



Suggested guidelines for using systemic antimicrobials in bacterial skin infections (1): diagnosis based on clinical presentation, cytology and culture

L. Beco, E. Guaguère, C. Lorente Méndez, C. Noli, T. Nuttall, M. Vroom

Systemic antimicrobials are critically important in veterinary healthcare and resistance is a major concern. Antimicrobial stewardship will be important in maintaining clinical efficacy by reducing the development and spread of antimicrobial resistance. Bacterial skin infections are one of the most common reasons for using systemic antimicrobials in dogs and cats. Appropriate management of these infections is therefore crucial in any policy for responsible antimicrobial use. The goals of therapy are to confirm that an infection is present, identify the causative bacteria, select the most appropriate antimicrobial, ensure that the infection is treated correctly, and to identify and manage any underlying conditions. This is the first of two articles that will provide evidence-led guidelines to help practitioners address these issues. This article covers diagnosis, including descriptions of the different clinical presentations of surface, superficial and deep bacterial skin infections, how to perform and interpret cytology, and how to best use bacterial culture and sensitivity testing. The second article, to be published in a subsequent issue of *Veterinary Record*, will discuss therapy, including choice of drug and treatment regimens.

THE use of antibiotics in small animal practice has become an important and controversial issue. The general public is more aware that antibiotic resistance is of increasing concern in medical and veterinary healthcare, and that antibiotics should only be used where necessary. It is our goal as veterinarians to prevent misuse and promote responsible use of antibiotics to preserve their efficacy and minimise the development and spread of resistance. There are, however, few detailed and practical guidelines for responsible antimicrobial use in small animal practice. Skin diseases comprise 20 to 25 per cent of non-elective consultations, and are likely to account for a major proportion of antimicrobial use in small animal practice. The aim of these articles is therefore to outline a set of principles for the responsible use of antibiotics in the management of skin and soft tissue infections.

The key steps for a correct treatment of skin infections are:

- Correct diagnosis of pyoderma;
- Selecting an appropriate antibiotic;
- Ensuring the antibiotic is given at the correct dose and frequency until clinical cure; and
- Diagnosing and treating any underlying disease.

These articles will review the evidence underlying these principles. Part 1 will discuss diagnosis of pyoderma using clinical signs, cytology, and bacterial culture and antimicrobial sensitivity tests. Part 2 will review the choice of antimicrobials, emphasising the importance of using a first-, second- and third-line drug classification, and treatment regimens, including dose, frequency, duration, compliance and adverse effects. Recurrent pyodermas and clinic hygiene will also be briefly discussed.

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Correct diagnosis of pyoderma Clinical presentation

Pyodermas were traditionally classified according to the depth of bacterial infection (surface, superficial and deep) (Scott and others 2001). Surface and superficial pyoderma are restricted to the epidermis and do not penetrate below the basement membrane. These pyodermas are typically exudative and lesions include papules, pustules, epidermal collarettes, scales and crusts. Pruritus is often present. Deep pyodermas penetrate below the basement membrane into the dermis and deeper tissues. Lesions include haemorrhagic bullae, nodules, ulcers, and draining tracts with haemorrhagic or purulent discharge and crusts. Lesions are often painful, but pruritus is less common. However, while this classification is useful in determining the prognosis and expected duration of therapy, it is not helpful in diagnosis.



FIG 1: (a) Ventral body of a West Highland white terrier with bacterial overgrowth syndrome. Note the extensive alopecia, lichenification, erythema and hyperpigmentation. The affected skin was also seborrhoeic and malodorous. The underlying cause in this case was atopic dermatitis. (b) Intertrigo in a British bull dog with a screw tail. The folds of skin around the tail and tailbase are eroded, seborrhoeic and erythematous. Topical antimicrobial therapy was only partially effective and this dog required surgery to obliterate the skin folds

To help clinicians diagnose and treat pyoderma, a problem-based classification based on the clinical appearance of the lesions has been proposed (Noli 2003):

- Seborrhoeic pyoderma: erythema, erosions, exudation without pustules and collarettes;
- Papules, pustules, scaling, focal alopecia;
- Erosions and/or ulcers;
- Ulcers and draining sinus tracts; and
- Nodules and/or regional swelling.

Seborrhoeic pyodermas: erythema, erosion, exudation without pustules and collarettes

Bacterial overgrowth syndrome (Fig 1a)

Recently a condition called 'bacterial overgrowth syndrome' has been recognised in dogs (Pin and others 2006). It is characterised by diffuse erythema, scales and a greasy, keratoseborrhoeic exudate. Affected dogs are often pruritic and malodorous. The abdomen, interdigital skin and pinnae are most commonly affected. Allergic or endocrine dermatoses are common primary causes. Cytology of affected skin reveals an excessive number of bacteria with few to no neutrophils.

Intertrigo (Fig 1b)

Intertrigo is infection of skin folds caused by irritation and lack of ventilation. Intertrigo is common in facial folds, especially in brachycephalic dog and cat breeds, tail folds in bulldogs, vulval folds in obese

females and body folds in shar peis. However, any skin fold can be affected. The affected skin is moist, greasy and erythematous and may harbour a whitish malodorous exudate.

Papules, pustules, scaling, focal alopecia

Impetigo (Fig 2a)

Impetigo is characterised by epidermal pustules that are not centred on hair follicles. It affects mainly young dogs, especially if they are poorly managed (eg, malnourished, have endoparasites or are affected by viral diseases). Older immunosuppressed dogs can also exhibit similar lesions with large flaccid or tense pustules (bullous impetigo).

Bacterial folliculitis (Fig 2b)

Bacterial folliculitis is the most common form of canine pyoderma. It presents with small, hair follicle-associated erythematous macules, papules, pustules, epidermal collarettes, and patchy tufting of the coat followed by focal or multifocal alopecia (this is especially noticeable in short-coated dogs). Bacterial folliculitis is an uncommon cause of miliary dermatitis in cats.

Superficial spreading pyoderma (Fig 2c)

Superficial spreading pyoderma is characterised by large, spreading and coalescing epidermal collarettes, erythema and exfoliation. A mild exudate can be observed at the edge of the collarettes. This uncommon clinical presentation is mainly seen on the ventral body.

Erosions and/or ulcers

Pyotraumatic dermatitis (Fig 3a)

Pyotraumatic dermatitis is a superficial, exudative, highly pruritic and possibly painful bacterial infection caused by repeated self-trauma. The mildest clinical presentation consists of a well-defined area of moist skin and coat with mild surface erosion. This presentation may evolve into a superficial folliculitis or a deep furunculosis with alopecia, erythema, papules, erosion, exudation and ulceration. The affected skin becomes thickened and painful. It can be difficult to differentiate between superficial and deep forms, because some cases that clinically appear to be superficial nevertheless show deep lesions on histopathology (Holm and others 2004). In addition, clinically mild erosions may be associated with folliculitis or furunculosis hidden in the surrounding haired skin (satellite lesions).

Intertrigo

In severe cases of skin fold pyoderma the skin may be eroded or ulcerated, and very painful.

Mucocutaneous pyoderma (Fig 3b)

Mucocutaneous pyoderma is characterised by crusts and erosions localised to one or more mucocutaneous junctions including the lips,



FIG 2: (a) Bullous impetigo with a large, flaccid, non-follicular pustule in a Yorkshire terrier with hyperadrenocorticism. (b) Superficial folliculitis of the ventral body in a Staffordshire bull terrier with atopic dermatitis. Note the widespread macular erythema, papules, pustules and epidermal collarettes. The papules and pustules are small and associated with single hair follicles. (c) Superficial spreading pyoderma in a cocker spaniel with a primary keratinisation defect. There is extensive erythema, with large, spreading and coalescing epidermal collarettes, and exfoliation

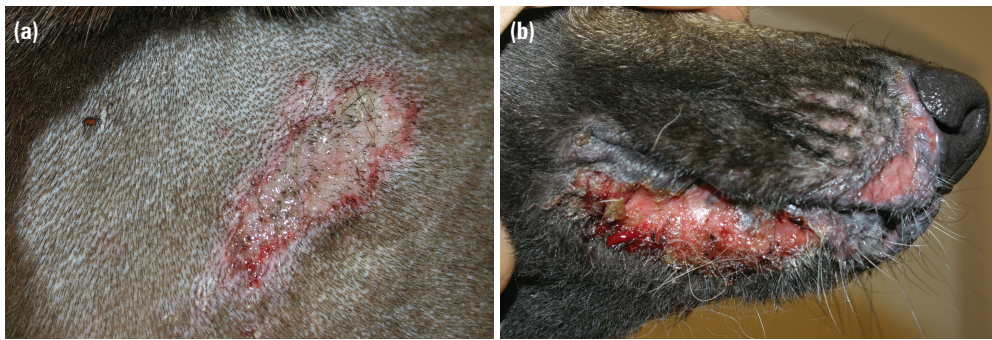


FIG 3: (a) Pyotraumatic dermatitis in a dog with tick infestation. The coat has been clipped to reveal the extent of the lesion. There is a well-demarcated area of moist erythema and erosion. Note the tick on the left side of the figure. (b) Mucocutaneous pyoderma with erythema, erosion and ulceration of the lips and muzzle in a German shepherd dog. These lesions are very similar to those seen with cutaneous lupus erythematosus or cutaneous epitheliotropic T cell lymphoma at this site

eyelids, vulva, prepuce and anus. German shepherd dogs seem to be predisposed to this. This form of pyoderma can be clinically and histopathologically mistaken for immune-mediated or neoplastic diseases, but can be differentiated by complete resolution following an appropriate antibiotic course.

Ulcers and draining sinus tracts

Furunculosis (deep pyoderma)

Furunculosis is associated with rupture of the hair follicles and the spilling of their contents into the dermis, creating a foreign body reaction and an infection in the dermis or subcutis. Clinical lesions include erythema, haemorrhagic bullae, draining sinus tracts, ulcers and crusts. The exudate may be purulent to bloody. The skin may be swollen and painful. Chronic lesions become fibrosed and firm.

Some forms of deep pyoderma remain localised and represent specific syndromes: callus pyoderma (Fig 4a), foreign body sinus, muzzle furunculosis or canine acne, and interdigital furunculosis (Fig 4b). Feline chin acne is a keratinisation disorder associated with comedones and furuncle formation (Fig 4c).

Other deep pyodermas are widespread and may cause malaise and systemic illness. German shepherd dog deep pyoderma is a unique

papules, pustules, haemorrhagic bullae, ulcers and crusts on the dorsum and dorsal flanks. It has been associated with bathing or grooming (Hillier and others 2006).

Nodules and/or regional swelling

Abscess

Abscesses are discrete, swollen, walled-off accumulations of pus and necrotic tissue. They will often burst, drain purulent fluid, and form crusts matted into the surrounding hairs. They may be painful. Cats are more predisposed to abscesses due to their fighting behaviour, but they can follow any penetrating wound.

Cellulitis

Cellulitis refers to diffuse infection and inflammation along tissue planes. This is often regional, although it may be poorly circumscribed. The skin is usually intact, but penetrating wounds may be found. These may drain a bloody serous to purulent fluid. The affected area is usually painful (Fig 5).

Necrotising fasciitis is a rare but severe form of cellulitis associated with dissemination of bacterial toxins (Naidoo and others 2005). There is rapid progression with severe cellulitis, swelling, necrosis,

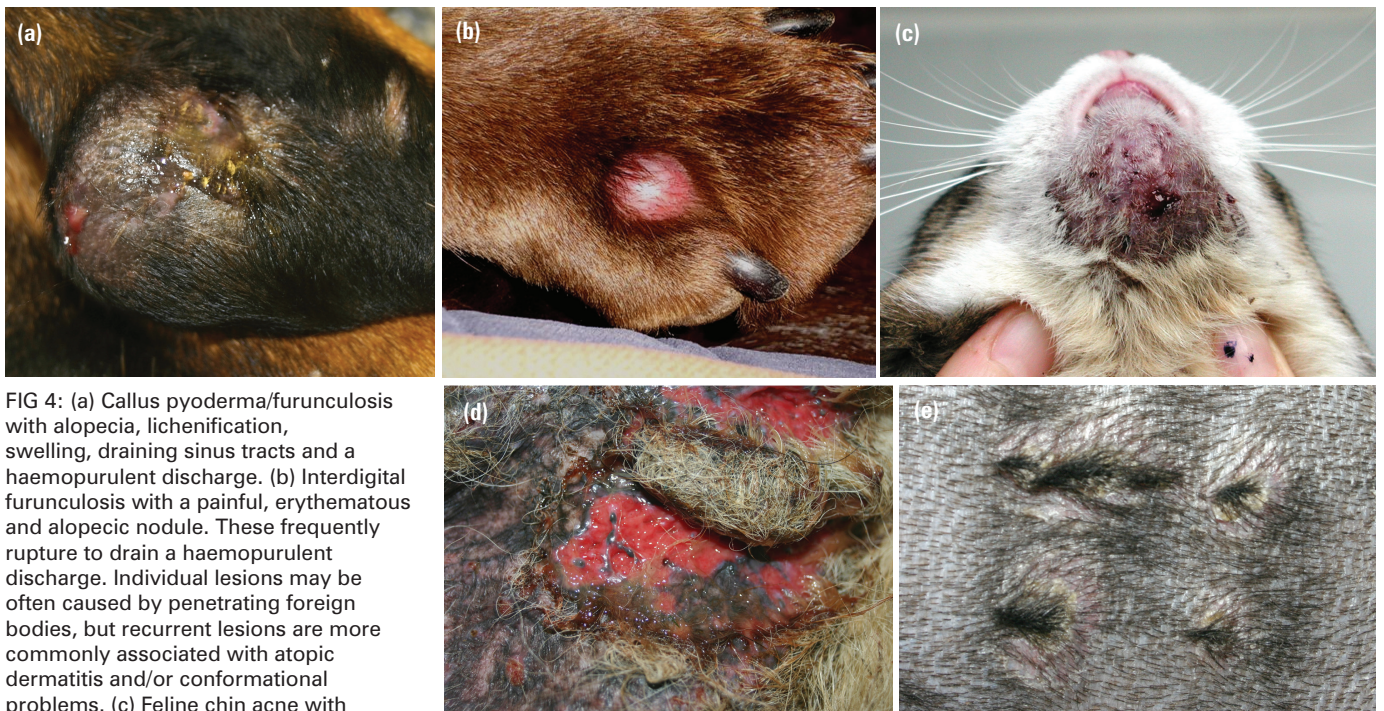


FIG 4: (a) Callus pyoderma/furunculosis with alopecia, lichenification, swelling, draining sinus tracts and a haemopurulent discharge. (b) Interdigital furunculosis with a painful, erythematous and alopecic nodule. These frequently rupture to drain a haemopurulent discharge. Individual lesions may be often caused by penetrating foreign bodies, but recurrent lesions are more commonly associated with atopic dermatitis and/or conformational problems. (c) Feline chin acne with multiple comedones, furuncles, alopecia, discharge and crusts. (d) German shepherd dog pyoderma. There is severe ulceration of the ventral abdomen with erythema, papules and nodules, necrosis and a purulent discharge. (e) Deep pyoderma on the dorsum of a dog due to *Pseudomonas* species. There are painful erythematous papules, pustules and crusts on the dorsum and dorsal flanks. The hair has been clipped for a better visualisation of the lesions



FIG 5: Severe cellulitis of the axilla and forelimb of a dog with ill-defined swelling, erythema, purpura and necrosis

infection of deeper tissues and septicaemia, which is often fatal. Affected dogs are usually dull to depressed and pyrexia.

Ancillary diagnostic procedures: cytology

The clinical signs are often highly suggestive of pyoderma, but the diagnosis must be confirmed using cytology and, where necessary, bacterial culture and antibiotic sensitivity testing. Antimicrobials should not be speculatively used on the basis of the clinical signs only (Weese 2005, May 2006, Nuttall and others 2008, Guardabassi and Fondati 2009).

Cytology is a simple, quick and minimally invasive technique, which can be performed on fully conscious animals with minimum risk and no lasting harm.

Cytological techniques

There are a number of different methods, some of which are better suited to certain conditions and situations than others (Mendelsohn and others 2006, Mueller 2009). Techniques include:

- Adhesive tape strips;
- Direct impression smears;
- Indirect impression smears;
- Needle cores and fine needle aspirates.

Adhesive tape strip cytology

Adhesive tape can be used to remove the outer layers of the stratum corneum and adherent microorganisms. Adhesive tape strip cytology is an excellent method to sample dry, greasy, scaling or eroded lesions. It is especially useful for irregular surfaces or restricted sites such as the interdigital skin. Tape strips are less useful with moist lesions, such as pustules, exudates, erosions or ulcers, as material may fail to adhere to the tape.

Direct and indirect impression smears

Impression smears are especially useful for moist or seborrhoeic lesions that will not stick to adhesive tape. Direct impression smears are made by applying the microscope slide directly to the lesion, such as an erosion, underside of a crust or ruptured pustule. It may be necessary to gently debride the surface to reveal representative cells. Indirect impression smears are appropriate when the slide cannot be apposed to the skin and adhesive tape strips are unsuitable. Material can be collected and transferred to microscope slides using cotton buds, spatulas, scalpel blades, and so on.

Needle cores

Needle cores (ie, needle insertion and rotation and/or re-position) are useful for cutaneous masses and enlarged lymph nodes. It can be difficult to obtain material by simple needle cores from very firm or fluid-filled lesions. These may need aspirating using a syringe, which can harvest more material and cells, but also causes more trauma, damaged cells and haemorrhage. This may make it difficult to interpret the samples.

Staining of cytology samples

Modified Wright-Giemsa stains such as Rapi-Diff or Diff-Quik are suitable for practice use. These stains are quick and easy to use, and can be used to reliably identify inflammatory cells and microorganisms. Heat fixation before staining is not necessary (Toma and others 2006). Some adhesive tapes, however, dissolve or turn opaque in the fixative. Adhesive tapes should be used with the eosinophilic and basophilic stains only, although some brands will still be affected by the stains. An alternative method involves placing a drop of the basophilic stain only on the preparation and then placing the adhesive tape or coverslip on top. This results in very intense staining of microorganisms, but gives a monochromatic stain where it may be more difficult to identify inflammatory cells (Toma and others 2006). Other stains such as Gram and Ziehl-Neelsen can be used to more precisely identify bacteria, but are more time consuming and difficult, and are rarely performed in practice laboratories.

Interpretation of cytological preparations (Mendelsohn and others 2006)

Inflammatory cells

Neutrophils predominate in most cases of pyoderma. Degenerate or toxic neutrophils are a good indication of infection (Fig 6a). They appear swollen and have indistinct nuclei with an open and disrupted chromatin pattern (karyorrhexis). Nuclear streaming is common, as the cells are fragile and vulnerable to trauma. Non-degenerate neutrophils appear to be smaller, with dark, shrunken nuclei (pyknosis) and nuclear streaming is less common. They are more usually associated with sterile inflammation, but there is no exact differentiation between degenerate and non-degenerate neutrophils – both may be seen in the same smear and their presence or absence should not be relied on to exclude the possibility of an infection.

Macrophages, containing phagocytosed microorganisms, degenerate cells and other debris, are often seen in chronic and/or deep pyoderma (Fig 6b). Multinucleate giant cells are much larger than other cell types seen on cytology, and have multiple nuclei, ranging from two or three to 10 or more in very large cells. Large numbers of macrophages and/or giant cells (ie, granulomatous or pyogranulomatous inflammation) could be consistent with mycobacterial or fungal infections. Low to moderate numbers of lymphocytes, plasma cells and eosinophils are seen in most inflammatory reactions and are of little diagnostic significance.

Bacteria

All bacteria that take up modified Wright-Giemsa stains are basophilic, that is, they stain blue-purple. This does not reflect whether they are Gram-positive or Gram-negative. Their identity can therefore only be inferred from morphology and knowledge of the likely organisms on most cytology preparations. Full identification will require further tests and culture.

Bacterial overgrowth syndrome is characterised by large numbers of bacteria, often of several different forms, with no or only minimal numbers of inflammatory cells (Fig 6c). Bacteria are also readily seen with other surface and superficial infections (Fig 6a). They may, in contrast, be difficult to detect in deep pyodermas, particularly if there is a lot of fibrosis and scarring. The presence of intracytoplasmic bacteria is a definite indicator of infection (Fig 6a) (Pappalardo and others 2002). Extracellular bacteria, however, particularly in low numbers, may simply be contaminants from the surface of the skin.

Staphylococci are relatively large cocci that often form diploid or irregular arrangements of two to eight organisms (Scott and others 2001, Pappalardo and others 2002, Mendelsohn and others 2006). Streptococci are smaller and often appear to form chains. Micrococci and enterococci are also small, but form irregular groups. Rod bacteria (bacilli) are easily differentiated from cocci; common species recovered from the skin include *Pseudomonas*, *Proteus* and coliforms. Mycobacteria and some related forms do not take up Wright-Giemsa stains, but pyogranulomatous inflammation and the presence of small, clear, rod-shaped vacuoles in macrophages is suggestive. Clear rod-like shapes may also be highlighted against stained background debris.

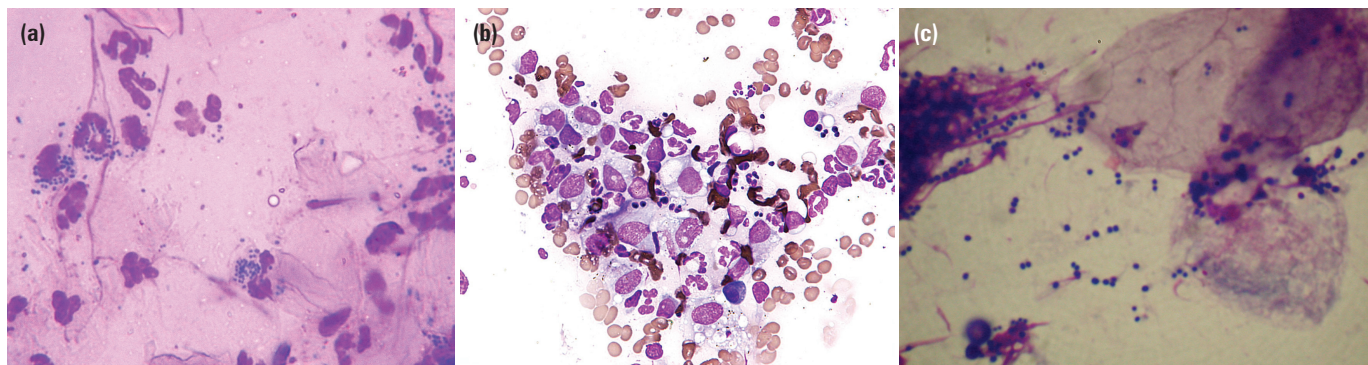


FIG 6: (a) Tape strip cytology of a cutaneous pyoderma in a dog stained with a modified Wright-Giemsa stain. Immersion oil. x 1000. There are several degenerate neutrophils with swollen, pale and fragmenting nuclei, with some nuclear streaming from ruptured cells. There are numerous blue-staining coccoid bacteria. These are most likely to be staphylococci – they are the most common cause of skin infections, and they typically form pairs or small groups on cytology. Extracellular bacteria may simply be contaminants, but the presence of intracellular phagocytosed bacteria confirms the diagnosis of infection in this case. (b) Indirect impression smear of material expressed from a furuncle stained with a modified Wright-Giemsa stain. Immersion oil. x 1000. The cytology is dominated by large, activated macrophages with a pale, foamy cytoplasm. Several cells have ingested debris, including dead or dying neutrophils. Other cells include degenerate neutrophils, lymphocytes, plasma cells and erythrocytes. In contrast to Fig 6a, bacteria are sparse and hard to find. (c) Tape strip cytology of canine bacterial overgrowth syndrome stained with Haemacolor. Immersion oil. x 1000. There are large numbers of bacteria (mostly large cocci forming pairs and groups) and one round budding *Malassezia* yeast (bottom left). Photograph 6c: Stefano Toma

Potential mistakes in interpreting cytology specimens

It is important that samples are taken from representative lesions. Several lesions should be sampled to look for consistent findings. In addition, the diagnosis should not be based on a single finding, and it is important to look for diagnostic changes and patterns that are consistent over the whole preparation. Cytology preparations yield relatively few cells, and while positive findings are useful, negative results should be interpreted with care. Clinicians should therefore be familiar with the cells, organisms and inflammatory patterns likely to be seen with samples from the skin and associated structures. Clinicians should also be aware of the limitations of these techniques and use cytology as a support and adjunct, and not a replacement, for microbial culture and histopathology.

Bacterial culture and antimicrobial sensitivity testing

When to culture

Bacterial culture and antimicrobial sensitivity testing is not necessary in all situations. Cytology can be a quick, easy and cost-effective way to detect the presence of infection and identify the likely microorganisms. Some microbes, such as staphylococci, have a relatively predictable pattern of antimicrobial sensitivity and empirical selection of treatment is often successful (Weese 2005, May 2006, Nuttall and others 2008, Guardabassi and Fondati 2009). Empirical antibiotic therapy is appropriate when all of the following apply (Loeffler and others 2005, Weese 2005, May 2006, Nuttall and others 2008, Guardabassi and Fondati 2009):

- Non-life threatening infection;
- First episode of a skin infection;
- Clinical lesions are consistent with a surface or superficial pyoderma;
- Cytology is consistent with a staphylococcal infection; and
- No reason to suspect antibiotic resistance.

Bacterial culture and antimicrobial sensitivity testing, however, are necessary when any of the following apply (Loeffler and others 2005, Weese 2005, May 2006, Nuttall and others 2008, Guardabassi and Fondati 2009):

- Life-threatening infections, as the first choice antibiotic must be effective;
- Clinical lesions are consistent with a deep pyoderma;
- The clinical signs and cytology are not consistent with each other;
- Rod-shaped bacteria are seen on cytology, as their antibiotic sensitivity is not predictable and may be limited;
- Empirical antibiotic therapy does not resolve the infection as expected; or

- Where antibiotic resistance is more likely:

- After one or more broad-spectrum antibiotic courses;
- Non-healing wounds;
- Postoperative and other nosocomial infections; or
- The owner or animal has recent healthcare contacts.

Cytology versus culture

It is helpful to perform cytology as well as taking material for culture. Most cultures are qualitative rather than quantitative. Cultured organisms may or may not be involved in the infection, particularly if they are normal commensal organisms. Cytology, in contrast, yields quantitative data including the number of organisms involved, whether they have been phagocytosed, and their relationship to cutaneous cells and structures. The relative abundance and likely importance of different organisms revealed by cytology can be useful when culture detects multiple species with differing antimicrobial sensitivity patterns.

Prior antibiotic therapy

Antibiotic treatment may result in false-negative cultures (Scott and others 2001). If possible, samples should be taken 48 hours after the last dose of oral antibiotics or beyond the appropriate dose interval for parenteral antibiotics. If appropriate withdrawal times are not possible but cytology indicates the presence of bacteria, prolonged and/or enriched cultures may be necessary to decrease the chance of false-negative cultures. It is therefore important to note recent or ongoing antibiotic therapy on the microbiology laboratory submission form.

Obtaining material for culture

Material for culture can be obtained by a variety of means depending on the lesions involved. It is important to obtain representative samples and avoid surface contamination that may not be relevant. Primary lesions, such as intact pustules, furuncles and nodules, and the leading edge of ulcers, should be selected where possible (Scott and others 2001). It may be necessary to carefully debride crusts to expose deeper lesions. It can be useful to take several samples if there are multiple lesions, especially if the cytology findings differ.

Bacteriology swabs

A variety of bacteriology swabs are available but standard cotton-tipped swabs in transport medium for aerobic and anaerobic culture are best for routine clinical use. Swabs taken without transport medium are only really suitable for on-site laboratories where the sample will be rapidly processed or placed in culture medium. Special swabs for anaerobic or other fastidious organisms can be used if these species are suspected.

Swabs can be taken directly from the skin surface, although there is a risk that this may simply reveal commensals or secondary,

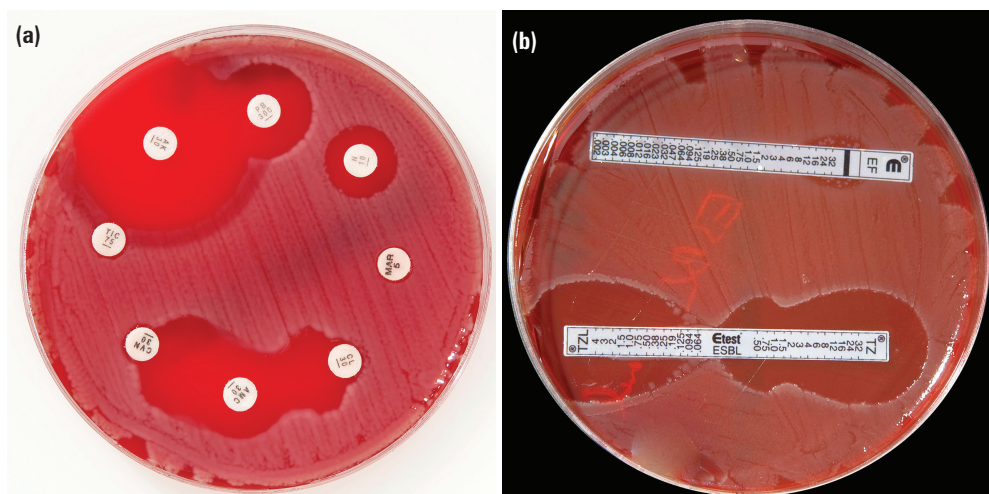


FIG 7: (a) Kirby-Bauer disc diffusion test. An isolate of *Escherichia coli* has been incubated with several antibiotic impregnated discs. The zones of inhibition around each disc will be measured and compared to agreed standards to determine whether the isolate is sensitive or resistant to each antibiotic. (b) E-strip minimum inhibitory concentration (MIC) assay. An *E coli* isolate has been incubated with two e-strips; one impregnated with enrofloxacin (EF), and one with both ceftazidime alone (TZ) and ceftazidime in combination with clavulanic acid (TZL). The MIC for enrofloxacin is 8 to 12 µg/ml. In contrast, the isolate appears to be sensitive to even low concentrations of ceftazidime with or without clavulanic acid. Photographs: Dorina Timofte and Andrew Wattret, University of Liverpool

opportunistic invaders. This can be avoided by cleaning the skin with alcohol before sampling pustules or furuncles, although alcohol can penetrate the thin overlying stratum corneum of superficial pustules (Scott and others 2001). The alcohol should be allowed to evaporate before the sample is collected to avoid inhibiting subsequent bacterial culture. Intact pustules can be ruptured with a sterile needle to expose fresh pus that can then be collected using a swab. It can be difficult to find intact pustules in dogs and cats, and samples may need to be taken from the edge of an epidermal collarette or the underside of the crust of a recently ruptured pustule. In cases of erythematous papulopustular dermatitis, good samples can be obtained by swabbing material from a papule debrided with a sterile needle. Fresh material can be expressed from draining sinus tracts and intact furuncles by digital pressure. Moistening the swab with sterile saline may enhance recovery of organisms. Taking swabs from both the nares and perineum when screening animals for staphylococcal carriage will reduce the chance of a false-negative culture (Fazakerley and others 2009).

Biopsy

Biopsies are preferable for deeper lesions, as bacteria on the skin surface may not be representative of the deeper organisms. The skin surface should be prepared with alcohol to reduce contamination before biopsy, and sterile instruments and gloves should be used. Local anaesthetics may be bactericidal (Sakuragi and others 1996), although local anaesthetics did not inhibit staphylococcal growth in a surgical wound model (Kose and others 2010). Nevertheless, it may be better to use a ring block, local nerve block or general anaesthesia rather than infiltrate the biopsy site. Punch biopsies of 4 to 6 mm are suitable for lesions affecting the epidermis and dermis, but full-thickness wedge biopsies are necessary for deeper lesions in the subcutis or underlying tissues.

Culture techniques

Kirby-Bauer disc diffusion tests

Kirby-Bauer disc diffusion tests use antibiotic impregnated paper discs (Jorgensen and Turnidge 2007, Schwarz and others 2010). A known quantity of bacteria are subcultured onto suitable agar plates in the presence of these discs. If the bacteria are susceptible to an antibiotic, a clear halo where the bacteria cannot grow develops around the disc (the zone of inhibition) (Fig 7a). The zone of inhibition is compared to agreed standards (Clinical Laboratory and Standards Institute: www.clsi.org, British Society for Antimicrobial Chemotherapy: bsac.org.uk, European Committee on Antimicrobial Susceptibility: www.eucast.org), to determine whether the bacteria are susceptible or resistant to a particular antibiotic. Samples may also be reported as having intermediate sensitivity, but these are best regarded as resistant in practice. The size of the zone of inhibition by itself is meaningless and cannot be used to determine how susceptible or resistant a bacterial isolate is to an antibiotic. Disc diffusion tests may also give misleading results; for example, cefoxitin and amoxicillin-clavulanate susceptibility or resistance in vitro is poorly predictive of the presence of *mecA*-positive (meticillin-resistant) coagulase-positive staphylococci (Weese 2005, Bemis and others 2006, Nuttall and others 2008).

Minimum inhibitory concentrations

Minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that completely inhibits growth of a microorganism. There are various means to determine the MIC, but

broth dilution methods that culture a known quantity of bacteria with doubling dilutions of each antibiotic are most common (Schwarz and others 2010). E-strips (E-test; bioMérieux UK) are paper strips impregnated with antibiotics at differing concentrations along the strip. They are used in a similar way to Kirby-Bauer discs, but produce an elliptical zone of inhibition with the MIC read as the concentration where the edge of the zone of inhibition touches the strip (Fig 7b) (Jorgensen and Turnidge 2007).

MICs are usually tested and reported in µg/ml ranges as the tests assume that the antimicrobial will be administered systemically. The isolate will be reported as susceptible, intermediate or resistant to the antimicrobials based on accepted breakpoints. These breakpoints are established for specific antimicrobials, organisms and disease conditions; clinicians should not use MICs from epidemiological surveys to determine whether an isolate is susceptible or resistant (Schwarz and others 2010). If the isolate is reported as susceptible then it is likely that systemic treatment will exceed the MIC in the target tissue.

The efficacy of concentration-dependent drugs with post-antibiotic effects (PAE) (type 1 drugs, eg, fluoroquinolones and aminoglycosides) depends on the peak concentration in the target tissue exceeding the MIC (McKinnon and Davis 2004). The larger the peak concentration:MIC ratio, the better the efficacy; this ratio is therefore an important predictor of antibiotic efficacy (currently 8 to 10:1 is thought to be optimal). The efficacy of time-dependent antibiotics with no PAE (type 2 drugs, eg, penicillins, cephalosporins and macrolides) depends on maximising the duration of exposure, that is, the concentration should exceed the MIC for at least 70 per cent of the dosing interval (McKinnon and Davis 2004). Increasing the dose and peak concentration may increase the time antimicrobial levels are above the MIC, but will not enhance the efficacy by itself. Type 3 antibiotics (eg, tetracyclines and some lincosamides) have mixed properties showing time-dependent killing with moderate PAE (McKinnon and Davis 2004). The ideal dosing regimen for these antibiotics maximises the amount of drug received. Depending on the organism, tissue and dose, once or twice daily administration can be appropriate.

Care should be taken with antibiotics where the susceptibilities are close to the breakpoint, as they may not achieve therapeutic concentrations in the target tissues (McKinnon and Davis 2004, Jorgensen and Turnidge 2007, Cole and others 2009, Schwarz and others 2010). Isolates with intermediate susceptibilities should be regarded as resistant, as it is unlikely that the MIC will be exceeded at the target tissue (Cole and others 2009). However, if the distribution to target tissues is known, it may be possible to calculate whether an increased dose

would be effective. Using topical therapy, which delivers mg/ml antibiotic concentrations, can also overcome apparent in vitro resistance (Cole and others 2009).

Other tests

Further testing may be necessary to confirm the identity, characteristics and antimicrobial susceptibility of bacterial isolates where simple culture and biochemical methods may be inadequate or misleading (Abraham and others 2007). These may include PBP2a latex bead agglutination tests, *mecA* PCR and SCC_{mec} typing for methicillin-resistant staphylococci (MRS), and PCR for extended-spectrum beta-lactamase (ESBL) *Escherichia coli*. The results of these tests must be taken into account when considering therapy; for example, MRS and ESBL *E. coli* will be resistant to penicillins and cephalosporins even if in vitro tests suggest that they will be sensitive (Weese 2005, Nuttall and others 2008). Many MRS, furthermore, exhibit inducible clindamycin resistance in vivo despite apparent in vitro sensitivity. Inducible clindamycin resistance should therefore be assessed using D-zone or PCR tests (Rich and others 2005). If in doubt, clinicians should discuss the implications of the laboratory findings with a microbiologist.

Conflicts of interest

The authors are all recognised specialists in veterinary dermatology who were given an independent brief to develop a comprehensive

guide to the use of systemic antimicrobials in bacterial skin infections. The meetings of the authors to produce these guidelines were sponsored by Pfizer Animal Health. However, the guidelines are exclusively the opinion of the authors. The treatment options may include off-label or off-cascade suggestions. The authors believe that any decision on treatment protocols for a particular case remains the complete responsibility of the prescribing veterinarian. In particular, veterinarians must be aware of relevant medicines legislation and whether it is legal to administer certain treatments in their country of work.

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