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INTRODUCTION

Why should these tests be performed?

Antimicrobial testing in veterinary practice is an essential tool for the treatment of infectious diseases and for monitoring therapeutic success, especially in livestock. The on-going, dramatic increase in resistant bacterial strains further emphasizes the need for target-orientated therapy rather than a shot-gun approach.

How is it done?

The two major approaches to antimicrobial susceptibility testing that evolved during the last four decades are the diffusion method and the dilution method. The disk agar diffusion method is a highly standardized and an acceptable qualitative test used for the determination of in vitro susceptibility of most clinical isolates. Many diagnostic laboratories now prefer to report results qualitatively as susceptible (S), intermediate (I) or resistant (R). Broth dilution techniques are regarded as the benchmark, but are not routinely performed by all laboratories due to the cumbersome nature of the tests. Semi-automated microtitration systems have overcome some constraints of broth dilution tests, but their cost will preclude their routine use in veterinary laboratories.

There are two basic methods used for doing the disc diffusion test. The Kirby-Bauer method is the method standardized and described by the National Committee for Clinical Laboratory Standards (NCCLS). It is based on the measurement of zones of inhibition of bacterial growth on an agar surface. The Stokes method compares the inhibition zone sizes when the test and control organisms are inoculated on the same plate.

The National Committee for Clinical Laboratory Standards (NCCLS), 771 East Lancaster Avenue, Villanova, Pennsylvania, USA, is a non-profitable, educational organization that provides a communication forum for the development, promotion, and use of national and international standards. Founded in 1968 and accredited by the American National Standards Institute, NCCLS is based on the principle that voluntary consensus standards are essential for maintaining the performance of the clinical laboratory at the high level necessary for quality patient care. Universal adoption of the Kirby-Bauer procedure for disk diffusion susceptibility testing in veterinary laboratories should be encouraged. It will contribute to harmonization of antimicrobial susceptibility testing in veterinary medicine.

SELECTION OF BACTERIA

Direct sample antimicrobial test

In emergencies, clinicians may request that a direct sample antimicrobial test be done. It is however, subject to many variables, which should be taken into account when interpreting your results:

- Sample has an unknown concentration of bacteria.
- Sample may contain more than one organism, including contaminants or normal commensals.
- Certain bacteria present in a sample may have a faster growth rate than other bacteria.

Should you decide to make use of this method, only samples that have been taken with great care from body sites not usually colonized by bacteria e.g. pleural fluid should be selected. Before inoculating the sample, examine a stained smear of the sample material under a light microscope to find out if bacteria are present in sufficient numbers and that only one morphological type is present. This test is repeated after isolating the suspect bacteria in pure culture.

**After culturing the bacteria**

Before performing the test, one should distinguish between contamination, normal microflora and pathogens. Only pathogens should be selected. Thus a good knowledge of the normal microflora and expected pathogens of the diverse organ systems is a prerequisite.

To select what antimicrobials to use in the test, a provisional identification of the organism can be made, by colony characteristics, Gram's stain, catalase and oxidase reactions.

**QUALITATIVE ANTIMICROBIAL SENSITIVITY TESTING**

**The Kirby-Bauer disc diffusion method as recommended in NCCLS document M31-P**

**Medium**

Mueller-Hinton agar is recommended and only this medium or an acceptable equivalent should be used. For fastidious organisms such as *Streptococci*, which are unable to grow on Mueller-Hinton agar, 5% defibrinated blood can be added. Pour the medium into Petri dishes to a depth of 4 mm per circular 90 mm dish. Before use, the plates should be checked for droplets of moisture. If present on the surface of the agar, plates should be dried in a laminar flow cabinet for a few minutes with the lids ajar. The pH of the medium should be checked at the time of preparation and should be within the range 7.2 - 7.4.

**Inoculation**

Touch at least 4 morphologically similar colonies growing on a non-selective agar plate with a wire loop. Transfer to a test tube containing 4 ml tryptone soya broth or tryptose phosphate broth or soybean-casein digest broth. Incubate for two or more hours at 35 - 37°C until a visible turbidity appears that exceeds that of the 0.5 MacFarland standards.

Plates should be inoculated within 15 minutes of inoculum preparation. A sterile, non-toxic swab is dipped into the suspension and the excess fluid removed by rotation of the swab against the side of the tube above the fluid level. The swab is streaked in three directions over the entire surface of the agar.
An alternative method is available to standardize the inoculum of bacteria that do not grow well in broth cultures, e.g. *Actinobacillus pleuropneumoniae*. The colonies are again picked from a non-selective medium such as blood agar, but directly transferred into saline and adjusted to match the turbidity standard.

**Susceptibility disks**

Apply disks to the dried plates using sterile forceps or an antibiotic disk dispenser. Press down gently to ensure even contact with the medium. Place six to a maximum of eight disks on a 90 mm plate.

**Incubation**

Plates should be incubated aerobically, except for capnophilic bacteria such as *Histophilus somni* or *Trueperella (Arcanobacterium) pyogenes*. The latter can be incubated in a candle jar, and the results reliably reported if the control organisms gave acceptable values under those circumstances.

Plates should be incubated inverted for 16 - 18 hours at 35 - 37 C, but 24 hours for the staphylococci. The latter procedure will help with the detection of methicillin-resistance (see later).

**Results**

The resulting zone diameters are measured to the nearest millimeter with calipers or a millimeter rule held on the back of the plate. The zone edge should be taken as the area showing no obvious visible growth, ignoring faint growth, or tiny colonies that can be detected only with difficulty at the edge of the zone. The interpretation of each zone is done with the aid of a published table and the pathogen placed into one of the following categories (See Figure 1)

- **Susceptible**: the infection may respond to the treatment at the normal dosage.
- **Intermediate**: the pathogen may be inhibited by attainable concentrations of the antimicrobial agent provided a higher dosage is used, or the pathogen is in a certain body site, such as urine, where the drug is physiologically concentrated.
- **Resistant**: the bacterium is not inhibited by the usually achievable systemic concentrations of the antimicrobial agent and treatment with this agent cannot be considered.
Figure 1: Schematic example and evaluation of a sensitivity test, agar diffusion method.

Updated zone diameter interpretive standards for veterinary medicine can be found in NCCLS document M31-P.

**Controls**

Three control organisms should be tested regularly using the standard procedure.

- *Staphylococcus aureus* ATCC 25923
- *Escherichia coli* ATCC 25922
- *Pseudomonas aeruginosa* ATCC 27853

Zone sizes should be recorded and if the standard deviation, or range, or mean of five successive observations is outside the limits given by the NCCLS, the techniques should be investigated for sources of error.
Beta lactamase (penicillinase) producing staphylococci

Beta lactamases are enzymes produced by some bacteria and are responsible for its resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems. Some lactamase producing staphylococci yield zones of inhibition that creates a false impression of sensitivity. Those organisms can, however, be recognized as penicillinase producers because the colonies at the edge of the zone are large and well developed, and there is no gradual fading away of growth towards the disk as there is with the control strain. These strains must be reported as resistant, also against cephalosporins, regardless of the cephalosporin zone size.

Methicillin-resistant (heteroresistant) *Staphylococcus* is more likely to be detected with oxacillin disks and can be missed with methicillin or cloxacillin disks. The inoculum should also be prepared the alternative way (see Inoculation). Heteroresistant *Staphylococci* are usually resistant to multiple antibiotics, e.g. aminoglycosides, macrolides, clindamycin, tetracyclines and lactams. The latter observation can also serve as a clue to resistance to penicillinase-resistant penicillins such as cloxacillin, dicloxacillin, methicillin, nafcillin and oxacillin.

**Summary**

The following sources of error in disk diffusion testing should be considered:

- Use of a medium other than Mueller-Hinton agar alters zone sizes.
- Changes in agar depth alter zone sizes produced by some antimicrobial drugs.
- A fall in pH of the medium alters zone sizes produced by some antimicrobial drugs.
- Inoculum size is an important determinant of zone diameter.
- Antibiotic disks must be properly stored to avoid deterioration. Freeze containers at -14°C or lower except the current working stock that should be kept between 0 – 8°C.
- Incubation must be maintained at 35-37°C.
- Premature reading of zones may produce incorrect susceptibility patterns.

![Figure 2: Influence of inoculum size on the resulting zones of inhibition](image)
QUANTITATIVE ANTIMICROBIAL SUSCEPTIBILITY TESTING

The standard used to measure bacterial susceptibility to a drug in vitro is the minimum inhibitory concentration (MIC), the drug concentration that just inhibits the visible growth of an organism. The conventional tests for determining the MIC include the broth microdilution method and agar incorporation tests.

**Dilution techniques**

Tests are performed using serial twofold dilutions of the antimicrobial agent in broth inoculated with a standardized suspension of bacteria. The broth is examined for visible growth after 18 hours of overnight incubation at 35–37°C.

The lowest concentration of antimicrobial drug that completely inhibits visible bacterial growth is the MIC. The minimum bactericidal concentration (MBC) can be determined by sub culturing all tubes that showed no evidence of growth onto antibiotic-free agar. After additional overnight incubation the lowest concentration of antibiotic which showed sterility on subculture is the minimum bactericidal concentration.

The microdilution broth method makes use of sterile, plastic 96-well plates. When these plates are purchased from commercial sources, they usually have dried antimicrobial agents in its wells. The agents are put into solution by adding diluent and inoculum together, or separately, to each well. A typical series of two-fold dilutions will run for example from 64 μg/ml to 0.5 μg/ml. As with the disk diffusion test, broth media, inoculum standardization, incubation and interpretation of results are described in NCCLS document M31-P.
Materials

- Agar plates containing a fresh pure culture of the bacteria to be tested.
- An example of a commercial microdilution method is VetMIC™ consisting of dried two-fold dilutions of the required antimicrobials in a 96-well plate. (Department of Antibiotics, National Veterinary Institute (SVA), Sweden. You can also make up your own antibiotic dilutions.
- Transparent covering tape provided together with the VetMIC™ plates
- 12 chamber (for us it is more convenient with 4 chambers, we pour the inoculum into Petri dishes and fill the pipette from these) multi-channel automatic pipette that deliver 50 µl volumes
- Sterile Petri dishes or “V” channels to pour the inoculum into
- Single chamber automatic pipette than can deliver 1-10 µl volumes
- Sterile pipette tips
- Inoculation loop that delivers approximately 1 µl
- Viewing device in the form of a rack with an enlarging mirror
- Bench light
- 0.5 MacFarland Standard
- Cation-adjusted Mueller-Hinton broth (for members of the family Pasteurellaceae HTM broth is preferred)
- Sterile horse serum
- Sterile distilled water with 0.02 % Tween 80
- MIC score sheet

Procedure

Preparation of the bacterial inoculum

Standard procedure

- Touch 3 to 5 colonies of bacteria from cultures < 7 days of age and transfer to 5 ml cation-adjusted Mueller-Hinton broth (CAMHB).
- Incubate at 35-37°C until the suspension reaches a turbidity approximating 0.5 MacFarland standard (for most bacteria 2 to 6 hours). This results in a suspension containing approximately 10⁶ CFU/ ml.
- Vortex well and transfer 1-10 µl to 10ml CAMHB to give a bacterial concentration of approximately but not above 5 x 10⁵ CFU/ml.
Alternative procedure

- Alternatively material from at least 3 to 5 colonies from an 18 to 24-hour agar plate culture can be directly inoculated into 2 ml sterile deionized/distilled water with 0.02% Tween 80 and the turbidity adjusted to a 0.5 MacFarland standard.
- Immediately vortex well and transfer 1-10 µl to 10ml CAMHB to give a bacterial concentration of approximately but not above 5 x 10^5 CFU/ml.
- In the case of *M. haemolytica*, the above alternative method and *Haemophilus* test medium (HTM) is recommended.
- Unless the broth batch and serum has been successfully tested for performance regarding MICs of trimethoprim and sulphonamides, 0.2 IU/ml thymidine phosphorylase should be added to the broth to be used for inoculation of the plates when these antimicrobials are tested.

Inoculation procedure

- Inoculate 50 µl of the bacterial suspension into each well of the microtitre plates using a multi-channel automatic pipette.
- Seal the wells with a transparent covering tape.
- 10µl from the inoculum is streaked onto blood agar to check for purity.
- Stack up to a maximum of 4 plates and place in the incubator.
- Incubate in an air incubator at 35 - 37°C for 18 to 24 hours.
- After incubation the inoculum control is checked for purity and the MIC is read as the lowest concentration inhibiting visible growth.
- To ensure reproducibility and comparability of results, established quality control procedures should be followed.

Reading the MIC

The MIC is read as the lowest concentration of antimicrobial that completely inhibits visible growth, or for sulphonamides the lowest concentration that inhibits 80% of growth. The 96-well plate is placed on top of the viewing device. A bench lamp giving indirect light in a dark room facilitates reading. Bacterial growth is easily detected in the mirror mostly as a pellet on the bottom of the well. For certain bacterial isolates the growth may appear only as increased turbidity. The results are recorded on a MIC score sheet (See Figure 3).
Figure 3: VetMIC™

Panel for monitoring of resistance in bacteria

50 μl/well gives concentrations (μg/ml.) as below

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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<tr>
<td></td>
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<td>0.5</td>
<td>16</td>
<td>0.25</td>
<td>0.5</td>
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</tbody>
</table>

Am  Ampicillin  Tc  Oxytetracycline
Ce  Ceftiofur  Su  Sulfamethoxazole
Ef  Enrofloxacin  T  Trimethoprim
Nal Nalidixic acid  Gm  Gentamicin
Va  Vancomycin  Nm  Neomycin
Em  Erythromycin
Cm  Chloramphenicol

Note: No growth in the tri-cit control wells implies that the strain is sensitive to the citric acid included in the buffer used for Am. In such cases, reading of MIC for Am is not relevant.

Repeat testing with 100μl per well to dilute the citric acid. Note! The concentration in the wells will be half of that noted above. Reading of MICs will have to be adjusted accordingly.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Animal owner</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>
Interpretation of results

From a clinical point of view, the ultimate criterion of antimicrobial susceptibility is the clinical response. To facilitate interpretation, the results of susceptibility tests are often categorised as sensitive (S) or resistant (R) with an intermediate (I) group in between. Breakpoints for these categories are set depending on the pharmacodynamics and toxicity of the drugs, on population distribution of relevant microbes and on clinical experience.

In monitoring programmes, microbiological cut-off values may be preferred. In this case the cut-offs are based on the distribution of the population of the bacterial species tested. Isolates that significantly deviate from the normal, susceptible, population are designated as resistant.

QUALITY CONTROL PROCEDURES

Quality control organisms

At least one quality control organism should be included in each test round and several when a new batch of CAMHB is tested. For these panels *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 are suitable control strains. When results do not fall into the ranges according to the Committee for Clinical Laboratory Standards (NCCLS) the reason for the deviation is clarified and if needed the test is redone. Pure cultures of the quality control bacteria should be used in the test. As it is possible that these organisms can mutate on repeated sub-culture, it is essential that fresh cultures are made on a monthly basis from lyophilised or frozen cultures.
Bacterial growth control

To test for bacterial viability the bacterial inoculum is added to one or two wells without antimicrobials. It also serves as a turbidity control for determining end points.

**Purity control**

A sample of each bacterial inoculum is streaked onto Columbia agar (Oxoid, CA Milsch) containing 5 - 7% horse, sheep or bovine blood and incubated overnight at 37°C to detect mixed cultures and to provide freshly isolated colonies should retesting be necessary.

**Testing for correct inoculum density**

It is necessary to routinely test whether the correct inoculum size is being used in the test. This can be achieved by placing 10 ul of the final inoculum in 10 ml Normal saline. 100ul is taken from this dilution and spread onto an agar plate using a sealed, bent Pasteur pipette that has been flamed in 100% alcohol. A result of 10 to 50 CFU should be present. If not the inoculum concentration should be adjusted accordingly. This inoculum control can also be read for purity control and replace the above described procedure.

**Sterility testing**

Test samples of each batch of CAMHB broth for sterility by incubating four broth vials at 37°C for 48 hours and check for absence of growth (turbidity). A batch is discarded if contaminants are present.

**Criteria for rejection**

1. When the cultures have proven to be mixed after using either the purity control or testing for correct inoculum size methods. This may be due to the inadvertent use of a mixed culture or the use of non-sterile equipment and media.

2. If the recorded MIC for the quality control strain deviates by one or more dilutions from the accepted range the reasons for this should be clarified. If there is an obvious reason for the out-of-control result (contamination, wrong broth, temperature etc.), the QC strain should be retested (and if appropriate other strains as well). If there is no obvious reason for the out-of-control result, the implicated antimicrobial is monitored for five consecutive days. Please note that cultures that have repeatedly been sub-cultured may have mutated and thus bacterial cultures with a low passage should be used. Whenever an out-of-control result for a QC strain is recorded, careful assessment of whether to record or invalidate the results for field isolates should be made on an individual basis. (this is basically an abstract of the instructions provided in NCCLS M7-A6)

3. When the bacterial controls on the plate have not grown within the expected time frame, it may be due to problems with the CAMHB, incubation temperatures or the atmospheric conditions provided.
Storage

VetMIC™ plates – sealed at room temperature until the expiry date.

Once prepared, CAMHB should be stored at 4 °C for a maximum of one month

Reference strains are stored on tryptic soy slants at 2 – 8 °C for up to 2 weeks. Thereafter fresh slants are prepared. Whenever aberrant results occur, a new stock culture is obtained. Check that this matches what is written under “quality control procedures”. For long term storage of reference strains they are either lyophilized or stored in 50 % foetal calf serum in broth or 10 % glycerol in broth and stored at -60°C or lower.

Agar incorporation (agar dilution) tests

These tests are similar to the broth dilution method, with the substitution of liquid for solid media. Mueller-Hinton which supports the growth of most pathogens is used. Plates contain serial twofold dilutions of antibiotic. Standardized suspensions of bacteria are applied to the agar surface with a calibrated loop or the metal pins of an inoculum-replicating device. This allows simultaneous testing of up to 35 strains per 9 cm plate. Inoculum size is less critical and interpretation of the result, as growth or no growth, is easier than measuring and interpreting zones of inhibition.

Several laboratories are now using one or two critical concentrations of each drug required in the so-called "breakpoint" method. It is directly related to MIC measurement. The antimicrobial concentration used (breakpoint concentration) is that which can be achieved at the site of infection during a course of treatment. The results are read as sensitive or resistant where one concentration is used and as sensitive, intermediate or resistant where two concentrations are used. A series of control bacteria of known sensitivity should be included on each plate. MIC's cannot be determined by this method. Updated minimum inhibitory concentration breakpoints for veterinary medicine have been published in NCCLS document M31-P.

New methods for determining MICs

Developments in the field of antimicrobial testing have been limited in recent years. During the late 1980s, the E test was developed in Sweden by Bolmström and Eriksson. The test was launched commercially by AB Biodisk in 1991. It provides a methodology which combines the principle of the agar diffusion test and the determination of MIC values. This test can be used for slow growing bacteria and anaerobes. This test shows a high degree of correlation with MIC values obtained by the classical MIC method. According to the manufacturer's claims the E test is not influenced by the inoculum density or pre-diffusion time. The E test is an antibiotic gradient immobilised onto a thin plastic strip that is directly calibrated with a continuous MIC scale in µg/ml. A strip is placed onto an inoculated agar plate, incubated and the MIC read.
Figure 4: Epsilon-test (schematic). The resulting zone of inhibition (white area) crosses the antibiotic gradient (scale on carrier strip) at the minimum inhibitory concentration (here ca. 0.125 µg/ml).

Applicability of this test to veterinary microbiology is presently limited, due to the high cost of the strips and lack of availability of disks impregnated with veterinary-specific chemotherapeutics.

APPLICATION OF DILUTION METHODS IN VETERINARY MEDICINE

A full MIC usually consists of 15 dilutions. Preparation of media is therefore time consuming and costly in labour. A microtitration method is less time consuming but the cost of a new sterile plate for each batch of tests is a disadvantage. These methods are not suitable for routine testing in veterinary laboratories. The need for MIC’s, however, will occasionally arise when a disk test is equivocal and the animal is critically ill. The E-test may fulfil that objective without the labour and cost implications of the conventional dilution methods. Antimicrobial susceptibilities of aerobes and fastidious organisms such as anaerobes, Haemophilus and beta-haemolytic Streptococci can be quantified with equal accuracy and precision.
SELECTION OF ANTIMICROBIAL DRUGS

Selection of antibiotic disks for use in sensitivity tests depends on availability of drugs and clinical considerations. Occasionally a clinician may specifically request the inclusion of certain drugs, but in general the choice of disks is left to the consulting laboratory. As only twelve antimicrobial agents are usually tested simultaneously, the species approach has been found the most practical. Sensitivity panels for equines, production animals, companion animals and poultry can accommodate the full spectrum of drugs currently used in veterinary medicine (Table 1).

Table 1: A guideline for the selection of antibiotics to be tested in the different animal species

<table>
<thead>
<tr>
<th>Dogs, cats and horses</th>
<th>Cattle and sheep</th>
<th>Cage and aviary birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin-clavulanate (e.g. Synulox, Augmentin)</td>
<td>Tiilmicosin (Mycotil)*</td>
<td>Enrofloxacin*</td>
</tr>
<tr>
<td>Ampicillin (better) or amoxycillin</td>
<td>Cefllofur (Excenel)*</td>
<td>Danofloxacin</td>
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<tr>
<td>Cephalothin or cepalexin or cephradine</td>
<td>Danofloxacin (Advocin)*</td>
<td>Gentamicin</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Enrofloxacin (Baytril)*</td>
<td>Amikacin</td>
</tr>
<tr>
<td>Trimethoprim-potentiated sulphonamides (e.g. Cotrimoxazole</td>
<td>Florfenicol (Nufluor)*</td>
<td>Piperacillin</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole)</td>
<td>Tetracycline*</td>
<td>Cefotaxime (Clavoran)</td>
</tr>
<tr>
<td>Enrofloxacin (Baytril)</td>
<td></td>
<td>Lincospectin</td>
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<td>Erythromycin</td>
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<td>Tetracycline*</td>
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<tr>
<td>Gentamicin</td>
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<td>Apramycin*</td>
</tr>
<tr>
<td>Lincomycin or clindamycin</td>
<td></td>
<td>Florfenicol</td>
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<tr>
<td>Cloxacillin (use an oxacillin disk)</td>
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<td></td>
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<tr>
<td>Orbifloxacin (cats and dogs)</td>
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<tr>
<td>Penicillin G</td>
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<td>Streptomycin</td>
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<td>Tetracycline</td>
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* Cattle in feedlots

<table>
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<th>Additional drugs that can be included for resistant Gram-negative organisms</th>
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<tbody>
<tr>
<td>Amikacin</td>
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<td>Tobramyacin</td>
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<td>Ticarcillin</td>
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<tr>
<td>Carbenicillin</td>
</tr>
<tr>
<td>Polymyxin (local use only)</td>
</tr>
<tr>
<td>Framycetin (local use only)</td>
</tr>
<tr>
<td>Neomycin (for eyes and ears)</td>
</tr>
</tbody>
</table>

* Commercial poultry
The following guidelines may be helpful to select agents known to be both effective and representative of other compounds in their class.

- In view of the similar spectrum of ampicillin and amoxicillin, only one of these agents need be tested routinely.
- Oxacillin diffusion disks are preferred to cloxacillin or methicillin due to the superior stability of oxacillin disks. Methicillin-resistant strains may be missed with cloxacillin or methicillin disks. The Nitrocefin test can also be done.
- A cephalothin disk has a similar antibacterial spectrum to cefaclor, cephalirin, cefazolin, cephradine, cephalixin and cefadroxil. These are first generation cephalosporins with a spectrum of activity directed mostly at Gram-positive organisms. Cefotaxime can represent ceftazidime, ceftriaxone and cefotaxime. These third generation cephalosporins are mainly used against serious Gram-negative infections.
- All tetracycline derivatives are closely related and only tetracycline hydrochloride need be tested routinely.
- Either lincomycin or clindamycin can be included in the panel.
- Erythromycin is a suitable representative for macrolide drugs, e.g. oleandomycin, tylosin, tilmicosin and spiramycin. Tiamulin must be tested separately.
- Aminoglycosides differ significantly in their antimicrobial spectrum. Members such as gentamicin, streptomycin, kanamycin, tobramycin, amikacin are therefore tested individually. The same applies to quinolones such as nalidixic acid, enrofloxacin, danofloxacin and ciprofloxacin.
- Sulphisoxazole is a suitable representative for all the sulphonamides, as is trimethoprim-sulphamethoxazole for trimethoprim-potentiated sulphonamides.
- Either polymyxin B or polymyxin E (colistin) can be included in the panel.
- Chloramphenicol and nitrofurantoin should not be tested for food animals. An alternative is florfenicol.

SUSCEPTIBILITY TESTING OF ANAEROBIC BACTERIA

The Working Group on Anaerobic Susceptibility Testing of the NCCLS published a review in the Journal of Clinical Microbiology, vol. 26, no. 7, July 1988. In this publication the essential recommendation was that good quality specimen collection, transport and processing for presumptive identification of anaerobes should receive precedence over routine susceptibility testing of anaerobes. Anaerobes should not be tested by the disk diffusion method. A standard document has been published by the NCCLS (T11-T2) which describes methods such as agar dilution, broth microdilution, broth macrodilution and broth disk elution for anaerobic sensitivity testing.

The Working Group agreed that susceptibility testing is generally not needed for isolates from sick animals, unless there is a lack of response to an empirical regimen with persistence of the infection. The fact that several antimicrobial drugs have consistently proved effective against anaerobes should limit the requirement for testing of individual animal isolates. The settings where testing of anaerobes should be done are:
• To determine patterns of susceptibility of anaerobes to new drugs
• To monitor susceptibility patterns in countries or cities.

REFERENCE


APPENDIX

**McFarland 0.5 Turbidity Standard**

Solution A: 1.75 g BaCl2.2H2O Make up to 100 ml with dist water

Solution B: 1.0 ml H2SO4 (Analar grade, sp. grav. 1.84)

Make up to 100 ml with dist water

Stock solution 0.5 ml solution A

99.5 ml solution B

Shake vigorously and dispense into a 5-10 ml glass vial with rubber stopper. Store in the dark at room temperature and replace 3 months after preparation. The turbidity standard should always be agitated before use.

Verify the correct turbidity of the standard by using a spectrophotometer with a 1 cm light path and matched curvette to determine the absorbance. The absorbance at 625 nm should be 0.08 to 0.10.

**Cation-adjusted Mueller-Hinton broth:**

Use this solution if CAMHB is not commercially available. It is used to ensure that MIC values obtained for the aminoglycosides (and tetracyclines) correspond to those obtained on Mueller-Hinton agar.

**Magnesium stock solution:**

Dissolve 8.36 g of MgCl2.6H2O in 100 ml deionised water. This solution will contain 10 mg of Mg2+

**Calcium stock solution:**

3.68 g of CaCl2.2H2O is dissolved in 100 ml deionised water. This will give a final concentration of 10 mg Ca2+/ml
Mueller-Hinton broth is prepared as the manufacturer directs, autoclaved at 121 °C for 20 minutes and chilled to 4°C or an ice bath if it is to be used the same day.

With stirring 1 ml of chilled Mg²⁺ and 2 ml Ca²⁺ stock solutions is added to 1 litre of chilled Mueller-Hinton broth.

**Haemophilus Test Medium**

Use for members of the *Pasteurellaceae* e.g. *Mannheimia haemolytica*

To one litre of Mueller-Hinton broth (Oxoid), add:

- 15 µg/ml β-NAD (Sigma, Merck, RSA)
- 15 µG BOVINE HEMATIN/ML (SIGMA, MERCK, RSA)
- 5 MG YEAST EXTRACT/ML (OXOID)
- 1 % foetal calf serum (Highveld Biological, Sterilab)