Antimicrobial susceptibility profiles of *Staphylococcus intermedius* isolates from clinical cases of canine pyoderma in South Africa

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Successful treatment of canine pyoderma has become compromised owing to the development of antimicrobial resistance with accompanying recurrence of infection. Canine skin samples submitted to a veterinary diagnostic laboratory for microbiological culture and sensitivity between January 2007 and June 2010, from which *Staphylococcus intermedius* was isolated, were selected for this investigation. Antimicrobial resistance of *S. intermedius* was most prevalent with reference to ampicillin followed by resistance to tetracycline and then potentiated sulphonamides. In general, antimicrobial resistance was low and very few methicillin-resistant isolates were detected. Temporal trends were not noted, except for ampicillin, with isolates becoming more susceptible, and potentiated sulphonamides (co-trimoxazole), with isolates becoming more resistant. In general, both the Kirby–Bauer disc diffusion and broth dilution minimum inhibitory concentration tests yielded similar results for the antimicrobial agents tested. The main difference was evident in the over-estimation of resistance by the Kirby–Bauer test for ampicillin, co-trimoxazole, penicillin and doxycycline. Knowledge of trends in bacterial resistance is important for veterinarians when presented with canine pyoderma. Analysis of antimicrobial susceptibility profiles of *S. intermedius* isolated from canine pyodermas will guide veterinarians' use of the most appropriate agent and encourage prudent use of antimicrobials in companion animals.

Introduction

Staphylococcus species are facultatively anaerobic, Gram-positive, coccal bacteria that belong to the family Micrococcaceae (Rich 2005). They are mostly harmless commensals of the skin and mucous membranes, but are potentially pathogenic to humans and many other animal species (Vanni *et al.* 2009). Nearly all cases of pyoderma in dogs are caused by *Staphylococcus intermedius* (DeBoer 2006). The name *S. intermedius* was proposed for isolates that differed from *Staphylococcus aureus* in various biochemical reactions and with regard to cell wall composition (Hajek 1976). The name *Staphylococcus pseudintermedius* is now given to the canine-specific strain of *S. intermedius*. For the purpose of this study, these canine-specific isolates will be referred to as *S. intermedius*. *Staphylococcus intermedius* is also an important cause of wound infections, otitis externa, cystitis and ocular and respiratory disease. Similar to *S. aureus* colonisation seen in humans, healthy dogs frequently carry *S. intermedius* as part of their normal microflora. It is a transient inhabitant of the skin and hair coat. Reservoir sites include the oral and nasal cavities as well as the perineum and anus (Hartmann *et al.* 2005). Although *S. intermedius* is not usually isolated from humans owing to its host-specificity for canine corneocytes, transmission between humans and their pets has been demonstrated (Fitzgerald 2009).

Since the introduction of antimicrobials, *Staphylococcus* species have shown rapid development and increased spread of resistance, particularly in nosocomial infections (Werckenthin *et al.* 2001). Antimicrobial resistance is of increasing concern in both veterinary and human medicine as it has led to treatment failures and hence increased morbidity, mortality and treatment costs (Pellerin *et al.* 1998). Owing to increased attention to small-animal welfare antimicrobial agents are increasingly prescribed for pets, including preparations formerly reserved for human use and the treatment of human infections (Guardabassi, Schwartz & Lloyd 2004b). Deep pyoderma associated with *S. intermedius* is possibly the most common reason for administration of antimicrobials in dogs (Guardabassi, Loeber & Jacobson 2004a). The choice of an antimicrobial is usually empirical. This approach (i.e. prescribing drugs without the use of microbiological culturing and sensitivity testing) is probably a major contributor to the emergence of resistant staphylococci strains (Lilenbaum *et al.* 2000). Antimicrobial resistance is, however, complex and involves various bacterial species, resistance mechanisms, transfer mechanisms and reservoirs (Guardabassi *et al.* 2004b).

Awareness and monitoring of antimicrobial resistance in veterinary staphylococcal isolates is required as the development of resistance in animal pathogens can result in treatment failure in individual patients and resultant zoonotic risk to pet owners. Moreover, increases in resistance to antimicrobial classes that are important in human medicine may result in the withdrawal of previously available antibacterial agents from veterinary use (Loeffler *et al.* 2007).

The *in vitro* susceptibility of a pathogen to an antimicrobial agent can be assessed by disc diffusion or by measuring the minimum inhibitory concentration (MIC), both of which indicate the lowest drug concentration capable of inhibiting the growth of the bacterium under investigation. Susceptibility testing is controlled with regard to medium, atmosphere and temperature conditions and incubation duration (Blondeau 2009) to facilitate suitable incubation conditions.

The Kirby–Bauer disc diffusion method is a flexible and relatively inexpensive technique that is commonly used in diagnostic laboratories. Dilution methods offer flexibility in the sense that standard media used to test for frequently encountered organisms can be supplemented or replaced with alternative media to allow for accurate testing of fastidious bacteria, which may otherwise not be reliably surveyed by disc diffusion. Dilution methods can also be adapted for automation. The flexibility of dilution testing is also evident in the reporting formats that may be used. Results can be reported quantitatively (µg/mL) or categorically (susceptible, intermediate and resistant), although the two approaches can also be used simultaneously (Murray *et al.* 1999).

Materials and methods
Sampling

Canine skin samples submitted to Vetdiagnostix Veterinary Pathology Services for microbiological culture and sensitivity testing and from which *S. intermedius* was isolated were selected for this investigation. A total of 319 samples from male and female dogs of various ages and breeds from across South Africa were included in this study. Duplicates were not excluded. Samples included skin swabs, skin biopsies, skin abscess and pustule swabs, and fine needle aspirates.

Identification of *Staphylococcus intermedius*

Following growth on 5% sheep blood agar after 24 h incubation at 37 °C, *S. intermedius* was identified on the basis of colony characteristics, catalase production, Gram's stain, lack of pigment production, delayed acid production from D-mannitol, slow or weak maltose production and positive Deoxyribonuclease (DNase) reaction on DNase agar (Quinn *et al.* 1994). All media used were quality controlled using *S. aureus* ATCC 25923. The canine-specific strain of *S. intermedius* (now officially known as *S. pseudintermedius*) can be determined accurately only by DNA sequencing. The isolates found in this study will therefore be referred to as *S. intermedius*, as phenotypic typing cannot reliably distinguish the canine-specific species from others.

Antimicrobial susceptibility tests

Bacteria identified as *S. intermedius* in this study were tested for antimicrobial susceptibility by the disc diffusion method on Mueller Hinton agar. A suspension of the test organism in sterile saline (0.5 McFarland) was evenly spread onto the Mueller Hinton agar plates, after which the plates were disked and incubated at 37 °C for 18 h –24 h (CLSI 2008).

All isolates were tested against the following antimicrobial agents: ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), amoxicillin–clavulanic acid (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), neomycin (30 µg), oxacillin (1 IU), tetracycline (30 µg) and trimethoprim–sulphamethoxazole (co-trimoxazole) (25 µg). The isolates collected between June 2009 and June 2010 were tested against the following additional antimicrobial agents: amikacin (30 µg), ceftiofur (30 µg), clindamycin (2 µg), doxycycline (30 µg), erythromycin (15 µg), imipenem (10 µg), marbofloxacin (5 µg), penicillin (10 IU), rifampicin (5 µg) and ticarcillin (75 µg). After measuring the zones of inhibition, the strains were classified as sensitive or resistant to the drugs tested according to standards and criteria set out by the Clinical and Laboratory Standards Institute (CLSI 2008). The strains showing intermediate results were classified as sensitive or resistant depending on whether the reading was closer to the sensitive or the resistant cut-off.

In addition, the MIC results for the aforementioned antimicrobial drugs were determined for the *S. intermedius* isolates collected between June 2009 and June 2010. The laboratory does not routinely make use of the MIC method and therefore retrospective data were not available for isolates. The broth microdilution method was used and commercial COMPAN1F Sensititre MIC plates were obtained for this purpose (Trek Diagnostics). A 0.5-McFarland suspension of the test organism was prepared in sterile saline and 10 µL of this suspension was added to 990 µL of cation-adjusted Mueller Hinton broth. Chilled calcium and magnesium ion stock solutions were added at 0.1 mL per litre for each desired increment of 1 mg/L in the final concentration in the adjusted Mueller Hinton broth (CLSI 2008). An aliquot of 100 µL of this inoculated broth suspension was then pipetted into each of the 96 wells on the commercial microtitre plate, which was incubated at 37 °C for 18 h – 24 h.

Data analysis

Microsoft Excel was used to calculate, compare and graph the resultant parameters. The statistical analysis is descriptive, using numerator or denominator data.

Results
Susceptibility testing

Staphylococcus intermedius isolates showed greatest antimicrobial resistance to ampicillin, followed by tetracycline and then potentiated sulphonamides (Table 1 and Figure 1). In general, demonstrable antimicrobial resistance was low. Very few methicillin-resistant isolates were detected. Temporal trends
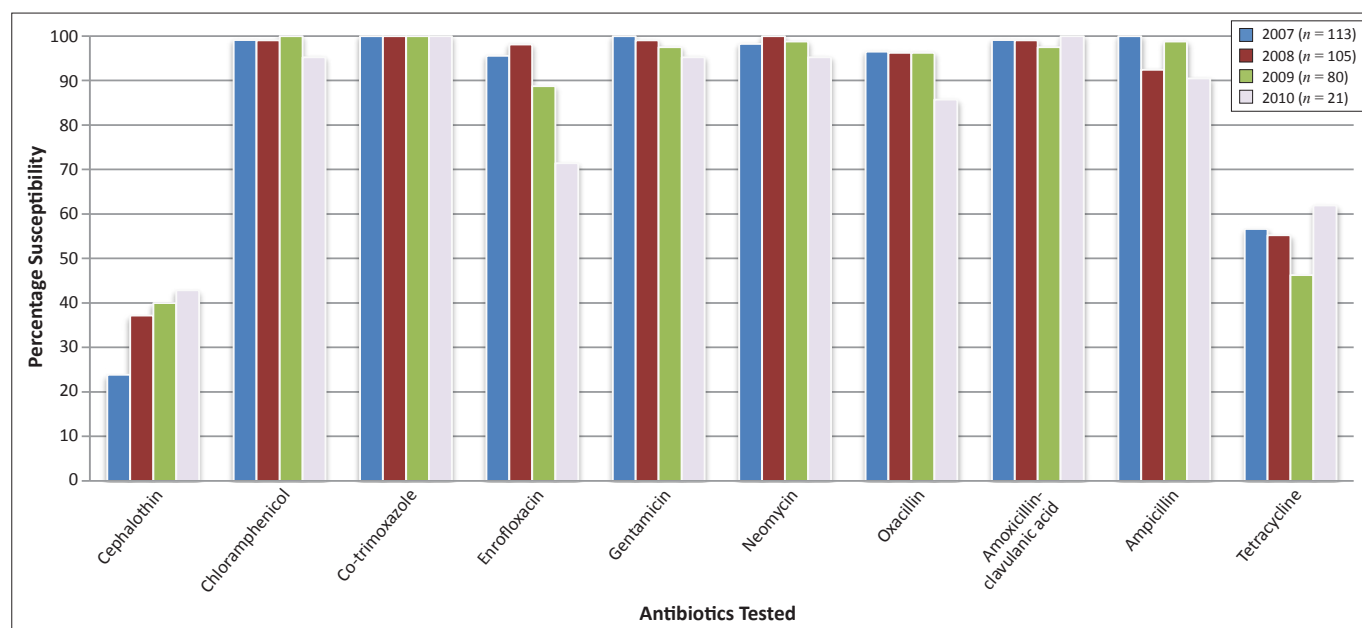
Source: Breakpoint zone of inhibition diameters derived from CLSI, 2008, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard, 3rd edn. (CLSI document M31 – A3), Clinical and Laboratory Standards Institute, Philadelphia

FIGURE 1: Percentage antimicrobial susceptibility of *Staphylococcus intermedius* isolates from canine skin samples for the period 2007–2010.

TABLE 1: Percentage antimicrobial susceptibility of *Staphylococcus intermedius* isolates from canine skin samples for the period 2007–2010.

Antimicrobial	2007 (n = 113)	2008 (n = 105)	2009 (n = 80)	2010 (n = 21)
Ampicillin	23.89	37.14	40.00	42.86
Cephalothin	99.12	99.05	100.00	95.24
Chloramphenicol	100.00	100.00	100.00	100.00
Co-trimoxazole	95.58	98.10	88.75	71.43
Enrofloxacin	100.00	99.05	97.50	95.24
Gentamicin	98.23	100.00	98.75	95.24
Neomycin	96.46	96.19	96.25	85.71
Oxacillin	99.12	99.05	97.50	100.00
Amoxicillin–clavulanic acid	100.00	92.38	98.75	90.48
Tetracycline	56.64	55.24	46.25	61.90

n, given as a number.

were not noted, except for ampicillin, with isolates becoming more susceptible, and potentiated sulphonamides (co-trimoxazole), with isolates becoming more resistant.

Kirby–Bauer and minimum inhibitory concentration data for the period 2007–2010

In general, both the Kirby–Bauer and broth dilution MIC tests yielded similar results for the antimicrobial agents tested (Table 2 and Figure 2).

Table 3 shows the percentage MIC distribution of isolates for each dilution as well as the MIC_{50} (median) and MIC_{90} values. The percentage resistance represented in the table was based on published breakpoints (CLSI 2008). Using the MIC method, all of the tested isolates were found to be completely sensitive to ticarcillin, oxacillin, amoxicillin–clavulanic acid, imipenem, ceftiofur, chloramphenicol, doxycycline, gentamicin, amikacin and co-trimoxazole. Of the isolates tested, 2% – 40% showed some level of resistance to erythromycin, penicillin, ampicillin, enrofloxacin, clindamycin and marbofloxacin. The highest level of resistance was against erythromycin.

TABLE 2: Comparison of percentage antimicrobial susceptibility as indicated by Kirby–Bauer and broth dilution minimum inhibitory concentration data for the period 2007–2010.

Antimicrobial	Kirby–Bauer	Broth dilution MIC
Amikacin	82.92	100.00
Ampicillin	34.14	87.80
Amoxicillin–clavulanic acid	92.68	100.00
Clindamycin	97.56	97.56
Co-trimoxazole	75.61	95.12
Enrofloxacin	97.56	100.00
Erythromycin	95.12	100.00
Gentamicin	97.56	100.00
Imipenem	100.00	100.00
Penicillin	34.14	68.29
Rifampicin	100.00	100.00
Chloramphenicol	100.00	100.00
Marbofloxacin	97.56	100.00
Doxycycline	51.21	100.00
Ticarcillin	97.56	100.00
Ceftiofur	97.56	100.00
Oxacillin	100.00	100.00

MIC, minimum inhibitory concentration.
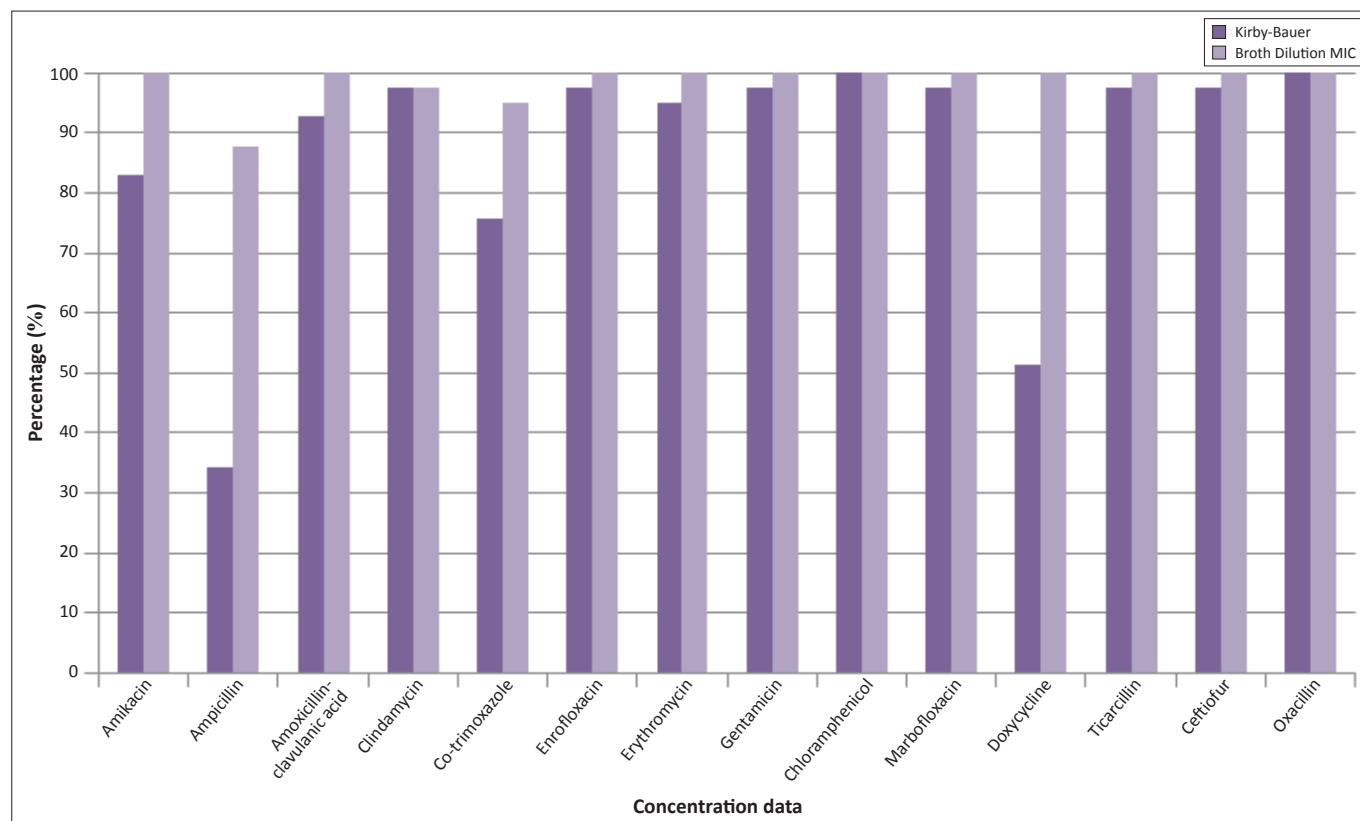
FIGURE 2: Comparison of percentage antimicrobial susceptibility as indicated by Kirby–Bauer and broth dilution minimum inhibitory concentration data for the period 2007–2010.

Discussion

The antimicrobial resistance predicament in human medicine has brought to light various aspects of the use of these substances in animals. There is, however, little useful information on antimicrobial resistance and hence optimal usage in companion animals (Prescott *et al.* 2002).

Susceptibility testing

Antimicrobial resistance of *S. intermedius* was generally low, with the greatest resistance recorded against ampicillin, tetracycline and potentiated sulphonamides. Very few methicillin-resistant isolates were detected. Temporal trends were not noted, except for isolates becoming more susceptible to ampicillin and more resistant to potentiated sulphonamides (co-trimoxazole). Pellerin *et al.* (1998) and Hartmann *et al.* (2005) similarly showed that resistance was most commonly observed to penicillin, tetracycline and sulphamethoxazole-trimethoprim (co-trimoxazole). These drugs should not be used without prior susceptibility testing (Aarestrup 2006). *S. intermedius* is less resistant to erythromycin, clindamycin, cephalexin, oxacillin, amoxicillin–clavulanic acid, enrofloxacin, marbofloxacin and gentamicin, with most strains being susceptible to these drugs (Pellerin *et al.* 1998). Werckenthin *et al.* (2001) found that resistance to penicillin and tetracycline is common in *S. intermedius* and is on the increase. Resistance to most other antimicrobials, particularly newer-generation antimicrobial agents such as the fluoroquinolones is still comparatively low.

Kirby–Bauer and minimum inhibitory concentration data

In general, both the Kirby–Bauer and broth dilution MIC tests yielded similar results for the antimicrobial agents tested. The main difference between the two tests was evident in the over-estimation of resistance by the Kirby–Bauer test for ampicillin, co-trimoxazole, penicillin and doxycycline. This could be related to the instability of these particular drugs *in vitro*. Inoculum densities may also have played a role, with denser inocula producing smaller zone sizes for the drugs tested.

The Kirby–Bauer method remains a convenient, low-cost means of conducting antimicrobial susceptibility tests and is widely used in veterinary laboratories. The test provides qualitative results that categorise isolates as susceptible, intermediate or resistant. Almost all veterinary-specific agents are available in the antimicrobial-impregnated discs. However, low-volume veterinary-specific agents may be available only from the pharmaceutical manufacturer. Smaller veterinary laboratories may experience difficulties standardising the inoculum used in this method; however, commercial systems are available for this purpose (Aarestrup 2006).

Based on the results of the MIC method, all of the isolates tested were found to be completely sensitive to ticarcillin, oxacillin, amoxicillin–clavulanic acid, imipenem, ceftiofur, chloramphenicol, doxycycline, gentamicin, amikacin and co-trimoxazole. Of the isolates tested, 2% – 40% showed

TABLE 3: Percentage minimum inhibitory concentration distribution of *Staphylococcus intermedius* strains isolated in the period June 2009 – June 2010.

Antimicrobial	Susceptibility (%)	Samples with MIC less than tested range (%)	Concentration of antimicrobial tested (µg/mL)											MIC$_{50}$	MIC$_{90}$
			0.06	0.125	0.25	0.5	1	2	4	8	16	32	64		
Penicillin	68.29	43.90	9.76†	14.63†	12.20‡	7.32‡	7.32‡	0.00‡	2.44‡	2.44‡	-	-	-	0.06	0.5
Ampicillin	87.80	80.49	-	-	7.32†	4.88‡	2.44‡	2.44‡	2.44‡	0.00‡	0.00‡	-	-	<0.125	0.5
Ticarcillin	100.00	100.00	-	-	-	-	-	-	-	0.00‡	0.00‡	0.00‡	0.00‡	<8.00	<8.00
Oxacillin	100.00	100.00	-	-	0.00†	0.00†	-	0.00†	0.00‡	-	-	-	-	<0.25	<0.25
Amoxicillin–clavulanic acid	100.00	100.00	-	-	-	-	-	-	0.00†	0.00‡	0.00‡	0.00‡	-	<4/2	<4/2
Imipenem	100.00	100.00	-	-	-	-	-	-	-	-	-	-	-	<1	<1
Ceftiofur	100.00	63.41	-	-	34.15†	2.44†	0.00†	0.00‡	0.00‡	0.00‡	0.00‡	-	-	<0.25	0.25
Erythromycin	53.66	39.02	-	-	-	14.63†	7.32‡	17.07‡	21.95‡	0.00‡	-	-	-	0.5	4
Clindamycin	97.56	97.56	-	-	-	0.00†	0.00‡	0.00‡	2.44‡	-	-	-	-	<0.5	<0.5
Chloramphenicol	100.00	14.63	-	-	-	-	-	-	78.05†	7.32†	0.00‡	-	-	4	4
Doxycycline	100.00	48.78	-	-	-	-	-	17.07†	34.15†	0.00‡	-	-	-	<2	4
Enrofloxacin	92.68	68.29	-	-	17.07†	7.32†	4.88†	2.44‡	-	-	-	-	-	<0.25	0.5
Marbofloxacin	97.56	9.76	-	-	65.85†	19.51†	2.44†	2.44‡	-	-	-	-	-	0.25	0.5
Gentamicin	100.00	90.24	-	-	-	-	7.32†	2.44†	0.00‡	0.00‡	-	-	-	<1	<1
Amikacin	100.00	87.80	-	-	-	-	-	-	7.32†	4.88‡	0.00‡	0.00‡	-	<4	4
Co-trimoxazole	100.00	82.93	-	-	-	12.20†	0.00†	4.88†	0.0‡	-	-	-	-	<0.5/9.5	0.5/9.5

MIC, minimum inhibitory concentration

†, MIC within the tested range

‡, Resistance to the particular antimicrobial, based on the various breakpoints used.

The concentration range for amoxicillin–clavulanic acid was 4/2 µg/mL – 32/16 µg/mL. The concentration range for co-trimoxazole was 0.5/9.5 µg/mL – 2/38 µg/mL.

some level of resistance to the following antimicrobials: erythromycin, penicillin, ampicillin, enrofloxacin, clindamycin and marbofloxacin. The highest level of resistance was shown to erythromycin. The genes that confer erythromycin resistance in canine staphylococci are almost exclusively *ermB* genes. The increase in resistance to the lincosamides, lincomycin, clindamycin and erythromycin may be attributed to the increased use of these drugs in the last decade (Pellerin *et al.* 1998).

The MIC method may be performed in a variety of ways. This method can be used either to provide a quantitative result or to categorise the organism as susceptible or resistant. Standardised methods for testing more fastidious organisms such as anaerobes and *Campylobacter* species have been developed. The MIC method is preferred for use in surveillance or epidemiological investigations as it allows for calculation of summary statistics. Of the various MIC formats used, the broth microdilution method is most widely used and is available in a variety of commercial systems as either dry or frozen panels. It permits testing of a wide range of antimicrobials on a small scale. However, these MIC panels can be inflexible and using custom panels could incur additional cost for a laboratory. Furthermore, not all veterinary-specific antimicrobial agents are available on all panels. Laboratories involved in surveillance programmes or epidemiological studies usually prefer to test a smaller number of antimicrobial agents for an extended number of dilutions. Many diagnostic laboratories choose to use a breakpoint panel. Breakpoint panels allow the laboratory to test a larger number of compounds with dilution ranges spanning the interpretive criteria or breakpoints for each agent (Aarestrup 2006).

Limitations of the study
Recommendations

Bacteria that exhibit antimicrobial resistance will continuously evolve. The bacterial resistance dilemma in human medicine has highlighted the rapid emergence of community-acquired resistance. Data on antimicrobial resistance and trends in antimicrobial use in companion animal practice are still relatively limited. Advice to veterinarians on antimicrobial use needs to be agreed on and continuously monitored and revised. Active and effective infection control programmes need to be implemented in veterinary hospitals to minimise the spread of resistant organisms or their resistance genes. Owing to the limited data available it is imperative that veterinary clinical microbiologists agree on standards for reporting and monitoring resistance and the relationship with trends in antimicrobial use.

Conclusion

Antimicrobial resistance patterns largely reflect evolving patterns in the use of antimicrobial drugs. Laboratory reports of resistance often result from treatment failures rather than treatment successes, as animals that have been treated previously will be more likely to yield resistant bacteria

than those that have not. Antimicrobial resistance in canine bacterial pathogens is possibly of less concern than in human pathogens as pets are exposed to antimicrobial agents for shorter periods and less frequently. Pets are also less likely to be hospitalised. Euthanasia is often a preferred option in chronically ill pets and financial constraints restrict the use of agents such as imipenem (Prescott *et al.* 2002). *S. intermedius* isolates from dogs have developed increased resistance to some drugs but decreased resistance to others. These changes reflect the evolving patterns in antimicrobial use over time. It is the opinion of the authors that antimicrobial resistance is not yet at a critical stage but should be monitored carefully. More information is needed on antimicrobial resistance and its molecular basis in canine medicine.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

J.P. (University of Pretoria) and M.v.V. (University of Pretoria) were the supervisors of the project. C.A.B. (Vetdiagnostix) was responsible for conducting the research, including all experimental procedures, and writing the article.

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