

Research Article

Comparison of antibacterial activity of Ciprofloxacin and Cephalexin against Some common bacterial Species isolates from donkey wounds around the vicinity of Tandojam Sindh Pakistan

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Abstract

The results obtained in the current study have increased knowledge on bacterial infections of skin wounds in equines around the vicinity of Tandojam Sindh Pakistan and compare with two different antibiotics (Ciprofloxacin and Cephalexin) against some common pathogens. During this experiments, a total of 50 wound samples were collected from donkey wounds. Specimens were brought in a cold chain. All 50 samples were found contaminated with different bacterial organisms including *Staphalococcus aureus* (45%), *Escherichia coli* (35%), *Shigella* (33%) and *Salmonella* spp (25%). The isolated bacterial organisms were checked for their susceptibility against Cephalexin and Ciprofloxacin. *S. aureus* (18mm), showed higher sensitivity against Cephalexin (18mm) and followed by *Escherichia coli*, (15mm), *Shigella* (13mm), and *Salmonella* spp. (13mm) respectively. Whereas Ciprofloxacin showed more significant activity against *S. aureus* (24mm), *E. coli* (20mm), *Shigella* (15mm), and *Salmonella* spp. (18mm). The results of this

study revealed that both antibiotics Ciprofloxacin and Cephalexin showed higher sensitivity against the isolated microorganisms. *S.aureus* showed greater susceptibility against Cephalexin, followed by *Escherichia coli*, *Shigella*, and *Salmonella* spp. In contrast, Ciprofloxacin showed higher activity against *Staphylococcus aureus* and *E. coli*, and minimum for *Shigella* and *Salmonella* spp.

Keywords: Antimicrobial effect; Antibiotics; Donkey wound; Gram negative; Gram positive bacteria

Introduction

The Donkey is a domesticated animal of the family *Equidae*. It has been used as a working animal from last 5000 years. There are more than 41.5 million donkeys in the world [1]. Several studies indicated that the antibiotic has a notable effect on donkey wound healing. Ciprofloxacin was introduced in 1980 and was first introduced in marketed in 1987 [2]. It is one of the most effective and safe medications on the list of global health organizations [3]. Ciprofloxacin is one of the best fluoroquinolones for wide clinical use and was previously known for its activity against a wide range of pathogens [4]. In previous studies, it was reported that fluoroquinolone was among the most commonly proposed class of antimicrobial drugs in communities and hospitals [5,6]. Cephalexin is effective against gram-positive and some gram-negative bacteria that disturb the growth of the cell wall of the bacteria. Cephalexin is the first generation cephalosporin, it is also known as beta-lactam antibiotic. Cephalexin has been used to treat some infectious diseases of the urinary tract, bones, joints, middle ear, and skin. It can also be used to prevent bacterial endocarditis, pneumonia and throat infection [7]. Other researchers have suggested that *Salmonella* was highly sensitive to ciprofloxacin and less sensitive cephalexin [7].

There are different kinds of open wounds; incised wound, laceration, abrasion, avulsion, puncture wound, penetration wound and gunshot wound [8]. Skin wound of a donkey can develop ideal condition where microorganisms can grow, especially

in the wound cause by saddle (saddle sore), it may lead to secondary infection by providing suitable condition for the growth of microorganism. The typical signs of wound on equine skin include erythematic, pain, hotness and pyogenic discharge. Other symptoms in an advances stage include slow healing, friable granulation at the base of the lesion, odor, exudate and the pain increase when these wound disrupt due to reoccurrence of any injury [9, 10].

The open wound can be infected by contaminating wide range of microorganism such as bacteria, fungi and parasites [9]. The microorganisms, which are commonly involve in septic wound (infected wound), are gram positive such as hemolytic *streptococcus* β : *Streptococcus pyogenes* and *Staphylococcus aureus* and gram-negative aerobic, such as *Pseudomonas aeruginosa*. In contrast, facultative anaerobes include, *Escherichia coli*, *Klebsiella* and *Proteus*, fungal organisms such as *Candida* species and molds (*Aspergillus* species) [8].

Several studies indicated that the antibiotic has a notable effect on wound healing. Therefore, very little work has been conducted on bacteria, which are isolated from donkey's wounds. Therefore, considering the importance of the donkey as a domesticated member of the horse family, *Equidae*, this study has been designed to isolate and identified the bacterial species from the wound and the antibacterial effect of Ciprofloxacin and Cephalexin against the bacterial isolates.

Materials and Methods

Study population and sampling

In this study, a total of 50 different samples of saddle sore and other types of skin wounds from 50 donkeys were collected. Skin around the wound were disinfected by 70% alcohol to prevent from extraneous contamination. Samples were collected by using sterile swab and transported in a cold chain to the Department of Veterinary Pharmacology, SAU, Tandojam. Samples were cultured and processed for identification by using morphological characteristics and biochemical properties. Followed by antisusceptibility test (AST) was determined by disc diffusion method described by Rind and Khan [11].

Bacterial isolation and identification

Primary culture

Nutrient broth and Nutrient, MacConkey and Blood agar media were prepared and swab samples were cultured aerobically and anaerobically on these media and incubated at 37°C for 24 hrs. Colony characteristics were observed and smears were prepared, stained by Gram's staining and examined under the microscope for the identification of cell morphology.

Sub-culture

Further purification of cultures was prepared by sub culturing the well-separated colony on respective medium and repeated it severally. The purity of the samples were checked by examining stained smear. Followed by, the pure culture was grown onto agar slants and incubated at 37°C for 24hrs. and stored at 4°C. Further, the bacterial isolates were identified based on colony characteristic, microscopic morphology and biochemical properties such as catalase, oxidase, indole, coagulase, and triple sugar iron simmon citrate.

Staphalococcus aureus, *Escherichia coli*, *Shigella* and *Salmonella* spp were isolated from the donkey's wounds. Efficacy of selected anti-bacterial agents (Ciprofloxacin

and Cephalexin) was determined against gram positive and gram-negative bacteria isolates.

Detection of *Staphylococcus aureus*

For the detection of *Staphylococcus aureus*, wound sample were sub cultured on the nutrient agar. Growth plates were observed for such as yellow golden colonies; further confirmation was done by Gram's staining and biochemical reactions of the isolates.

Detection of *E. coli*

For the detection of *E. coli*, wound samples were subcultured on the MacConkey's agar which is typical for *E.coli* growth. Growth plates were observed for bacterial growth, which included pink colonies on MacConkey's agar, and further confirmation was done by Gram's staining and biochemical test of the isolates.

Detection of *Salmonella* and *Shigella* Spp

For the detection of *Salmonella/ Shigella* spp., wound samples were subcultured on the *Salmonella /Shigella* agar (SS Agar), which is specific for *Salmonella* and *Shigella* spp. Growth plates were observed for typical colonies such as for *Shigella*, as transparent or translucent colorless colonies. Colony characteristic may appear with or without black centers, and further confirmation was done by Gram's staining and biochemical test of the isolates.

Determination resistance profile of antibiotic Susceptibility Testing (AST) and Multiple antibiotic resistance (MAR) Index

Disk diffusion method was used to determine the AST test against some selected antibiotics of isolated bacteria [11]. The isolated bacteria were cultured by swabbing on the nutrient agar overnight and commercial antibiotic disk (HiMedia Laboratories, India) were placed on the swabbed plate aseptically and incubated at 37°C for 24 hrs. The clear zones were formed around the disc which were recorded in mm (millimeter) by using Hi Antibiotic Zone Scale (HiMedia). The MAR

index were determined for each bacterial species;

MAR Index= $\frac{\text{No. of antibiotic to which isolates are resistant}}{\text{No. of antibiotic test}}$

Results

In the current study, we compare the antibacterial activity of two standard antibiotics against some common pathogenic bacterial isolates from donkey wound.

The percentage prevalence of bacterial organisms isolated from donkey wound

A total of 50 wound samples were examined and all were recorded positive for different organisms. Out of 50 samples, 45, 35, 33, and 25 number were found positive for *Staphylococcus aureus*, *Escherichia coli*, *Shigella* and *Salmonella* spp. respectively (Table 1). All organisms were identified on their morphological, cultural characteristics and staining reactions. Organisms were further confirmed by their biochemical reactions.

Table 1. The number and percentage prevalence of bacterial organisms isolated from donkey wounds

Bacterial species	Total No. of wound sample	No. of positive wound sample	Percentage (%)	No. of negative Wound sample	Percent age (%)
<i>Staphylococcus aureus</i>	50	45	90%	5	10%
<i>Escherichia coli</i>	50	35	70%	15	30%
<i>Shigella</i> spp	50	33	66%	17	35 %
<i>Salmonella</i> spp	50	25	50%	25	50%

Table 2. The zones of inhibition were observed and recorded with the following annotations

Description	Significance	Sensitivity level
Absence of clear zone	No sensitivity	–
Clear zone with 1-2mm	Weakly sensitivity	+
Clear zone with 3-7mm	Moderately sensitivity	++
Clear zone with 7-11mm	Quite sensitivity	+++
Clear zone with 12-15mm or above	Highly sensitivity	++++

Susceptibility of isolated organisms against different antibiotics

It provides the basis to test the sensitivity, and the zone of inhibition around disk determined resistances according to reference standards. The following antibiotic discs were used: Ciprofloxacin and Cephalexin, as shown in (Table 2).

Antibiotics i-e Cephalexin and Ciprofloxacin were used during this study; both are broad-spectrum antibiotics as this work against gram-negative as well as gram-positive bacteria. For Cephalexin, *S. aureus* showed greater susceptibility (18 mm), followed by *E.coli* (15 mm), *Shigella* and *Salmonella* (13 mm) respectively, whereas Ciprofloxacin produced maximum sensitivity for *S.aureus* and *E.coli* (20 to 24 mm) minimum for *Shigella* and *Salmonella* spp (15 to 18 mm). With respect to individual pathogens, antibiotics showed sensitivity against gram-positive as well as gram-negative isolate (Table 3).

Table 3. Zones of inhibition produced by different antibiotics against bacterial isolates

Zones of inhibition (In mm) against Antibiotics				
Bacterial species	Ciprofloxacin	Sensitivity	Cephalexin	Sensitivity
<i>Staphylococcus aureus</i>	24mm	Highly sensitive (+++)	18mm	Highly sensitive (++++)
<i>Escherichia coli</i>	20mm	Highly sensitive (++++)	15mm	Highly sensitive (++++)
<i>Shigella</i> spp	18mm	Highly sensitive (++++)	13mm	Highly sensitive (++++)
<i>Salmonella</i> spp	15mm	Highly sensitive (++++)	13mm	Highly sensitive (++++)

Discussion

The study was conducted to investigate and isolate the bacteria which are associate with equine wounds around the vicinity of Tandojam Sindh Pakistan. The results of this study shows that, 4 different species of aerobic and anaerobic bacteria namely: *S. aureus*, *E. coli*, *Shigella* and *Salmonella spp* were isolated from the different wound of donkeys. These findings of this studies were agreement to the previous study by [11] who isolated different species of aerobic and anaerobic bacteria from the non-surgical (injured) and surgical wounds located on body surface of animals.

Similar findings were also reported by [12] they reported most prevalent bacterial species was *Staphylococcus spp* from equine wounds . Four bacterial species were recognized from wound samples of donkey's wounds. *Staphylococcus aureus*, *Escherichia coli* *Salmonella* and *Shigella spp* and their incidence percentages recorded were 90%, 70%, 66% and 50% respectively (Table 2). This finding could be related to bacterial organisms from wound samples of animals by various workers throughout the world. However, the comparison of the present figures (Fig. 1 & 2) can be compared with the results of [11]. They isolated and characterized bacterial species from non-surgical and surgical wounds located on skins and hides of domestic animals. Their results showed *Staphylococcus aureus* as most common organisms, followed by *Escherichia*

coli and *Bacillus spp.* from buffaloes, cattle, sheep and goat wounds, another study showed that *Staphylococcus*, isolates are the common pathogens from injuries of dogs [12]. The finding of our results may be associate with the pathology of *Staphylococcus spp*, which are capable of producing various toxin and enzyme, which may facilitate the colonization and invasion of infection better than other bacteria.

The results of this study shows that, the prevalence of *salmonella spp* was 50.0%. Similar bacterial isolates were also investigated by [13] with 95% and CI range from 3.93 to 26.07 with reasonable explanation. This may be because *Salmonella spp* and *Streptococcus spp* are less virulent and cause less severe and non-invasive infection but variable numbers of organism may be found on the skin lesion. However, the least prevalent bacteria on the skin wound are *Pseudomonas spp*, *E. coli*, *Clostridium spp*, *Shigella spp* and *Klebsiella spp*. Similar results were also reported on the wounds of equines by [10, 13, 14]. It is reported that, the growth of these bacteria are inhibited either due immunity of an animal against these bacteria, or micro-environment of the wound and competitive inhibition by other virulent microbes (*Staphylococcus spp*) [15].

In this study, the contaminated skin wound were most commonly found at the back area and both sides of abdomen. The findings are agreement to previous study by [16] which may be due to, the back and both sides of

abdomen are constantly covered with saddles and harness for a longer period of time in a day. The profuse sweating resulting from strenuous work during the warm hours of the day and continuous abrasive movement of poor saddle and harness macerates the skin over these areas and this may lead to abrasion and result in damaging the skin barrier.

In addition, the longtime covering of saddle and harness increases the warmth of these regions of the body which may lead to deprivation of air at these region and this may create suitable microenvironment that would be favorable for the growth of microorganism. The donkeys are highly susceptible to the similar infections as compare to horse this may be due to different managemental practices and variation in number of tested sample in the two investigated area. The results of our finding are in contrast of the findings reported by [16]. It was reported that, not all the bacteria, which were isolated from a wound, are susceptible to the single antibiotic. The antibiotic sensitivity pattern of the bacteria, which were isolate, showed resistant against one or multiple antibiotic drugs [15] Similar results were also reported in the present study with the most resistant and susceptible bacteria was *Shigella* and *S. aureus* respectively. Moreover, it is also reported in this study that, ciprofloxacin was highly effective antibiotic against all the bacterial

isolates and less effective against cephalixin whereas it is reported by [15] that, the highly sensitive antibiotics which are effective against the bacterial isolates from the wound were gentamicin, chloramphenicol, amikacin, gatifloxacin and sparflaxacin. The appropriate choice of antibiotic for wound infection is extend spectrum ciprofloxacin (Fluoroquinolone). Moreover, The highest prevalent bacteria which was isolated from the wound was *S.aureus* (30.7%) and the pattern of sensitivity of Ciproflaxcin was 78.9% against the *S. aureus* which was being considered as active antibiotic against the bacterial speies [17]. It was also reported in this study that, the zone of inhibition was larger against *S.aureus* (20-25mm). The results of present study was agreement to previous study by [10]. He reported that, the larger zones of growth inhibition was produced by Tetracycline, ampicillin and ciprofloxacin against the *S aureus* and *S.pyogenes*. Similar research was carried out, and it was found that most effective antibiotic for gram positive as well as gram negative is Meropenem followed by Cefoperazone with 100% efficacy. It is reported that, all the isolates which were resistant to Doxycycline and the rate of susceptibility against erythromycin and ciprofloxacin was 54.55% [18]. The current study also showed agreement with previous studies [19, 20].

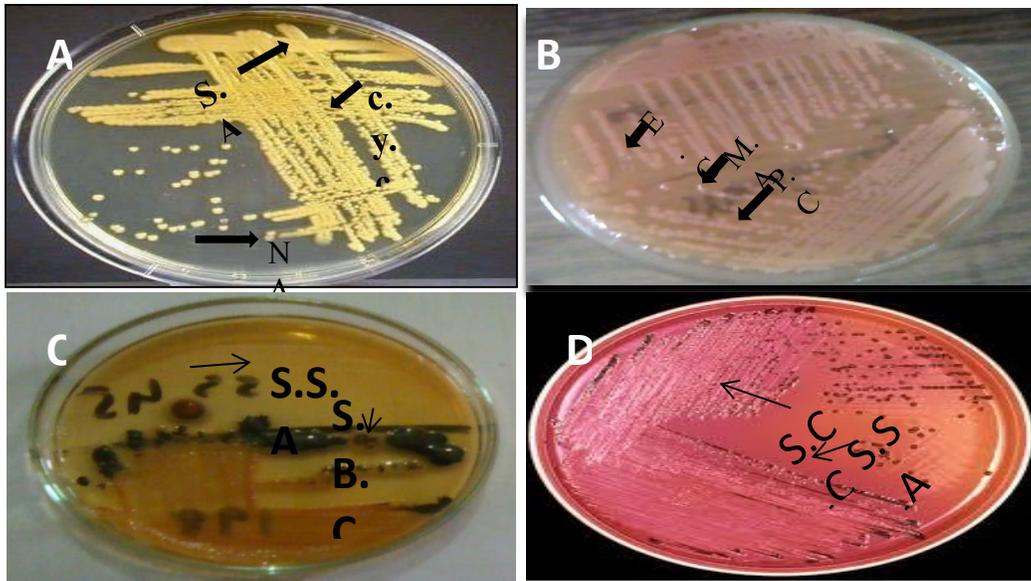


Figure 1. Plate-(a) showing the culture characteristics *Staphylococcus aureus*(S.A) golden yellow colonies (CYC) on nutrient agar (NA) plate- (b) showing the culture characteristics *Escherichia coli* (EC) pink colonies (PC) on MacConkey's agar (MA). Plate- (c) showing the culture characteristics *Salmonella* black center (SBC) black colonies (BC) on S.S agar. Plate- (d)is showing the culture characteristics of *Shigella* colorless colonies (SCC) on S.S agar.

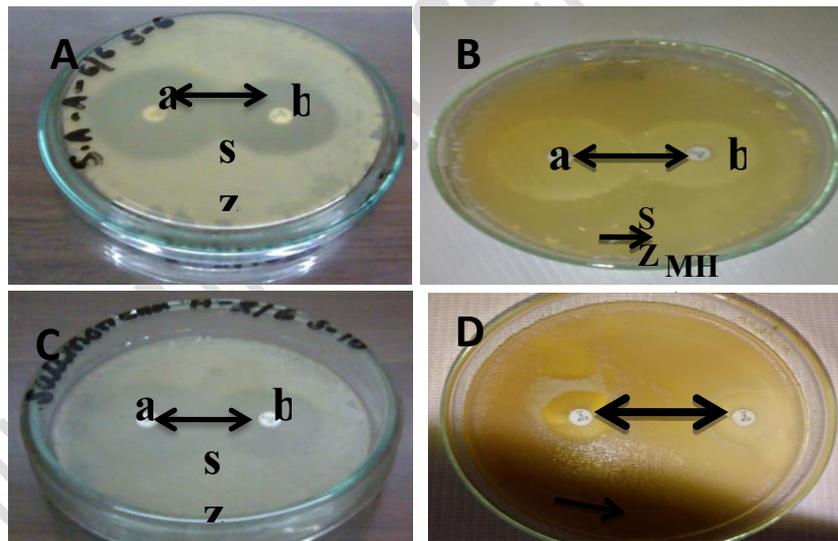


Figure 1. Plate (a) - showing sensitivity zones (SZ) produced by two different antibiotics against *Staphylococcus aureus* on Muller- Hinton agar plate (MHA) (a) Ciprofloxacin and (b) Cephalexin Plate - (b) showing sensitivity zones (SZ) produced by two different antibiotics against *Escherichia coli* on Muller- Hinton agar plate (MHA) (a) Ciprofloxacin and (b) Cephalexin Plate- (c) showing sensitivity zones (SZ) produced by two different antibiotics against *Salmonella* on Muller- Hinton agar plate (MHA) (a) Ciprofloxacin and (b)Cephalexin Plate- (d) showing sensitivity zones (SZ) of inhibition produced by two different antibiotics against *Shigella* on Muller- Hinton agar plate (MHA) (a) Cephalexin and (b) Ciprofloxacin

Conclusion

It is concluded that, *Staphylococcus aureus* were the most prevalent bacteria in equine wound followed by *Escherichia coli*, *Shigella*, and *Salmonella* spp. It is also concluded that the bacterial species, which were isolated, are either resistant to single or multiple antimicrobial drug resistance. Ciprofloxacin showed higher sensitivity against the *S. aureus* as compared to cephalexin. The variety of organisms were observed in this study support the need to obtain culture specimens from infected wounds for microbiological evaluation and antibiotic susceptibility determination so that adapted chemotherapy can be prescribed. We also suggest that we should educate the owner on the public health and improve their managemental practices, which will help enhance wound healing.

Authors' contributions

Conceived and designed the experiments: S Arbab & R Buriro, Performed the experiments: S Bhugio, AH Shah & Hanifullah, Analyzed the data: S Bhugio, AH Shah & D Kalhor, Contributed reagents/materials/analysis tools: IA Ujjan, S Tunio, WA Vistro & AN Khoso, Wrote the paper: S Arbab, MA Memon & M Rajput.

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