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Nationwide analysis of methicillin-resistant staphylococci in cats and dogs: resistance patterns and geographic distribution

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Objective
This study aims to quantify the frequency and resistance patterns of 3 methicillin-resistant staphylococci (MRS), *Staphylococcus aureus* (MRSA), *Staphylococcus pseudintermedius* (MRSP), and *Staphylococcus schleiferi* (MRSS), in companion animals, using historical culture and susceptibility data from a national diagnostic laboratory.

Methods
Samples from cats and dogs across the US, between 2019 and 2022, were analyzed. Methicillin-resistant isolates identified according to Clinical and Laboratory Standards Institute VET01S (5th ed) were included. Data included location, patient species, sampling site, year, and susceptibility results for various panels of antimicrobials.

Results
There were 110,423 MRSP, 5,618 MRSA, and 20,934 MRSS isolates identified. Methicillin-resistant *S pseudintermedius* was predominantly found in dogs (96.2%), with skin and soft tissue being the most common sites. Methicillin-resistant *S aureus* and MRSS were also primarily isolated from dogs, with significant yearly, regional, and species-specific differences in antimicrobial susceptibility observed. This study highlights high resistance levels in MRSP isolates, while MRSA and MRSS showed relatively higher susceptibility to several antimicrobials.

Conclusions
This study provides insight into the distribution and antimicrobial resistance patterns of MRSA, MRSP, and MRSS in companion animals in the US. Resistance rates for enrofloxacin, marbofloxacin, and chloramphenicol may be higher than reported in this analysis due to recent changes in MIC breakpoints in the Clinical and Laboratory Standards Institute VET01S (7th ed). The findings underscore significant geographical and temporal variations in resistance, emphasizing the need for tailored antimicrobial stewardship programs.

Clinical Relevance
The prevalence of MRS in companion animals poses treatment challenges and potential zoonotic risks. This study provides nationwide insight that was not previously available.

Keywords: antimicrobial resistance, multidrug resistance, staphylococci, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococci are opportunistic bacteria that are naturally found in the microbiota of healthy dogs, cats, and humans.[1] *Staphylococcus pseudintermedius* is a major concern for a range of infections in companion animals, including pyoderma, otitis, and urinary tract infections.[1–3] Other species, including *Staphylococcus aureus* and *Staphylococcus schleiferi*, have also been frequently isolated from clinically healthy and diseased dogs and cats.[2,4,5] With skin infections being one of the most common infections in companion animals, the increase in antimicrobial-resistant staphylococci strains poses a concern in the veterinary field.[6,7]

Methicillin resistance is conferred by the *mecA* gene and renders bacteria resistant to most β-lactam antimicrobials, which are heavily dependent on in human and animal medicine.[4,8,9] Isolates are defined as multidrug resistant (MDR) if they exhibit resistance to at least 1 antimicrobial in 3 or more different

Received September 7, 2024
Accepted December 20, 2024
Published online February 10, 2025

doi.org/10.2460/ajvr.24.09.0253

classes of antimicrobials.[10,11] Multidrug resistance is a rising concern that is often found to be associated with staphylococci isolates, particularly the isolates exhibiting methicillin resistance.[2,4,12] In 2021, 98% of the methicillin-resistant *S pseudintermedius* (MRSP) isolates obtained from veterinary diagnostic laboratories across the US were MDR.[13] The increase in MRSP, methicillin-resistant *S aureus* (MRSA), and methicillin-resistant *S schleiferi* (MRSS) isolates limits the treatment plans available for staphylococci infections.[14–16]

While MRSA is recognized as a major pathogen in human health, MRSP and MRSS are the primary pathogens of concern for skin and soft tissue infections in cats and dogs.[17] However, MRSA strains have been increasingly isolated from healthy dogs and cats, while MRSP and MRSS have been found colonizing and infecting humans.[17–19] Companion animals are especially relevant to this discussion as the close relationship between humans and their pets may promote the cross-colonization and transmission of staphylococci.[20,21] Recent studies[21–23] have found that humans and companion animals may serve as reciprocal reservoirs and spread strains of staphylococci to one another. Seeing that roughly 45% and 26% of households in the US own dogs and cats, respectively, and that these numbers have been growing over the past couple of years,[24] the zoonotic potential of MDR staphylococci is a significant public health concern.

This study investigates the distribution of methicillin-resistant staphylococci (MRS) isolates submitted for culture and susceptibility testing across the US and the resistance patterns of MRSP, MRSA, and MRSS isolates to select antimicrobial drugs.

Methods

Historical data from specimens sampled from dogs and cats submitted to IDEXX Laboratories for culture and susceptibility testing from 2019–2022, inclusive, were obtained. Data were included for isolates of *S pseudintermedius*, *S aureus*, and *S schleiferi* that were phenotypically identified as methicillin resistant. The identification of these isolates was performed using matrix-assisted laser desorption ionization–time of flight.[25] Antimicrobial susceptibility testing was performed with the with the VITEK2 system (bioMérieux). All staphylococcal isolates were classified as methicillin resistant or susceptible using oxacillin test results. Cefoxitin susceptibility results were not available for this study. For each isolate, available information included the location of the submitting clinic (county level), patient species, sampling site, year, and antimicrobial susceptibility test results for all antibiotics tested. Antimicrobials tested for > 98% of identified MRSP isolates were included. In addition, a subset of antimicrobials was selected a priori based on expected testing patterns or their importance in treating MDR infections. Multidrug resistance was defined as nonsusceptibility to at least 1 antimicrobial in 3 or more classes.[10,11]

Repeat observations were defined by the following criteria: originating from the same patient, isolated from the same sampling site, identified as the same bacterial species, and identified within a 90-day period.[26] If repeat observations were present, only the first result was included. Data were analyzed according to the Clinical and Laboratory Standards Institute (CLSI) M39 guidelines for the *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*.[26] Results were reported as susceptible, intermediate, or resistant, with laboratory testing and interpretation conducted following the standards outlined in the CLSI VET01S (5th ed): *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*.[27] Where applicable, species-specific breakpoints were used. Cat-specific breakpoints were available only for fluoroquinolone antibiotics; all other cat interpretations used dog-specific breakpoints. When neither cat- nor dog-specific breakpoints were available, human breakpoints, borrowed from CLSI M100 (30th ed),[28] were applied.

The VITEK2 system contains an inducible clindamycin resistance (ICR) test. Isolates identified as ICR positive were recorded as clindamycin resistant, regardless of MIC value. This test and classification were performed before data transfer from the lab. The number of ICR isolates could not be determined from available data sources.

Categorical comparisons were performed using χ^2 tests. To account for the large sample size, which could result in many statistically significant findings, the Cohen *h* effect size metric was included to assess the magnitude of the observed effects. The Cohen *h* effect sizes were interpreted as follows: ≥ 0.2 was considered small, ≥ 0.5 was considered moderate, and ≥ 0.8 was considered large. For comparative analyses, categories with < 30 isolates were excluded.

Relative comparisons between states were made by standardizing the number of MRSP isolates observed by the cat and dog population. This population was approximated using the *2002 AVMA Pet Ownership and Demographic Sourcebook*[24] and 2022 US Census data.[29] The national average of MRSP isolates per 100,000 animals was determined, and the percentage deviation from this mean was calculated for each state. To further assess geographical differences, states were categorized into 4 regions per the US Census Bureau[30] for comparison.

All statistical analysis was performed using R Statistical Software.[31]

Results

Methicillin-resistant S pseudintermedius

Methicillin-resistant *S pseudintermedius* was isolated from 110,423 samples: 20,397 (18.5%) in 2019, 26,809 (24.3%) in 2020, 30,922 (28.0%) in 2021, and 32,295 (29.2%) in 2022. After 2019, the proportion of isolates classified as methicillin resistant remained stable: 29.5% (20,069/68,125) in 2019, 33.6% (26,451/78,783) in 2020, 33.3% (30,567/91,728) in 2021, and 33.7% (31,953/94,914) in 2022. Isolates were obtained from 106,205 (96.2%) dogs and 4,218 (3.8%) cats. The most common sites were skin and soft tissue (56,197 [50.9%]), ear (21,271 [19.3%]), urinary tract (14,789 [13.4%]), and procedural site

(2,668 [2.4%]). Virtually all isolates identified as MRSP were classified as MDR (99.98%).

Susceptibility to the following antimicrobials was evaluated: amikacin (tested in 110,417 isolates), chloramphenicol (110,421), clindamycin (110,400), doxycycline (110,082), enrofloxacin (110,412), gentamicin (110,422), marbofloxacin (110,418), minocycline (109,005), and trimethoprim-sulfamethoxazole (107,612). Nitrofurantoin was also included despite only 14,307 isolates tested. Nitrofurantoin testing is typically only performed on urinary isolates; therefore, a subset analysis was likely based primarily on the source, not initial susceptibility results. Rifampin (89,210) was also included. This inclusion was based on the relatively high testing and the potential relevance of the drug for treating MDR infections. Azithromycin (95,943) was also included for exploratory analysis, given limited previous information about the susceptibility of MRSP to this antimicrobial.

Overall and yearly susceptibility results are presented **(Figure 1)** and described in detail **(Table 1)**. There were statistically significant annual variations

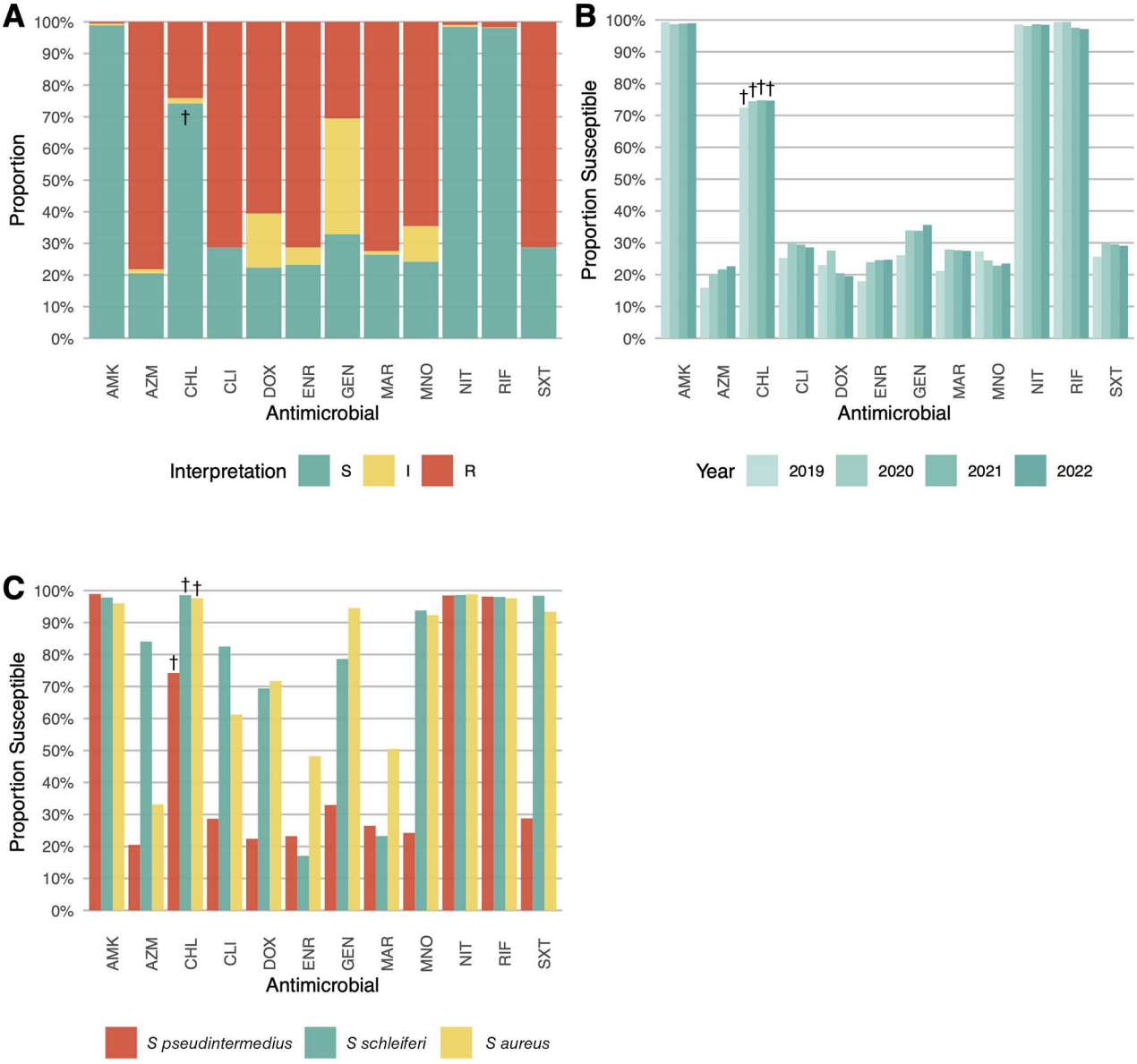

Figure 1—A—Stacked bar chart of susceptible (S), resistant (R), intermediate (I), interpretations for MRSP isolates from cats and dogs across various antibiotics. B—Grouped bar chart of annual susceptible proportions (2019–2022) for the same antibiotics for methicillin-resistant *Staphylococcus* pseudintermedius (MRSP) isolates. C—Grouped bar chart of susceptible proportions for *Staphylococcus aureus*, MRSP, and *Staphylococcus schleiferi* across the same antibiotics. †The most recent Clinical Laboratory Standards Institute VET01S guidelines have considerably lowered the breakpoints for chloramphenicol compared to the breakpoints used in this analysis. As a result, the susceptibility rates reported here are likely overestimated. AMK = Amikacin. AZM = Azithromycin. CHL = Chloramphenicol. CLI = Clindamycin. DOX = Doxycycline. ENR = Enrofloxacin. GEN = Gentamicin. MAR = Marbofloxacin. MNO = Minocycline. NIT = Nitrofurantoin. RIF = Rifampicin. SXT = Trimethoprim-sulfamethoxazole.

Table 1—Percent susceptible (S), resistant (R), intermediate (I), total isolates, and Cohen *h* for effect size in the proportion of S between *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, and *Staphylococcus schleiferi* for commonly tested antibiotics.

	S aureus				*S pseudintermedius*				*S schleiferi*					
	S (%)	I (%)	R (%)	Total	S (%)	I (%)	R (%)	Total	S (%)	I (%)	R (%)	Total	*P* value	Effect size
Amikacin	96	3.42	0.59	5,618	98.92	0.55	0.53	110,417	97.81	1.02	1.17	20,930	***	
Azithromycin	33.17	1.37	65.46	5,191	20.52	1.28	78.2	95,943	84.04	1.25	14.71	20,405	***	Large
Chloramphenicol†	97.58	0.28	2.14	5,618	74.24	1.69	24.08	110,421	98.53	0.51	0.97	20,932	***	Medium
Clindamycin	61.22	0.05	38.72	5,617	28.63	0.05	71.31	110,400	82.51	0.1	17.39	20,934	***	Large
Doxycycline	71.75	13.53	14.72	5,551	22.4	17.07	60.54	110,082	69.41	11.88	18.7	20,617	***	Large
Enrofloxacin	48.23	5.88	45.9	5,617	23.21	5.52	71.27	110,412	17.07	25.54	57.39	20,933	***	Medium
Gentamicin	94.54	0.14	5.32	5,618	32.94	36.55	30.51	110,422	78.61	14.74	6.64	20,934	***	Large
Marbofloxacin	50.52	0.46	49.02	5,618	26.46	1.11	72.43	110,418	23.25	2.39	74.36	20,934	***	Small
Minocycline	92.35	0.41	7.24	5,593	24.23	11.24	64.53	109,005	93.77	1.43	4.8	20,896	***	Large
Nitrofurantoin	98.82	0.71	0.47	425	98.46	0.59	0.95	14,307	98.6	0.8	0.6	501		
Rifampicin	97.59	0.04	2.37	4,604	98.1	0.21	1.69	89,210	98.01	0.3	1.7	17,557	*	
SXT	93.33	0	6.67	5,503	28.75	0.01	71.23	107,612	98.35	0.01	1.64	20,321	***	Large

SXT = Trimethoprim-sulfamethoxazole.

†The most recent Clinical Laboratory Standards Institute VET01S guidelines have considerably lowered the breakpoints for chloramphenicol compared to the breakpoints used in this analysis. As a result, the susceptibility rates reported here are likely overestimated. ***$P \leq .001$. *$P \leq .05$.

in susceptibility for most antimicrobials. From 2019 to 2022, there was an increase in susceptibility to azithromycin ($P < .0001$), chloramphenicol ($P < .0001$), clindamycin ($P < .0001$), enrofloxacin ($P < .0001$), gentamicin ($P < .0001$), marbofloxacin ($P < .0001$), and trimethoprim-sulfamethoxazole ($P < .0001$) and decreases in susceptibility to doxycycline ($P < .0001$), minocycline ($P < .0001$), and rifampin ($P < .0001$).

Isolates were obtained from all 50 states and the District of Columbia, ranging from 37 (Wyoming) to 18,830 (California) isolates, with a median of 1,083. Relative differences in the number of MRSP isolates per 100,000 dogs and cats were noted at the state level **(Figure 2)**. The following states showed MRSP observations at least 50% greater than the national average: Wisconsin (99.1% greater than the national average), Maine (90%), the District of Columbia (86.7%), Connecticut (83.9%), New Hampshire (79.9%), Ohio (73.1%), California (63.9%), Massachusetts (63.4%), Illinois (59.9%), and Arizona (54.4%). Conversely, the following states showed MRSP observations at least 50% below the national

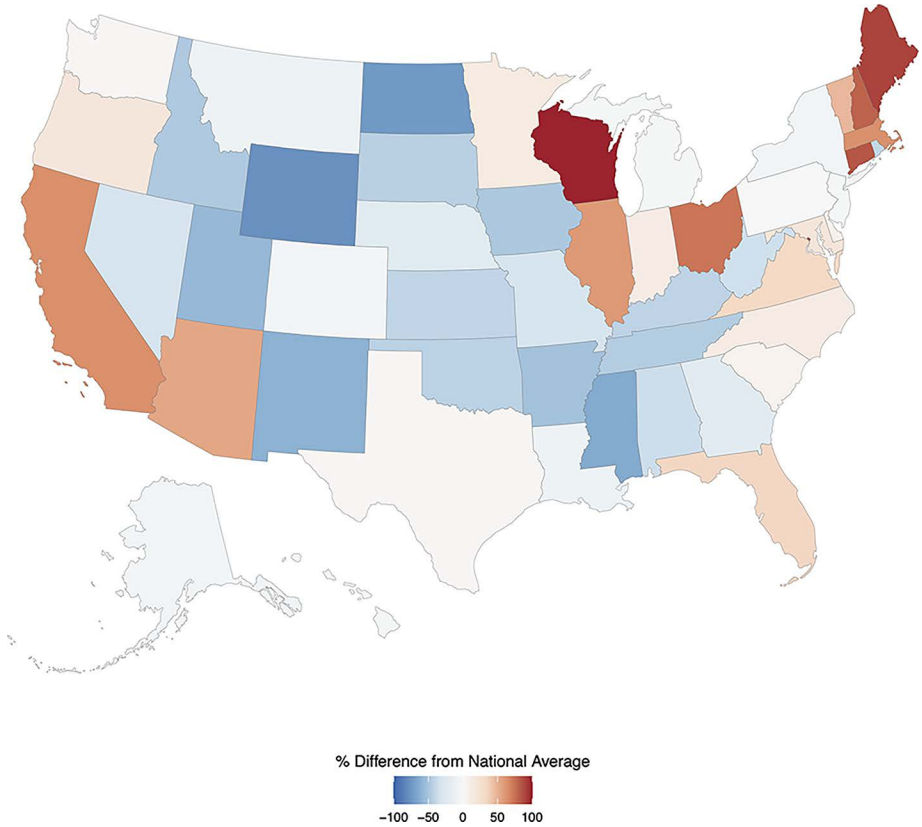

Figure 2—Map of the US showing relative differences in observed MRSP isolates per 100,000 cats and dogs between 2019 and 2022, compared to the national average, with each state color coded by this relative difference.

average: Wyoming (77.9% less than the national average), North Dakota (−73.5%), Mississippi (−64.3%), New Mexico (−61%), Utah (−57%), and Arkansas (−52.1%). There were significant differences in susceptibility to most antibiotics at the census region level. Only chloramphenicol was found to have a notable effect size between the regions based on the Cohen *h* **(Table 2)**. The proportion of susceptible isolates for select antimicrobials is presented at the state level **(Figure 3)**, excluding Wyoming (n = 19) and North Dakota (25) in some instances due to small sample sizes.

Statistical differences in susceptibility between canine and feline isolates for the main antimicrobials were observed. No appreciable effect size was noted for any antimicrobial. These results are presented **(Supplementary Material S1)**. Similarly, statistical differences were noted in the antimicrobial susceptibility between common isolation sites; however, the only notable effect size was observed with doxycycline **(Supplementary Material S2)**.

Methicillin-resistant S aureus

A total of 5,618 MRSA isolates were identified from 50 states and the District of Columbia, ranging from 4 (Rhode Island and Wyoming) to 584 (California) per state with a median of 69. Methicillin-resistant *S aureus* was isolated from 3,835 (68.3%) dogs and 1,783 (31.7%) cats. The most common sources were skin and soft tissue (3,207 [57.1%]), urinary (449 [8.0%]), and ear (n = 443 [7.9%]). The number of MRSA isolates observed per year was as follows: 1,178 (21.0%) in 2019, 1,242 (22.1%) in 2020, 1,580 (28.1%) in 2021, and 1,618 (28.8%) in 2022. The proportion of *S aureus* isolates classified as methicillin resistant remained relatively stable over the years: 20.6% (1,160/5,643) in 2019, 19.6% (1,236/6,296) in 2020, 20.5% (1,568/7,637) in 2021, and 20.2% (1,615/7,994) in 2022. Virtually all isolates identified as MRSA were MDR (99.95%).

All 5,618 MRSA isolates were tested for susceptibility to amikacin (96.0% susceptible), chloramphenicol (97.6% susceptible), gentamicin (94.5%), and marbofloxacin (50.5% susceptible). Subsets of isolates were tested against minocycline (n = 5,593 [93.3% susceptible]), nitrofurantoin (425 [98.8%]), rifampicin (4,604 [97.6%]), and trimethoprim-sulfamethoxazole (5,503 [93.3%]). Chloramphenicol susceptibility results should be interpreted cautiously, as the latest CLSI VET01S (7th ed)[32] guidelines have considerably lowered the breakpoints.

Methicillin-resistant S schleiferi

A total of 20,934 MRSS isolates were identified between 2019 and 2022; 20,529 (98.1%) from dogs and 405 (1.9%) from cats. Isolates were predominantly from skin and soft tissue (10,345 [49.4%]) and ear (8,284 [39.6%]). The MRSS isolates were identified from all states except for Wyoming, plus the District of Columbia. The MRSS observations per state ranged from 3 (North Dakota and South Dakota) to 3,962 (California), with a median of 141.5. The number of MRSS isolates observed per year was as follows: 4,983 (23.8%) in 2019, 5,120 (24.5%) in 2020, 5,330 (25.5%) in 2021, and 5,501 (26.3%) in 2022. The proportion of *S schleiferi* isolates classified as methicillin resistant was similar over the 4 years: 34.3% (4,934/14,393) in 2019, 32.8% (5,067/15,461) in 2020, 31.1% (5,279/16,975) in 2021, and 31.1% (5,458/17,526) in 2022. Subspecies identification was not reported. Virtually all isolates identified as MRSS were MDR (99.9%).

All 20,934 isolates were tested for susceptibility to clindamycin (82.5% susceptible), gentamicin (78.6%), and marbofloxacin (23.3%). Most were tested for susceptibility to amikacin (n = 20,930 [97.8% susceptible]), chloramphenicol (20,932 [98.5%]), minocycline (20,896 [93.8%]), and trimethoprim-sulfamethoxazole (20,321 [98.4%]), with smaller subsets tested for susceptibility to nitrofurantoin (501 [98.6%]), and rifampicin (17,557 [98.0%]). Chloramphenicol susceptibility results should be interpreted cautiously, as the latest CLSI VET01S (7th ed)[32] guidelines have considerably lowered the breakpoints.

Table 2—Percent susceptible MRSP isolates for commonly tested antibiotics by US Census regions, observed between 2019 and 2022.

	Midwest		Northeast		South		West			
	S (%)	Total	S (%)	Total	S (%)	Total	S (%)	Total	*P* value	Effect size
Amikacin	98.9	25,109	99	19,102	98.9	36,363	98.9	29,843		
Azithromycin	22	21,714	18.5	16,651	19.8	31,924	21.6	25,654	***	
Chloramphenicol†	79.8	25,109	75	19,102	77.7	36,364	64.9	29,846	***	Small
Clindamycin	27.3	25,107	26	19,102	26.1	36,357	34.6	29,834	***	
Doxycycline	23.8	25,088	18.2	18,990	23.5	36,182	22.6	29,822	***	
Enrofloxacin	25.9	25,108	24	19,101	20.2	36,363	24.1	29,840	***	
Gentamicin	34.4	25,109	30.8	19,102	33.2	36,365	32.8	29,846	***	
Marbofloxacin	29.2	25,107	26.5	19,102	23.2	36,363	28.1	29,846	***	
Minocycline	26.2	24,852	22.2	18,675	26.3	35,669	21.4	29,809	***	
Nitrofurantoin	98.2	3,361	98.6	2,434	98.4	4,383	98.7	4,129		
Rifampicin	97.4	21,332	98.3	13,991	98	28,486	98.8	25,401		
SXT	29.3	24,493	27.1	18,647	25.9	35,306	32.8	29,166	***	

†The most recent Clinical Laboratory Standards Institute VET01S guidelines have considerably lowered the breakpoints for chloramphenicol compared to the breakpoints used in this analysis. As a result, the susceptibility rates reported here are likely overestimated. ***$P \leq .001$.

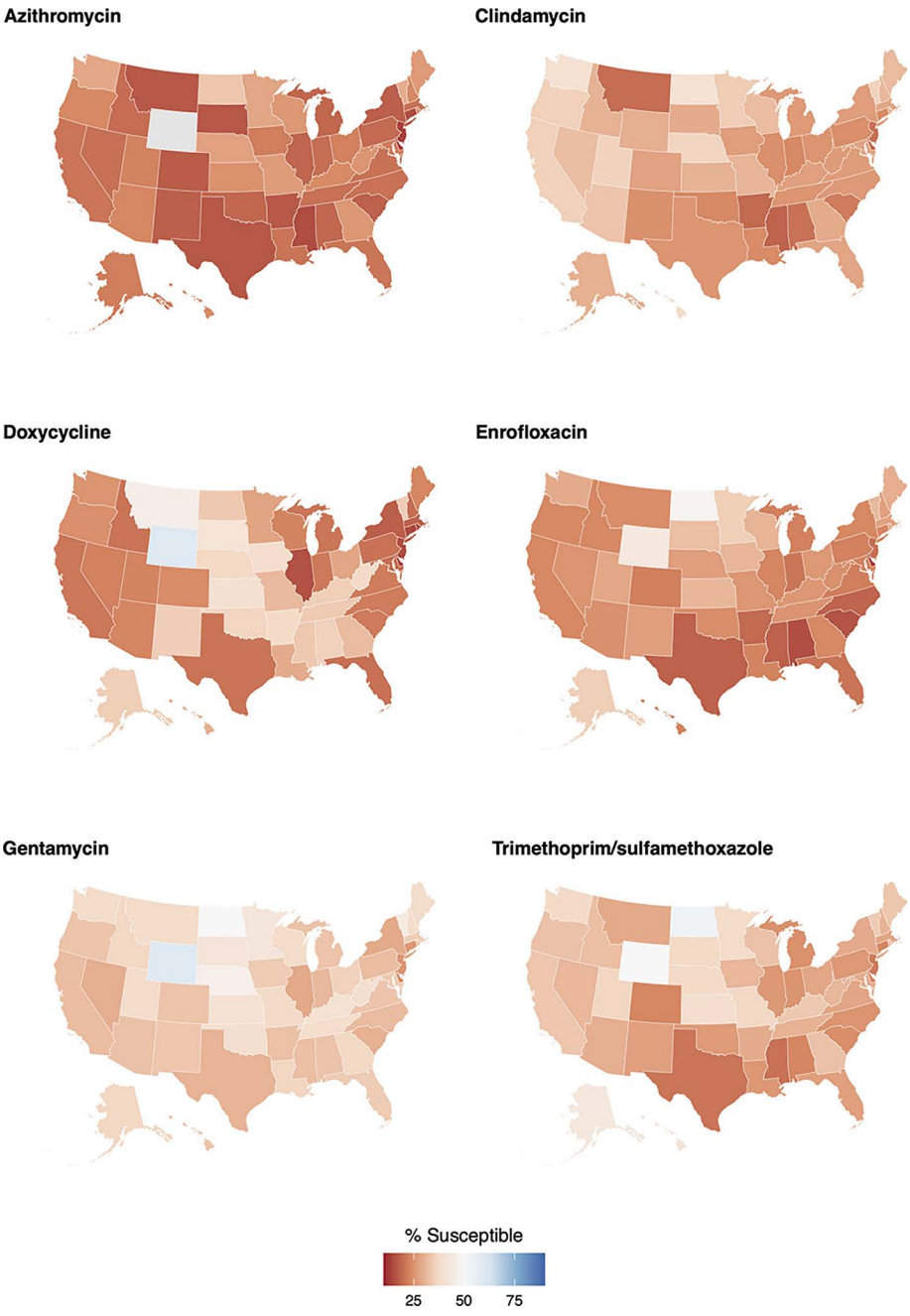

Figure 3—Maps of the proportion of MRSP isolates observed to be resistant to selected antibiotics, with states color coded by resistance levels.

Discussion

Although MRSP, MRSA, and MRSS have been well described in companion animal veterinary medicine, there is a lack of epidemiological evidence quantifying their prevalence and resistance patterns at a national, multiyear scale in the US. This study leveraged 4 years of culture and susceptibility data from a commercial diagnostic laboratory to estimate the frequency of MRSP, MRSA, and MRSS isolates observed at the state and national levels and to explore the resistance of these isolates to other antimicrobials.

The MRSP, MRSA, and MRSS strains were isolated from companion animal samples in all 50 states and the District of Columbia, except for MRSS, which was not observed in Wyoming during the 4 years studied. All 3 MRS species exhibited a year-over-year increase in the samples observed. However, the proportion of isolates classified as methicillin-resistant relative to the number of isolates tested remained stable, with only a small increase for *S pseudintermedius* between 2019 and 2020. Among the 3 MRS species, MRSP was the most frequently isolated, with 110,423 isolates collected from 2019 to 2022. Most of these isolates were from dogs, with skin and soft tissue being the predominant sampling sites. These findings are consistent with knowledge about MRSP and its associated diseases in veterinary medicine.[33,34]

Comparing the relative occurrence of isolated MRSP at the state level revealed a higher tendency to observe MRSP isolates along the East and West

coasts and the states surrounding the Great Lakes. Central states were consistently at or below the national average in observed MRSP. These central states are typically warmer, drier, and less densely populated. Other explanatory factors could include regional variation in veterinary practices, antibiotic use, culture submission patterns, or patient population structure, but these hypotheses were not directly evaluated in this study. While this work aims to describe the frequency of MRSP, it does not attempt to explain the underlying cause for the observed variation. Explaining the underlying cause of the geographic variation would require a dedicated analysis and the inclusion of external data sources beyond historical laboratory data. Therefore, further research is needed to elucidate the underlying causes of this pattern.

Historical susceptibility testing data of submitted MRSP samples revealed significant annual, geographical, and patient-specific differences. Notably, susceptibility appears to have increased for azithromycin, chloramphenicol, clindamycin, enrofloxacin, gentamicin, marbofloxacin, and trimethoprim-sulfamethoxazole, while decreasing for doxycycline, minocycline, and rifampin. Updates to the CLSI breakpoint guidelines (7th ed)[32] have introduced more stringent criteria for classifying staphylococci isolates as susceptible to chloramphenicol, enrofloxacin, and marbofloxacin, so these results should be interpreted with caution. The reasons for these trends are unclear. Sampling bias may have played a factor as laboratory datasets tend to bias toward isolates from animals that have failed initial treatment and are, therefore, more likely to be resistant.[35] However, this concern is likely less relevant when evaluating a population like MRS, which is specifically selected for the presence of antimicrobial resistance. It is also possible that changes in resistance rates are related to shifts in the relative abundance of different MRS strains, which can have different susceptibility patterns. Genomic data were not available for these isolates.

Susceptibility rates for all but 3 antimicrobials, amikacin, nitrofurantoin, and rifampicin, varied significantly at the census region level, although effect sizes were negligible for all but chloramphenicol. While geographical differences in resistance were evident, a clear spatial trend was not easily discerned, unlike other resistance patterns reported previously in the US, which showed an apparent southeast-to-northwest pattern.[36] Differences in resistance between cats and dogs were also observed, as well as differences among the sample collection sites. Many of these differences were slight but still notable. These differences emphasize the need for regionally localized and syndromic-specific antibiograms.[37] Since enhanced antibiograms are often inaccessible, culture and susceptibility testing remains essential for identifying viable treatment options for MDR infections.[37]

The MRSA and MRSS isolates were less commonly observed than MRSP. This is expected as *S aureus* infrequently causes disease in cats and dogs compared to *S pseudintermedius*.[34,38] The MRSA isolates were observed at about 5% the frequency of MRSP, while the MRSS isolates were observed at about 20% the frequency of MRSP. Like MRSP, MRSA was predominantly isolated from dogs and most frequently associated with skin and soft tissue infections.

The MRSS isolates were also predominantly from dog samples and were distributed across all states except Wyoming. Consistent with previous knowledge, MRSS isolates were predominantly observed in skin and ear samples,[34] with only 11% of samples coming from all other sources combined. Observed MRSS isolates showed appreciable levels of susceptibility to all antimicrobials evaluated besides fluoroquinolones. There were significant geographical differences in the number of MRSS isolates observed per state, with some states having as few as 3 and others nearly 4,000. This variation could be due to differences in sample submission or be indicative of regional differences in prevalence. A more random sampling strategy of healthy and diseased animals would be necessary to distinguish the driver of this variation.

Comparing susceptibility patterns among MRSP, MRSA, and MRSS indicated statistically significant differences for all antimicrobials except nitrofurantoin. Azithromycin, clindamycin, doxycycline, gentamicin, minocycline, and trimethoprim-sulfamethoxazole had large effect sizes (Cohen $h \geq 0.8$), indicating substantial differences in susceptibility among the species. Methicillin-resistant *S pseudintermedius* generally exhibited lower susceptibility than MRSA and MRSS, particularly for 8 of the 12 antimicrobials tested. These differences in susceptibility suggest that polymicrobial infections of multiple MRS strains could have limited available treatment options. Amikacin, nitrofurantoin, and rifampicin susceptibly remained high across all MRS species.

These data underscore the therapeutic challenges of treating MRSP. The susceptibility of MRSP isolates to commonly used first-line antimicrobials, such as clindamycin, trimethoprim-sulfamethoxazole, and doxycycline, was generally low. Nitrofurantoin (limited to the treatment of cystitis), rifampin (limited by concerns about hepatotoxicity), and aminoglycosides such as amikacin and gentamicin (limited by nephrotoxicity and ototoxicity concerns) were the only antimicrobials with susceptibility rates exceeding 50%. Although susceptibility to rifampin was high, its use as a standalone treatment is generally discouraged due to the rapid development of resistance. To mitigate this risk, it is advised to combine rifampin with another antibiotic showing in vitro efficacy.[39] Consequently, the availability of lower-tier, oral treatment options for MRSP infections is severely restricted, complicating patient management and raising significant antimicrobial stewardship concerns.

The major limitation of this study is the nature of the data used. Diagnostic laboratory data, while beneficial in providing broad geographical coverage and a level of standardization in laboratory protocol and reporting, are typically skewed toward recurrent and difficult-to-treat infections.[40,41] As there is both a time and financial cost associated with culture and

susceptibility testing, samples are more likely to be included in this data if there has been at least one failed treatment with a first-line antimicrobial, thus producing a sampling bias toward resistant isolates. In Europe, < 33.33% of companion animal practitioners report regularly submitting samples for culture and susceptibility testing.[35] Although similar data for the American context are lacking, there would be little reason to anticipate a different result. Furthermore, this study lacked access to raw MIC data. Instead, susceptibility interpretations were provided by the commercial diagnostic laboratory. These interpretations were generated using consistent breakpoint guidelines[27] and adhered to laboratory standards and reporting guidelines.[26] However, the absence of raw MIC data limits the ability to independently verify the interpretations or analyze trends in MIC distributions. While the diagnostic laboratory employs validated instrumentation and protocols monitored for compliance with CLSI standards, it is acknowledged that reliance on preinterpreted data places trust in the laboratory's processes and validation procedures. This should be considered when interpreting the findings of this study.

Furthermore, multiple other diagnostic laboratories exist throughout the US, and thus, geographical coverage by any single laboratory may be inconsistent. These data also do not distinguish the level of care the submitting clinic provides (ie, community practice or tertiary animal hospital). Several steps were taken to mitigate the influence of these limitations. The MRSP isolates were mapped as a relative metric, standardized by animal population in each state. While the absolute value of MRSP isolates is likely incorrect, comparing states to the national average should yield a more representative result. However, clustering may still be present in areas of tertiary care clinics. As for assessing the resistance patterns, since this analysis focused solely on methicillin-resistant isolates, which are likely to not respond to initial treatment, the influence of sampling bias should be reduced. This is because most patients with MRS would fall into the difficult-to-treat category, making them more likely to be captured in the data. Comparison of these results with results from other commercial and regional laboratories, sentinel clinics, and random surveillance sampling could provide more accurate estimates of the true prevalence through an integrated approach.

A further limitation is the nature of the breakpoints used and the lack of available MIC data for this study. The MIC values were interpreted before data transfer from the lab; therefore, raw MIC values were unavailable. Susceptibility interpretations used in this study were based on the CLSI guidelines available during data generation. However, since then, there have been substantial changes to breakpoints for interpreting fluoroquinolone and chloramphenicol susceptibility in *S pseudintermedius* from dogs. A notable change in the latest VET01S guidelines (7th ed)[32] is the reduction of the susceptible breakpoint for chloramphenicol in staphylococci isolated from dogs, which was lowered from < 8 µg/mL to < 2 µg/mL. This change means isolates with MIC values of 4 to 8 µg/mL, classified as susceptible under the VET01S (5th ed)[27] guidelines (used in this analysis), would now be interpreted as resistant. As a result, resistance rates for chloramphenicol in this study are likely considerably underestimated. However, retrospective analysis to reclassify these data is impossible due to the lower dilution limit of 4 µg/mL on the VITEK2 cards used during testing. Dog-specific fluoroquinolone breakpoints have been lowered in the latest CLSI VET01S guidelines (7th ed).[32] As a result, some isolates classified as susceptible in this study, which applied CLSI breakpoints (5 ed),[26] would now likely be considered resistant. However, due to the lack of access to raw MIC values and the limitations of the MIC dilution ranges used in testing, this assumption could not be directly evaluated. Given the already low observed susceptibility of MRS isolates to fluoroquinolones in this study, the clinical impact of this reclassification could be minimal.

This study provides insights into the prevalence and antimicrobial susceptibility patterns of MRSP, MRSA, and MRSS in companion animals. The findings underscore the need for continuous surveillance, region-specific antimicrobial stewardship programs, and species-specific treatment guidelines to manage MRSA infections in veterinary practice effectively.

Acknowledgments

None reported.

Disclosures

Dr. Plum and D. Szlosek were employed by IDEXX Laboratories Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The following AI-powered tools were used in the preparation of this manuscript: Notion.AI for note-taking; Semantic Scholar for literature searches; and Grammarly.AI for spelling and grammar.

Funding

The research was funded by the authors' departments. Dr. Sobkowich was also supported by funding provided to the IDEXX Chair of Emerging Technologies and Preventive Healthcare.

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Supplementary Materials

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