Research Article

Prevalence and antimicrobial sensitivity of bacterial isolates from canine wound infection in Tandojam, Sindh

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Abstract

A total of 100 canine wound samples of accidental wound (n=40), pyoderma (n=16), tail wound (n=15), localized anal wound (n=10), otitis (n=8), lacerated mammary wound (n=6) and post-surgical wound (n=5) were taken from Clinic of Surgery and Obstetrics and brought to the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam to isolate and identify bacterial species responsible for canine wound infection and their susceptibility to various antibiotics. Samples were confirmed through morphological, culture and biochemical tests. *Staphylococcus aureus* (67%) was found to be the predominant isolate. It was followed by *Pseudomonas aeruginosa* (50%), *Escherichia coli* (40%), *Streptococcus pyogenes* and *Pasteurella canis* (38%), *Bacillus cereus* (36%) and *Micrococcus luteus* (25%). *Staphylococcus aureus* was isolated and identified as 40 (55%), 10 (90%), 8(62.5%), 6(66.6%), 15(86.6%), 16(62.5%) and 5(80%) from accidental wound, localized anal wound, otitis, lacerated mammary wound, tail wound, pyoderma and post- surgical wounds respectively. Antibiotics Erythromycin and Ofloxacin were sensitive to *S. aureus*. *B. cereus* was sensitive to Ampicillin and Gentamicin. *P. canis* was sensitive to Ampicillin, Enrofloxacin and Ofloxacin. *E. coli* was sensitive to Enrofloxacin and Ofloxacin. *M. luteus* was sensitive to the Ampicillin and Enrofloxacin. *P. aeruginosa* and *S. pyogenes* were sensitive to Enrofloxacin.

Keywords: Antibiotic; Canine; Sensitivity; *Staphylococcus*; Wound

Introduction

Bacterial wound infection is a critical problem and the treatment of wound infections remain a significant concern in

dogs. Wounds can be classified as acute and chronic. The acute type of wounds either upsetting, distressing or surgically treated through the healing process but chronic

wounds are not resolved over a long time [1]. It is reported that bacterial infections persist more than 80% are likely to involve biofilms [2]. Biofilm bacteria are resistant to antimicrobial agents and host immune responses that results in chronic infection [3]. The bacteria adhere to the surface of wound by attaching to the exposed extracellular matrix, multiply and develop into microcolonies, these biofilms once established, resist the action of antimicrobials and evade the host defense mechanism [4]. Most dermal wounds are populated with aerobic and anaerobic microorganisms that occur mainly from mucosal surfaces such as oral cavity and gut. Wound due to bacterial infection and resistance to antibiotics are the common post-surgical and scientific challenges. Wounds of bacterial contaminations are the frequent hospital acquired infections causing more than 80% mortality [5]. *Escherichia*, *Enterococci*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Proteus* and *Acinetobacter* are the most frequent bacterial genera infecting wounds [6, 7]. Wound infections are identified as the most unfavorable problem especially in the presence of foreign materials that increases the risk of serious infection even with relatively minute bacterial infection [8]. Antimicrobial resistance is a very complex problem involving various bacterial species, resistance mechanisms, transfer mechanisms and reservoirs. Research have revealed that antimicrobial use in pet animals contributes to the selection of antimicrobial resistant and possess threat to the people because of transmission of resistant zoonotic microorganism via the food chain and oblique switch of resistant genes from animals to man [9]. Resistant bacteria isolated from pets are observable by using their absence as key organisms which have been reported to understand resistance gene organization [10]. For example, methicillin resistance *Staphylococcus aureus* of canine

origin may be more common than reported [11]. Excessive use of antibiotics together with the length of time cause a significant antibiotic resistance to wound infecting bacteria [12] that later increase the problems and costs of treatment [13].

Applications for proper preventive measures to control antimicrobial resistant microorganisms and surveillance protocols which include patterns of antimicrobial resistant in human are the key component in decreasing rate of infection. Bacterial culture and antimicrobial susceptibility testing of clinical site of surgical infections (SSIs) in veterinary practice is essential for the diagnosis of bacterial infection and to guide the therapy towards rational antimicrobial use. Culture based treatments also help to facilitate surveillance efforts [14]. Surgical procedures are successful with early decontamination and tension free closure in companion animals [15]. Therefore, present study is planned to evaluate the microbial profile of wounds in dogs and select appropriate drug for their better management practices.

Materials and methods

Isolation and Identification of microbes

A total of 100 canine wound samples of accidental wound (n=40), pyoderma (n=16), tail wound (n=15), localized anal wound (n=10), otitis (n=8), lacerated mammary wound (n=6) and post-surgical wound (n=5) were taken from Clinic of Surgery and Obstetrics and brought to the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. Samples were processed for the bacterial culture by inoculating swabs into agar plates. i.e Nutrient agar, Macconkey's agar, Blood agar and incubated at 37°C for 24h to separate the colonies for each kind of bacteria present in culture. Morphological characteristics were noted and confirmation of morphological difference among them was done by Gram

staining. Biochemical Tests such as Catalase, Oxidase, Coagulase, Indole, Triple Sugar Iron Test, Simmon Citrate Test, Voges Proskauer Test, Nitrate Reduction Test were done for further identification.

Antibiotic sensitivity test

The antibiotic sensitivity test was performed by method described by [16]. Pure colonies were taken by wire loop and inoculated in to 5ml normal saline. Sterile cotton swab was taken and dipped into the suspension and rotated several times with a firm pressure on the inside wall of the tube. Sterile swab was streaked over the entire surface of Muller Hinton agar in three different directions to make sure an even distribution of the inoculum. After 3-5 minutes of drying, the antibiotic disks were placed there. Plates were incubated for 24 hours at 37°C. The inhibitory zone diameters gained around the discs were measured after incubation for 24h at 37°C and evaluated. Following antibiotics were used: Ampicillin, Amikacin, Erythromycin, Enrofloxacin, Gentamicin, Kanamycin, Neomycin, Ofloxacin, Penicillin, Streptomycin and Tetracycline.

Results

Prevalence of bacterial isolates from canine wound

Results of the bacterial isolates from canine wound are described in (Table 1). The predominant isolate from different kinds of wound infection of dogs was found to be *Staphylococcus aureus* (67%). It was followed by *Pseudomonas aeruginosa* (50%), *Escherichia coli* (40%), *Streptococcus pyogenes* and *Pasteurella canis* (38%), *Bacillus cereus* (36%) and *Micrococcus luteus* (25%). *S. aureus* was isolated and identified as 40 (55%), 10 (90%), 8(62.5%), 6(66.6%), 15(86.6%), 16(62.5%) and 5(80%) from accidental wound, localized anal wound, otitis, lacerated mammary wound, tail wound, pyoderma and post-surgical wound respectively. *P. aeruginosa* became a main

cause of infection in majority of wounds except otitis in which the *E. coli* and *P. canis* had been predominant. In addition, *M. luteus* was observed to be least in all the wounds except otitis where *S. pyogenes* become least predominant. Prevalence of isolated bacterial species from canine wound is summarized in (Table 1).

Antibiotics sensitivity

The antibiotics sensitivity against bacterial isolates from canine wound is presented in (Table 2).

Staphylococcus aureus

Erythromycin and Ofloxacin were highly sensitive against *Staphylococcus aureus*. Ampicillin, Amikacin, Enrofloxacin, Penicillin, Gentamicin, Streptomycin and tetracycline were quite sensitive. Kanamycin was moderately sensitive. Neomycin was not sensitive against this bacterial specie.

Bacillus cereus

Ampicillin and Gentamicin were highly sensitive against *Bacillus cereus*. Amikacin, Erythromycin, Penicillin and Streptomycin were quite sensitive. Kanamycin, Neomycin, Ofloxacin and Tetracycline were moderately sensitive. Enrofloxacin was not sensitive against *Bacillus cereus*.

Pasteurella canis

Ampicillin, Enrofloxacin and Ofloxacin were highly sensitive against *Pasteurella canis*. Amikacin, Erythromycin, Gentamicin, Kanamycin, Neomycin and streptomycin were found quite sensitive. Erythromycin, Penicillin and Tetracycline were not sensitive against *Pasteurella canis*.

Escherichia coli

Enrofloxacin and Ofloxacin were highly sensitive against *Escherichia coli*. Ampicillin, Amikacin, Erythromycin, Gentamicin, Streptomycin and Tetracycline were quite sensitive. Kanamycin and Neomycin were moderately sensitive. Penicillin was not sensitive against *Escherichia coli*.

Table 1. Prevalence of bacterial isolates from canine wound

Type of wound	No of sample	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>M. luteus</i>	<i>P. aeruginosa</i>	<i>S. pyogenes</i>	<i>P. canis</i>
Accidental wound	40	22 (55%)	09 (22.5%)	12 (30%)	07 (17.5%)	14 (35%)	13 (32.5%)	08 (20%)
Pyoderma	16	10 (62.5%)	08 (50%)	06 (37.5%)	05 (31.2%)	10 (62.5%)	06 (37.5%)	07 (43.7%)
Tail wound	15	13 (86.6%)	08 (53.3%)	07 (46.6%)	04 (26.6%)	11 (73.3%)	05 (33.3%)	07 (46.6%)
Localized anal wound	10	09 (90%)	04 (40%)	06 (60%)	03 (30%)	06 (60%)	07 (70%)	04 (40%)
Otitis	08	05 (62.5)	03 (37.5%)	05 (62.5%)	04 (50%)	04 (50%)	02 (25%)	05 (62.5%)
Lacerated mammary wound	06	04 (66.6%)	02 (33.3%)	02 (33.3%)	01 (16%)	02 (33.3%)	03 (50%)	05 (83.3%)
Postsurgical wound	05	04 (80%)	02 (40%)	02 (40%)	01 (20%)	03 (60%)	02 (40%)	02 (40%)
Total	100	67 (67%)	36 (36%)	40 (40%)	25 (25%)	50 (50%)	38 (38%)	38 (38%)

Micrococcus luteus

Ampicillin and Enrofloxacin were highly sensitive against *Micrococcus luteus*. Amikacin, Gentamicin, Kanamycin, Neomycin, Penicillin, Streptomycin and Tetracycline were quite sensitive against this bacterial specie. Ofloxacin and Erythromycin were not sensitive against *Micrococcus luteus*.

Pseudomonas aeruginosa

Enrofloxacin was highly sensitive against *Pseudomonas aeruginosa*. Ampicillin, Amikacin, Erythromycin, Gentamicin,

Kanamycin, Neomycin, Ofloxacin, Streptomycin were quite sensitive. Tetracycline was found moderately sensitive. Penicillin was not sensitive against *Pseudomonas aeruginosa*.

Streptococcus pyogenes

Enrofloxacin was highly sensitive against *Streptococcus pyogenes*. Amikacin, Gentamicin, Neomycin, Ofloxacin Penicillin, Streptomycin and Tetracycline, were quite sensitive. Ampicillin and Erythromycin were moderately sensitive. Kanamycin was not sensitive against *Streptococcus pyogenes*.

Table 2. The antibiotic sensitivity of bacterial isolates from canine wound

Bacterial species	Antibiotic discs used	Zone around Discs	Identification of sensitivity	Degree of sensitivity
<i>Staphylococcus aureus</i>	Ampicillin	10mm	+++	Quite sensitive
	Amikacin	08mm	+++	Quite sensitive
	Erythromycin	14mm	++++	Highly sensitive
	Enrofloxacin	08mm	+++	Quite sensitive
	Gentamicin	10mm	+++	Quite sensitive
	Kanamycin	04mm	++	Moderately sensitive
	Neomycin	0mm	-	No sensitivity
	Ofloxacin	15mm	++++	Highly sensitive
	Penicillin	10mm	+++	Quite sensitive
	Streptomycin	08mm	++	Quite sensitive
	Tetracycline	10mm	+++	Quite sensitive
<i>Bacillus cereus</i>	Ampicillin	15mm	++++	Highly sensitive
	Amikacin	10mm	+++	Quite sensitive
	Erythromycin	08mm	+++	Quite sensitive
	Enrofloxacin	0mm	-	No sensitivity
	Gentamicin	15mm	++++	Highly sensitive
	Kanamycin	05mm	++	Moderately sensitive

	Neomycin	04mm	+++	Moderately sensitive
	Ofloxacin	04mm	++	Moderately sensitive
	Penicillin	08mm	++	Quite sensitive
	Streptomycin	08mm	++	Quite sensitive
	Tetracycline	05mm	++	Moderately sensitive
<i>Pasteurella canis</i>	Ampicillin	16mm	++++	Highly sensitive
	Amikacin	09mm	+++	Quite sensitive
	Erythromycin	0mm	-	No sensitivity
	Enrofloxacin	13mm	++++	Highly sensitive
	Gentamicin	09mm	+++	Quite sensitive
	Kanamycin	09mm	+++	Quite sensitive
	Neomycin	08mm	+++	Quite sensitive
	Ofloxacin	14mm	++++	Highly sensitive
	Penicillin	00mm	-	No sensitivity
	Streptomycin	10mm	+++	Quite sensitive
	Tetracycline	0mm	-	No sensitivity
<i>Escherichia coli</i>	Ampicillin	08mm	+++	Quite sensitive
	Amikacin	08mm	+++	Quite sensitive
	Erythromycin	08mm	+++	Quite sensitive
	Enrofloxacin	14mm	++++	Highly sensitive
	Gentamicin	10mm	+++	Quite sensitive
	Kanamycin	04mm	++	Moderate sensitive
	Neomycin	04mm	++	Moderate sensitive
	Ofloxacin	15mm	++++	Highly sensitive
	Penicillin	0mm	-	No sensitivity
	Streptomycin	08mm	+++	Quite sensitive
	Tetracycline	08mm	+++	Quite sensitive
<i>Micrococcus luteus</i>	Ampicillin	15mm	++++	Highly sensitive
	Amikacin	10mm	+++	Quite sensitive
	Erythromycin	00mm	-	No sensitivity
	Enrofloxacin	15mm	++++	Highly sensitive
	Gentamicin	08mm	+++	Quite sensitive
	Kanamycin	08mm	+++	Quite sensitive
	Neomycin	08mm	+++	Quite sensitive
	Ofloxacin	0mm	-	No sensitivity
	Penicillin	10mm	+++	Quite sensitive
	Streptomycin	08mm	+++	Quite sensitive
	Tetracycline	10mm	+++	Quite sensitive
<i>Pseudomonas aeruginosa</i>	Ampicillin	08mm	+++	Quite sensitive
	Amikacin	10mm	+++	Quite sensitive
	Erythromycin	08mm	+++	Quite sensitive
	Enrofloxacin	14mm	++++	Highly sensitive
	Gentamicin	08mm	+++	Quite sensitive
	Kanamycin	08mm	+++	Quite sensitive
	Neomycin	08mm	+++	Quite sensitive
	Ofloxacin	08mm	+++	Quite sensitive
	Penicillin	00mm	-	No sensitive
	Streptomycin	08mm	+++	Quite sensitive
	Tetracycline	07mm	++	Moderately sensitive

<i>Streptococcus pyogenes</i>	Ampicillin	05mm	++	Moderately sensitive
	Amikacin	10mm	+++	Quite sensitive
	Erythromycin	04mm	++	Moderately sensitive
	Enrofloxacin	15mm	++++	Highly sensitive
	Gentamicin	08mm	+++	Quite sensitive
	Kanamycin	0mm	-	No sensitivity
	Neomycin	10mm	+++	Quite sensitive
	Ofloxacin	11mm	+++	Quite sensitive
	Penicillin	08mm	+++	Quite sensitive
	Streptomycin	08mm	+++	Quite sensitive
	Tetracycline	08mm	+++	Quite sensitive

Discussion

Rearing of dogs and other pet animals has become increasingly popular in modern society. In this context it is important to note that respiratory diseases pose a major health problem in dogs and other pet animals [17, 18]. Bacteria involved in respiratory infections in dogs and cats are usually *Escherichia coli*, *Klebsiella spp.*, *Streptococcus spp.* and *Staphylococcus spp* [19].

In our study the most dominant isolates from accidental wound, pyoderma, tail wound, localized anal wound, otitis, lacerated mammary wound and post-surgical wounds was *S. aureus* followed by *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyogenes*, *Pasteurella canis*, *Bacillus cereus* and *Micrococcus luteus*. Similar results are supported by other authors [20] who reported *Staphylococcus intermedius* (20%), *Enterococcus spp.* (15%), *Coagulase-negative staphylococci* (13%), *Escherichia coli* (13%) as most common aerobic isolates. Whereas, [21] reported *Staphylococcus intermedius* (23%), *Escherichia coli* (18%), non-lactose fermenting coliforms (14%) and *Pseudomonas spp* (14%) as the most commonly pathogenic isolates from wound infections. While, [22] reported the higher prevalence of *Pasteurella canis* and *Staphylococcus aureus*. This is in accordance with our finding in which *Staphylococcus aureus* and *Pasteurella canis* were most common. In our study *Staphylococcus aureus* (67%) was found to be the predominant

isolate. It was followed by *Pseudomonas aeruginosa* (50%), *Escherichia coli* (40%), *Streptococcus pyogenes* and *Pasteurella canis* (38%), *Bacillus cereus* (36%) and *Micrococcus luteus* (25%). *Staphylococcus aureus* was isolated and identified in 40 (55%), 10 (90%), 8(62.5%), 6(66.6%), 15(86.6%), 16(62.5%) and 5(80%) samples from accidental wound, localized anal wound, otitis, lacerated mammary wound, tail wound, pyoderma and post-surgical wound respectively. This is similar to previous reports by [23] who reported higher prevalence of *Staphylococcus aureus* (33%), *Escherichia coli* (18.9%), *Klebsiella pneumonia* (16.7%), *Proteus vulgaris* (11.4%), *Streptococcus spp* (9.2%) and *Pseudomonas aeruginosa* (9.6%) in dogs.

A study by [24] reported that the isolates *Staphylococcus intermedius*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pasteurella spp*, *Corynebacterium spp* and *bacillus spp.* are persistently causative agent of wound infection in dogs. This suggests that canine wounds are polymicrobial in nature and containing pathogens which are significantly prevalent in chronic wounds. In our study *Staphylococcus* was found in synergistic relation with other bacteria in infected wounds. This agrees with the findings of previous studies [1]. Whereas, [25] reported that bacteria like *Staphylococcus* never occurs singly and commonly found synergistically with others. The synergism of bacteria might lead to greater pathogenesis

than individual inputs. This is in accordance with [24] who reported that the *Staphylococcus* (65.7%) was found to be the most predominant isolate from traumatic wounds, while *Staphylococcus intermedius* as most prevalent bacteria in dog infected wounds [26]. The increased prevalence of *Staphylococcus intermedius* might be due to occurrence as commensal on skin and mucus membranes. *Pseudomonas aeruginosa* (75%) was found to be the main agent isolated from postsurgical wounds which show the similarity with previous reviews [27]. The genus *Staphylococcus* was most commonly isolated in all type of wounds. This is much like preceding reports in human and equines [28]. The reason for above outcomes may be the microbial isolates have strong affinity for that specific site. The composition and variation of microbial agents in wounds are affected by many factors such as depth of affected tissue, localization, tissue perfusion, intensity, quality and strength of host antimicrobial immune response [25].

Antimicrobial resistance is a critical health problem in companion animals with systemic antimicrobial therapy driving development of AMR across the host spectrum [29, 30]. The antibiotics are mostly resistant due to misuse of prescribed medication [31, 32]. In our study erythromycin and ofloxacin were found highly sensitive against *Staphylococcus aureus*. Ampicillin and Gentamicin were found highly sensitive against *Bacillus cereus*. Ampicillin, Enrofloxacin and Ofloxacin were found highly sensitive against *Pasteurella canis*. Enrofloxacin and Ofloxacin were found highly sensitive against *Escherichia coli*. Ampicillin and Enrofloxacin were found highly sensitive against *Micrococcus luteus*. *Pseudomonas aeruginosa* and *Streptococcus pyogenes* were observed as highly sensitivity to Enrofloxacin. These results are in agreement with results obtained by [33] who showed that Gentamicin was the most sensitive

antibiotic followed by Enrofloxacin, Ciprofloxacin and Amoxicillin. While [34] found that *Staphylococcus* spp. were predominant and sensitive to Gentamicin and Enrofloxacin but were generally resistant to Lincomycin and Oxytetracycline. whereas, [35] investigated that *Escherichia coli*, *Proteus* spp, *Staphylococcus aureus* and *Streptococcus pyogenes* were major isolates from canine infections. Majority of the isolates were highly sensitive to Gentamicin. Lowered sensitivity was noticed towards Ampicillin. Gentamicin, Chloramphenicol, Cefixime, Ciprofloxacin were sensitive against *Escherichia coli*, whereas, Amikacin, Tetracycline, Kanamycin, Vancomycin, Erythromycin and Novobiocin showed their infectivity against these isolates [36]. Thus, our findings are in agreement with above workers.

Conclusion

It was concluded from present study that the maximum prevalence of canine wound infection is recorded from accidental wound, pyoderma, tail wound, localized anal wound, otitis, lacerated mammary wound and post-surgical wounds. *Staphylococcus aureus* was predominant specie followed by *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Streptococcus pyogenes*, *Pasteurella canis* and *Micrococcus luteus*, respectively. Antibiotics should be taken into consideration for effective treatment of canine wound infections.

Authors' contributions

Conceived and designed the experiments: DH Kalhoro, SH Abro & AN Tuino, Performed the experiments: DH Kalhoro & MI Ansari, Analyzed the data: MS Kalhoro, GM Lochi & MA Chandio, Contributed materials/ analysis/ tools: SH Abro, A Kaka & S Kumbhar, Wrote the paper: DH Kalhoro & MQ Mazari.

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