

Chapter 28

Antimicrobial Susceptibility Testing

The aim of the exercise is to find antibiotics which will be useful in eliminating an infection caused by an isolated organism in a given clinical situation. Selection of antibiotics, method of testing, and reporting of results are all important.

Methods of Choosing an Antibiotic

Empirical.

Depends on knowledge of likely pathogens and their probable susceptibilities.

May be applied:

Before, or without, culture.

Based on microscopy of a specimen.

Based on confirmed or presumptive identification of isolate.

Based on susceptibility testing.

Choice of an Antibiotic

Appropriate to organism.

Appropriate to clinical condition.

Appropriate to patient:

Age:

Neonate: chloramphenicol, sulphonamides, cotrimoxazole contraindicated.

Children: tetracyclines, quinolones contraindicated.

Elderly: cotrimoxazole, clindamycin contraindicated.

Pregnancy: see table below.

Breastfeeding: chloramphenicol, quinolones, sulphonamides, azithromycin, tetracyclines, cotrimoxazole contraindicated.

Genetic factors: sulphonamides in glucose-6-phosphate dehydrogenase deficient infants.

Interaction with other drugs:

Antibiotic potentiating or diminishing effect of other drug.

Other drug potentiating or diminishing effect of antibiotic.

Antibiotic increasing side-effect of other drug.

Other drug increasing side-effect of antibiotic.

Clinical condition of patient:

Renal failure: polymyxin B, nalidixic acid, sulphonamides, cotrimoxazole, tetracycline contraindicated.

Liver failure: sulphonamides, cotrimoxazole contraindicated.

Dialysis: nalidixic acid contraindicated.

Availability for treatment:

Hospital or outpatient.

Patient compliance.

Remote areas.

Government regulation.

Choosing Antibiotics to Test

Above considerations +:

Able to be tested by method used. Antibiotic may not be testable because:

Intrinsic qualities of antibiotic, eg, poor diffusibility, need for acidification to become active.

Not so far standardised or incapable of standardisation for method.

If it is known which antibiotic the patient is being, or will be, treated with, this should be tested if at all appropriate.

In mixed infections with multiple organisms, all possible efforts should be made to find a single antibiotic appropriate for treating all significant organisms.

ANTIBIOTIC USE IN PREGNANCY

Safe	Probably Safe	Safety Not Established	Likely to Cause Ill-Effects	Absolutely Contraindicated
Amoxicillin	Amoxicillin-clavulanate	Cefpirome	Capreomycin	
Ampicillin	Azithromycin	Ciprofloxacin	Clofazimine	
Benzathine penicillin	Aztreonam	Clarithromycin	Cotrimoxazole	
benzylpenicillin	Cefaclor	Colistin	Doxycycline	
Cephalexin	Cefepime	Cycloserine	Framycetin	
cephalothin	Cefotaxime	Dapsone	Sodium fusidate	
chloramphenicol	Cefotetan	Dicloxacillin	Gentamicin	
clindamycin	Cefoxitin	Enoxacin	Gramicidin	
cloxacillin	Cefpodoxime	Imipenem	Methacycline	
Erythromycin	Ceftazidime	Meropenem	Minocycline	
ethambutol	Ceftriaxone	Metronidazole	Neomycin	
hexamine	Cephmandole	Norfloxacin	Netilmicin	
Isoniazid	Cephazolin	Ofloxacin	Rifampicin	
lincomycin	Flucloxacillin	Teicoplanin	Silver sulphadiazine	
Nalidixic acid	Piperacillin	Ticarcillin	Streptomycin	
nitrofurantoin	Piperacillin-tazobactam	Ticarcillin-clavulanate	Sulphacetamide	
Phenoxymethyl penicillin	Roxithromycin	Trimethoprim	Sulphadiazine	
Procaine penicillin	Spectinomycin	Vancomycin	Sulphamethoxazole	
			Tetracycline	
			Tobramycin	

Testing

A successful outcome to therapy depends on the definition of a susceptible isolate as one where there has been a prior correlation with a favourable clinical response. All methods may give false susceptible results for some organisms showing intrinsic resistance, which may not be detected— eg., *Klebsiella* and ampicillin [see table of intrinsic resistances below]. Within limits, specificity is more important than sensitivity— ie, no false susceptibles, even at the expense of missing some that could be susceptible.

Intrinsic/Easily Induced Resistances

Organism	Report Resistant to
<i>Acinetobacter</i>	all cephalosporins
<i>Enterobacter</i> , <i>Serratia</i> , <i>Citrobacter</i> , <i>Aeromonas</i> , <i>Providencia rettgeri</i> , <i>Providencia stuartii</i> , <i>Morganella morganii</i>	ampicillin, cephalosporins, augmentin, ticarcillin
<i>Proteus vulgaris</i> , <i>Proteus penneri</i>	ampicillin, cephalosporins, ticarcillin, nitrofurantoin, tetracycline
<i>Proteus mirabilis</i>	tetracycline, nitrofurantoin, colistin
<i>Klebsiella</i>	ampicillin, ticarcillin
<i>Yersinia enterocolitica</i>	ampicillin
<i>Pseudomonas aeruginosa</i>	ampicillin, cephalothin, chloramphenicol, cotrimoxazole, tetracycline, augmentin
<i>Stenotrophomonas maltophilia</i>	ampicillin, augmentin, all cephalosporins, ciprofloxacin,

Methods

A standard method should be used.

Agar dilution is regarded as the 'gold standard', but results are influenced by agar, do not reflect high mutation rates, are somewhat time-consuming and prone to 'clerical' errors. Also, it is not applicable to bactericidal studies. Mast AdaTab system may be used to make accurate solutions; 'in house' solutions are less stable and more difficult to control.

The most suitable method overall appears to be broth microdilution (for both aerobes and anaerobes). It is a reference or standardised method which yields MICs directly over a wide range, does not require antimicrobial dilutions, has an alterable incubation routine, is uninfluenced by agar or diffusion rate, has divalent cation content controllable by performance testing or media supplementation, gives results which reflect high mutation rates by bacteria, is applicable to urgent direct susceptibility testing, is fairly easily individualised, and is applicable to bactericidal studies. Preparation of inocula directly from growth on agar plates gives as reproducible results as preliminary growth in broth. Commercially available products are convenient and accurate but relatively expensive and restricted to the range supplied by the manufacture. Of the commercial products available, Flow's MPS is the most accurate (95-97%) and reproducible (91-100%), while the Autobac MIC is the automated system with the best performance, giving 95% agreement with agar dilution. There are, however, problems in detecting methicillin resistance with these systems, the API Unispect KB being the only system giving results comparable with Kirby-Bauer. Broth dilution methods also have problems with sulphonamides, trimethoprim and aminoglycosides.

The Vitek semi-automated form of the broth microdilution method can produce results for Enterobacteriaceae in a minimum of 4 hours and for staphylococci in a minimum of 6 hours, allowing 70% of Vitek tests to be reported the same day. Because of this and because of its convenience when handling large numbers of isolates, it is widely used in larger laboratories. It is also the most accurate (specificity 93%) routine method for testing methicillin susceptibility, while also showing high sensitivity (96%). However, it has problems with testing ampicillin, cephalosporins and augmentin against Enterobacteriaceae, and all antibiotics against *Pseudomonas*. Also, the relatively large inoculum needed may result in false results due to mixed cultures, which may not be detected by the operator.

Problems with other automated systems include cephalothin against *Enterococcus*, aerobic Gram negative bacilli and MRSA, and kanamycin against *Pseudomonas aeruginosa* with Autobac 1; enterococcus with cephalothin, penicillin, gentamicin and kanamycin, *Enterobacter* with β -lactam drugs, and *Serratia* against colistin with MS-2.

The API - ATB uses a sloppy agar but is otherwise similar to manual broth microdilution methods.

Broth macrodilution methods are laborious, time-consuming and require careful technique. They have the disadvantages that antimicrobial dilutions are required, they are not applicable to urgent direct susceptibility testing, and are not easily individualised.

Agar diffusion is in many ways the least desirable. MICs are not directly obtainable, incubation routine is not alterable, it is influenced by agar and diffusion rate, results do not reflect high mutation rates by bacteria, and it is not applicable to bactericidal studies. However, no antimicrobial dilutions are required, it is applicable to urgent susceptibility testing, and antimicrobial tests are easily individualised. Because of this, agar diffusion methods are probably still the most widely used overall. They cannot be used for slow-growing organisms or for poorly diffusing antibiotics or for those whose activity depends on conditions which cannot be duplicated in the method.

Agar disc diffusion methods depend on finding break-points for each antibiotic by plotting MICs against zone size. A properly calibrated disc test is, in fact, a highly accurate, reproducible method of determining an MIC. If a susceptible isolate is defined as one where there has been a prior correlation with a favourable clinical response, the test predicts a successful outcome to antimicrobial therapy. If agar disc diffusion is used, a standard method such as Kirby-Bauer (NCCLS) or Bell's CDS method should be used.

The Kirby-Bauer (NCLS) method uses a single disc concentration for each antibiotic and finds the zone size corresponding to the MIC for susceptible organisms. Zone sizes may vary for different classes of organisms (eg, ampicillin with Enterobacteriaceae and with Staphylococci). If the category 'intermediate' is reported, this should indicate that the test result is equivocal. A more appropriate term is 'indeterminate', requiring an alternative test. A

'moderately susceptible' result should be reported to indicate susceptibility under certain conditions. Enterococci, other streptococci and non-penicillinase-producing, penicillin-susceptible organisms, when tested against penicillin or ampicillin, should be reported as 'moderately susceptible' rather than as 'intermediate'; this applies especially to enterococci, which for blood or serious invasive tissue infections require high dosage of penicillin or ampicillin, generally combined with an aminoglycoside for improved therapeutic response and bactericidal action. For streptococci, staphylococci and other penicillin-susceptible organisms, 'susceptible' means 'very susceptible'. When an intermediate result is obtained with staphylococci, the strains should be further investigated to determine if they are heteroresistant. The method is sensitive (> 96%) in testing for methicillin resistant staphylococci but its specificity is only 50%.

The CDS method attempts to find a disc concentration for each antibiotic which will give a susceptible zone size of 6 mm. Unfortunately, this is not always possible. It is easier to use than Kirby-Bauer and less prone to 'false susceptible' results but some antibiotics which can be tested by Kirby-Bauer cannot be by CDS. The method is only used in Australia and New Zealand.

The Stokes method compares zone sizes obtained for a test organism with those for a control organism. It is now rarely used in Australia because it is somewhat more troublesome to use and, in many cases, is less accurate than other disc diffusion methods. It does, however, show a specificity of 88% in testing staphylococci for methicillin resistance, while also having high sensitivity (> 96%).

The E-test uses a strip with a gradient of antibiotic. This allows the direct reading of the MIC (highly comparable to that obtained by dilution methods). The method is simple to use but expensive and is not useful for detecting extended broad spectrum beta-lactamase production.

Within limits, zone sizes in disc diffusion susceptibility testing are a function of inoculum density, lower densities producing larger zones. Depending on relative diffusion rates and stability characteristics of the antimicrobial and growth characteristics of the organism at room temperature and at incubation temperature, prediffusion prior to incubation may increase or decrease zone sizes; an increase is usual but by no means universal. In some cases, zone sizes may diminish with prolonged incubation, presumably because the drug has had a bacteriostatic effect and, with the passage of time, it has either leached out of the organism or has been metabolised, allowing the resumption of growth. Increased agar concentration decreases the diffusion rate of the drug and produces smaller zones. The depth of agar is also important, smaller volumes (< 17 mL for a 9 cm plate) giving larger zone sizes.

Antibiotic discs must be stored under the correct conditions. For β -lactams and some other antibiotics, this means in the freezer, not in the refrigerator.

Staphylococci must be incubated at 35°C, not at 37°C. Using a 0.5 U benzylpenicillin disc, susceptible strains of *Staphylococcus aureus* have a zone of inhibition of around 12 mm, while resistant strains have 0-1 mm zones. Rare strains with low penicillinase activity give zones of 4-5 mm with a sharp edge (MIC = 0.06 mg/L). *Staphylococcus saprophyticus* produces low levels of non-inducible penicillinase and gives zones of 5-7.5 mm with a 0.5U benzylpenicillin disc.

Resistance to methicillin is mainly due to the presence of altered PBP2a, which has poor affinity for methicillin and all other β -lactams, including imipenem and cephalothin. Therefore, resistance to methicillin implies resistance to all β -lactams. Only a minority of strains show 'homogenous' resistance, with all cells appearing to be resistant to high levels of methicillin. The majority of clinical isolates are thermosensitive heterogenous strains. These strains contain methicillin susceptible organisms that have the usual characteristics of nonheteroresistant *Staphylococcus aureus*, and methicillin resistant organisms which grow more slowly and may escape detection under ordinary conditions of culture and temperature. Only 1 in 10⁴ to 1 in 10⁷ organisms in such a population is resistant. Reducing the incubation temperature from 37°C to at least 35°C and increasing the osmolality of the culture medium by adding sodium chloride enhance expression of resistance to penicillinase-resistant penicillins in this subpopulation. Strains of *Staphylococcus aureus* have been described that require special culture conditions of temperature and osmolality but longer incubation periods (48 h) for expression of resistance to penicillinase resistant penicillins. These strains have been called acquired-resistant *Staphylococcus aureus*. They produce large quantities of β -lactamase and

may be rendered susceptible by adding clavulanic acid. Some studies were unable to find clinical justification either for routine screening for acquired-resistant strains or for reporting these strains as methicillin resistant. Infections caused by acquired-resistant strains of *Staphylococcus aureus* appeared to respond well to therapy with the penicillinase-resistant penicillins and at least as well as to therapy with other agents, including vancomycin. The solution may be to regard only amoxicillin-clavulanate resistant isolates as showing resistance to penicillinase-resistant penicillins.

Heterogenous strains of coagulase negative staphylococci also occur but these do not show resistance at 30°C nor on mannitol salt agar at 35°C. The CDS method is not reliable for testing.

Definitive testing for methicillin resistance can be performed using PCR or the Mastalex kit for detection of PBP2a.

If a tetrazolium dye is incorporated into the medium, results can be read in 1-3 hours, with identical results to standard methods.

In general, susceptibility tests should be performed on media as minimal as is required for growth. The zone sizes obtained with aminoglycosides, particularly when testing *Pseudomonas aeruginosa*, are very medium dependent because of variations in divalent cation content. With *Pseudomonas* species tested against aminoglycosides, the degree of susceptibility obtained varies inversely with the concentration of calcium and magnesium ions in the medium. Organisms in the intermediate category may be either susceptible or resistant when tested by dilution methods and should therefore more properly be classified as 'indeterminant'. A number of media have been specially formulated for susceptibility testing. In most applications, they are quite comparable, though some may be found incapable of supporting the growth of some organisms which will grow well on others. With organisms requiring blood or serum for growth, normal susceptibility medium + lysed blood should be used, the lysing process inactivating sulphonamide inhibitors present in whole blood. Media used for sulphonamide and trimethoprim testing should also be as thymidine free as possible. For those organisms requiring chocolate blood, susceptibility tests may be carried out using such media, but in such a case, sulphonamides and trimethoprim should be reported as susceptible if any diminution of growth in the vicinity of the disc is observed. Generally, however, Muller-Hinton or similar agar supplemented with 5% lysed horse blood and 1% IsoVitalex or comparable supplement and adjusted to pH 7.2 should be used. Susceptibility tests should not be performed on media containing antibiotics.

Cases most likely to yield unacceptable results by whichever method is used include *Enterobacter* testing with cefamandole, where discrepant (usually false susceptible) results are generally due to mutant resistant subpopulations or depressed β -lactamase activity requiring induction or other technical modifications, and the clinically irrelevant ampicillin and cephalothin; *Proteus/Providencia* testing against clinically irrelevant nitrofurantoin; *Serratia* testing against clinically irrelevant polymyxins; *Pseudomonas aeruginosa* testing against gentamicin and the clinically irrelevant kanamycin and chloramphenicol; enterococci against erythromycin and the clinically irrelevant cephalothin, clindamycin and aminoglycosides; *Staphylococcus aureus* against erythromycin and methicillin; coagulase negative staphylococci against penicillin and tetracycline.

The correlation of cephalothin MIC with the zone size using a disc diffusion test gives a continuous distribution of susceptibility and, therefore, cephalothin cannot be used for disc testing. Susceptibility of *Staphylococcus aureus* to cephalothin can be inferred from susceptibility to methicillin. Susceptibility of Enterobacteriaceae (except those which produce a Class I chromosomal β -lactamase) to cephalothin can be inferred from susceptibility to ampicillin.

The following antimicrobials should be tested (others whose susceptibility/resistance can be inferred from those tested shown in brackets; see also tables below):

Staphylococci: benzylpenicillin (phenoxymethylpenicillin, phenethicillin, amoxicillin, ampicillin and analogues, azlocillin, carbenicillin, mezlocillin, piperacillin, ticarcillin; in CDS, test and report ampicillin (extrapolate benzylpenicillin, amoxicillin and cephalothin) for *S.saprophyticus*), methicillin (CDS; cannot test *S.saprophyticus*—always report sensitive) or oxacillin (NCCLS) (amoxicillin-clavulanate, cephalosporins (staphylococci exhibiting resistance must be reported resistant to all cephalosporins, because in most cases they are clinically ineffective), cloxacillin, dicloxacillin, flucloxacillin, oxacillin, ticarcillin-clavulanate), cephalixin (CDS *S.saprophyticus* in urines only), erythromycin (clindamycin, lincomycin; do not report for urinary or blood culture isolates), tetracycline (all

tetracyclines; do not report for urinary or blood isolates); cotrimoxazole or trimethoprim (test and report for urinary isolates only; in CDS, sulphafurazole and trimethoprim are tested separately), vancomycin (MRSA and coagulase negative staphylococci from sterile sites only), rifampicin (MRSA only), fusidic acid (MRSA only), ciprofloxacin (MRSA and urine isolates only; report as norfloxacin in urinary isolates), nitrofurantoin (urine isolates only), chloramphenicol (isolates from eye infections only)

Enterococci: no cephalosporins (always report as resistant); ampicillin (amoxycillin, ampicillin analogues, apalcillin, azlocillin, mezlocillin, benzylpenicillin, piperacillin), cotrimoxazole or trimethoprim (NCCLS only), vancomycin (report if allergic to penicillin, resistant to ampicillin or if from peritoneal dialysates or other sterile sites), gentamicin (high level resistance in blood culture isolates only; NCCLS: brain heart infusion agar plate with 500 µg/mL gentamicin or 120 µg disc of gentamicin; CDS: use 200 µg disc), nitrofurantoin (urinary isolates only)

Streptococci: incubate *Streptococcus pneumoniae* and *Streptococcus milleri* in 5% CO₂; penicillin (NCCLS agar dilution and automated, CDS) or oxacillin (NCCLS disc methods to test pneumococci for relative resistance to penicillin) (penicillin (all relatively resistant pneumococci should have MIC determined, eg., by, E test), ampicillin and amoxycillin (report only if also reporting *Haemophilus influenzae*), ticarcillin, piperacillin, azlocillin, cephalothin and cephalexin (if MIC > 0.06 mg/L, report as resistant)), erythromycin (not CSF or urine), tetracycline (doxycycline, minocycline; *Streptococcus pneumoniae* respiratory or blood), cotrimoxazole or trimethoprim (NCCLS: only report on resistant pneumococci; CDS: Group B streptococci from urine only), chloramphenicol (resistant *Streptococcus pneumoniae* and eye isolates only), cefotaxime or ceftriaxone (NCCLS: test and report for CSF and blood isolates only; CDS: test and report for resistant *S.pneumoniae* only), vancomycin (if allergic to penicillin or for blood cultures, serious nosocomial infections or resistant strains), nitrofurantoin (CDS urinary only)

Enterobacteriaceae and Other Gram Negative Rods: ampicillin (amoxycillin; *Enterobacter*, *Serratia*, *Citrobacter freundii*, *Acinetobacter*, *Proteus vulgaris*, *Proteus penneri*, *Providencia*, *Morganella morganii* either report all isolates resistant regardless of result or, if susceptible, issue actual result with comment such as 'may result in selection of resistance during therapy'; all isolates of *Aeromonas* should be reported as resistant to all penicillins), amoxycillin-clavulanate (report if β-lactamase producer; report result for organisms listed under ampicillin similarly as for ampicillin), cephalothin (NCCLS only; CDS: extrapolate from ampicillin) and cephalexin (CDS: urinary isolates only; report result for organisms listed under ampicillin similarly as for ampicillin), cefotaxime (ceftriaxone, cefmenoxime, ceftazidime, ceftizoxime, moxalactam; report result for organisms listed under ampicillin similarly as for ampicillin; do not report for urinary isolates unless resistant to amoxycillin, amoxycillin-clavulanate and cephalexin; strains of *Aeromonas* demonstrating presence of inducible cephalosporinase by flattening of inhibitory zone around a cefotaxime 5 µg disc adjacent to an imipenem 10 µg disc or showing resistant mutants which appear as colonies within the zone of inhibition around disc containing any cephalosporin, cephamycin or aztreonam should be regarded as resistant to aztreonam, cefotaxime, cefotetan, ceftaxitin, ceftazidime, ceftriaxone and cephalexin), cotrimoxazole or trimethoprim (CDS: sulphafurazole and trimethoprim are tested separately), gentamicin, amikacin (test only if resistant to gentamicin, report only if resistant to other aminoglycosides), norfloxacin (test for urinary isolates only, report only for organisms listed under ampicillin or multi-resistant urinary isolates), ciprofloxacin (report for organisms listed under ampicillin and multi-resistant non-urinary isolates), nitrofurantoin (test and report for urinary isolates only; report resistant for *Proteus*, *Morganella morganii*, *Providencia*, *Serratia*), tetracycline (not urinary isolates or faecal isolates other than *Vibrio*), ticarcillin (multi-resistant organisms, hospital patients or on request only), tobramycin (multi-resistant organisms, hospital patients or on request only), ceftazidime (multi-resistant organisms, hospital patients or on request only); strains of *Aeromonas* which produce mutant colonies within the zone of inhibition should be regarded as resistant to imipenem irrespective of size of inhibitory zone

***Pseudomonas aeruginosa* and *Burkholderia*:** ceftazidime (cefperazone, cefsulodin; isolates from blood cultures, cystic fibrosis patients or on request only), ticarcillin or piperacillin (apalcillin, azlocillin, mezlocillin), gentamicin, tobramycin (report only if resistant to gentamicin), amikacin (test and report only if resistant to other aminoglycosides) ciprofloxacin (do not report for urinary isolates unless resistant to norfloxacin or on request), norfloxacin (urinary isolates only), imipenem (on request only), polymyxin B (colistin; isolates from external ear infections only)

***Haemophilus influenzae*, *Moraxella catarrhalis*:** benzylpenicillin (CDS: test for *Mcatarrhalis* only; do not report, but extrapolate ampicillin/amoxycillin result; possibly all resistant in clinical practice), ampicillin (amoxycillin,

i.v. benzylpenicillin; CDS: *Hinfluenzae* only (check all susceptible isolate for beta-lactamase production and report resistant if positive), extrapolate from penicillin result for *Mcatarrhalis*, cefaclor (CDS: *Hinfluenzae* only non-encapsulated strains), amoxicillin-clavulanate (NCCLS: dilution methods only; CDS: *Haemophilus influenzae* only for non-encapsulated strains, report if a β -lactamase producer), cefpodoxime (CDS *Mcatarrhalis* only; do not report), cefotaxime (ceftriaxone, ceftazidime; *Hinfluenzae* only; report if a β -lactamase producer and invasive isolate; CDS: extrapolate from cefpodoxime for *Mcatarrhalis*), tetracycline (NCCLS; CDS: *Hinfluenzae* non-encapsulated strains only), cotrimoxazole, chloramphenicol (invasive *Haemophilus* only; may be better to test for chloramphenicol acetyl transferase, using a commercial enzyme detection kit or by the 'clover leaf' method), rifampicin (NCCLS only; test and report for invasive *Haemophilus*), erythromycin (test and report only for *Mcatarrhalis*), β -lactamase test

Stenotrophomonas maltophilia: may appear susceptible to antimicrobials on in vitro testing to which it has a high rate of mutation to resistance (cotrimoxazole is considered the antimicrobial of choice); ticarcillin-clavulanate, piperacillin-tazobactam, ceftazidime, imipenem, gentamicin, tobramycin and cotrimoxazole are tested in NCCLS, though the clinical efficacy of ticarcillin-clavulanate and piperacillin-tazobactam is uncertain and imipenem, gentamicin and tobramycin are always reported as resistant; in CDS, sulphafurazole is tested and reported

Listeria monocytogenes: penicillin (for CDS, extrapolate from ampicillin), erythromycin (NCCLS only), ampicillin (amoxicillin), no cephalosporins (report resistant), gentamicin, chloramphenicol (NCCLS only; report only for CSF isolates), vancomycin (NCCLS only; report if resistant to penicillin or if peritoneal dialysate isolate)

Neisseria meningitidis: penicillin, chloramphenicol, cefotaxime or ceftriaxone, rifampicin (NCCLS only; report only if testing and only if resistant), ciprofloxacin (NCCLS only; report only if testing and only if resistant)

Neisseria gonorrhoeae: β -lactamase test; penicillin, ceftriaxone, spectinomycin, tetracycline (not testable by disc diffusion methods)

Guidelines for Antibiotic Susceptibility Testing and Reporting Using CDS System

Staphylococcus (Sensitest, air, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
benzylpenicillin ¹	0.5 U	penicillin, ampicillin, amoxycillin		? 0.06 mg/L
methicillin ¹	5 ?g	dicloxacillin, flucloxacillin, cephalosporins ²	augmentin, cloxacillin	? 4 mg/L
erythromycin ³	5 ?g	erythromycin	azithromycin, roxithromycin, lincomycin, clindamycin	? 0.5 mg/L
tetracycline ³	30 ?g	tetracycline	all tetracyclines	? 4 mg/L
ciprofloxacin ^{4,5}	2.5 ?g	norfloxacin		? 1 mg/L
sulphafurazole	300 ?g	cotrimoxazole ⁶		? 64 mg/L
trimethoprim ⁵	5 ?g	trimethoprim, cotrimoxazole ⁶		? 2 mg/L
nitrofurantoin ⁵	200 ?g	nitrofurantoin		? 32 mg/L
vancomycin ^{7,8}	5 ?g	vancomycin		? 4 mg/L
rifampicin ⁷	1?g	rifampicin		? 0.5 mg/L
fusidic acid ⁷	2.5 ?g	fusidic acid		? 0.5 mg/L
Chloramphenicol ^{8,9}	30?g	chloramphenicol		? 8 mg/L
ampicillin ⁴	5 ?g	penicillin, ampicillin, amoxycillin		? 0.5 mg/L
cephalexin ⁴	100 ?g	cephalexin		? 16 mg/L
gentamicin	10 ?g	gentamicin		? 1 mg/L
kanamycin	50 ?g	kanamycin		? 8 mg/L
teicoplanin	15 ?g	teicoplanin		? 8 mg/L

Notes:

1. Not *S.saprophyticus*.
2. Ceftazidime is considered inactive.
3. Not for blood or urinary isolates.
4. *S.saprophyticus* only.
5. Urine isolates only.
6. Report as susceptible to cotrimoxazole unless resistant to both sulphafurazole and trimethoprim.
7. Methicillin resistant *S.aureus* only.
8. Zone size for susceptible isolates = 2 mm.
9. Isolates from eye infections only.

Streptococcus (Blood Sensitest, air¹, 35 °C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
benzylpenicillin ²	0.5 U	penicillin, ampicillin, amoxycillin	cephalosporins ³	? 0.25 mg/L
ampicillin	5 ?g	see note 2		? 2 mg/L
erythromycin ⁴	5 ?g	erythromycin	azithromycin, roxithromycin, lincomycin, clindamycin	? 0.5 mg/L
tetracycline ⁴	30 ?g	tetracycline	all tetracyclines	? 4 mg/l
nitrofurantoin ⁵	200 ?g	nitrofurantoin		? 32 mg/L
vancomycin ^{6,7}	5 ?g	vancomycin		? 4 mg/L
ceftriaxone ⁸	5 ?g	ceftriaxone		? 2 mg/L
chloramphenicol ^{7,9}	30 ?g	chloramphenicol		? 8 mg/L
cotrimoxazole ¹⁰	25 µg	cotrimoxazole		? 0.5/9.5 mg/L

Notes:

1. Incubate *S.pneumoniae* and *S.milleri* in 5% CO₂.
2. If resistant and isolate not from CSF, test ampicillin and, if susceptible, report 'isolate shows reduced susceptibility to penicillin but may, in clinical practice, respond to higher doses of penicillin, ampicillin or amoxycillin'.
3. Groups A, B, C, F and G only. Ceftazidime is considered inactive against Gram positive organisms.
4. Not CSF or urinary isolates.
5. Urinary isolates only.
6. If allergic to penicillin or for blood cultures, serious nosocomial infections or resistant strains.
7. Zone size for susceptible isolates = 2 mm.
8. Resistant *S.pneumoniae* isolates only.
9. Resistant *S.pneumoniae* isolates and isolates from eye infections only.
10. *Streptococcus pneumoniae* and Group B streptococci only.

Enterococci (Blood Sensitest, air, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
nitrofurantoin ¹	200 ?g	nitrofurantoin		? 64 mg/L
vancomycin ^{2,3}	5 ?g	vancomycin		? 4 mg/L
ampicillin ⁴	5 ?g	benzylpenicillin, ampicillin, amoxycillin		? 2 mg/L
gentamicin ⁵	200 ?g	see note 6		? 512 mg/L

Notes:

1. Urinary isolates only.
2. If allergic to penicillin, resistant to ampicillin or from sterile sites.
3. Zone size for susceptible isolates ? 2 mm. Hazy zone edge indicates possible low level resistance (VanB type) even if zone size > 2 mm.
4. Zone < 4 mm = resistant. Perform a cefinase test for ?-lactamase on strains with an annular radius of 4-6 mm. ?-lactamase positive strains are reported as resistant. ?-lactamase negative strains are reported as 'reduced susceptibility to ampicillin'.
5. Blood culture isolates only. Zone size for susceptible isolates ? 4 mm.
6. Report susceptible/resistant as 'no/high level resistance to gentamicin, which may affect synergy with penicillins, demonstrated'.

Enterobacteriaceae, Vibrionaceae and Acinetobacter (Sensitest, air, 35°C)^{1,2,3}

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
tetracycline ⁴	30 µg	tetracycline	all tetracyclines	? 4 mg/L
ciprofloxacin ⁵	2.5 µg	ciprofloxacin		? 1 mg/L
sulphafurazole	300 µg	cotrimoxazole ⁶		? 64 mg/L
trimethoprim	5 µg	trimethoprim (urines only) cotrimoxazole ⁶		? 2 mg/L
nitrofurantoin ⁷	200 µg	nitrofurantoin		? 32 mg/L
norfloxacin ⁷	10 µg	norfloxacin		? 4 mg/L
gentamicin	10 µg	gentamicin		? 1 mg/L
cephalexin	100 µg	cephalexin		? 16 mg/L
ampicillin	25 µg	ampicillin, amoxycillin	cephalothin ⁸ , piperacillin, ticarcillin	? 8 mg/L
augmentin	60 µg	augmentin ⁹		< 16/8 mg/L
tobramycin ⁵	10 µg	tobramycin		< 1 mg/L
ceftazidime ⁵	10 µg	ceftazidime		? 4 mg/L
cefotaxime ⁵	5 µg	cefotaxime		? 1 mg/L
amikacin ⁵	30 µg	amikacin		? 4 mg/L
aztreonam ⁵	30 µg	aztreonam		? 8 mg/L
cefipime ⁵	10 µg	cefipime		? 1 mg/L
cefotetan ⁵	30 µg	cefotetan		? 8 mg/L
cefoxitin ⁵	30 µg	cefoxitin		? 8 mg/L
cefpime ⁵	10 µg	cefpime		? 2 mg/L
cefpodoxime ⁵	10 µg	cefpodoxime		? 2 mg/L
ceftriaxone ⁵	5 µg	ceftriaxone		? 1 mg/L
cephazolin ⁵	30 µg	cephazolin		? 16 mg/L
chloramphenicol	30 µg	chloramphenicol		? 8 mg/L
enoxacin	10 µg	enoxacin		? 4 mg/L
imipenem ⁵	10 µg	imipenem		? 4 mg/L
kanamycin	50 µg	kanamycin		? 8 mg/L
meropenem	5 µg	meropenem		? 2 mg/L
nalidixic acid ⁷	30 µg	nalidixic acid		? 4 mg/L
netilmicin ⁵	30 µg	netilmicin		? 2 mg/L
tazocin	55 µg	tazocin ¹⁰		? 16/2 mg/L
timentim	85 µg	timentim ¹⁰		? 32/2 mg/L

Notes:

1. Certain organisms exhibit intrinsic resistance or easily inducible resistance to certain antibiotics, that may not be detected on disc testing. In such cases, the organisms involved should always be reported as resistant regardless of the result of disc testing. The relevant organism/antibiotic combinations are listed in the table of **Intrinsic/Easily Induced Resistances** earlier in the chapter.
2. Multi-resistant isolates (especially *Klebsiella*) should be tested for extended broad spectrum beta-lactamase production by Casal's 'keyhole' method.
3. *Yersinia enterocolitica* is incubated in air at 30°C.
4. Not urinary isolates or faecal isolates other than *Vibrio*.
5. Multi-resistant organisms, hospital patients or on request only.

6. Report as susceptible to cotrimoxazole unless resistant to both sulphafurazole and trimethoprim.
7. Urinary isolates only.
8. Not for *Acinetobacter*.
9. If ampicillin resistant. If an ESBL is present, report for isolates from urine only.
10. If an ESBL is present, report resistant.

Listeria monocytogenes (Blood Sensitest, air, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
ampicillin	5 ?g	benzylpenicillin, ampicillin, amoxycillin		? 1 mg/L
gentamicin	10 ?g	gentamicin		? 1 mg/L

Pseudomonas, Burkholderia (Sensitest, air, 35°C)¹

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
ciprofloxacin ²	2.5? g	ciprofloxacin		? 1 mg/L
norfloxacin ³	10? g	norfloxacin		? 4 mg/L
gentamicin ⁴	10? g	gentamicin		? 4 mg/L
ticarcillin	75? g	ticarcillin		? 32 mg/L
tobramycin ⁴	10? g	tobramycin		? 4 mg/L
ceftazidime ⁵	10? g	ceftazidime		? 4 mg/L
imipenem ⁶	10? g	imipenem		? 4 mg/L
polymyxin b ^{4,7}	300U	colistin, polymyxin B		? 1 mg/L
amikacin ⁶	30 ?g	amikacin		? 16 mg/L
aztreonam ⁶	30 ?g	aztreonam		? 8 mg/L
cefipime ⁶	10 ?g	cefipime		? 2 mg/L
cefpime ⁶	10 ?g	cefpime		? 2 mg/L
meropenem ⁶	5 ?g	meropenem		? 2 mg/L
netilmicin ^{4,6}	30 ?g	netilmicin		? 8 mg/L
piperacillin ⁶	50 ?g	piperacillin		? 16 mg/L
sulphafurazole ⁸	300 ?g	cotrimoxazole		? 64 mg/L
trimethoprim ⁸	5 ?g	trimethoprim (urines only), cotrimoxazole ⁹		? 2 mg/L
tazocin ⁶	55 ?g	tazocin		? 16/2 mg/L
timentim	5 ?g	timentim		? 32/2 mg/L

Notes:

1. Regardless of results on disc testing, these organisms should be considered resistant to all other antibiotics listed under 'Enterobacteriaceae, Vibrionaceae and Acinetobacter'.
2. Not urinary isolates unless resistant to norfloxacin or on request.
3. Urinary isolates only.
4. Zone size for susceptible isolates ? 4 mm.
5. Isolates from blood cultures, cystic fibrosis patients or on request only.
6. On request only.
7. Isolates from external ear infections only.
8. Not *Pseudomonas aeruginosa*.
9. Report as susceptible to cotrimoxazole unless resistant to both sulphafurazole and trimethoprim.

Stenotrophomonas maltophilia

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance
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			May Be Inferred
sulphafurazole	300 ?g	cotrimoxazole	

Haemophilus (HTM agar, CO₂, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
ampicillin ¹	5 ?g	ampicillin, amoxycillin		? 1 mg/L
cefotaxime ²	0.5 ?g	cefotaxime, ceftriaxone	ceftazidime	? 0.25 mg/L
ceftriaxone ²	0.5 ?g	cefotaxime, ceftriaxone	ceftazidime	? 0.25 mg/L
tetracycline	30?g	tetracycline	all tetracyclines	? 4 mg/L
cotrimoxazole	25?g	cotrimoxazole		? 1/19 mg/l
cefaclor	30?g	cefaclor		? 4 mg/L
augmentin ³	15?g	augmentin		? 2 mg/L
chloramphenicol ⁴	10?g	chloramphenicol		? 2 mg/L
cefpodoxime	10 ?g	cefpodoxime		? 2 mg/l
ciprofloxacin	2.5 ?g	ciprofloxacin		? 1 mg/L

Notes:

1. Check all isolates susceptible by CDS for beta-lactamase production and report resistant if positive.
2. Isolates from CSF and other serious infections only.
3. If beta-lactamase positive.
4. Isolates from CSF, serious systemic infections or eye infections only.

Moraxella (Branhamella) catarrhalis (Blood Sensitest, O₂, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
benzylpenicillin ¹	0.5 U	penicillin, ampicillin, amoxycillin		? 0.25 mg/L
erythromycin	5 ?g	erythromycin	azithromycin, roxithromycin,	? 0.5 mg/L
tetracycline	30 ?g	tetracycline	all tetracyclines	? 4 mg/L
cotrimoxazole	25 ?g	cotrimoxazole		? 1/19 mg/L
cefaclor	30 ?g	cefaclor, augmentin		? 4 mg/L
cefpodoxime	10 ?g	cefpodoxime		? 2 mg/l
ciprofloxacin	2.5 ?g	ciprofloxacin		? 1 mg/L

Notes:

1. Probably all resistant in clinical practice.

Campylobacter (Blood Sensitest, microaerophilic, 42°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
Erythromycin ¹	5 ?g	Erythromycin	Azithromycin, roxithromycin,	? 0.5 mg/L
Tetracycline	30 ?g	Tetracycline	All tetracyclines	? 4 mg/L
Ciprofloxacin	2.5 ?g	Ciprofloxacin		? 1 mg/L
Gentamicin	10 ?g	Gentamicin		? 1 mg/L

Notes:

1. Zone size for susceptible isolates ? 4 mm.

Neisseria meningitidis (Blood Sensitest, CO₂, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
Benzylpenicillin	0.5 U	Penicillin	Ampicillin, amoxycillin	? 0.25 mg/L
Cefotaxime	0.5 ? g	Cefotaxime		? 0.25 mg/L
Ceftriaxone	0.5 ? g	Ceftriaxone		? 0.25 mg/L
Chloramphenicol	10 ? g	Chloramphenicol		? 2 mg/L
Ciprofloxacin	2.5 ? g	Ciprofloxacin		? 1 mg/L
Rifampicin	1 ? g	Rifampicin		? 0.5 mg/l

Pasteurella multocida (Sensitest, air, 35°C)

Disc Tested	Potency	Antibiotics Reported	Other Antibiotics Whose Susceptibility/Resistance May Be Inferred	MIC for Susceptible Strains
Ampicillin	5 ? g	Penicillin, ampicillin, amoxycillin		? 1 mg/L
Ciprofloxacin	2.5 ? g	Ciprofloxacin		? 1 mg/L
Tetracycline	30 ? g	Tetracycline	All tetracyclines	? 4 mg/L

Note that, while tetracycline is used as the class disc for all tetracyclines, certain organisms may be more susceptible to doxycycline and/or minocycline than to tetracycline.

CDS SUSCEPTIBILITY METHOD: Use only approved media. Plates should be dried before use. Using standard nichrome wire, touch the tops of 2 colonies and transfer to 5 mL sterile saline. Flood the plate with the suspension, making sure the surface is covered. Tilt the plate and remove the excess suspension with a pipette. Invert the plate and allow to dry at room temperature with the lid partially removed for 45 minutes. Place no more than 6 discs on each plate. Incubate at 35°C for approximately 18 hours in air (except *Haemophilus*, *Streptococcus milleri* and *Streptococcus pneumoniae*, which are incubated in CO₂).

Points to Watch in Using CDS

The method must be followed precisely.

The inoculum density is important; a higher density ? smaller zones, lower density ? larger zones.

Using the wrong medium, or the wrong atmosphere, for an organism can completely invalidate the result.

A wet surface when discs are placed on the plate may affect zone size or make zone sizes unreadable.

Prediffusion prior to incubation usually gives increased zones.

Prolonged incubation may give decreased zones.

Reporting

Class Agents: either report the class agent if this is clearly understood by medicos to include all members of the class (eg, tetracycline) or the most appropriate member if the connection is less obvious or the patient is known to be treated with a particular antibiotic.

Surrogate Agents: remember that some antibiotics stand in for others; eg, cephalothin cannot be tested by CDS but susceptibility to cephalothin can be inferred from the result for methicillin for Staphylococci and from ampicillin for Enterobacteriaceae.

Remember intrinsic resistances.

Remember contraindications.

Antibiotic Therapy for Anaerobes

No satisfactory, standardised methods exist for determination of the susceptibility of anaerobic isolates to antibiotics. Penicillin is the treatment of choice for all Gram positive anaerobes except *Clostridium difficile*, for anaerobic Gram negative cocci, and for beta-lactamase negative anaerobic Gram negative bacilli. Metronidazole is the treatment of choice for *Clostridium difficile*, for beta-lactamase positive anaerobic Gram negative bacilli and for most other anaerobes where patient penicillin hypersensitivity is a problem (erythromycin may be more useful for anaerobic Gram positive cocci). Most anaerobes are susceptible to clindamycin and chloramphenicol, but these will rarely be used unless the other choices are completely inappropriate. In situations of mixed aerobic and anaerobic infections, particularly those involving aerobic Gram negative bacilli, augmentin or timentim may be appropriate. The broth-disc technique may be used for antibiotic susceptibility tests on anaerobes, but tetracycline, and perhaps erythromycin, yield discrepant results.

Beta-lactamase: With organisms where β -lactamase production is the sole, or main, method of resistance to β -lactams, β -lactamase testing may substitute for disc testing. This is particularly important for *N.gonorrhoeae* and *Bacteroides*, which cannot be tested by disc methods. Staphylococci and *Haemophilus* may show susceptible zones for β -lactams on disc testing but produce β -lactamase which can inactivate these antibiotics. The apparent susceptibility of these isolates must, therefore, be checked by β -lactamase testing. The acidimetric paper strip or disc test is reliable only for *Haemophilus*, *Neisseria*, *Staphylococcus* and *Moraxella catarrhalis*. Exposure times should be at least 10-15 minutes before calling a result negative. The paper disc test utilising a chromogenic cephalosporin as the substrate and indicator can be used for the same organisms and also for certain anaerobes and for *Enterococcus*. These are organisms producing group IIa β -lactamase (plasmid mediated penicillinases inhibited by clavulanic acid). Exposure times are 1 minute for *Haemophilus*, *Neisseria* and *Moraxella catarrhalis*, 30 minutes for anaerobes and 60 minutes for *Staphylococcus* (for greater sensitivity, test growth from zone edge of oxacillin or methicillin disc; weak β -lactamase activity detected in the majority of strains of *Staphylococcus saprophyticus* is of little clinical significance; store discs in freezer but use at room temperature and moisten disc slightly before use) and *Enterococcus*. Testing for β -lactamase activity in other organisms gives misleading information of no clinical significance. *Enterobacter*, *Serratia*, *Citrobacter*, *Acinetobacter*, *Providencia*, *Proteus vulgaris* and *Morganella* produce chromosomally determined group I β -lactamases under the control of one or more regulatory genes which initiate production of the enzyme on exposure to the antimicrobial (ie, induction). These regulatory genes have a high rate of spontaneous mutation (1 in every 10^5 - 10^6 organisms). Because most susceptibility tests use inocula of 10^4 organisms, resistance may not be apparent on in vitro testing. This group can spontaneously mutate to become high level β -lactamase producers (hyperproduction of β -lactamase can occur at levels hundreds of times greater than normal). This renders them resistant to most β -lactam antimicrobials (except imipenem). If these organisms are treated with β -lactam antimicrobials, there is a high risk of selecting out the β -lactamase-producing organisms, which then persist, resulting in treatment failure. For this reason, organisms in this group should be reported resistant to all β -lactam antimicrobials (all penicillins, all cephalosporins, all cephamycins, aztreonam) except imipenem, regardless of susceptibility test results. *Pseudomonas aeruginosa* can also produce inducible β -lactamase, but the frequency of mutation is much lower (1 in 10^9). Therefore, agents such as piperacillin and ticarcillin are still therapeutically useful if they appear susceptible in vitro. Enterobacteriaceae may also produce group IIb β -lactamase (cephalosporinases, including extended broad spectrum β -lactamases active against third generation cephalosporins and monobactams. These are plasmid mediated (mainly involving plasmids of the TEM and SHV series) and are characteristically susceptible to inhibition by clavulanic acid. They have almost invariably been found in *Klebsiella pneumoniae* isolates, though rare instances have been reported in *Escherichia coli*, *Salmonella*, *Citrobacter freundii* and *Enterobacter*. *Klebsiella* isolates from blood cultures or serious hospital infections (eg, ICU/CCU patients), multi-resistant Enterobacteriaceae (ie., resistant or only moderately susceptible to third generation cephalosporins or aztreonam or resistant to aminoglycosides), isolates of Enterobacteriaceae from patients who have been treated with third generation cephalosporins or aztreonam, and Enterobacteriaceae which show resistant colonies within the zones of inhibition of cefotaxime, ceftazidime or aztreonam should be tested for production of extended broad spectrum β -lactamase by Cassal's test. If positive, the isolate should be regarded as resistant to all

cephalosporins and penicillins except amoxicillin-clavulanate and ticarcillin-clavulanate. The Vitek ESBL card detects 96% of these strains, but the E test only 65%.

Note that antibiotic susceptibility may be useful as an aid to, or check on, identification. For instance, *Pasteurella* and *Kingella* are always susceptible to penicillin. An oxidase negative and/or large-celled Gram negative bacillus which appears penicillin susceptible should be viewed with suspicion unless it has been identified as belonging to a species which includes penicillin susceptible strains. It should be verified that it is in fact Gram negative (by repeat Gram stain and/or string test and/or vancomycin susceptibility). If this is indeed so, oxidase test and penicillin susceptibility test should be repeated. Likewise, a tetracycline susceptible *Proteus mirabilis* demands checking of identification, susceptibility or both, as does an ampicillin susceptible *Morganella morganii*. Similar considerations apply to nalidixic acid, polymyxin or colistin susceptible Gram positive organisms or resistant Gram negatives. Again, enterococci producing zones ≥ 30 mm for ampicillin or ≥ 28 mm for penicillin are quite unusual and the speciation should be reexamined. Other resistances which should be checked include ampicillin resistant *Enterococcus*, penicillin resistant *Neisseria meningitidis*, rifampicin or chloramphenicol resistant *Haemophilus influenzae*, penicillin resistant *Streptococcus pyogenes*, penicillin or chloramphenicol resistant *Streptococcus pneumoniae*, vancomycin resistant *Staphylococcus*.

In the case of mixed infections, every effort should be made to find antibiotics to which all significant isolates are susceptible in common.

Baker et al's indicator broth kit is rapid, simple and inexpensive, gives 80-100% agreement with Kirby-Bauer and may be useful, especially for small laboratories and 'field' conditions.

Acquired resistance by chromosomal mutation differs from plasmid mediated resistance in that it forms a single resistance determinant in a single strain. Plasmids may code for multiple antibiotics and may be transferred by conjugation from one bacterium to another. Under some circumstances, bacteria may be 'cured' of plasmids and lose their resistance. On the other hand, a loss mutation reversing chromosomally acquired resistance is relatively rare. Resistance by either mechanism is not induced by presence of the agent; rather a resistant population is selected by death of sensitive strains. Since acquired chromosomal resistance involves only a single agent (at a time), use of multiple antimicrobials may prevent the emergence of resistant strains. This is less likely to be true with strains showing plasmid mediated resistance since a single plasmid frequently codes resistance to several antimicrobials.

Bacterial tolerance is failure of an antimicrobial to kill the organism, not just inhibit growth. It is due to inhibition or depletion of the autolytic enzyme system within the cell. Tolerance to penicillin has been proposed as one possible explanation for the failure of response of some streptococcal infections to penicillin therapy. This is particularly important in endocarditis, where a bactericidal effect appears essential to cure. Addition of an aminoglycoside usually produces a synergistic effect and reduces the MBC to a value close to the MIC. Tolerance has also been observed in staphylococci. Penicillin tolerance is usually reflected in vitro by a significant discrepancy between the MIC and the MBC. The ratio of MBC to MIC selected for the definition of tolerance has varied from study to study but a value of $\geq 32:1$ is most commonly used.

Isolates of *Streptococcus pneumoniae* with MICs in the range of 0.1-1.0 mg/L should be reported as being of intermediate or reduced sensitivity or as being moderately resistant to penicillin. Estimates of the prevalence of pneumococci in this group have ranged from zero to 35%. Individuals with pneumonia caused by these organisms may respond to conventional therapy with penicillin, whereas individuals with meningitis have responded irregularly, perhaps because of the inconsistent penetration of penicillin into inflamed meninges.

SUGGESTED MINIMUM QUALITY CONTROL PROCEDURES

- ? checking inoculum preparation
- ? checking new batches of media
- ? weekly outcome testing with control strains of *Staphylococcus aureus*, *Staphylococcus aureus* (methicillin heteroresistant), *Escherichia coli*, *Escherichia coli* (ampicillin resistant), *Pseudomonas aeruginosa*, *Haemophilus influenzae*
- ? participation in an outside quality assurance program
- ? any reasonable procedures recommended by equipment manufacturers not included in the above
- ? 'eternal vigilance' for unusual results

