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Original Article

Characterization, distribution, antimicrobial resistance and resistance risk factors in staphylococci isolated from cats from 2001 to 2014

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Abstract

Relatively few studies have been published describing the patterns of staphylococcal isolation and antimicrobial resistance over time in cats. The objective of this retrospective study was to determine the frequency, location, characteristics and antimicrobial resistance profiles of staphylococci isolated by the Louisiana Animal Disease Diagnostic Laboratory between the years 2001 and 2014. All feline staphylococcal isolates were classified phenotypically. Isolates corresponding to known or possibly pathogenic species (*Staphylococcus intermedius* group (SIG) and *Staphylococcus aureus* (SA)) as well as *Staphylococcus epidermidis* (SE) and non-speciated coagulase-negative staphylococci (CNS) were further evaluated to determine antimicrobial resistance patterns. A total of 519 staphylococci were isolated. The largest percentage of isolates was CNS, representing 39.3% of the total, while SIG, SE, SA and non-speciated coagulase positive staphylococci (CPS) represented 18.1%, 10.2%, 8.3% and 7.3%, respectively. Methicillin resistance (MR) was identified in 57.1% of SA and 20.5% of SIG. Resistance to 3 or more antimicrobial classes (multidrug resistance; MDR) was demonstrated in 54.5% of SA and 23.9% of SIG. The prevalence of MDR increased over time in both SIG and SA, while the prevalence of MR increased over time in SIG. An increase in mean antimicrobial resistance score over time was seen in SIG. This study demonstrates a high and increasing prevalence of MDR in SIG and SA, as well as increasing prevalence of MR in SIG isolated from cats.

Keywords: Antimicrobial resistance, Cat, methicillin resistance, multidrug resistance, staphylococcus.

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Introduction

Staphylococci are gram-positive cocci commonly isolated from the skin and other organs and can represent either normal resident flora or opportunistic pathogens. Clinical disease is most commonly associated with coagulase positive species (CPS) of staphylococci, notably *Staphylococcus pseudintermedius* and *S. aureus* (Devriese *et al.* 2005; Morris *et al.* 2006). Less is known about the pathogenic potential of coagulase negative staphylococcal species (CNS). The clinical relevance of their isolation is often considered questionable and they may be dismissed as contaminants. However, recent evidence suggests that some CNS (most notably *S. schleiferi* ssp. *schleiferi*, *S. epidermidis* and *S. lugdunensis*) may be associated with clinical infection in multiple host species, including humans, dogs and cats (Rook *et al.* 2012; Misic *et al.* 2015; Seng *et al.* 2017; Yamada *et al.* 2017).

Until recently, treatment of staphylococcal infections in small animals had been relatively rewarding, as numerous safe and readily available systemic antimicrobials demonstrated good efficacy *in vivo* and *in vitro*. However, in recent years, several studies have demonstrated an alarming increase in antimicrobial resistance in staphylococci isolated not only from humans but also from several veterinary

species, including dogs and horses (Magiorakos *et al.* 2012; Weese & Yu 2013; Beever *et al.* 2015; Priyantha *et al.* 2016). The most well-described pattern of antimicrobial resistance in staphylococci is methicillin resistance (MR). Methicillin resistance is generally associated with the presence of the *mecA* gene (Weese & van Duijkeren 2010). This gene codes for the production of an altered penicillin-binding protein (penicillin-binding protein 2a; PBP2a), which is poorly bound by beta-lactam antimicrobials (Weese & van Duijkeren 2010). While *mecA* does not directly affect susceptibility to other antimicrobial classes, MR isolates frequently demonstrate resistance to multiple classes of antimicrobials (Weese & van Duijkeren 2010).

In contrast to humans, dogs, and horses, relatively little data is available regarding the prevalence and risk factors associated with the development of staphylococcal antimicrobial resistance in cats. Many of the published studies focus on surveillance of healthy pet cats, or on antimicrobial resistance profiles of a single staphylococcal species (typically *S. pseudintermedius* or *S. aureus*)(Davis *et al.* 2014; Vincze *et al.* 2014; Bierowiec *et al.* 2016).

The purpose of this retrospective study was to describe the frequency, source, characteristics and antimicrobial resistance profiles of staphylococci isolated from cats at the Louisiana Animal Disease Diagnostic Laboratory (LADDL) between 2001 and 2014.

Materials and methods

Case selection

The electronic database of the LADDL was queried to identify all aerobic bacterial isolates obtained from cats between 2001 and 2014, excluding necropsy specimens. These samples were submitted from Louisiana and Texas. From this list of isolates, the overall total number of feline staphylococcal cultures was determined. Further analysis was restricted to those staphylococcal cultures for which antimicrobial sensitivity testing was performed. This included the determination of resistance pattern (i.e. MR and/or multidrug resistance), mean resistance score, changes in resistance patterns over time, and resistance in cultures submitted from in-house versus outside patients (Fig. 1).

Identification of bacterial isolates and susceptibility testing

Isolates were acquired and identified using routine aerobic culture methods. Isolates were plated onto a blood agar plate containing 5% sheep blood and MacConkey agar using a sterile loop. Thioglycollate broth was also inoculated using a sterile swab, loop or pipette. After incubation at 35–37°C for 24–48 h, plates were examined for growth. The number of colony-forming units per mL (CFU mL^{-1}) were

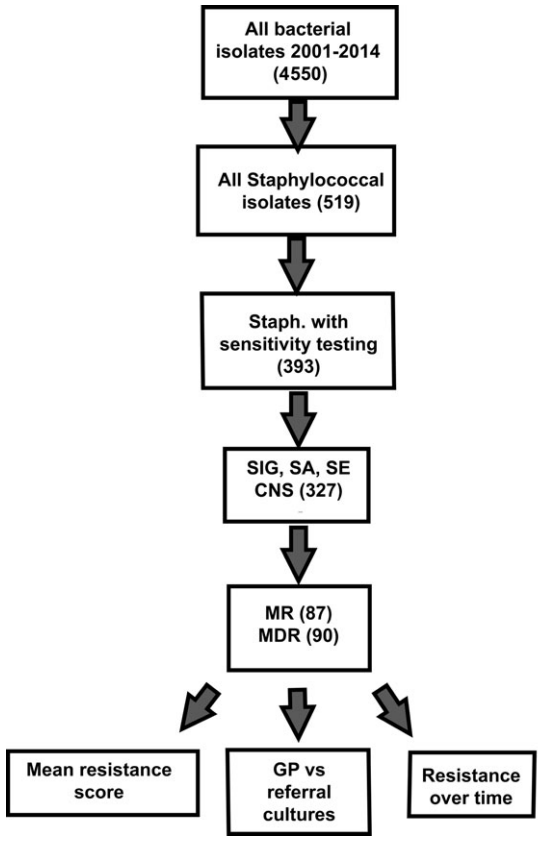

Fig. 1. Sample analysis outline. SIG, *Staphylococcus intermedius* group; SA, *Staphylococcus aureus;* SE, *Staphylococcus epidermidis;* CNS, Coagulase negative *Staphylococcus;* MR, Methicillin resistant; MDR, Multidrug resistant; GP, General practitioner.

determined by counting the number of isolated colonies and multiplying by the dilution, with one colony representing 10^3 organisms mL^{-1}. Suspect colonies were usually opaque, off-white to yellow in colour, ≥1 mm in diameter, and surrounded by a zone of haemolysis on the blood agar plate. There was no growth on the Maconkey agar. Identification of isolates suspected of being a *Staphylococcus* consisted of a gram stain, a catalase test, a tube coagulase test, fermentation of mannitol salt and maltose, and API Staph-Ident (bioMerieux, Marcy L'etoile, France). In the early years of the study, some isolates were not identified beyond the genus or as CPS. Species identification of CNS was generally not performed with the exception of *Staphylococcus epidermidis* (SE).

Specific identification was performed as follows: *S. intermedius* group isolates were identified using the following criteria: coagulase positive, negative for fermentation of mannitol salt and negative for maltose; *S. aureus*: yellow pigmented colony morphology, coagulase positive, positive for fermentation of mannitol salt and positive for maltose; *S. epidermidis*: coagulase negative with consistent API Staph results. If there was no growth on the blood agar plates and the thioglycollate broth was turbid, indicating possible growth, the broth was subcultured onto a blood agar plate.

Determination of antimicrobial susceptibility was performed using the disk diffusion and microbroth dilution method testing procedures in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M31A3 2nd and 3rd editions; CLSI Vet01-S2 2013) with the exception that oxacillin was used to determine methicillin resistance in all staphylococcal species throughout the time evaluated by the study.

The standard LADDL sensitivity panel evaluates up to 9 classes of antimicrobials (*β*-Lactams, macrolides, lincosamides, fluoroquinolones, aminoglycosides, amphenicols, sulphonamides, tetracyclines and polymixins). Extended panels were available upon clinician request. Doxycycline testing was not instituted by the laboratory until 2007. The laboratory also did not specifically test for the tetracycline resistance genes, *mecA* gene, or use the *D*-test to identify inducible resistance between the lincosamides and macrolides. Intermediate susceptibility to an antimicrobial was classified as resistant.

Resistance scoring

Methicillin resistance was defined as resistance to oxacillin as determined by disk diffusion analysis. Each *Staphylococcus* spp. isolate was assigned a resistance score from 0 to 9 based on the number of drug classes reported as resistant. An isolate was considered multidrug resistant (MDR) if it demonstrated resistance to three or more classes of antimicrobials, regardless of whether or not the isolate was resistant to *β*-lactams in general, or to methicillin specifically. Pan-drug resistance was not observed but would have included isolates resistant to all nine antimicrobial classes (score = 9). This classification and scoring scheme was adapted from one previously developed by Magiorakos *et al.* (2012).

Submission source

Staphylococcal isolates submitted by general practitioners and in-house from the University referral centre were compared to determine whether there was a significant difference in the occurrence of MR or MDR between these two source types.

Statistical analysis

All analyses were conducted with SAS® (version 9.4, SAS Institute Inc., Cary, NC, USA) statistical software. The chi-square test was used for contingency table analysis of frequency data. For stratified, multiway tables the Cochran-Mantel-Haenszel test was used. For 2×2 tables, Fisher's exact test was performed, together with calculation of odds ratios. When year was included in the table, the Cochran-Armitage test for trend was used to evaluate yearly resistance trends. Metric response variables were tested with t tests and Wilcoxon Rank Sum tests, as appropriate, for two-group comparisons. Finally, analysis of variance was conducted for factorial arrangements of treatments with respect to isolate score. All tests were considered significant at $P \leq 0.05$.

Results

All bacterial isolates

Between 2001 and 2014, 4550 feline bacterial cultures were performed by the LADDL. Staphylococcal isolates made up 11% (519/4550) of the total number of bacterial isolates.

All staphylococcal isolates

A total of 519 staphylococcal isolates were cultured during the study period (Fig. 1). The largest percentage of isolates were classified as non-speciated coagulase negative (CNS; 204/519, 39.3%). *Staphylococcus intermedius* group isolates represented 18.11% (SIG; 94/519) of the total, followed by *S. epidermidis* (SE; 53/519; 10.2%), *S. aureus* (SA; 43/519; 8.29%), non-speciated coagulase positive staphylococci (CPS; 38/519; 7.32%) and staphylococci not classified beyond genus determination (unclassified *Staphylococcus*; USC; 87/519; 16.76%).

Of the 519 staphylococci, 393 (75.5%) had susceptibility data available for further analysis. These included 159 CNS, 88 SIG, 47 SE and 33 SA (totalling 327; Fig. 1). The remainder of the isolates (CPS and USC) were relatively few in number. Furthermore, these designations were phased out in the latter half of the study, as isolates were more consistently identified to the species level. Because of this non-homogenous distribution, these isolates were not considered for further statistical analysis.

Methicillin resistance

When the four most commonly identified isolate types (SIG, SA, SE and CNS; total 327 cultures) were considered as a group, 26.6% (87/327) were MR. Individually, MR was identified in 20.5%, 36.4%, 21.3% and 29.6% of SIG, SA, SE and CNS, respectively. There was no significant difference between these four groups with regards to the frequency of MR overall (Table 1).

Multidrug resistance

As a group, 27.5% (90/327) of the most commonly identified isolates were resistant to 3 or more antimicrobial classes and were considered multidrug resistant. Individually, MDR was identified in 20.5%, 54.5%, 34% and 23.9% of SIG, SA, SE and CNS, respectively, with SA isolates significantly ($P = 0.001$) more likely to demonstrate multidrug resistance than were the other three isolate types (Table 1).

Association of methicillin resistance with multidrug resistance

When the 327 staphylococcal isolates were evaluated as a whole, a significant association ($P < 0.0001$) between MR and MDR was appreciated (Table 2). This association was also seen when the staphylococcal species were evaluated individually. None of the isolate types were significantly more likely to

Table 1. Methicillin-resistance (MR) and multidrug resistance (MDR; resistance to ≥3 antimicrobial classes)

	Total % (*n*)	MR % (*n*)	MS % (*n*)	MDR (resistant to ≥ 3 antimicrobial classes) % (*n*)	Resistant to < 3 antimicrobial classes % (*n*)
All SIG, SA, SE and CNS	100 ($n = 327$)	26.6 ($n = 87$)	73.4 ($n = 240$)	27.5 ($n = 90$)	72.5 ($n = 237$)
Staphylococcus intermedius group (SIG)	100 ($n = 88$)	20.5 ($n = 18$)	79.5 ($n = 70$)	20.5 ($n = 18$)	79.5 ($n = 70$)
Staphylococcus aureus (SA)	100 ($n = 33$)	36.4 ($n = 12$)	63.6 ($n = 21$)	54.5 ($n = 18$)***	45.5 ($n = 15$)
Staphylococcus epidermidis (SE)	100 ($n = 47$)	21.3 ($n = 10$)	78.7 ($n = 37$)	34 ($n = 16$)	66 ($n = 31$)
Unspeciated coagulase negative Staphylococcus (CNS)	100 ($n = 159$)	29.6 ($n = 47$)	70.4 ($n = 112$)	23.9 ($n = 38$)	76.1 ($n = 121$)

Multidrug resistance was seen significantly more often in SA isolates compared to other isolate types (***$P < 0.001$). N/S, not significant; SIG, *Staphylococcus intermedius* group; SA, *Staphylococcus aureus;* SE, *Staphylococcus epidermidis;* CNS, Coagulase negative *Staphylococcus.*

Table 2. Association of methicillin resistance (MR) with multidrug resistance (MDR)

	Total %	Methicillin resistant		Methicillin sensitive	
		Total MR %	MR/MDR %	Total MS %	MS/MDR %
All SIG, SA, SE and CNS	100 (n = 327)	100 (n = 87)	70.1 (n = 61)***	100 (n = 240)	13.3 (n = 32)
Staphylococcus intermedius group (SIG)	100 (n = 88)	100 (n = 18)	66.7 (n = 12)***	100 (nn=70)	12.9 (n = 9)
Staphylococcus aureus (SA)	100 (n = 33)	100 (n = 12)	91.7 (n = 11)**	100 (n = 21)	33.3 (n = 7)
Staphylococcus epidermidis (SE)	100 (n = 47)	100 (n = 10)	90 (n = 9)***	100 (n = 37)	18.9 (n = 7)
Unspeciated coagulase negative *Staphylococcus* (CNS)	100 (n = 159)	100 (n = 47)	61.7 (n = 29)***	100 (n = 112)	8 (n = 9)

Isolates demonstrating MR were significantly more likely to also be MDR than were methicillin sensitive isolates (**$P < 0.01$; ***$P < 0.001$). N/S, not significant; SIG, *Staphylococcus intermedius* group; SA, *Staphylococcus aureus;* SE, *Staphylococcus epidermidis;* CNS, Coagulase negative *Staphylococcus.*

demonstrate concurrent MR/MDR than were the other types.

Changes in resistance patterns over time

The distribution of MR and MDR staphylococci was evaluated over all years of the study, to determine whether the prevalence of either MR or MDR changed significantly over time. Overall, no significant increase in MR or MDR resistance was observed in the group of the four most commonly identified isolates.

When evaluated separately, neither SE nor CNS demonstrated significant differences in MR or MDR over time (Appendix S1 and S2). In contrast, SIG demonstrated a significant trend towards increases in both MR and MDR over time ($P < 0.001$). When considered alone, SA demonstrated a significant increase in MDR, but not MR, over time ($P < 0.01$).

Calculation of the mean antimicrobial resistance score was performed on individual staphylococcal isolate types as an additional parameter by which global antimicrobial resistance could be evaluated (Appendix S3). Due to the low number of isolates identified, statistical analysis was not able to be performed for SA or SE. No significant difference between the years was found in the mean resistance score for CNS. Because only one isolate of SIG was reported in 2014, statistical analysis was not possible for the entire time period covered by the study. However, when the 2014 data were eliminated from consideration, a significant increase in mean antimicrobial resistance score could be demonstrated ($P = 0.029$; Appendix S3; Fig. 2).

Differences in antimicrobial resistance between submission sources

There was no significant difference in MR between samples submitted from general practices and university referral centre overall, or for SA, SE or CNS individually (Table 3). The occurrence of MDR SIG isolates also did not differ significantly between practice types. However, SIG obtained from the university referral practice were found to have a significantly higher occurrence of MR when compared to those obtained from general practices.

Discussion

The current work represents a large-scale retrospective description of staphylococcal isolation patterns and antimicrobial susceptibility profiles in samples obtained from cats between 2001 and 2014. The most frequently isolated staphylococci were CNS, SIG, SE and SA. The prevalence of MDR was demonstrated to increase over time in both SIG and SA, while the prevalence of MR increased over time in SIG. Furthermore, SIG demonstrated an increase in overall antimicrobial resistance (as evaluated by an antimicrobial resistance score) over the period of this study.

This work also demonstrated an increase in the prevalence of MR in SIG isolated from patients of

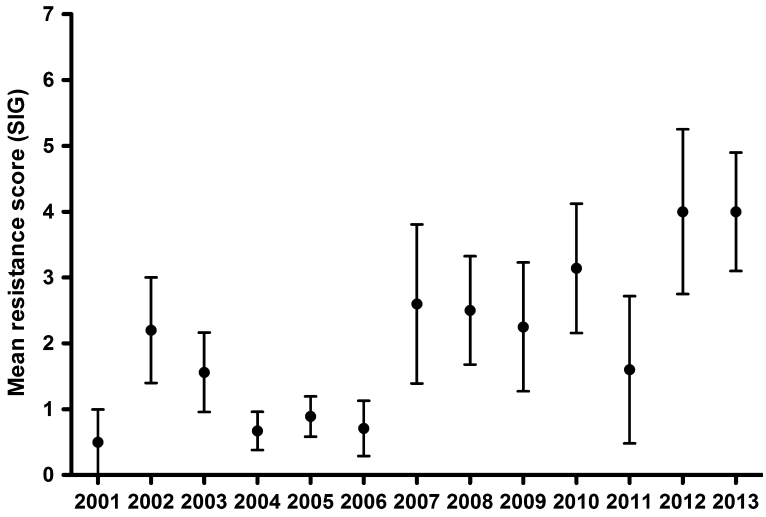

Fig. 2. Mean and standard deviation antimicrobial resistance scores over time for (a) *Staphylococcus intermedius* group (SIG) isolates.

the university referral hospital as compared with samples submitted by general practice veterinarians. These results are similar to those reported in previous studies (Abbott *et al.* 2010; Beever *et al.* 2015). The reason for this increase is not certain but may be related to the greater relative chronicity, complexity and antimicrobial administration history of cases admitted to the referral centre.

The demonstration of increased antimicrobial resistance over this period in SIG and SA (which represent the two staphylococcal species for which pathogenic potential has been best characterized in veterinary species) is particularly disturbing. Over the past 15 years, numerous studies have demonstrated an emergence and increasing prevalence of methicillin and multidrug resistance in staphylococci obtained from not only humans, but also from several veterinary species including horses and dogs (Magiorakos *et al.* 2012; Weese & Yu 2013; Beever *et al.* 2015; Priyantha *et al.* 2016). However, to date, relatively few studies have been published evaluating the prevalence of antimicrobial resistance in staphylococci isolated from cats, with many of those studies representing surveillance of normal cats, or of cats housed in proximity to humans with methicillin-resistant SA (Lilenbaum *et al.* 1999; Morris *et al.* 2012; Gandolfi-Decristophoris *et al.* 2013; Bierowiec *et al.* 2016).

The lack of feline samples is particularly problematic when attempting to discern changes in antimicrobial resistance over time. To the authors' knowledge, only three studies have evaluated chronologic changes in antimicrobial resistance in feline staphylococci. This lack may be due to the relatively small number of staphylococci isolated from cats (and a lower number of cultures submitted overall) as compared to other species, such as dogs. In the first study, Dorsch et. al. evaluated the prevalence of staphylococci (among other bacteria) isolated from urine samples over a ten-year period but failed to demonstrate an increase in the prevalence of antimicrobial resistance over this time (Dorsch *et al.* 2015). Regrettably, this manuscript did not report the actual prevalence rates for staphylococcal antimicrobial resistance, making comparison to the current work difficult. In the second study, Couto *et al.* (2016) demonstrated an overall increase in the prevalence of antimicrobial resistance in samples obtained from several animal species (including cats) between 1999 and 2014, but did not provide specific information for feline-source isolates. Finally, Beever *et al.* (2015) evaluated 14 555 SIG cultured from small animal patients in the United Kingdom (UK) between 2003 and 2012, of which 583 were obtained from cats, and of those, only three isolates (0.5%) demonstrated MR. This low prevalence rate is in contrast to the overall

Table 3. Comparison of occurrence of methicillin-resistance (MR) and multidrug resistance (MDR) by sample source

	General practitioner	University	General practitioner		University		General practitioner		University	
	Total %	Total %	MR %	MS %	MR %	MS %	MDR %	Low %	MDR %	Low %
All SIG, SA, SE and CNS	100 (n = 195)	100 (n = 132)	25.1 (n = 49)	74.9 (n = 146)	28.8 (n = 38)	71.2 (n = 94)	27.2 (n = 53)	72.8 (n = 142)	28 (n = 37)	72 (n = 95)
Staphylococcus intermedius group (SIG)	100 (n = 54)	100 (n = 34)	13 (n = 7)	87 (n = 47)	32.4* (n = 11)	67.6 (n = 23)	18.5 (n = 10)	81.5 (n = 44)	32.4 (n = 11)	67.6 (n = 23)
Staphylococcus aureus (SA)	100 (n = 15)	100 (n = 18)	20 (n = 3)	80 (n = 12)	50 (n = 9)	50 (n = 9)	40 (n = 6)	60 (n = 9)	50 (n = 9)	50 (n = 9)
Staphylococcus epidermidis (SE)	100 (n = 31)	100 (n = 16)	22.6 (n = 7)	77.4 (n = 24)	18.8 (n = 3)	81.3 (n = 13)	35.5 (n = 11)	31.3 (n = 20)	31.3 (n = 5)	68.8 (n = 11)
Unspeciated coagulase negative Staphylococcus (CNS)	100 (n = 95)	100 (n = 64)	33.7 (n = 32)	66.3 (n = 63)	23.4 (n = 15)	76.6 (n = 49)	27.4 (n = 26)	72.6 (n = 69)	18.8 (n = 12)	81.3 (n = 52)

Staphylococcus intermedius group bacteria cultured from patients of a university referral centre were significantly more likely to demonstrate MR than were SIG obtained from samples submitted by general practitioners (*$P < 0.05$). N/S, not significant; SIG, *Staphylococcus intermedius* group; SA, *Staphylococcus aureus;* SE, *Staphylococcus epidermidis;* CNS, Coagulase negative *Staphylococcus.*

prevalence rate of MR in SIG reported in cats in this study (20.5%). The reason for this difference is unclear, but may include such factors as geographic differences in the spread of resistant clones (a slower spread to the relatively isolated UK might be expected), different antimicrobial prescribing practices and different sources of guidelines for susceptibility testing (i.e. CLSI in the United States and the British Society for Antimicrobial Chemotherapy in the UK). In addition, at least some of the difference may have come from the use of cefoxitin disks as a surrogate for oxacillin between 2007 and 2011 in one of the source laboratories for the Beever manuscript. Although this was the recommended practice at the time, cefoxitin has subsequently been demonstrated to underestimate the prevalence of MR in SIG, and its use is no longer recommended for this species (Bemis *et al.* 2006; CLSI, 2013).

An additional factor of concern raised in this study is the increase in the number of antimicrobial classes to which resistance was demonstrated in this study. Both SIG and SA demonstrated a significant trend for an increase in the occurrence of MDR (defined as resistance to 3 or more antimicrobial classes) over time. While this is a disturbing finding, the trend for increased resistance to even more classes of antimicrobials is truly alarming. In this study, resistance to seven or more antimicrobial classes was first demonstrated in 2007, with additional cases demonstrated in 2008, 2009, 2010, 2012 and 2013. This pattern is very similar to that recently demonstrated in dogs (Magiorakos *et al.* 2012).

Because of its retrospective nature, this study does have limitations. First, it was not possible to confirm the pathogenic role of all of the identified isolates. While the pathogenic potential of certain species (notably SIG and SA) is generally accepted, the role of other staphylococcal species (particularly CNS) in the development of disease is less well characterized. In humans, SE is a common nosocomial pathogen, and clinically significant infections with other CNS species (including *S. lugdunensis*, *S. schleiferi* spp. s*cheleiferi* and *S. simulans*) have recently been reported (Hernández *et al.* 2001; Otto 2012; Marchant *et al.* 2013; Shields *et al.* 2016; Seng *et al.* 2017). In veterinary medicine,

CNS were historically considered to have low pathogenic potential, and isolation of these bacteria was often dismissed as indicative of contamination. However, recent evidence suggests that CNS may also be opportunistic pathogens in non-human species, including cats. For example, *S. felis* has been associated with cystitis, otitis externa, and dermatitis, *S. haemolyticus* with cystitis, and *S. epidermidis* with cystitis, abscesses and conjunctivitis (Higgins & Gottschalk 1991; Patel *et al.* 2002; Litster *et al.* 2007; Kern & Perreten 2013; Worthing *et al.* 2018). Additionally, colonization with *S. schleiferi* spp. *schleiferi* has been reported in association with inflammatory skin disease in cats (Abraham *et al.* 2007; Griffeth *et al.* 2008). Furthermore, *S. felis* has been demonstrated to harbour genes coding for putative virulence factors, including exfoliative toxin (*siet*), biofilm-associated protein (*bap*), and staphylococcal complement inhibitor (*scn*), thus supporting its potential to act as a true pathogen (Worthing *et al.* 2018). Finally, evidence exists suggesting that horizontal transfer of genetic elements coding for antimicrobial resistance (including *mecA* and the mupirocin resistance gene *mupA*) between CNS and coagulase positive staphylococci such as SA is possible (Hurdle *et al.* 2005; Berglund & Söderquist 2008; Fluit *et al.* 2013; Otto 2013). If this proves to be the case, then CNS may serve as potential reservoirs for spread of antimicrobial resistance genes, suggesting that antimicrobial resistance in these bacteria may be clinically relevant independent of direct pathogenic potential.

Second, molecular confirmation of the staphylococcal species identified in this study was not performed, and in many cases, only a limited characterization (by coagulase properties or by species) was performed. Along similar lines, demonstration of antimicrobial resistance genes (such as *mecA* or *tetK*) was not performed, and characterization of resistance profiles was carried out based upon disk diffusion data only. While the lack of these data is unfortunate, neither molecular confirmation of species nor identification of specific resistance cassettes is likely to be clinically relevant. It must be kept in mind that this manuscript was not intended to provide a detailed microbiological analysis but rather was meant to be a broad survey of the isolation patterns and susceptibility profiles of staphylococci isolated from cats.

Third, antimicrobial susceptibility to methicillin was evaluated using oxacillin for all staphylococcal species during the time covered by this study. This practice was not in strict agreement with veterinary CLSI guidelines, which were revised in 2008 to recommend cefoxitin as a replacement for oxacillin for the evaluation of MR in staphylococci (CLSI, 2008). Cefoxitin remains the preferred test agent for SA and most CNS. However, the guidelines were changed again in 2013 to recommend oxacillin as the preferred test agent for SIG (CLSI, 2013). Relative to cefoxitin, oxacillin has the potential to overestimate MR associated with *mecA* in CNS and underestimate MR associated with *mecA* in SA (CLSI, 2006; El Dine *et al.* 2009). Thus, some degree of imprecision in the overall prevalence results presented here for SA and CNS may be present. Although strict and timely adherence to this particular guideline would have been ideal, a change in testing procedures during the evaluation period would have introduced additional variables into the analysis of chronologic changes in MR in the current report. A similar issue was reported by Beever *et al.* (2015), in which accurate determination of changes in the frequency of MR was complicated by these changing guidelines.

Finally, these samples were obtained from a relatively limited geographic area (Louisiana and Texas). The results of previous studies evaluating antimicrobial susceptibility patterns have often differed considerably depending upon the geographic region. For this reason, the results of this study may not necessarily be representative of global susceptibility patterns. Ideally, similar investigations should be performed at other institutions (or as multi-centre collaborative works) to determine whether these results represent a local or global phenomenon.

Conclusion

This work has described the frequency and source of the major staphylococcal species (SIG, SA, SE and CNS) isolated from feline samples submitted to the

LADDL between 2001 and 2014. We have demonstrated a relatively high prevalence of both MR and MDR in all four species/groups. No significant difference between the species was found for the prevalence of MR, but SA was significantly more likely to demonstrate MDR than the other species evaluated here. The prevalence of MR, but not MDR was found to be significantly higher in samples submitted from the university referral centre relative to those submitted by general practitioners. The occurrence of both MR and MDR was found to increase over the time period covered by the study in SIG. A similar increase in MDR, but not MR, was also demonstrated for SA. Finally, a significant increase in overall antimicrobial resistance (as measured by the mean antimicrobial resistance score) was demonstrated in SIG.

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Conflict of interest

The authors claim no conflict of interest.

Contribution

MJL collated and interpreted the resistance data and wrote much of the manuscript. AFR assisted in collation and interpretation of the resistance data and provided information regarding laboratory methodologies. MTK performed statistical analysis and assistance. CMP-H oversaw the project, assisted with data collation and interpretation and assisted with manuscript writing and revision.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journals author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Methicillin resistance (MR) and sensitivity from 2001 to 2014.

Appendix S2. Multidrug resistance (MDR) from 2001 to 2014.

Appendix S3. Antimicrobial resistance score, mean and standard deviation from 2001 to 2013.