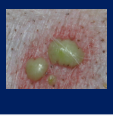
# Prevalence of methicillin resistance in Staphylococcus *pseudintermedius* isolates from dogs with skin and ear infections in South Africa

Prior, C.*, Karma, M., Moodley, Y, Leisewitz, A.

* Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa



## ABSTRACT

*Staphylococcus pseudintermedius* (SP) is an important opportunistic pathogen, frequently associated with dermatitis and otitis in small animals. The emergence and rapid expansion of methicillin resistance is problematic due to multi-drug resistance and reduced treatment options. We aimed to 1) evaluate standard laboratory methods used at five regional veterinary labs in South Africa (RSA) to identify SP; 2) determine if there is association between resistance to first and second tier antibiotics and presence of *mecA*; and 3) determine if there is an association between methicillin resistant *Staphylococcus pseudintermedius* (MRSP) and previous antibiotic use. Sixty-eight clinical samples from five geographically dispersed laboratories in RSA were collected over a 24-month period. SP were detected by standard laboratory methods and antimicrobial susceptibility testing by disc diffusion. Possible MRSP isolates were flagged when resistance to oxacillin was observed. All isolates were confirmed to as SP and genotyped for the *mecA* gene by PCR. Fifty-seven isolates were confirmed to be SP (83.8%) while 49 (85%) carried *mecA*. Important risk factors for *mecA* positive carriage were previous hospital admission, pruritus and previous antibacterial failure. This study provides evidence that there is a high prevalence of *mecA* positive carriage (85% of samples) in MRSP pyoderma and otitis in dogs in South Africa. There is an urgent need for better laboratory diagnosis of MRSP and surveillance of dogs presenting pyoderma and otitis in South Africa. Moreover, diligent antibiotic stewardship will be crucial to prevent a deterioration in this situation in South Africa.

## INTRODUCTION

SP is the most frequent bacterial pathogen isolated from canine skin and ear infections, (Griffeth et al., 2008) and is a leading cause of pyoderma, which accounts for 20% of ear infections in dogs presenting to veterinarians (Cole et al., 2006). The worldwide spread of MRSP has become a significant animal health problem (Hensel et al., 2016). In South Africa alone, there are an estimated 9.2 million dogs living in households (Canine zone, 2019) and given the close interaction between humans and their pets, there is an increased risk for communal spread of multidrug resistant bacteria (MDR), particularly in animals that are treated for life threatening diseases, which can become zoonotic (Hartantyo et al., 2018). As the prevalence of MDR bacteria continues to increase, misuse, abuse and overuse of antimicrobials remains the key factor for selection of MRSP strains in healthy dogs, representing a huge challenge for effective veterinary treatment (Rota et al., 2013).

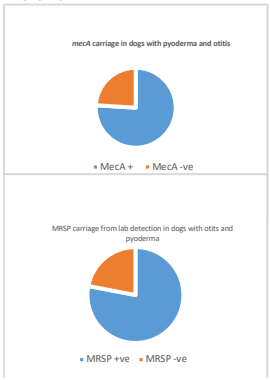
## MATERIAL & METHODS

AIM: **1) Evaluate standard laboratory methods used at five regional veterinary labs in South Africa (RSA) to identify SP 2) Determine if there is association between resistance to first and second tier antibiotics and presence of the gene *mecA* 3) Determine if there is an association between MRSP and previous antibiotic use.** The SP isolates were tested by PCR-restriction fragment length polymorphism (PCR-RFLP) according to Bannoehr *et al.* on a 320-bp fragment of the *pta* gene (Bannoehr et al., 2009) and PCR protocol by Sasaki *et al.* on a 926-bp fragment of the *nuc* gene (Sasaki et al., 2010) to confirm their identity. Veterinarians who consented to participate in this study completed a questionnaire for each dog diagnosed with pyoderma and/or otitis externa. Information on each questionnaire included a dog identification number that blinded the investigator to owner identity, sampling site, clinical signs, gender, age (in months), breed, sterilization status and treatments used (such as glucocorticoids and topical and/or systemic antimicrobials).

## RESULTS

### Fig. 1- *mecA* carriage in dogs with pyoderma and otitis

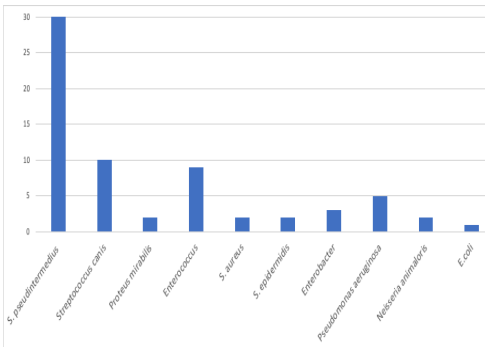
1. **Detection of *mecA*** via PCR is the gold standard for the diagnosis of methicillin resistance (Schissler et al., 2009)
2. The **disc diffusion** via standard lab methods identified **77.9% (53/68)** isolates to be methicillin resistant while *mecA* positive PCR in this study identified **72.1% (49/68)** of the methicillin resistant isolates




### Fig. 2- Mixed Infections: implications of MRSP and other Staphylococci in dogs with pyoderma and otitis externa

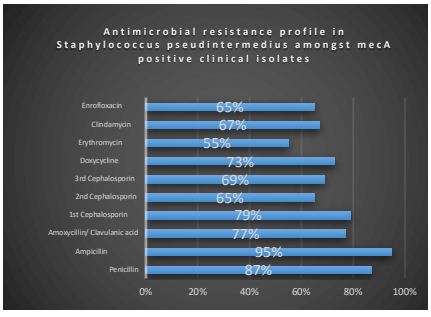
3. ***S. pseudintermedius*** was the most frequently isolated organism in canine pyoderma and otitis cases – 83.8% (57/68). Including coagulase-negative *S. epidermides* – 7% (4/57)

4. **Mixed infections** that included MRSP and other non-Staphylococci bacteria such as *Streptococcus canis*, *Enterococcus* spp, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Neisseria animaloris*, *Proteus mirabilis* and *Escherichia coli*




### Fig. 3- Antimicrobial resistance

5. The degree of **multi-resistance** in this study, 49.1% (28/57)
6. High incidence of **amoxicillin resistance 70.1% (40/57)**
7. Levels of resistance to **enrofloxacin** were especially high amongst the *mecA* positive isolates **at 65% (32/49)**
8. No statistically significant association amongst *mecA* positive isolates and 2nd tier antibiotic in this study with only 51% (25/49) of the isolates having a history of **second tier antibiotic use** (p>0.1)




### Fig. 4- Risk factors of *mecA* carriage in dogs with pyoderma and otitis

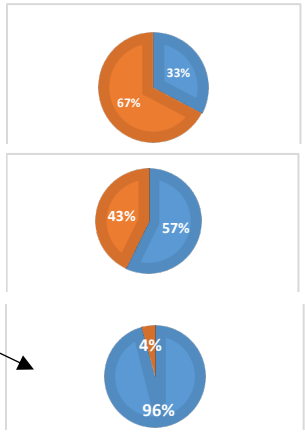
9. Regarding the different risk factors that were investigated in this study, findings showed that variations in *mecA* occurrence rates in dogs can be ascribed to a number of factors, namely,

**- Hospital admission**

**- Pruritis**

**- Antibiotic failure**

Univariable analysis of risk factor variables from animals was based on *mecA* positive isolates from PCR as this is considered the gold standard for the defining MRSP




## DISCUSSION

To the researcher's knowledge, this is the first study conducted in South Africa reporting on the occurrence of antimicrobial resistant MRSP, carrying the *mecA* gene. The study examined resistance trends as well as risk factors associated with MRSP isolates, which were recovered from canine otitis and pyoderma cases. A total of 68 presumptive MRSP clinical isolates were collected from laboratories of which 83.8% (57/68) were identified as true SP, using molecular methods. The remaining 16% (11/68) of isolates were classified as staphylococci, which comprised of *S. aureus* and *S. epidermides*. Detection of *mecA* via PCR is the gold standard for the diagnosis of methicillin resistance (Schissler et al., 2009). The disc diffusion method identified 77.9% (53/68) isolates to be methicillin resistant while *mecA* was identified in 72.1% (49/68) of the methicillin resistant isolates. Antimicrobial resistance was prevalent in this study. All isolates were resistant to at least one antimicrobial drug. The degree of multi-resistance in this study, 49.1% (28/57). Regarding the different risk factors that were investigated in this study, findings showed that variations in *mecA* occurrence rates in dogs were associated with a number of factors, namely, hospital admission (33%), pruritis (57%), and antibiotic failure (96%). Univariable analysis of risk factor variables from animals was based on *mecA* positive isolates from PCR.

## CONCLUSION

**This study provides evidence on the high prevalence of *mecA* positive isolates in pyoderma and otitis clinical isolates taken from dogs in South Africa. Important risk factors for *mecA* positive carriage include hospital admission, pruritus and antimicrobial failure. Methicillin-resistant isolates were significantly more likely to exhibit non ß-lactam resistance, especially to doxycycline, clindamycin, sulphamethoxazole/trimethoprim and enrofloxacin.**

**REFERENCES**: Hartantyo, S. H. P., M. L. Chau, L. Fillon, A. Z. B. Ariff, J. S. L. Kang, K. T. Aung & R. A. Gutierrez, 'Sick pets as potential reservoirs of antibiotic-resistant bacteria in Singapore', *Antimicrobial Resistance and Infection Control*, 7 (2018)
Bannoehr, J., A. Franco, M. Iurescia, A. Battisti & J. R. Fitzgerald, 'Molecular diagnostic identification of Staphylococcus pseudintermedius', *J Clin Microbiol*, 47, 2 (2009), 469-471.
Sasaki, T., S. Tsubakishita, Y. Tanaka, A. Sakusabe, M. Ohtsuka, S. Hirotaki, T. Kawakami, T. Fukata & K. Hiramatsu, 'Multiplex-PCR method for species identification of coagulase-positive staphylococci', *J Clin Microbiol*, 48, 3 (2010), 765-769.
Canine zone. 2019. Pet Insurance Has Become A "Must-have" For All Pet Owners. [Online]. [Accessed 20 July 2020]. Available from: https://www.magzter.com/stories/Animals-and-Pets/Canine-Zone/Pet-Insurance-Has-Become-A-Must-have-For-All-Pet-Owner
Qekwana, D. N., J. W. Oguttu, F. Sithole & A. Odoi, 'Patterns and predictors of antimicrobial resistance among Staphylococcus spp. from canine clinical cases presented at a veterinary academic hospital in South Africa', *BMC Vet Res*, 13, 1 (2017), 116.Rota, A., C. Milani, M. Corrò, I. Drigo & S. Börjesson, 'Misuse of antimicrobials and selection of methicillin-resistant *Staphylococcus pseudintermedius* strains in breeding kennels: genetic characterization of bacteria after a two-year interval', *Reprod Domest Anim*, 48, 1 (2013), 1-6.