Gram-negative coccobacilli include *Bordetella* and *Haemophilus*, which cause respiratory infections.

Gram-positive cocci include staphylococci and streptococci, which cause infections in skin, ears, joints, urine, and are common causes of surgical site infections; and enterococci, which cause infections in many sites and have a wide range of intrinsic resistance. Gram-positive rods include *Actinomyces* (which often cause soft tissue abscesses or pyothorax), *Corynebacterium* (which can cause ear infections) and clostridia.

Antibiotic susceptibility testing is performed with disc diffusion or broth dilution tests following strict protocols. In disc diffusion tests, the isolate is uniformly streaked on specified agar and antibiotic discs applied. After incubation the zone sizes of bacterial growth are measured and compared to published breakpoints. For broth dilution testing a known concentration of isolate suspension is incubated with different dilutions of antibiotics. The minimum concentration of antibiotic that inhibits bacterial growth is determined and again compared to published break points. For both methods breakpoints are set by EUCAST and CLSI, taking into account multiple factors such as resistance mechanisms, pharmacokinetics, pharmacodynamics and clinical outcome data. Breakpoints differ for different bug–drug combinations and are regularly updated, so it is important for the laboratory to ensure correct break points are being used.

KEY LEARNING OBJECTIVES

- Outline the techniques employed in identifying bacteria

- Recognize the common types of bacteria isolated from different sites
- Be able to list methods used for susceptibility testing

MULTIPLE CHOICE QUESTIONS

- Which of the following is not part of the Enterobacteriaceae family?
 - Pasteurella multocida*
 - Enterobacter aerogenes*
 - Klebsiella pneumoniae*
 - Proteus mirabilis*
- Which of the following is the gold standard method for identifying bacteria?
 - Automated biochemical testing, e.g. using Vitek
 - Colorimetric biochemical testing, e.g. using API strips
 - MALDI-TOF analysis
 - Visual assessment of colonies with catalase, oxidase tests and Gram stain
- Which of the following is the most common cause of joint infections?
 - Enterococcus* spp.
 - Staphylococcus* spp.
 - Escherichia coli*
 - Pseudomonas aeruginosa*

Which antibiotic should I test for and which should I use?

Elizabeth Villiers

Intrinsic resistance refers to resistance resulting from the normal physiology or structure of the organism and does not depend on the organism being exposed to the antibiotic. For example, enterobacteriaceae and non-fermenting Gram-negative bacteria are intrinsically resistant to clindamycin, erythromycin and fusidic acid. *Pseudomonas aeruginosa* is also intrinsically resistant to amoxicillin-clavulanate acid, first- to third-generation cephalosporins, tetracyclines, trimethoprim-sulphomamide and chloramphenicol. Intrinsic resistance tables are found at http://www.eucast.org/expert_rules_and_intrinsic_resistance/.

Antibiotics for which there is known intrinsic resistance are not tested, and a resistant result is issued. Susceptibility for some drugs is inferred from results of drugs in the same class, e.g. for *Streptococcus*, benzylpenicillin susceptibility is used to predict susceptibility to all penicillins and cephalosporins.

For staphylococci there are two important resistance mechanisms: beta lactamase production and alteration of the drug-binding site, penicillin-binding protein (PBP). Beta lactamase leads to resistance to ampicillin/amoxicillin but susceptibility to amoxicillin clavulanate, cefalexin and higher-generation cephalosporins. Methicillin-resistant staphylococci (MRS) have altered PBP which renders them resistant to ALL beta lactam antibiotics (including carbapenems), the only exception being ceftazoline (which should not be used in animals). Beta lactamase production is evaluated with benzylpenicillin, whilst methicillin resistance is evaluated with oxacillin or ceftiofur. MRS may be resistant only to beta lactams, or may be resistant to drugs in other classes due to concurrent expression of other resistance genes.

Streptococci generally have very low levels of resistance. Conversely enterococci are intrinsically resistant to cephalosporins, clindamycin, erythromycin and sulphomamides and may acquire resistance to penicillins and tetracyclines, with more frequent resistance to fluoroquinolones.

Beta lactamase resistance is problematic in Gram-negative bacteria which are often resistant to amoxicillin and cefalexin. Some produce extended spectrum beta lactamases rendering them resistant to penicillins and first- to third-generation cephalosporins. These enzymes are encoded on plasmid genes, which move between bacteria

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via conjugation. Multiple antibiotic-resistant genes are often co-localized on the same plasmid, which means resistance to other antibiotics such as aminoglycosides, trimethoprim–sulphonamides, tetracyclines and fluoroquinolones and occasionally carbapenems can develop.

The choice of antibiotic should be based on the susceptibility testing including the minimum inhibitory concentration (MIC), the site of infection and the prescribing cascade. Where two or more suitable drugs are available, the drug with the MIC furthest away from the breakpoint is selected. Antibiotics which concentrate at the site of infection should be chosen, for example, drugs concentrated in urine (beta lactams, fluoroquinolones, or potentiated sulphonamides) should be used for UTIs, and the latter two used for prostatic infections since these cross the blood–prostate barrier. The lowest generation effective drug in a given class should be selected (e.g. cefalexin over cefovecin). For serious infection, especially sepsis, bactericidal drugs may be selected over bacteriostatic drugs.

KEY LEARNING OBJECTIVES

- Outline the difference between intrinsic and acquired resistance
- List important resistance mechanisms in Gram-positive and Gram-negative bacteria
- Select the best antibiotic from the susceptibility results

MULTIPLE CHOICE QUESTIONS

1. Which of the following antibiotics would NOT be part of a panel for *Pseudomonas aeruginosa*?
- (A) Gentamycin (C) Marbofloxacin
(B) Cefovecin (D) Polymixin

2. Which is the most important resistance mechanism for staphylococci?
- (A) Production of altered PBP
(B) Production of extended spectrum beta lactamase
(C) Production of efflux pumps
(D) Reduced membrane permeability
3. Susceptibility results for an *Escherichia coli* isolated from an area of cellulitis in a dog are given in Figure 1. Which antibiotic would be the best choice?
- (A) Marbofloxacin
(B) Amoxicillin–clavulanate
(C) Amoxicillin
(D) Trimethoprim–sulphonamide

Antibiotic	MIC result	Breakpoint		
		Sensitive	Inter-mediate	Resistant
Amoxicillin	≤2	≤8		≥16
Amoxicillin clavulanate	≤2	≤8		≥16
Marbofloxacin	0.5	≤1	2	≥4
Cefovecin	2	≤2	4	≥8
Gentamycin	4	≤2	4	≥8
Trimethoprim sulphonamide	≤20	≤40		≥80
Doxycycline	16	≤4	8	≥16

Figure 1: Susceptibility results.

Beyond microscopy: using molecular testing on routine cytology samples

Nick Carmichael

qPCR analysis can be undertaken on a wide range of liquid cytology submissions with the aim of identifying the presence of infectious agents and quantifying the amount of the agent present in the original sample. The latter information can be useful in assessing the significance of the qPCR result.

PCR analysis additionally has greater sensitivity than conventional culture techniques, does not depend on the presence of viable organisms and works well in identifying a range of both DNA and RNA viral infections.

Multiple qPCRs can be run on the same sample simultaneously allowing rapid identification and quantification of co-existing infectious agents at the same time. These techniques are now being applied across a range of exotic species and can be useful in health screening and quarantine programmes.

PARR is a technique which is used to determine if a lymphoid population is clonal (i.e. derived from a single neoplastic lymphoid cell) and to determine if the clonal lymphoid population is of T cell or B cell origin. This technique can be applied to unstained or prestained cytology slides derived from lymph nodes, cavity effusions, internal organs, bone marrow and/or peripheral blood. While the specificity of PARR is very high for identification of lymphoma the sensitivity is lower, particularly in cats, and as such negative PARR results do not entirely exclude the presence of lymphoma.

Immunocytochemistry involves the use of specific antibodies directed against target antigens on or within cells. These antibodies bind to their target antigen and can then be detected using a variety of systems which make the bound antibody (and therefore the presence of the