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

target antigen) visible on microscopy. While this technique is well established within histopathology its application to routine prestained cytology slides is relatively new.

Applications include determination of the immunophenotype in lymphoma, differentiation of mesenchymal and epithelial cells in the context neoplasia and identification of amelanotic melanoma.

These molecular techniques now allow the advantages of fine needle aspirate cytology in terms of minimal tissue invasion, speed and convenience to be coupled with a significant enhancement of the diagnostic information available from a single sample.

KEY LEARNING OBJECTIVES

- Develop an understanding of quantitative polymerase chain reaction (qPCR) for infectious agents and the application of this technology to effusions and washing fluids in order to increase their diagnostic yield
- Appreciate the applications of PCR for antigen receptor rearrangement (PARR) in relation to the identification of lymphoma and determination of its immunophenotype
- Understand the potential application of immunocytochemical analysis of cytology slides to assist characterization of cell populations which cannot be definitively identified based on morphological features alone

MULTIPLE CHOICE QUESTIONS

1. Which one of the following statements is FALSE regarding PARR analysis of cytology slides in the context of canine lymph node aspirates
 - (A) PARR analysis cannot be undertaken on cytology slides stained with Diff Quik in practice

- (B) PARR analysis may occasionally identify lymphoma cases as being a dual immunophenotype containing clonal DNA sequences of both T and B cell origin
 - (C) Samples containing a high proportion of lysed or disrupted lymphocytes are unsuitable for PARR analysis
 - (D) PARR analysis generally reliably differentiates between lymphoid hyperplasia and neoplasia
2. Which of the following statements regarding qPCR analysis of BAL samples in cats is UNTRUE?
 - (A) Results for *Mycoplasma felis* can be influenced by oropharyngeal contamination of the sample
 - (B) Rapid analysis of samples for *Bordetella* and *Mycoplasma* spp. is essential for reliable qPCR results
 - (C) Co-infections of respiratory viral and bacterial infections are not uncommonly detected in symptomatic cats
 - (D) qPCR has a much higher sensitivity for the identification of *Mycoplasma* spp. than conventional culture
3. Which one of the following statements is FALSE with regard to immunocytochemistry undertaken on cytological preparations?
 - (A) It is useful for the differentiation of B cell versus T cell lymphoma
 - (B) It is useful for the differentiation of epithelial versus mesenchymally derived cells
 - (C) It is useful for the differentiation of benign versus malignant neoplasia
 - (D) It is useful for the differentiation of amelanotic melanoma versus soft tissue sarcoma

Diagnosis and classification of canine lymphoma from FNA samples with a combination of cytology and flow cytometry

Elizabeth Villiers

Lymphoma is classified according to the World Health Organization system, which identifies several types of

high- and low-grade T and B cell lymphoma. The most common types are diffuse large B cell lymphoma (DLBCL), high-grade T cell lymphoma (HGTCL), T zone lymphoma (TZL) and marginal zone lymphoma. TZL has the longest median survival time, followed by DLBL, whilst HGTCL has the shortest survival times when standard protocols are used. There are different treatment protocols for low- and high-grade lymphoma and recent studies have shown HGTCL may respond better to protocols with L-asparaginase and lomustine, whilst standard CHOP protocols with doxorubicin are less effective. These different types of lymphoma can usually be identified with a combination of flow cytometry and cytology.

Samples for flow cytometry are collected into a cell medium or a mix of saline and plasma. Several fine-needle aspiration samples are injected into one vial, mixing carefully to prevent clotting. Aliquots of cells are labelled with lineage-specific antibodies tagged with a fluorescent dye. The cells pass through a laser beam causing the laser light to scatter at different angles,

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