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Study on the nasal colonization of methicillin-resistant *Staphylococcus aureus* (MRSA) in dogs at Central Veterinary Hospital, Kathmandu, Nepal

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Abstract
Methicillin-Resistance *Staphylococcus aureus* (MRSA) is any strain of *S. aureus* that has developed resistance to beta-lactam antibiotics. Dogs are reported to be colonized with MRSA and may become potential transmission sources to other animal species and humans. This study aimed to detect methicillin-resistant *Staphylococcus aureus* (MRSA) in dogs brought to the Central Veterinary Hospital (CVH), Kathmandu, Nepal. This study was based on the cultural characteristics, morphology, biochemical, and drug sensitivity tests of the bacteria of interest. Nasal swabs from 450 dogs were taken at CVH, transported to the Central Veterinary Lab (CVL) in charcoal agar, and incubated in mannitol broth. Characterization, isolation, and identification of *Staphylococcus aureus* were performed in the Blood Agar, Nutrient Agar, and biochemical tests (catalase, coagulase, trehalose, and VP). Positive isolates were suspended in peptone water via standard methods. The antibiotic sensitivity test followed Kirby Bauer's disk diffusion method. MRSA was categorized on the basis zone of inhibition of the cefoxitin (CX 30) antibiotic (CLSI, 2012). The overall prevalence of MRSA in dogs was found to be 4%. The Prevalence of MRSA among males and females; and among various age groups were statistically non-significant ($p>0.05$). The prevalence of MRSA among the dogs with and without antibiotic courses was 4.1% and 3.9% respectively. No significant difference was found between the sensitivity of Cefoxitin, Ceftriaxone, Enrofloxacin, Erythromycin, Tetracycline, and Amoxicillin ($p>0.05$) antibiotics. The prevalence of MRSA in dogs registered at CVH is 4%. This indicates MRSA is an emerging pathogen and a threat to public health. Thus, it is necessary to evaluate MRSA before prescribing any antimicrobial agents to minimize the associated risk factors and concept for the development of appropriate strategies and judicious antibiotic therapy.

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Introduction

The origins and rapid spread of antimicrobial resistance (AMR) is a major enigma globally because it creates limitations in the ability of antimicrobial drugs to suppress the pathogens in infected humans and animals (EUCAST, 2019) [8]. Antimicrobials are beneficial therapeutic agents regularly used in humans as well as in veterinary medicine. However, the non-therapeutic use of antimicrobials could be the major factor in the development of resistivity by microorganisms (CDC, 2019) [4]. Inappropriate and overuse of antibiotics for non-bacterial infections such as colds and other viral infections and their inadequate doses may lead to problems in the selection of alternative drugs (Limmathurotsakul *et al*., 2019) [18].

The other major factor in the growth of antibiotic resistance is the spread of resistant strains of bacteria from person to person or from non-human sources in the environment.

Those Staphylococcal infections are frequently treated with antimicrobials and consequence of which antimicrobial resistance has developed out of which the Methicillin-Resistance *Staphylococcus aureus (*MRSA) is a critical one. Methicillin-resistant *Staphylococcus aureus (*MRSA) is the strain of *S. aureus* that is resistant to all available penicillins and other β-lactam antimicrobial drugs (David *et al*., 2010; Kumar *et al*., 2011) [7, 15]. Thus, Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global, major cause of healthcare, community, and livestock-associated infections, creating enormous disease burdens and leading to substantial efforts in preventive measures (Chambers et.al., 2009; Knock *et al*., 2013; McKenna, 2010; Pandey *et al*., 2024) [5, 13, 21, 24]**.** The most important factor that determines the resistance is the genetic factor of the bacteria. MRSA has the mecA gene that is located on a genetically mobile chromosomal determinant termed staphylococcal cassette chromosome mec (SCCmec). The mecA gene codes for the modified penicillin-binding protein 2a (PBP2a) that is in the cell wall with low binding affinity to β-lactams (Morgan, 2008) [23]. The reduction in binding affinity of antibiotics PBP2a allows the cell wall synthesis to continue. The production of the beta-lactamase enzyme by the blaZ gene which is responsible for drug inactivation. MRSA, initially emerging as a nosocomial pathogen, has evolved to resist multiple antibiotics and is now prevalent in both hospital and community settings, causing significant concerns in veterinary medicine across various animal species. MRSA, predominantly a human pathogen, has been detected in animals, with molecular typing revealing widespread inter-transmission between humans and animals, indicating companion animals as potential reservoirs. Considering identical molecular factors and related reports of interspecies sharing of MRSA, it should be considered a serious matter of zoonotic importance. The public should be aware of health concerns because pet animals, especially dogs are in close contact with their owners, risking them to the transmission of pathogenic bacteria (Gurdabassi *et al*., 2004) [10].

The study aims to determine the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in dogs registered at CVH, Kathmandu, Nepal. Specifically, it seeks to detect the overall presence of Staphylococcus species in these dogs, determine the nasal colonization of *S. aureus* (coagulase-positive *Staphylococcus* spp.), compare MRSA prevalence in dogs with and without an antibiotic course, and determine the antibiotic sensitivity profile of *S. aureus*.

Materials and Methods

The nasal swabs were collected from the dogs in Central Veterinary Hospital (CVH) Tripureshwor, Kathmandu which were brought for treatments and vaccinations. This is a cross-sectional study. A total of 450 dogs of any sex, any breed, and any age that were brought to CVH were included during the study period.

Sampling and study procedure

Dog samples were collected from the Central Veterinary Hospital Tripureshwor. The samples were collected in nasal swabs and the individual dogs were the sample unit. Nasal swabs were taken from all the dogs. The Swab was inserted approximately 2 cm into the nares. The swab was rubbed against the anterior nasal mucosa for three seconds. Using the same swab it is repeated for other nares. The swab was placed back for transportation charcoal media (Himedia) was used. After taking nasal swabs it was inoculated in charcoal media kept in a cool box and transported to the Central Veterinary Laboratory, Tripureshwor, Kathmandu.

Isolation of *Staphylococcus aureus*

Nasal swabs were inoculated in mannitol salt broth soon after they arrived at the lab and then incubated at 37^0 C for 24 hours. After that cultured in blood agar and incubated at 37 ^{0}c for 24 hrs. Golden, yellow-colored colonies showing beta-hemolysis were suspected as Staphylococci. Then, Gram's staining was done. Gram-negative samples were discarded. Samples that showed gram-positive cocci were further inoculated on nutrient agar and incubated for 24 hrs. at 37^0c for pure culture isolation. After inoculation, colony morphology was observed. Staphylococcus produces whitish to yellow colonies on nutrient agar. Again, gram staining was done and samples having Gram-positive cocci were used for further analysis. All the gram-positive cocci samples were further subjected to a catalase test. All the catalase-positive samples were further subjected to a coagulase test. Then, the Trehalose fermentation test and finally the Vogas-Proskauer (VP) test **(**Reynolds, 2011) [25].

Identification of Methicillin Resistant *Staphylococcus aureus* (MRSA)

S. aureus isolated from the above procedure was first suspended on peptone water and incubated for 4 hours at 37^0C. Turbidity of bacterial suspension was maintained at 0.5 Mc Farland by further incubation if turbidity was lower and the addition of normal saline if turbidity was higher. Then an antibiotic sensitivity test was done on MHA according to the Kirby Bauer disk diffusion method. Cefoxitin (having a zone of inhibition less than 21mm) was identified as MRSA.

Antibiotic Sensitivity Test (AST)

An antibiotic sensitivity test was done for *S. aureus* according to the Kirby Bauer disk diffusion method. Briefly, 0.5 Mc Farland of bacterial suspension was inoculated to Muller Hilton Agar, and the antibiotic discs were Cefoxitin (Cx), Ceftriaxone (CTR), Enrofloxacin (Ex), Tetracycline (TE), Ciprofloxacin (CIP), and Amoxicillin (AMOX). Then, plates were incubated at 37^0C for 18 hours and the zone of inhibition was measured with the help of zone scale. Antibiotics were classified as resistant, intermediate, and sensitive based on the zone of inhibition produced by each antibiotic disc.

Data Analysis

Data entry was done in Ms-Excel 2010. For prevalence determination, bar graphs, and pie charts Ms-Excel 2010 were used. Data were analyzed by Open EpiVersion 3.03 software.

Results

Out of 450 nasal samples from dogs, 12% were *S. aureus*, 5% were Staphylococcus species excluding *S. aureus*, 10% were other gram-positive (Gm+) bacteria, and 6% were gram-negative (Gm-) bacteria Out of 450 samples tested, 18 samples were found to be resistant to Cefoxitin. Thus, the prevalence of MRSA was found to be 4% (Figure 1).

Out of 87 samples from dogs below four months of age 3 (3.4%) were found to be positive for MRSA, 180 samples

between four to twelve months of age 9 (4%) dogs were found to be positive and 183 samples above twelve months of age 6 (3.3%) dogs were positive for MRSA.

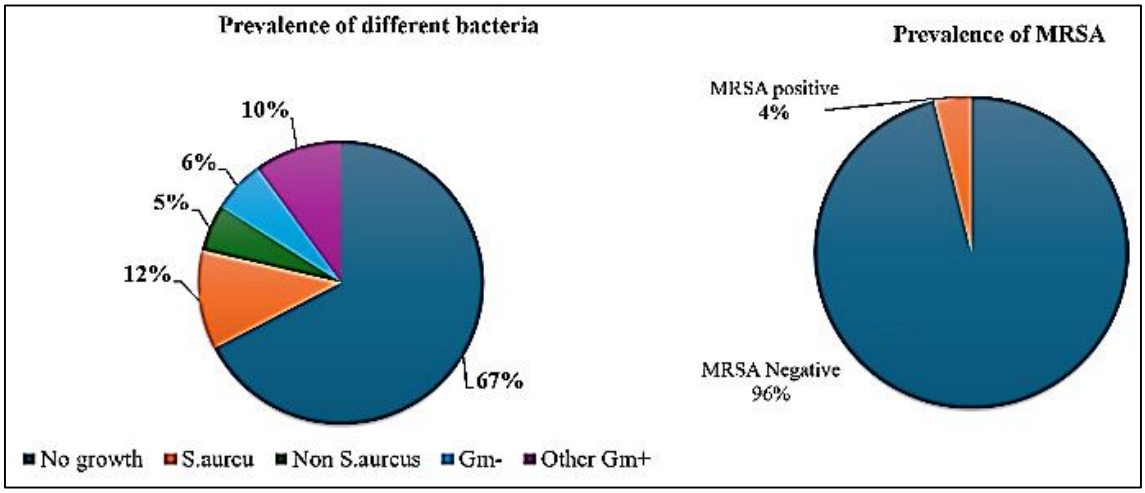

Fig 1: Prevalence of difference species of bacteria with MRSA colonized in the nasal canal of dogs

Out of 237 samples from male dogs, 12 (5%) were found to be positive for MRSA and out of 213 samples from female dogs 6 (2.8%) were found to be positive for MRSA.

Out of 219 samples from dogs that were on antibiotic course 9 were found to be MRSA positive and out of 231 samples from dogs that were not on any antibiotic course 9 were found to be MRSA positive.

The different antibiotics were used for antibiotic sensitivity tests. These were Cefoxitin (Cx10), Ceftriaxone (CTR30), Enrofloxacin (Ex15), Tetracycline (TE30), Ciprofloxacin (CIP5) and Amoxicillin (AMOX30). Among them, Ceftriaxone, Enrofloxacin, and tetracycline were found to be sensitive whereas Cefoxitin, Ciprofloxacin, and amoxicillin were found to be resistant antibiotics for isolated *Staphylococcal* species (Figure 2).

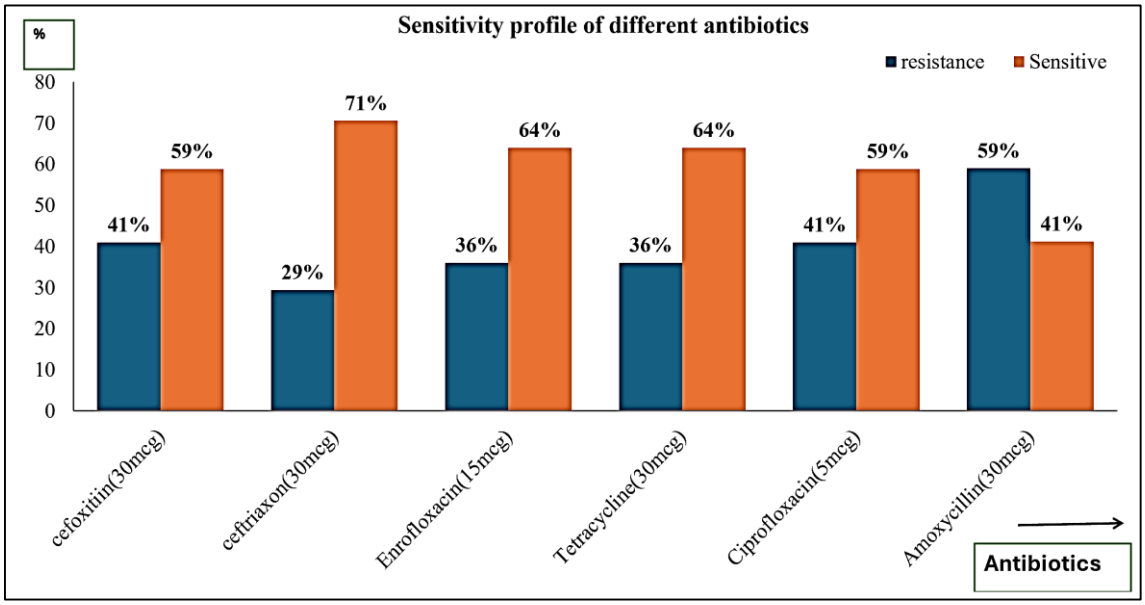

Fig 2: Diagram showing the sensitivity profile of difference antibiotic for *S. aureus*

Discussion

Methicillin-resistant *Staphylococcus aureus* infection (MRSA) is becoming increasingly prevalent worldwide. Nowadays, the whole medical industry is in serious jeopardy. MRSA-causing pathogens are not limited to veterinary animals but also have the potential to affect human species, making MRSA a significant zoonotic hazard. Methicillin was discovered to be resistant to treating nosocomial infections caused by *Staphylococcus aureus* shortly after its invention. Since then, several cases of MRSA have been documented in cattle and pets. Extensive antibiotic usage in companion animals, dogs, and cats that come into close contact with people can lead to the spread of MRSA germs (Gurdabasi *et al*, 2004) [10]. Many studies on MRSA indicate that MRSA is an important nosocomial pathogen that is not confined to hospitals and can be propagated by healthy humans and animals in the community.

In this study, 12% of *S. aureus* were colonized in the nares of dogs, which is less than the findings of Griffeth *et al* (2008) [9] who found that 15%, 14%, and 16% of prevalence that might be due to a lower sample Our findings for *S. aureus* infection was also concurrent with the findings of Middleton

(2005) [22] who found that 11%.

This study revealed that the prevalence of MRSA in dogs in Kathmandu is 4%. The finding of this study was similar to that of who reported a 5% prevalence of MRSA in dogs of Godawari VDC, Lalitpur, Nepal. The finding of this study was also like Kottler *et al* (2010) [14] reported a 3.41% prevalence in dogs in the USA. However reported that 56.2% (18/32). Similarly, Griffeth *et al* (2008) [9] reported a 0% prevalence in community dogs in the US. Found the same result in the dogs from Veterinary Hospital in Denmark.

The prevalence rates of MRSA found in several studies were lower compared to that of the current study. Kwon *et al*. (2006) [16] reported MRSA in 1.9% of hospitalized dogs in Korea. Malik *et al*. (2006) [20] reported MRSA in 0.8% (2/252) of dogs and cats in Australia. According to Rich and Robert (2004) prevalence rate of MRSA is 0.4% (1/255) in the UK and a very low rate in Hong Kong reported by Boost *et al*. (2011) [3] among dogs. Boost *et al*. (2007) [2] reported 0.7% of MRSA in dogs in the community in China. The regulations for the usage of antibiotics and the molecular characterization to detect MRSA might be the reason for a lower rate of prevalence.

Aklilu *et al*. (2010) [1] reported that 10% of MRSA in the University Veterinary Hospital was like Loeffler *et al*. (2005) [19] from hospitalized dogs in the UK which were found 9%. Those reports are found to be higher than our finding rate for MRSA in dogs. This might be because the collected samples from their studies were from all diseased dogs. Overall, the prevalence of MRSA in dogs varies widely across different regions and studies, likely due to differences in sample populations, antibiotic usage regulations, and detection methods, highlighting the ongoing need for vigilant monitoring and control measures to address this significant zoonotic threat.

Conclusion

In this research, the presence of *S. aureus*, *Staphylococcus* spp. (excluding *S. aureus*), and other Gram-positive and Gram-negative bacteria were found to be 12%, 5%, 10%, and 6% respectively in the colonized state inside the nasal passages of dogs of Kathmandu. Prevalence of MRSA in age groups below 4 months, between 4 to 12 months, and above 12 months of dogs were found to be 3.4%,4%, and 3.3% respectively. Male and female dogs were found to be positive with MRSA at 5% and 2.8% respectively. The dogs with antibiotic course 4.1% and without antibiotic course 3.9% were found to be positive with MRSA. The isolated *S. aureus* bacteria were resistant to Cefoxitin and Amoxycillin and more sensitive to Ciprofloxacin, Tetracycline, and Ceftriaxone.

Thus, irrespective of the mode of transmission either human to animals or vice versa, the high number of positive cases suggests that a high number of individuals serve as reservoir hosts for MRSA, who may then become the source of infections of colonization. Haphazard and indiscriminate use of antibiotics without proper prescription for treatment might be responsible for drug resistance.

Recommendation

Proper use of antibiotics should be done. Care must be taken in personal hygiene and sanitation. Prescription of antibiotics should be done only after AST. Research should be conducted on a larger scale to monitor MRSA in the country up to the molecular level for detection and confirmatory Epidemiological patterns of organisms. Public awareness should be given to the zoonotic importance of MRSA.

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