especially those with unusual or new resistance patterns to national reference centre(s);
- provide an early warning of new or changing resistance patterns.

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1 Second part of bench-level procedure manual on basic bacteriology: Guidelines for antimicrobial susceptibility testing. Unpublished WHO document WHO/LAB 87.1

2 Guidelines for antimicrobial susceptibility tests. WHO document LAB/79.3

3 Report Scientific Group on the control of bacterial resistance. Manila, Philippines, 1984, document LCP/CLR/002.

4 WHO Technical Report Series, 624, 1978 (Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria).

5 Antimicrobial resistance. Report of a Scientific Working Group. WHO, Geneva, 1982, document WHO/BVI/PHA/ANT/82.1.

#### National Central/Reference laboratories

Whilst ideally there should be one national central/reference laboratory for dealing with and coordinating activities relating to antimicrobial resistance in each country, it may be necessary to nominate different laboratories for dealing with different bacterial genera which are mainly of medical or veterinary importance.

There is general agreement that, for national and international surveillance of antimicrobial resistance, data should at least be obtained from a minimal list of bacterial species tested against a 'core' list of antimicrobial substances using agreed methods. These should be reviewed periodically.

The responsibilities and functions of such centres include:

- monitoring the performance of local laboratories;
- supplying appropriate reference strains, reagents and other materials;
- training of microbiologists and support staff;
- introducing specified standard methods for conducting susceptibility testing, recording and interpreting results;
- devising agreed methods for the easy and accurate flow of information between local, national and international centres using modern computer technology;
- Retesting a proportion of strains received from local laboratories, e.g. using a different range of antimicrobial substances or different concentrations of antimicrobial substances and for enzymes;
- research including the use of advanced molecular techniques;
- to provide expert advice to health professionals and others;
- to provide expert advice on research including the planning of special surveys and other programmes;

On a global level there is a need for an authority such as WHO to continue to use its prestige and influence to coordinate activities and to influence the actions of national governments in this field. Information collected from national reference centres needs to be collated, interpreted and then summarized in a readily assimilatable form to highlight trends and identify potential problems.

Other activities include bringing together experts in the field of antimicrobial resistance to pool experiences and plan strategies for consideration by national governments; organizing training courses and providing reference standards and protocols for susceptibility testing.

WHO has already taken a lead in these activities by convening meetings of experts, issuing guidelines on methodology, quality control, data reporting and on antimicrobial usage. These are continuing activities.

#### 4. REPORTING SYSTEMS

##### 4.1 Sources of information

The sources of information on antimicrobial resistance vary markedly between countries. Thus in some countries there may be a national network of both veterinary diagnostic laboratories and public health laboratories, as well as clinical laboratories situated in hospitals, universities and the private sector. The collection of data and planning of national surveys in such situations is relatively easy. In other countries, the number of veterinary diagnostic laboratories may be more limited and surveillance programmes may be more difficult to initiate and maintain.

Surveillance programmes may be carried out at three levels:-

##### 1) Clinical laboratory data

A great deal of information will be available from local laboratories but techniques may differ between laboratories thus making interpretation of data difficult.

##### 2) National/Central/Reference Centres

Reference laboratories receive specific species of micro-organisms and these may be subjected to more detailed tests, including further susceptibility tests. Consequently these results will be less subject to variations compared with those from the local laboratories.

##### 3) Special surveys

Surveys of bacteria colonizing healthy animals and humans, as well as their environment, provide a base line with which to assess trends, but these surveys are special investigations and seldom routinely carried out.

Any surveillance programme will need:-

- a) a central co-ordinator responsible for the implementation and design of the survey, the methodology, collection, collation of results and general day to day running. The responsibility may cover one or more species of bacteria;
- b) nominated local officers at each of the participating laboratories. They will be responsible for the quality control (media, technique and organisms), recording and interpreting the data and despatch of results.

##### 4.2 Veterinary laboratories

Although the indicator organisms may be isolated from animals and animal products at many possible laboratories, antimicrobial susceptibility tests are only routinely carried out at veterinary clinical laboratories.

The amount of information submitted with bacterial isolations or clinical material is usually limited and will seldom be as detailed as that received by hospital laboratories.

The minimum information must be:-

- 1) An accurate identification of the micro-organism, in appropriate cases, its serovar or phage type.
- 2) The laboratory reference number for the isolate.
- 3) The animal species, or material (eg. animal feed, water, milk, slurry) from which the organism was isolated and, in the case of material from overseas, the country of origin.
- 4) The nature of the specimen where appropriate, e.g. faeces, urine, pus, the actual tissue.
- 5) The antimicrobial substances against which the organism has been monitored and the resistance pattern, if any.

Additional information may include the animal owners' name and address.

#### 4.3 Medical and public health laboratories

Medical laboratories situated in hospitals and/or universities, provide a service to physicians for patients within their area. Public health laboratories which may also serve as hospital medical laboratories receive material from many sources, including man, animals, animal products, environmental samples, etc.

In the case of bacteria of human origin it is necessary to know in addition to bacterial species and sensitivity pattern:-

- 1) Patient's name and address, age and sex.
- 2) Nature of the specimen - faeces, urine, blood, pus, cerebro-spinal fluid or other specimen.
- 3) Clinical details.
- 4) Type of incidence, (sporadic, family outbreak, hospital outbreak, etc) and whether the patient has recently returned from abroad, and, if so, from which country.

Further details are given in the next section and in a report of a WHO consultation held in Geneva in 1982.<sup>1</sup>

The reporting system at its simplest could be periodic reports on a standard form containing the information listed above. A simple design is necessary so that the data can either be analysed easily or recorded and analysed by computer. Where national laboratory networks exist it may be possible to enter the information directly into a computer network for electronic transmissions.

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<sup>1</sup> Surveillance of antimicrobial resistance. Report of a WHO Consultation, Geneva, 1982, document BVI/PHA/ANT/82.2.

Analysis of the results may be either to provide simple summaries or more complicated analyses to expand and elaborate detailed data for delineating a local problem or possible epidemic.

In a surveillance programme relating to veterinary isolations the data for any one species of bacteria, serovar or phagetype should include:-

- 1) The percent resistant to an individual antimicrobial substance.
- 2) The percent of isolates from different animal species showing resistance to an individual antimicrobial substance.
- 3) The different resistance pattern of isolates from different animal species.

#### 4.4 Data Processing

##### 4.4.1 Principles

Laboratories participating in surveillance usually survey strains that they have already routinely isolated, speciated and susceptibility tested even if there were no surveillance. The extra effort that makes surveillance is data processing. The effort of data processing for each laboratory must thus be minimized and its benefit maximized, in order to enlist and sustain laboratory participation.

Primary data (inhibition zone size or MIC) are no harder to process and much better to analyse than resistance category data. Category breakpoints are arbitrary and changeable, while primary data provide confidence intervals, quality control and resistance mechanism profiling.

Data processing has to accommodate special populations and special needs of individual surveillance laboratories, but for optimal surveillance must also file all data into a universal file format so that primary data from any place and time can be compared with those from any other place and time.

##### 4.4.2 Data Entry

Data entry, the process by which laboratory susceptibility test results are encoded in electronic files, has evolved through several stages. Early surveys copied results on handwritten data sheets that were sent to one centre for expensive keypunch entry. These sheets were later replaced by hand-checked optical scanning forms that were machine-read at one data entry centre.

The growing availability of personal computers (PCs) has made PC data entry in each laboratory the preferred method, for several reasons. Technologist-friendly software allows rapid entry of results at the places where they are produced, minimizing transcription error and averting accumulation of data entry costs at one centre. PCs have the potential for interfacing with automatic zone or MIC readers. PC split-screen prompting can provide the operator with needed information to guide data entry. Data can be entered in a format that accommodates special local needs and yet files the data immediately in a universal file format. Entered results are immediately available for analysis on the same PC for the local needs of the entering laboratory (e.g. quality control or infection control).

4.4.3 Universal File Format

Programmes for surveillance of antimicrobial resistance all file similar data. A universal file format is thus easy to establish, and it makes possible integrated global surveillance of resistance. Such a file can be written in different computer languages and supported by different hardware but should be translatable between the computer languages used.

The categories of data included and the number of digits allocated to the field of each category in a representative programme, are as follows: (see also WHONET, Appendix 4)

Category of data	No of digits allocated for category field
1. Centre identification	4
2. Patient/animal/product identification	10
3. State if foreign travel involved/imported	2
4. Patient or host age	2
5. Patient or host sex	1
6. Patient hospital admission date	6
7. Patient/host/product location	7
8. Sample date	6
9. Sample type (site)	4
10. Species of isolate	5
11. Inhibition zone diameter or MIC value (alphanumerics used)	1 (per antimicrobial x 75 plus possible antimicrobials)
12. Biotype, auxotype, serotype, etc.	7
13. Programme version	1

In this example, allocation of approximately 130 digits or alphanumerics per isolate can be seen to encode the information needed for surveillance in most circumstances.

A centre might not have or might not choose to encode various categories of data, but what is encoded is then available for comparison with corresponding categories in other centres. Similarly, some centres might find reason to add categories, and these could ultimately be added to the universal file format if they gained wider acceptance.

A universal file format makes data analysis simple and flexible. The same analytical programmes are used by the individual centre to analyse its own infection control or quality control problems and by regional, national or international centres for surveillance. Increasing data storage capacity (removable cartridges or compact disks) may soon make it possible for each of the collaborating laboratories to have all of the data. This would mean that any one laboratory could perform on the whole surveillance data set any special analysis in which it might have a particular interest.

An important advantage of a universal file format is the preservation of the primary data through every level of analysis. In the pre-computer era, a laboratory might have tabulated the percent of its isolates categorized as resistant and passed these values on to a regional or national centre that would average them with others and then perhaps send them on to an international centre. Primary data, and hence the opportunity for further analysis of interesting results, would be lost each step of the way. Preservation of the complete primary data file, which has become progressively easier and cheaper, allows immediate re-examination of zone distribution, associated resistance, patient location etc. of any interesting observation that emerges.

#### 4.4.4 Data Analysis

The antimicrobial resistance detected in bacterial isolates in individual laboratories can be understood at many levels. At one extreme, it can be seen as the successive evolution of new antimicrobial resistance genes and their spread in mobile genetic elements through the world's bacterial populations. At the other extreme, it can be seen as a local data base to predict which antimicrobial substance is likely to eradicate the infection of a particular patient. Various possible uses of surveillance data by different groups are still applicable and more can be expected to emerge.

Such varied concepts and practical uses of antimicrobial resistance surveillance data require varied analyses. The data themselves are elaborate. Testing of twelve antimicrobial substances against twelve bacterial species generates 144 sets of values, any of which may have quality control problems or infection control significance. Examining all possible combinations of resistance to a set of antimicrobial substances seen in a species at a centre gives similar elaborate output; making comparisons over time or between centres generates further complexity. Analyses have to be detailed to find problems, and yet have to be summarized to track trends and provide overviews.

In view of these diverse needs, it is helpful that primary data on susceptibility test results once accumulated in a computer file can be analysed and reanalysed by a variety of programmes without much additional effort or expense. The 1982 Consultation Report<sup>1</sup> described nine basic methods for analysis of such data and four areas of interpretation to which these analyses could be projected; we can expect that more will continue to be devised.

A subset of five analytical programmes that is included in an existing software package for use by laboratories participating in surveillance of antimicrobial resistance (WHONET) is given in Annex 4.

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<sup>1</sup> Surveillance of antimicrobial resistance. Report of a WHO Consultation, Geneva, 1982, document BVI/PHA/ANT 82.2, 12.

5. OBJECTIVES IN ASSESSING REPORTING SYSTEMS IN THE PREVENTION AND CONTROL OF ANTIMICROBIAL-RESISTANT BACTERIA.

The ability to assess the efficacy and the accuracy of the reporting of emerging antimicrobial resistance and subsequently its prevention and control will depend upon a number of factors. These include:

- (a) the ability to collect data on resistance;
- (b) the quality of the data;
- (c) the ability correctly to analyse the data;
- (d) the ability to perform molecular and genetic studies to determine useful epidemiological markers;
- (e) the ability to perform adequate epidemiological studies when indicated; and,
- (f) the ability to influence public health policy.

To best achieve these aims, it is very important to:

- (a) carefully select a coordinator for each participant country;
- (b) carefully select an international coordinator; and
- (c) identify laboratories that can and will perform molecular studies on selected strains for use in determining molecular epidemiology.

5.1 National level

The coordinator within a country should:

- (a) be involved in selecting the laboratories to be reporting members; these should include:
  - large teaching hospitals;
  - large non-teaching hospitals;
  - small teaching hospitals;
  - small non-teaching laboratories;
  - public health laboratories;
  - veterinary diagnostic laboratories; and
  - veterinary central/reference laboratories.

- (b) be involved in determining methodologies;
- (c) supply reference materials, including standard reference organisms;
- (d) design report forms, preferably computerized;
- (e) determine the organisms and antimicrobial substances to be reported with special efforts to include newly-introduced antimicrobial substances.
- (f) ensure the receipt of data from all laboratories on a quarterly basis;
- (g) analyse the data for acceptability, ensuring inclusions of sufficient significant pathogens; quality control tests must be done and recorded at least weekly and results must be within control limits; assess results for changing or emerging resistance;
- (h) send the collected data and analysis to the international coordinator and to the participating laboratories;
- (i) contact laboratories that have testing problems or changing resistance patterns to discuss remedies and possible reasons;
- (j) develop methods for participating laboratories to save representative isolates of resistant organisms;
- (k) develop methods for acquiring antimicrobial usage data;
- (l) aid in determining if local epidemiological investigations are indicated;
- (m) determine if resistant organisms can be studied by more modern molecular methods within the country;
- (n) ensure that laboratories provide timely and acceptable data;
- (o) report emerging resistance to public health authorities and exert as much influence as possible to prevent and control emerging antimicrobial resistance; and
- (p) develop group meetings and publications as desired.

## 5.2 International level

The international coordinator should:

- (a) be actively involved (seeking advice as needed) in selecting country coordinators that are knowledgeable, responsive and prompt; those who do not meet these requirements should be replaced;
- (b) be involved with country coordinators in determining methodologies and reference materials to be used;

- (c) receive computerized quarterly reports from each country coordinator for collation, analysis, and distribution to the participating countries, other international organizations, and public health authorities;
- (d) ensure the publication of the worldwide data in the most appropriate journal(s);
- (e) collect antimicrobial usage data and correlate usage with worldwide emergence of resistance;
- (f) determine laboratories that can and will perform the most up-to-date studies on mechanisms of resistance and epidemiological markers; and
- (g) coordinate the collection of appropriate resistant strains from around the world for more detailed study by molecular methods and subsequent epidemiological investigations if indicated.

These are further discussed in Section 6.

The laboratories that agree to perform special studies on resistant organisms should have proven ability to perform the most up-to-date techniques to study proteins and nucleic acids, e.g. membrane typing, plasmid profiling, DNA probing, transposon characterization, ribotyping, etc.

#### 6. ROLE OF AN INTERNATIONAL WORKING GROUP AND INTERNATIONAL ORGANIZATIONS

Further effective collaboration and coordination is required between the medical, public, animal and environmental health sectors to solve common problems. Whilst this may exist at national level, there is also an urgent requirement at international level.

An international working group of an intersectoral nature could be formed to establish a system of monitoring and surveillance of antimicrobial resistance, to integrate the results into a global programme and to implement measures common to each of the sectors concerned so that data assessed are comparable. The group could achieve this through:

- (1) developing a world network of experts and institutions willing to engage in monitoring and surveillance of antimicrobial resistance, in collaboration with WHO and other international organizations (a list of FAO/WHO Collaborating Centres and of International Organizations is given in Annex I; a specimen questionnaire on monitoring and surveillance of antimicrobial resistance which has previously been used, is given in Annex II);
- (2) collecting current information from each of the sectors, including water and food safety authorities, to organize information exchange, and producing a report and/or circular letter on a regular basis for distribution to interested institutions and countries;

- (3) establishing methods for antimicrobial susceptibility testing common to all concerned sectors, and being responsible for epidemiological monitoring and surveillance and assisting WHO to establish prevention and control measures;
- (4) organizing meetings on epidemiological aspects of antimicrobial resistance, including molecular epidemiology, bacterial and genetic mechanisms of resistance, mode of transmission of resistant organisms, and research requirements;
- (5) organizing training courses, seminars, workshops on current topics and producing meeting reports;
- (6) taking initiatives, at international level, to develop a protocol for the proper use of antimicrobial substances that will be of maximum benefit to human and animal health, whilst protecting the safety of water, food and the environment;
- (7) collect data on antimicrobial usage and to correlate usage with emergency of resistance, nationally and globally;
- (8) advising on the establishment and provision of reference materials for antimicrobial susceptibility testing; and
- (9) developing coordinated computer programmes for the best use of available data for planning, management and assessment of international activities, and using electronic communication for improved effectiveness and efficiency in communication between participating parties.

International organizations should develop common ground for the planning and management of international cooperation on the prevention and control of antimicrobial resistance, and should be responsible for coordinating and financing the working group activities at global level.

## 7. RECOMMENDATIONS

- 7.1 An International Working Group of an intersectoral nature should be formed to establish a system of monitoring and surveillance of antimicrobial resistance and to integrate the results into a global programme.
- 7.2 National Central/Reference centres should be established to conduct surveillance on antimicrobial resistance using agreed methods and to report the results to the International Working Group.
- 7.3 In order to achieve these objectives, it is essential to have continued support from the World Health Organization, other international organizations and from national Governments.

Other recommendations of a scientific/technical nature will naturally follow when recommendations 1 to 3 are implemented.

BACKGROUND REFERENCES

WHO Technical Report Series, No. 616, 1978 (Neisseria gonorrhoeal and gonococcal infections: Report of a WHO Scientific Group).

WHO Technical Report Series, No. 624, 1978 (Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria).

WHO Technical Report Series, No. 673, 1982 (WHO Expert Committee on Biological Standardization), Annex 5: Requirements for susceptibility discs.

WHO Technical Report Series, No. 736, 1986 (WHO Expert Committee on Venereal Diseases and Treponematoses).

Report of an Informal WHO Consultation on Antibiotic Resistance in Agriculture, Geneva, January 1987 (WHO restricted document).

Elaboration of Guidelines for Monitoring and Surveillance of Antimicrobial Resistance in Clinical Medicine, Public Health, and in Agriculture - project document (VPH-RB0007, 5 October 1988).

Biological Substances. International standards for reference reagents. World Health Organization, Geneva, 1987.

ANNEX I

FAO/WHO Collaborating Centres and International Organizations

FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses, Institute of Veterinary Medicine (Robert von Osterag Institute), Postfach 330013, Thielallee 88/92, D-W-1000 Berlin 33

WHO Collaborating Centre for Phage Typing and Resistance of Enterobacteria, Division of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London, NW9 5HT, United Kingdom

WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, The State Medical Institute, Department of Microbiology, Asatiani Street 7, Tbilisi 380077, USSR

WHO Collaborating Centre for Hospital Infections and Antimicrobial Resistance, Central Research Institute of Epidemiology of the USSR, Ministry of Public Health, Novogireevskaya 3-a, Moscow 111123 USSR

WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, Brigham and Women's Hospital, Microbiology Laboratory, 75 Francis Street, Boston, Massachusetts 02155, USA

WHO Collaborating Centre for External Quality Assessment in Clinical Microbiology, University Hospital St Raphael, 33 Capocynervoer, 3000 Leuven, Belgium.

International organizations

International Office of Epizootics (OIE), 12 rue de Prony, 75017 Paris, France

Animal Production and Health Division, Food and Agriculture Organization of the United Nations (FAO), Via delle Terme di Caracalla, 00110 Rome, Italy

World Health Organization (WHO), Avenue Appia, 1211 Geneva 27, Switzerland

ANNEX II

(Sample questionnaire form)

REQUEST FOR INFORMATION OF ANTIMICROBIAL SUSCEPTIBILITY TESTS

We would be grateful if you could fill in the form given below and return it to Chief, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.

1. Name of institution: .....

.....

Address: .....

.....

Telephone: ..... Telex: .....

Name of principal investigator: .....

2. Please provide us with information on your antibiotic and/or antimicrobial resistance programme (and send us copies of your recent publications and/or documentation on the subject) as follows:

- routine diagnosis
- epidemiological surveillance
- research (please specify the subject)
- training and education
- any specific area related to agriculture

3. Indicate the species of bacteria tested:

- (a) Facultative aerobic Gram negative rods (Escherichia, Salmonella, Klebsiella, Yersinia, Vibrio, Pasteurella, Haemophilus.....)
- (b) Aerobic Gram-negative rods (Pseudomonas, Brucella, Bordetella);
- (c) Aerobic/microaerophilic motile curved Gram-negative bacteria (Campylobacter);
- (d) Spirochaetes (Treponema, Leptospira, Borrelia);
- (e) Anaerobic Gram-negative rods (Bacteroides);
- (f) Anaerobic Gram-positive rods (Clostridia);

- (g) Aerobic Gram-positive rods (Bacillus, Corynebacteria, Listeria);
- (h) Mycobacteria;
- (i) Mycoplasmas, Acholeplasmas;
- (j) Gram-positive cocci (Streptococci and Staphylococci);
- (k) Others.

4. Please indicate sources of bacteria tested:

Clinical materials and/or checking materials:

4.1 in man (adult, children, male, female .....  
.....  
.....)

4.2 in animals (cattle, calves, pigs, sheep, goats, horses, poultry  
.....  
.....  
.....)

4.3 Source of materials (blood, faeces, urine, milk, abscess, body  
fluid, discharges, post-mortem, swabs .....  
.....  
.....)

4.4 Food (vegetables, fish and shell fish, food of animal origin ....  
.....  
.....  
.....)

4.5 Sampled from environment (water, soil, bedding, floor, pens .....  
.....  
.....  
.....)

5. Antibiotics and/or antimicrobial substances used for testing (please circle, as appropriate/insert the names which are not in the list and provide information on the quantities used for the test (g):

Aminoglycosides

Gentamicin  
Kanamycin  
Streptomycin

Cephalosporins

Penicillins

Ampicillin  
Benzylpenicillin  
Methicillin

Other B-Lactams

Sulphonamides

Sulphadiazine  
Sulphamethazine

Tetracyclines

Chlortetracycline  
Doxycycline  
Methacycline  
Oxytetracycline

Others

Amphotericin B  
Bacitracin  
Chloramphenicol  
Erythromycin  
Flavomycin  
Ketoconazole  
Lincomycin  
Leucomycin  
Nalidixic acid  
Novobiocin  
Nystatin  
Oleandomycin  
Polymyxin B  
Rifampicin  
Spectinomycin  
Trimethoprim  
Tylosin  
Vancomycin  
Virginiamycin

Please indicate any other antimicrobial substances or antimicrobial substance you use.

5.1 Please provide details on quality control and the medium used for the test.

6. Do you conduct MIC estimations on representative strains?

7. Do you publish your results annually/periodically in the scientific press/annual reports?

8. Do you follow the procedures indicated in the Thirty-Second Report of the WHO Expert Committee on Biological Standardisation, Annex 5: "Requirements for antimicrobial susceptibility tests I. Agar diffusion test using antimicrobial susceptibility discs (Requirements for Biological Substances No. 26) (Technical Report Series, No. 673, WHO, Geneva, 1982)?

If you do not follow this procedure, what procedure is used?

9. Please provide your overall comments on antibiotic and/or antimicrobial susceptibility testing and indicate how you would like to see WHO develop its future activities in this area.

Thank you very much for your cooperation.

Signature: .....

Name (in print): .....

Date: .....

### ANNEX III

## GENETIC MECHANISMS OF ANTIMICROBIAL RESISTANCE

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### General principles

Basically there are three major types of mechanisms which produce antimicrobial-resistant bacteria: Antimicrobial substances are biochemically active substances which interact with molecules within the cells. So the first general principle in order to survive antimicrobial action is to lack the target site. This type of resistance called "natural resistance" defines the spectrum of the antimicrobial substance. Natural resistance is in some cases a criterion of a species and it is inherited vertically among the offspring. Natural resistance, as opposed to acquired resistance, cannot be influenced by monitoring programmes.

The second type of resistance requires at least one mutational event. It has the effect that, in bacteria which are in general susceptible to the antimicrobial substance, the target site of the antimicrobial substance is altered or in some way its penetration into the cell is diminished. The site of mutation is located on the bacterial chromosome and this type of resistance is consequently called "chromosomal resistance". Sometimes the resistance levels achieved can be extremely high especially when multiple mutational events are involved. Chromosomal resistance can be of clinical importance; however its mechanism of inheritance is also vertical and can be restricted to the species involved. This mechanism is important when there are ready means for disseminating such resistant strains, e.g. in hospitals or in animal husbandry. Once mutant strains have developed, they are likely to persist.

In contrast, the major public health problem in antibiotic resistance originates from the third type of mechanism - the possession of bacterial plasmids located within the cell but outside the chromosome. Plasmids carrying resistance genes are called R-factors and can either be passed on to the progeny of the cell or be exchanged among members of a bacterial population or, most importantly, even to other bacterial species. The process of plasmid transfer is called conjugation and the plasmids involved, transferable R-factors. These can occasionally carry non-transferable R-factors into other cells by mobilization.

Transferable R-factors are composed of two genetic units. One carries the genes important for conjugation and the second the R-determinants. They are responsible for the resistance phenotype and carry the individual resistance genes. Sometimes resistance genes can be spread by mechanisms other than conjugation. One process, called transformation, involves the uptake of free DNA into the cell and another, called transduction, uses bacteriophages as carriers of the DNA. An additional process of DNA spread called transposition leads to the integration of a resistance gene into another site on a DNA molecule, whether it is the chromosome, a plasmid or a bacteriophage. Resistance genes spread by this mechanism are called transposons.

Analysis of antibiotic-resistant bacteria has shown that there are several strategies by which resistance genes can prevent the action of the antibiotic. The first is the biochemical conversion of the antibiotic into an inactive compound. Secondly the level of the antibiotic within the cell can be kept low. The third strategy involves the alteration of the target site and finally a new target molecule which substitutes for the susceptible one can be synthesized.

Depending on the mechanism of action of the antibiotic, either of the strategies given above can be used; these are briefly described for the major families of antimicrobial substances.

#### Individual resistance mechanisms

##### Penicillins and Cephalosporins

These antimicrobial substances interact with binding proteins in the cells and interfere with cell wall synthesis. For the  $\beta$ -lactam antimicrobial substances several resistance mechanisms can be demonstrated. Whilst chromosomal resistance occurs in *Neisseria*, *Streptococcus*, *Staphylococcus* and *Pseudomonas*, resistance due to plasmid-encoded  $\beta$ -lactamases predominates. Their mode of action is the hydrolysis of the  $\beta$ -lactam ring. Numerous enzymes have been described and are still being investigated. The major groups are the triethylene melamine (TEM) type enzymes which are specified by plasmids in Gram-negative bacteria. Recently, mutant plasmid-mediated lactamases have been described which are able to inactivate third generation cephalosporins. Chromosome-encoded  $\beta$ -lactamases have also been described.

##### Tetracyclines

Tetracyclines inhibit protein synthesis by interacting with the 30S subunit of the ribosomes. The  $\beta$ -lactam antimicrobial substances along with the tetracyclines have been used for a long time, not only for therapy but also for growth promotion in animals. As a result, tetracycline resistance is widespread. Ten different resistance determinants have been described, 5 for Gram-negative and 5 for Gram-positive bacteria. Their major action is to influence the amount of the antibiotic in the cell. This is achieved by decreasing the influx and especially increasing the efflux of the drug. In a second mechanism of resistance, the ribosomes are protected from antibiotic action. Tetracycline resistance genes are often located on transposable elements and classes A, B and M determinants are disseminated widely among the bacterial population.

##### Aminoglycosides

Aminoglycosides exert multiple effects on the ribosomes and interfere with translation. Although chromosomal resistance can occur, most clinically significant resistance is due to enzymes specified by R-factors. The enzymes are situated in the periplasmic space and modify the antimicrobial substances in such a way that they no longer bind to the ribosomes.

The enzymes involved are aminoglycoside-N-acetyltransferase (AAC), aminoglycoside-N-phosphotransferase (APH) and aminoglycoside-O-nucleotidyltransferase (AAD). These enzymes show substrate specificity and the level of resistance can be dependant on the host strain.

### Chloramphenicol

Chloramphenicol acts on bacterial protein synthesis by interacting with the ribosome and blocking translation. In clinical isolates, resistance is mainly due to the production of chloramphenicol-acetyltransferase (CAT). In Gram-negative bacteria, at least three partly related CATs have been described but they differ from those enzymes described for Gram-positive bacteria. The type I CAT of Gram-negative species which has been studied intensively also confers resistance against fusidic acid.

### Sulphonamide and trimethoprim

The sulphonamides and trimethoprim exhibit their antimicrobial action by competitively binding to important bacterial enzymes. The enzyme blocked by sulphonamides is dehydropteroate synthetase and the one blocked by trimethoprim is dihydrofolate reductase. In both cases resistance is usually due to plasmid-encoded alternative enzymes which are not inhibited by either antimicrobial substance.

### Macrolide, lincosamides and streptogramins (MLS)

These antimicrobial substances inhibit the chain elongation step during protein synthesis and resistance is the result of the specified resistant gene modifying the 23S ribosomal RNA. This leads to the formation of ribosomes which are not susceptible to antimicrobial action (MLS resistance phenotype). At least seven rRNA methylases (ermA - G) have been defined. Impermeability to the drug and the production of esterases have also been described.

### Quinolones

Quinolones act on susceptible cells by interacting with a subunit of DNA gyrase which breaks and rejoins strands of DNA. The resistance to the oldest known agent-nalidixic acid, is due to chromosomal mutations in the *gyrA* gene. Resistance to the newer quinolones seems to be the result of rare chromosomal mutations. Alternations of the *gyrA* gene have been described as well as changes in the permeability of the cell wall. Cross resistance to other antimicrobial substances ( $\beta$ -lactams) has been observed in some species. Resistance levels are still low but the incidence is increasing rapidly in some bacterial species.

### Outlook for monitoring individual resistance genes

DNA-DNA hybridization is a valuable tool for monitoring the epidemiology of individual resistance genes. DNA-probes for  $\beta$ -lactam, aminoglycoside, MLS, tetracycline, chloramphenicol, and trimethoprim resistance genes are available. However, their use is confined to specialized laboratories.

The epidemiological use of nucleic acid probes will become more practical and widespread when sensitive, non-radioactively labelled probes become available.

ANNEX IV

WHONET

(A programme for entry and analysis of antimicrobial susceptibility test results)

WHONET is a set of programmes designed under WHO auspices for the entry and analysis of antimicrobial susceptibility test results in individual laboratories and also for comparison of results between laboratories differing in patient and isolate mix and in antimicrobial substances tested. WHONET utilizes a universal file format permitting summation and analyses of primary data from all of the participating laboratories. The programmes are written in BASIC and QuickBASIC and are supported by IBM compatible personal computers. They are written as executable files accessed from the DOS environment through a series of batch files.

Individualized version of WHONET adjusted for specific patient location, or host animal species and for the sets of antimicrobial substances tested by a particular laboratory can be formulated for participating laboratories from information entered in specification forms, as are the WHONET codes for specimen sites, isolate species and antimicrobial substances tested.

Individualized WHONET programmes are sent to the laboratories on diskettes. Copies of the laboratories' data diskettes may be sent to regional, national or WHO centres for multicentre surveillance analyses, which can also be run by any of the individual laboratories wishing to do so.

In addition to the data entry programme, each WHONET laboratory has three data analysis programmes.

RISINT tabulates for any species the percent of isolates categorized as resistant, intermediate or susceptible to each of the tested antimicrobial substances. This programme also performs two other types of analysis. It tabulates for any species the percent of its isolates having each value from 6mm to 42mm for the diameters of its zones of inhibition around each antimicrobial disk tested. It also produces histograms of the distributions of these values for specified antimicrobial substances.

SCATTINT plots a scattergram of the inhibition zone diameter value of isolates of any bacterial species to any pair of tested antimicrobial substances.

CLUSTINT tabulates the percentage of isolates of any bacterial species that are resistant to every possible combination of a set of antimicrobial substances.

WHONET also has report options such as line listings of isolates by species and line listings of isolates by species and by antimicrobial resistance patterns. In addition, each laboratory has the capability of editing files by any other criteria it wishes.

WHONET is designed to offer to each laboratory data management capabilities for laboratory quality control and centre infection control sufficient to justify the effort of data entry. Multicentre surveillance of antimicrobial resistance then becomes a byproduct with little added effort.

SPECIFICATIONS FOR A WHONET PROGRAMME

Please provide on the following forms the information needed to make a WHONET programme specifically for your medical centre.

1. Patient locations in hospital or clinic

Please list under LOCATION the names of patient locations in your centre, and add under ABBREVIATIONS the three letters or numerals you wish to use when entering the data. Then give each location a number representing the category that best describes it.

Location Categories are:

- |                   |                |              |
|-------------------|----------------|--------------|
| 1. Medicine       | 5. Paediatrics | 9. Community |
| 2. Surgery        | 6. Neonatal    | 10. Mixed    |
| 3. Intensive Care | 7. Emergency   | 11. Other    |
| 4. Obstetric/Gyn  | 8. Outpatient  |              |

2. Antimicrobial substances tested

List the antimicrobial substances tested in your laboratory in the sequence in which they are read. List the name of the substance and the disk number as given on the attached master list of susceptibility disks. Indicate by checks whether that disk is used to test Gram-positive isolates, Gram-negative isolates, Gram-negative urine isolates or Pseudomonas isolates. Breakpoints will be by the latest NCCLS standards unless you indicate otherwise.

Send completed forms to: Dr Thomas F. O'Brien, WHO Collaborating Centre for Surveillance of Antimicrobial Resistance Microbiology, Brigham and Women's Hospital, Boston, MA 02115, USA (FAX 617-732-4144)





SPECIMEN LIST

No.	Code	Specimen
01	no	Nose
02	th	Throat
03	sp	Sputum
04	br	Bronchial
05	ea	Ear
06	ey	Eyes
11	ur	Urine
12	bl	Blood
13	sf	CSF
14	lu	Lung/Pleural fluid
15	ab	Abd./Perit.fluid
16	ga	Gastric fluid
21	wd	Wound
31	og	Organ
41	st	Stool
51	gn	Genital
61	bo	Bone
90	ot	Other
92	di	Dialysis fluid
93	ar	Artificial
99	ct	Quality Control

ORGANISM LIST

No.	Code	Organism Name	No.	Code	Organism Name
1	sau	Staph. aureus	51	leg	Legionella
2	sep	Staph. epidermidis	52	him	Haemophilus influenzae
3	mic	Micrococcus	53	hph	Haemophilus parahaemolyticus
4	sar	Sarcina	54	hhe	Haemophilus haemolyticus
5	dxs	non-enterococcus Grp.D	55	vic	Vibrio cholerae
6	bse	B-haemolytic strep. Grp.E	56	hpi	Haemophilus parainfluenzae
7	lmo	Listeria monocytogenes	57	ha-	Haemophilus, unspecified
8	s--	Staph. unspecified	58	sha	Shigella sonnei
9	cdp	Diphtheroids, corynebacterium spp.	59		
10	spn	Strep. pneumoniae	60	pae	Pseu. aeruginosa
11	svi	Strep. viridans, A-hemolytic	61	ps-	Pseu. others
			62	pce	Pseu. cepacia
			63	pfl	Pseu. fluorescens, Pseu. putida.sp
12	bsc	B-haemolytic strep. Grp.B	64	pma	Pseu. maltophilia
13	bsb	B-haemolytic strep. others	65	ppi	Pseu. picketti
14	ent	Enterococcus	66	sat	Salmonella typhi
15	bsa	B-haemolytic strep. Grp.A	67	saa	Salmonella paratyphi A
16	bs-	B-haemolytic strep. others	68	sab	Salmonella paratyphi B
			69	sam	Salmonella typhimurium
17	rs-	Gamma strep, non-haemolytic	70	sa-	Salmonella other serotypes
			71	shc	Shigella boydii
18	ans	Anaerobic strep.	72	cam	Campylobacter spp
19	bef	B-haemolytic strep. Grp.F	73	ci-	Citrobacter spp
20	eco	E.coli	74	edw	Edwardsiella
21	arh	Arizona	75	shi	Shigella
22	aer	Aeromonas	76	bba	Bathesda ballerup
23	br-	Brucella spp	77	shd	Shigella dysenteriae
24	yer	Yersinia spp.	78	shb	Shigella flexnerii
25	bsg	B-haemolytic strep. Grp.G	79	ach	Achromobacter spp.
26			80	pac	Pasteurella-actinobacillus spp; Cardiobacterium hominis
27	klu	Kluyvera sp.	81	aan	Acinetobacter calcoaceticus var. anitratus (Herrella)
28			82	bbr	Bordetella bronchiseptica
29	Kl-	Klebsiella, others	83	nme	Neisseria meningitidis
30	en-	Enterobacter, others	84	mo-	Moraxella spp. (Mima polymorpha, var. oxicase)
31	ecl	Enterobacter cloacae	85	sma	Serratia marcescens
32	eae	Enterobacter aerogenes	86	cfr	Citrobacter freundii
33	slq	Serratia liquefaciens	87	ac-	Acinetobacter, unspecified
34	hal	Hafnia alvei	88	alw	Acinetobacter calcoaceticus var. Lwoffii (Mima)
35	kpn	Klebsiella pneumoniae	89	pst	Prov stuartii, Prov. alcalifaciens
36			90	se-	Serratia, others
37	eag	Enterobacter agglomerans	91	cdi	Citro. diversus, C. div-
38	kox	Klebsiella oxytoca			
39	erw	Erwinia, Pectobacterium			
40	pr-	Proteus, unspecified			

ORGANISM LIST (cont.)

No.	Code	Organism Name	No.	Code	Organism Name
41	pmi	Proteus mirabilis			levinia, C.amalonaticus
42			92	clo	Clostridium spp.
43	pvu	Proteus vulgaris	93	ne-	Neisseria sp.
44	mmo	Morganella morganii	94	bcs	Bacillus sp.
45	pre	Prov. rettgeri, Prov.	95	ngo	Neisseria gonorrhoeae
46			96	bac	Bacteroides spp.
47			97	fla	Flavobacterium spp.
48			98	eik	Eikenella corrodens
49			99	oth	Others
50					

MASTER LIST

NCCLS Standard Zone Cutoffs

No.	Code	Antibiotic Disk	Potency	Breakpoints
1	AMK	AMIKACIM	30g	15 - 16
2	ACA	AMOXICILLIN/CLAVULANIC	20/10g	14 - 17
3	AXS	AMOXICILLIN/SULBACTAM	10/10g	14 - 16
4	AMP	AMPICILLIN	10g	14 - 16
5	AMS	AMPICILLIN/SULBACTAM	10/10g	14 - 16
6	AZL	AZLOCILLIN	75g	15 - 17
7	ATM	AZTREONAM	30g	16 - 21
8	BAC	BACITRACIN	10units	9 - 12
9	CAR	CARBENICILLIN	100g	20 - 22
10	CFC	CEFACLOR	30g	15 - 17
11	MAN	CEFAMANDOLE	30g	15 - 17
12	FZN	CEFAZOLIN	30g	15 - 17
13	CID	CEFONICID	30g	15 - 17
14	CFP	CEFOPERAZONE	75g	16 - 20
15	FTX	CEFOTAXIME	30g	15 - 22
16	CTN	CEFOTETAN	30g	13 - 15
17	FOX	CEFOXITIN	30g	15 - 17
18	CAZ	CEFTAZIDIME	30g	15 - 17
19	ZOX	CEFTIZOXIME	30g	15 - 19
20	CRO	CEFTRIAZONE	30g	14 - 20
21	FRX	CEFUROXIME SODIUM	30g	15 - 17
22	FRA	CEFUROXIME AXETIL	30g	15 - 22
23	KEF	CEPHALOTHIN	30g	15 - 17
24	CHL	CHLORAMPHENICOL	30g	13 - 17
25	NOX	CINOXACIN	100g	15 - 18
26	CIP	CIPROFLOXACIN	5g	16 - 20
27	CLI	CLINDAMYCIN	2g	15 - 20
28	COL	COLISTIN	10g	9 - 10
29	DIB	DIBEKACIN		11 - 22
30	DOX	DOXYCYCLINE	30g	13 - 15
31	ENX	ENOXACIN	10g	15 - 17
32	ERY	ERYTHROMYCIN	15g	14 - 22
33	FOS	FOSFOMYCIN	50g	13 - 17
34	FUS	FUSIDIC ACID		12 - 11
35	GEN	GENTAMICIN	10g	13 - 14
36	IPM	IMIPENEM	10g	14 - 15
37	KAN	KANAMYCIN	30g	14 - 17
38	LIN	LINCOMYCIN		15 - 16
39	MET	METHICILLIN	5g	10 - 13
40	MEZ	MEZLOCILLIN	75g	18 - 20
41	MIN	MINOCYCLINE	30g	15 - 18
42	MOX	MOXALACTAM	30g	15 - 22
43	NAF	NAFCILLIN	1g	11 - 12

No.	Code	Antibiotic Disk	Potency	Breakpoints
44	NEG	NALIDIXIC ACID	30g	14 - 18
45	NEO	NEOMYCIN	30g	13 - 16
46	NET	NETILMIGIN	30g	13 - 14
47	FUR	NITROFURANTOIN	300g	15 - 16
48	NOR	NORFLOXACIN	10g	13 - 16
49	NOV	NOVOBIOCIN	5g	17 - 16
50	OFL	OFLOXACIN		12 - 19
51	OXA	OXACILLIN	1g	20 - 19
52	PEF	PEFLOXACIN	5g	17 - 21
53	PEN	PENICILLIN G	10 units	20 - 27
54	PPA	PIPEMIDIC ACID	20g	15 - 17
55	PIP	PIPERACILLIN	100g	18 - 20
56	POL	POLYMXIN B	300units	9 - 11
57	RIF	RIFAMPIN	5g	17 - 19
58	RIP	RIFAMPIN	30g	15 - 18
59	SPC	SPECTINOMYCIN		0 - 0
60	STR	STREPTOMYCIN	10g	12 - 14
61	SUL	SULFONAMIDES	250-300g	13 - 16
62	TET	TETRACYCLINE	30g	15 - 18
63	TIC	TICARCILLIN	75g	15 - 19
64	TCA	TICARCILLIN/CLAVULANIC	75/10g	15 - 19
65	TOB	TOBRAMYCIN	10g	13 - 14
66	TMP	TRIMETHOPRIM	5g	11 - 15
67	SXT	TRIMETHOPRIM/SULFAMETHO	1.25/23.75g	11 - 15
68	VAN	VANCOMYCIN	30g	10 - 11

Breakpoint exceptions for special species

Organism	Drug	Spec.	Zones
Gram positive sp.	AMP	all	22 - 29
Staphylococcus sp.	ACA	all	20 - 19
Staphylococcus sp.	AMP	all	29 - 28
Staphylococcus sp.	OXA	all	11 - 12
Staphylococcus sp.	PEN	all	29 - 28
Enterococcus	AMP	all	17 - 16
Enterococcus	PEN	all	14 - 15
Listeria monocytoge	AMP	all	20 - 19
Listeria monocytoge	PEN	all	19 - 20
Neisseria gonorrhoeae	PEN	all	20 - 19
Pseudomonas sp.	CAR	all	14 - 16
Pseudomonas sp.	MEZ	all	15 - 17
Pseudomonas sp.	PIP	all	15 - 17
Pseudomonas sp.	TIC	all	12 - 14
Pseudomonas sp.	TCA	all	12 - 14
Haemophilus sp.	ACA	all	19 - 20
Haemophilus sp.	AMP	all	22 - 24
Haemophilus sp.	AMS	all	19 - 20

Organism	Drug	Spec.	Zones
Haemophilus sp.	ATM	all	26 - 25
Haemophilus sp.	CFC	all	19 - 23
Haemophilus sp.	MAN	all	21 - 23
Haemophilus sp.	CID	all	21 - 23
Haemophilus sp.	FTX	all	26 - 25
Haemophilus sp.	CAZ	all	26 - 25
Haemophilus sp.	ZOX	all	26 - 25
Haemophilus sp.	CRO	all	26 - 25
Haemophilus sp.	FRX	all	21 - 23
Haemophilus sp.	CHL	all	26 - 28
Haemophilus sp.	CIP	all	21 - 20
Haemophilus sp.	IPM	all	16 - 15
Haemophilus sp.	RIF	all	17 - 19
Haemophilus sp.	TET	all	26 - 28
Haemophilus sp.	SXT	all	11 - 15

ANNEX V  
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