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STAPHYLOCOCCUS SPP. IDENTIFIED FROM DOGS WITH OTITIS EXTERNA AND THEIR ANTIMICROBIAL RESISTANCE

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Abstract

This study investigated the antimicrobial resistance of *Staphylococcus* species isolated from dogs with otitis externa. Out of the 71 samples collected, *Staphylococcus* spp. was isolated in 65% of cases. Isolation, identification of the species and antimicrobial susceptibility testing was carried out first using cultural microbiological methods and with the Vitek 2 system. The most common species identified were *S. pseudintermedius* (41.3%), followed by *S. aureus* (19.6%), *S. intermedius* (17.4%), and *S. schleiferi* (17.4%). Penicillin resistance was significant in *S. intermedius* (50%), *S. aureus* (44.4%), and *S. pseudintermedius* (31.6%). Clindamycin resistance was observed across all species. Multiple drug resistance (MDR) was observed in 13% of the strains, while no oxacillin resistance was noted. The study emphasizes the need for regular antimicrobial susceptibility testing to avoid ineffective treatments and control MDR strains.

• Introduction

Infections with multidrug-resistant bacteria are becoming an increasingly serious challenge, in veterinary medicine as well as in human medicine (11), having a significant impact on animal health and public health (2).

Inflammation of the external auditory canal, including the ear pinna, is known as otitis externa. It can be either acute or chronic (recurrent or persistent for at least 3 months). In the case of chronic inflammation, changes that may occur in the external auditory canal include dilation and glandular hyperplasia, hyperkeratosis, and epithelial hyperplasia (3).

Increased production of cerumen in the external auditory canal is usually the result of these changes, leading to increased humidity and local pH, making the ear more susceptible to secondary infections (3).

Inflammation, pain, ear discharge, head shaking and scratching of the ear are the most frequently observed clinical signs of otitis externa, and in prolonged cases, symptoms such as erythema, edema, pruritus, and an unpleasant odor may appear (20).

The pathogens most commonly involved in the etiology of otitis include species from the genus *Staphylococcus*, predominantly *S. pseudintermedius*, which is usually found in small quantities in healthy ears. Regarding gram-negative microorganisms, although they are not normally isolated from healthy ear canals, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* also play a role in the development of otitis (6,14).

• Material and method

Secretions and wax samples from the ears were collected from 71 dogs with otitis externa using sterile swabs.

For the primary bacteriological examination, the samples were inoculated into nutrient broth and incubated at 37°C for 24 hours. From each test tube containing the mixed bacterial culture, inoculations were then performed on selective media to isolate *Staphylococcus* spp. The inoculations were done using a sterile loop and streak plate technique on Chapman and Baird-Parker media, which had been previously poured into Petri dishes. The plates were maintained at 37°C for 24 hours under aerobic conditions. From each plate with these selective media, a characteristic colony was picked and examined by the Gram staining method, after which it was transferred onto blood agar plates for 24 hours at 37°C under aerobic conditions. *Staphylococcus* species identification was performed using the Vitek 2 Compact system, employing identification cards for Gram-positive bacteria. From each culture obtained on blood agar, a bacterial suspension was prepared in 3 ml of physiological saline in a 12 x 75 mm plastic tube.

Each bacterial suspension was adjusted to a density of 0.5 – 0.63 McFarland. Specific identification cards were used for bacterial strain identification, with each card containing 64 wells. Due to the various carbohydrates and enzymes present in these wells, the bacterial strains could be identified based on their biochemical characteristics. Due to the storage conditions of the testing cards, specifically refrigeration at 4°C, the cards were allowed to equilibrate to room temperature for 10 minutes before use. Afterwards, they were placed in the vacuum pressurization compartment alongside the bacterial suspension, with each tube of bacterial suspension positioned near the corresponding card, enabling transfer of the bacterial suspension to each individual card. The cards, along with the bacterial suspension, are maintained within the system at 33.5°C, with each well being periodically checked by the device's spectrophotometer. Each metabolic reaction generated by the enzymes on the card is recorded at 15-minute intervals, and upon completion of the process, which lasts between 8 and 24 hours, the results are displayed and interpreted via the AES system, with data stored on the computer attached to the device.

Antimicrobial susceptibility testing was also performed using the Vitek 2 Compact system, following the manufacturer's instructions.

• Results and discussions

Regarding the identification of species isolated from otic secretion samples collected from dogs with otitis, performed using the Vitek 2 system, the results are presented in Figure 1. Thus, *Staphylococcus* spp. was identified in 65% (n=46) of the collected samples. Specifically, the following *Staphylococcus* species were identified: *S. pseudintermedius* (n=19, 41.3%), *S. aureus* (n=9, 19.6%), *S. intermedius* (n=8, 17.4%), *S. schleiferi* (n=8, 17.4%), and *S. lentus* (n=2, 4.3%) (Fig. 1).

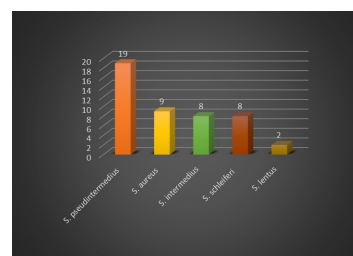


Fig. 1. The identified *Staphylococcus* species

In this study, the most frequently isolated species was *S. pseudintermedius* (41.3%), and our results are similar to those obtained in the study conducted by Hassan et al. (2023) (7), where *S. pseudintermedius* was isolated in 41.6%. Furthermore, our findings are comparable to those reported by Tesin et al. (2023) (20), where *S. pseudintermedius* was isolated in 54.7%. Additionally, our results are similar to those of the study by Popa et al. (2024) (15), where *S. pseudintermedius* was also the most frequently isolated species, at a rate of 30%. *S. pseudintermedius* is an opportunistic pathogen commonly associated with skin infections (13, 16), otitis externa [4, 13, 16, 18], abscesses, wound infections (4), and urinary tract infections in dogs (4, 18). It is a coagulase-positive staphylococcus, identified as a separate species from *Staphylococcus intermedius* in 2005 (18), and is included in the *Staphylococcus intermedius* group (SIG) along with four other species: *S. intermedius*, *S. delphini*, *S. ursi*, and *S. cornubiensis* (1,12).

According to the Vitek2 cards, the following antibiotics were used for testing: penicillin, oxacillin, gentamicin, tetracycline, ciprofloxacin, moxifloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, fusidic acid, tigecycline, rifampicin and trimethoprim + sulfamethoxazole. High resistance was observed with penicillin, particularly in *Staphylococcus intermedius* (n=4, 50%), *S. aureus* (n=4, 44.4%), and *S. pseudintermedius* (n=6, 31.6%). Additionally, high resistance was also observed with clindamycin, with all isolated species showing resistant strains to this antibiotic.

Regarding multidrug resistance (MDR), it was present in 6 strains (13%). The MDR strains belong to the species *S. pseudintermedius*, *S. aureus*, and *Staphylococcus intermedius*. Resistance to oxacillin was not identified in any of the strains.

In the present study, the *S. pseudintermedius* strains isolated from the otic secretions of dogs with otitis exhibited the highest resistance to penicillin and tetracycline. Our results are similar to those obtained in the study by Rosales et al. (2024) (17), where *S. pseudintermedius* also showed the highest resistance to penicillin and tetracycline.

In this study, *S. schleiferi* showed 25% resistance to clindamycin, which is somewhat different from the results obtained in the study by Rosales et al. (2024) (17), where *S. schleiferi* exhibited 6.3% resistance to clindamycin. Regarding *S. intermedius*, our study found high resistance to penicillin (50%), which is similar to the findings of Zamankhan Malayeri et al. (2010) (21), where *S. intermedius* also showed high resistance to penicillin (61.7%). In the present study, *S. lentus* exhibited 50% resistance to clindamycin. In the study by Zamankhan Malayeri et al. (2010) (21) *S. lentus* showed 100% resistance to ampicillin, ceftriaxone, oxytetracycline, and penicillin G.

• Conclusions

The study reveals a high prevalence of *Staphylococcus* bacteria in dogs with otitis externa, particularly the species *S. pseudintermedius*, which was isolated most frequently (41.3%). This is an opportunistic species, often involved in skin and ear infections in dogs.

Antimicrobial susceptibility testing showed that the bacterial strains exhibited variable resistance to different antibiotics, with high resistance to penicillin and clindamycin.

The emergence of MDR strains complicates clinical treatments. In this study, 13% of the strains were classified as multidrug-resistant, highlighting the need for careful management and continuous monitoring of bacterial susceptibility to prevent therapeutic failures.

The results suggest that antimicrobial susceptibility testing is essential before starting treatment in order to choose the appropriate antibiotic and reduce the risk of resistance development.