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Retrospective analysis of antibacterial susceptibility of *Staphylococcus* spp. isolated from clinical samples of dogs in Ontario, Canada

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Abstract

Antimicrobial resistance has developed as a major threat worldwide causing a serious risk to global public health. Infections with drug-resistant bacteria are quite challenging to treat with recommended antimicrobials. This retrospective study was conducted to analyse the antibacterial susceptibility pattern of Staphylococcus spp. bacteria isolated from canine patients from two veterinary hospitals in the Niagara Region in Ontario, Canada, from January 2015 to December 2021. The data included 1370 bacterial culture reports received from IDEXX laboratories, out of which 306 specimens (22.3%) were positive for Staphylococcus spp. The results showed that the most prevalent strain of Staphylococcus isolated is Staphylococcus pseudintermedius (48%), followed by methicillin-resistant Staphylococcus spp. (33%), Staphylococcus schleiferi (10%), Staphylococcus aureus (7%) and other Staphylococcus species (2%). The antibiotic susceptibility test results of Staphylococcal isolates revealed a high resistance pattern for beta-lactam antibiotics (32-80%), followed by tetracycline antibiotics (21-47%), macrolides (35-40%), chloramphenicol (15%), fluoroquinolones (28-29%) and the least resistance for amikacin (1%). The results also documented a high prevalence of multi-drug resistance in 55% of the Staphylococcus strains isolated. This study illustrates the antimicrobial resistance among Staphylococcus spp. from dogs in Ontario, Canada. Hence, the strict measures of antibiotic stewardship and judicious use of antimicrobials are highly mandated.

Keywords: *Antimicrobial resistance, Staphylococcus spp., methicillin-resistant, Canada*

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Antimicrobials are used extensively in human and veterinary patients, for treating infectious conditions. Consequently, antimicrobial resistance (AMR) is rising and has become a great challenge affecting global public health (Biswas *et al.,* 2002). Infections with multidrug-resistant (MDR) bacteria are becoming increasingly difficult to treat with recommended first-line antimicrobials (Frey *et al*., 2022). *Staphylococcus* species are of particular concern because they colonize a wide range of hosts. Infections with *Staphylococcus* bacteria are a leading cause of various infections in animals ranging from minor skin infections to major life-threatening diseases (Upadhyay *et al.,* 2020). Specifically, infections with methicillin-resistant *Staphylococcus* species are quite challenging to treat due to the MDR accrued by these pathogens (Sonola *et al.,* 2021). AMR and MDR are common One Health issues. The rise of methicillin-resistant *Staphylococcus* spp. infections is not only affecting our companion animals but also veterinarians who are involved in treating companion animals (Loeffler *et al.,* 2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported for its transmission from humans to animals (Baptiste *et al.,* 2005; Jordan *et al.,* 2011).

In animal medicine, veterinarians are responsible for preserving antimicrobial sensitivity through conscientious antimicrobial stewardship (Vivas *et al.,* 2019). Therefore, to sustain therapeutic effectiveness, it is essential to be aware of the prevalence of various species of *Staphylococcus* and to develop a better understanding of appropriate antimicrobial protocols. Few research studies assessed the prevalence of methicillin-resistant *Staphylococcus* spp. in first-opinion veterinary practices (Joffe *et al*., 2015). Further studies in Canadian first opinion practices would be beneficial to evaluate and monitor the emergence of methicillin-resistant *Staphylococcus* spp. in Canada. Hence, this retrospective study gathered data from two primary care veterinary hospitals in the Niagara Region in Ontario, Canada to investigate the AMR status of *Staphylococcus* bacteria isolated from canine patients diagnosed with conditions ranging from pyoderma, pruritis, post-surgical site infections (orthopaedic and soft tissue), urinary tract infections, abscesses, and other septicemic conditions.

Material and methods

In this retrospective study, we used the diagnostic laboratory reports of canine patients collected from two veterinary hospitals in the Niagara region of Ontario, Canada for the period of January 2015 to December 2021. A total of 1370 bacterial diagnostic culture reports of canine patients were retrieved from AVImark, a veterinary practice management software for the study period. The clinical samples were processed by IDEXX laboratories for bacterial isolation. The positive isolates were identified based on conventional diagnostic methods to characterise the isolates as respective *Staphylococcus* spp. The positive clinical samples of *Staphylococcus* spp. were subjected to antimicrobial susceptibility tests (AST) using the Kirby-Bauer disc diffusion method (Quinn *et al*., 1994) on Mueller-Hinton (MH) agar. The antimicrobial discs used were amoxicillin (10 µg), tetracycline (10 µg), azithromycin (15 µg), cefpodoxime (10 µg), cefovecin (30 µg), amoxicillin/potassium clavulanate (30 µg), erythromycin (10 µg), rifampin (15 µg), clindamycin (2 µg), penicillin (10 U), ciprofloxacin (5 µg), gentamicin (10 µg), clarithromycin (15 µg), enrofloxacin (5 µg), doxycycline (5 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (10 µg), imipenem (10 µg) and amikacin (30 µg). The zone of inhibition sizes was interpreted as susceptible, intermediate or resistant, based on the guidelines provided by the Clinical Laboratory Standards Institute (CLSI) guideline (CLSI, 2020). The AST results were tabulated onto the Microsoft Excel sheet. Descriptive statistics were used to analyze the AST results of *Staphylococcus* isolates of dogs. Prevalence rate, AMR and MDR values are expressed as percentages.

Results and discussion

Out of a sample size of 1370 canines, 306 patients (22.3%) tested positive for *Staphylococcus* spp. The prevalence of various *Staphylococcus* spp. is represented in Fig.1 as follows:

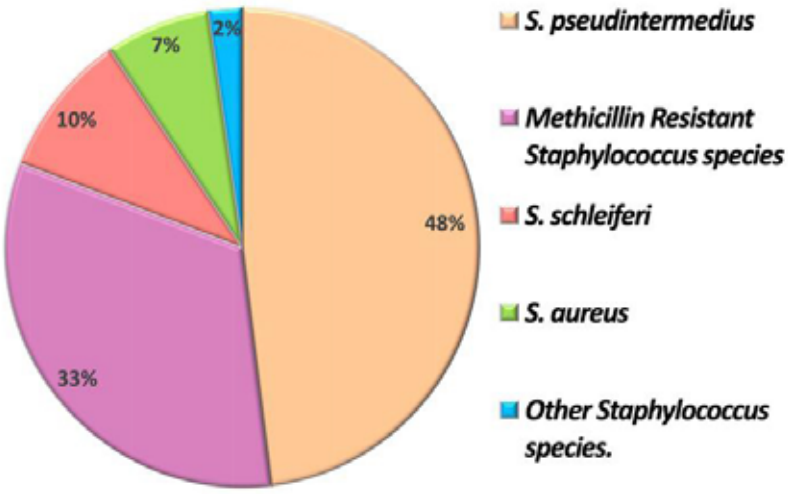

Fig. 1: Prevalence of *Staphylococcus* species from 306 canine patients testing positive for *Staphylococcus* infection

A high prevalence (101/306; 33%) of methicillin-resistant strains of *Staphylococcus* was documented during the study period (Fig. 1). This 33% includes a high percentage (28.43%; 87 isolates) of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), minimal occurrence (0.65%; 2 isolates) of methicillin-resistant *Staphylococcus aureus* (MRSA) and other methicillin-resistant *Staphylococcus* species (3.92%; 12 isolates). This study report revealed that MRSP prevalence was higher than both MRSA and other methicillin-resistant *Staphylococcus* species infections (Fig. 2).

Among the methicillin-resistant *Staphylococcus* strains, methicillin-resistant *S. pseudintermedius* (MRSP) was highly prevalent (87 isolates) as shown in Fig. 2. Other methicillin-resistant Staphylococcal strains

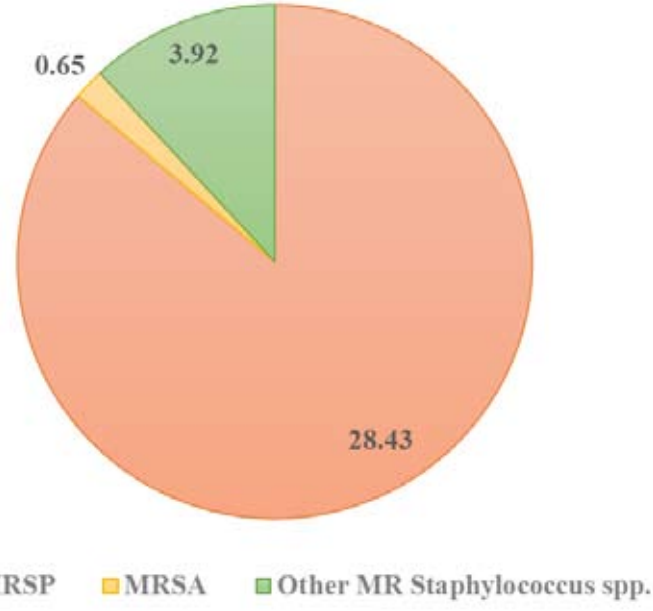

Fig. 2: Prevalence of methicillin-resistant *Staphylococcus* species within the sampled population

MR- Methicillin Resistant; MRSP – Methicillin-resistant *Staphylococcus pseudintermedius*; MRSA - Methicillin-resistant *Staphylococcus aureus*

comprised methicillin-resistant *S. aureus*, *S. schleiferi*, *S. hemolytic*us, *S. epidermidis* and *S. sciuri*.

The antibacterial sensitivity results of the canine staphylococcal isolates during the study period revealed a high AMR pattern for beta-lactam antibiotics, tetracycline antibiotics, macrolides, chloramphenicol, clindamycin, trimethoprim-sulfamethoxazole, and fluoroquinolones (Table 1). For aminoglycoside antimicrobials, the rate of resistance of *Staphylococcus* isolates to gentamicin is recorded as 29% however with amikacin, we observed only a minimal resistance (1%). Furthermore, 13% of *Staphylococcus* isolates were resistant to imipenem, a carbapenem-class antibacterial drug of good antibacterial efficacy. This is an important concern since imipenem is a last-resort drug for resistant bacterial infections. This study documented that 55% of *Staphylococcus* isolates exist as multidrug-resistant strains (resistance to 3 or more antimicrobial drugs).

In this retrospective study, the prevalence of *Staphylococcus* organisms in clinical cases of dogs was found to be 22.3% which is slightly less than the previous study reports (29% - 37%) conducted in healthy dogs to determine the presence of carriers during the period of 2016 to 2020 (Han *et al.,* 2016; Elnageh *et al.,* 2021).

Table 1: Antimicrobial Susceptibility Test (AST) Results of canine *Staphylococcus* spp. (%)

Antimicrobial Drug	% of Samples with Resistance
Amoxicillin	80.2
Tetracycline	46.5
Azithromycin	40.0
Cefpodoxime	36.9
Cefovecin	36.3
Cefalexin	35.6
Amoxicillin/K Clavulanate	35.4
Erythromycin	35.1
Rifampin	34.9
Clindamycin	31.9
Penicillin	31.5
Ciprofloxacin	29.2
Gentamicin	29.1
Clarithromycin	28.4
Enrofloxacin	27.7
Doxycycline	20.5
Trimethoprim-Sulfamethoxazole	19.0
Chloramphenicol	14.8
Imipenem	12.5
Amikacin	1.4

In 2015, the prevalence of methicillin-resistant *Staphylococcus* spp. from canine clinical cases within seven Canadian primary care veterinary practices was documented as 12.1% out of 149 staphylococcal-positive isolates (Vivas *et al.,* 2019). This retrospective study showed a marked increase in the prevalence of methicillin-resistant *Staphylococcus* spp. among the canine population (101/306; 33%). Within the methicillin-resistant *Staphylococcus* strains, MRSP was found to be the most prevalent strain (87%) and MRSA only accounted for 2% of clinical MR staphylococcal infections. The remaining 11% comprised of other methicillin-resistant *Staphylococcal* organisms, such as *S. schleiferi*, *S. hemolytic*us, *S. epidermidis* and *S. sciuri.* This is in contradiction to the previous study reporting a high incidence of methicillin-resistant *Staphylococcus aureus* in comparison to other methicillin-resistant *Staphylococcus* spp. (Weese, 2008). This study documented that the most prevalent staphylococcal strain is MRSP in Ontario, Canada. The high prevalence of MRSP among the *Staphylococcus* spp. population could be associated with a possible increase in the emergence of multi-drug-resistant organisms (Weese, 2008; Bean and Wigmore, 2016). This is in accordance with the study report stating that MRSP isolates are resistant to significantly more antimicrobial classes than MRSA (Worthing *et al.,* 2018).

This study evidenced that *S. pseudintermedius* is the most prevalent species of staphylococci (48% of 306 staphylococcal positive samples) in dogs within this Ontario region, with a high resistance rate to the recommended antimicrobials. Studies over time have been showing a recent increasing trend of antimicrobial resistance in *S. pseudintermedius* isolates (Malik *et al.,* 2006; Gottlieb *et al*., 2008; Bean and Wigmore, 2016). Several research studies conducted during the period spanning 1986 to 2003 initially documented no samples positive for either MRSA or MRSP and reported consistent antimicrobial sensitivity patterns of staphylococci cultured from canine patients (Lloyd *et al.,* 1996; Murphy *et al.,* 2009; Rubin *et al.,* 2011). Thereafter, several research studies in Ontario, Canada found an upward trend in the prevalence of methicillin-resistant

staphylococci from the years 2001-2010 (Jones *et al.,* 2007; Morris *et al.,* 2006; Beck *et al.,* 2012). In this study, MRSA showed the lowest prevalence among all the methicillin-resistant staphylococcus species (2%). This coincides with a study done previously in Toronto, Canada which reported an incidence of MRSA in only 1.7% of patients (Beck *et al.,* 2012). In 2018, an AMR research study confirmed that MRSP is more prevalent in veterinary settings while MRSA is more prevalent in human medical settings. This coincides with our study result indicating that *S. pseudintermedius* is in high prevalence rate in canines (Worthing *et al.,* 2018).

ABST data on canine *Staphylococcus* isolates of this study revealed a high resistance to beta-lactam antibiotics, tetracycline antibiotics, macrolides, cephalosporins and fluoroquinolones. Also, this study documented high evidence of MDR (resistance to 3 or more antimicrobial drugs) in 55% of the strains isolated. These results indicate a drastic increase in MDR as compared to a study conducted in 1996 where *Staphylococcus* spp. showed a good susceptibility (Bean and Wigmore, 2016). MRSP isolates specifically displayed a higher level of antimicrobial resistance than MRSA isolates (Weese, 2008; Ishihara *et al*., 2010; Beck *et al*., 2012). The increase in AMR development might be due to flaws in the protocol regarding antibiotic use within veterinary medicine (Weese, 2008). Even though MRSP is not a major zoonotic pathogen, the expansion of multidrug-resistant MRSP strains still presents a potential public health concern. Risk factors for MRSP-associated infection could be possibly linked to frequent veterinary visits and a history of hospitalisation; however, veterinarians should be aware that apparently, healthy dogs can also be carriers of MRSP (Ishihara *et al*., 2010; Nienhoff *et al.,* 2011).

The rise in prevalence of methicillin-resistant *Staphylococcus* spp. as well as MDR strains could be attributed to several components, such as lack of prophylactic control measures of infection spread, chronic or injudicious antimicrobial use, etc. (Hillier *et al.,* 2014; Lehner *et al*., 2014; Deyno *et al.,* 2017).

Prevention of MDR infections involves the appropriate use of antimicrobials, with recommended measures of proper disinfection, sanitation, personal hygiene and biosecurity measures (Dwyer, 2004). It should also be important to follow routine diagnostic measures of clinical samples and conduct ABST tests for the appropriate selection of the antibacterial drug (Han *et al*., 2016).

Conclusion

This retrospective study revealed a high level of AMR in canine *Staphylococcus* species within the Niagara region, in Ontario, Canada. The most prevalent species of *Staphylococcus* within this region were found to be *S. pseudintermedius* and methicillin-resistant *Staphylococcus* species. The high prevalence of MDR strains of *Staphylococcus* spp. needs to be addressed effectively as a serious problem for health care. AMR resulting in the progressive development of MDR is a One-Health problem. Therefore, responsible therapeutic decision-making regarding antimicrobial use protects animal, human and environmental health. Further, the molecular mechanisms associated with the plasmid-mediated antimicrobial resistance of *Staphylococcus* isolates of dogs in Ontario, Canada need to be investigated.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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