Clinical pathology

phagocytosed fat vacuoles. The fluid lipase concentration is higher than the serum lipase concentration.

Uroabdomen leads to an azotaemia with hyperkalaemia and often hyponatraemia. It typically leads to a transudate, or with longer-standing uroabdomen, inflammation may develop. The fluid creatinine:serum creatinine ratio is >2 and the fluid potassium:serum potassium is >1.4 .

Chylous effusions have a triglyceride concentration of >1.4 mmol/l and a cholesterol:triglyceride ratio of <1 (ratio calculated after converting to mg/dl). They form due to rupture or obstruction of, or increased pressure on, lymphatics or may be idiopathic. Cats with cardiac disease may have lymphocyte-rich effusions without elevated triglyceride.

Transudates are classified as protein-poor (protein <20 g/l), which usually form due to hypoalbuminaemia; and protein-rich (protein >20 g/l), which form due to increased hydrostatic pressure in veins or lymphatics due to cardiac disease, neoplasia, thrombosis or liver disease. They have low numbers of nucleated cells ($<5 \times 10^9$ /l) with a mix of macrophages, neutrophils, mesothelial cells and lymphocytes.

KEY LEARNING OBJECTIVES

- Outline the approach to evaluation of body cavity effusions in the practice laboratory
- Be able to distinguish transudates and exudates and identify their underlying cause
- Identify patterns in haematology and biochemistry accompanying effusions

MULTIPLE CHOICE QUESTIONS

1. A 12-year-old cat has an amber slightly cloudy pleural effusion.
Protein 35 g/l

Albumin 18 g/l

Globulin 17 g/l

Nucleated cells 3.8×10^9 /l

HCT <1

Cytology: 80% small lymphocytes, 15% macrophages, 5% neutrophils

What is the most likely cause of this effusion?

- (A) FIP
- (B) High-grade lymphoma
- (C) Cardiac disease
- (D) Ruptured thoracic duct

2. Which of the following would be expected in septic peritonitis:

- A Elevated serum protein and albumin
- B Effusion nucleated cell count $>13 \times 10^9$ /l
- C Predominance of macrophages in the abdominal effusion
- D Bacteria within neutrophils
- E Bacteria within macrophages
- (A) A, B, D
- (B) B, E
- (C) A, B, D
- (D) B, D

3. A 7-year-old Golden Retriever has a cranial mediastinal mass and a pleural effusion with a nucleated cell count of 28×10^9 /l, consisting of large lymphoid cells with a large nucleus containing stippled chromatin and two to three small nucleoli. Which of the following is causing this effusion?

- (A) Thymoma
- (B) Lymphoma
- (C) Cardiac disease
- (D) Ruptured thoracic duct

Nuts and bolts of culture and susceptibility testing

Elizabeth Villiers

In order to obtain accurate results in culture and susceptibility testing it is important to follow strict guidelines set out by regulatory bodies such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Swabs are plated out on to various agar plates using a streak technique, which, after incubation, produce individual colonies which are scooped off for analysis. A Gram stain and basic biochemical tests such as oxidase and catalase tests are performed. These preliminary

results, as well as the appearance of the colonies, will determine selection of subsequent biochemical tests which are performed using colorimetric strips or with automated systems such as the Vitek analyser. Results are compared to a database and algorithms used to generate the identification. Alternatively bacteria can be identified using matrix-assisted laser desorption/ionization time of flight analysis (MALDI-TOF).

Gram-negative rods are divided into those that ferment glucose (*Pasteurella* and *Enterobacteriaceae*) and non-fermenters. *Enterobacteriaceae* include *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter* and *Serratia*. These organisms commonly cause urinary tract, wound, soft tissue, body cavity and ear infections. Non-fermenters include *Pseudomonas aeruginosa* (a common cause of ear, eye and respiratory infections); *Acinetobacter* and *Burkholderia* (which can cause nosocomial infections). Non-fermenters have extensive intrinsic resistance to multiple antibiotics. Gram-negative cocci include *Neisseria* (which can cause oral infections) and *Moraxella*, which cause respiratory infections.

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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