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Research Article

Preputial Bacteria and Their Antimicrobial Susceptibility Patterns in Stallions within Maiduguri Metropolis, Nigeria

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ABSTRACT

The prepuce is a normal part of the external genitalia that forms the anatomical covering of the glans penis thereby internalizing the penis and decreasing irritation and contamination. The prepuce accommodates diverse microorganisms, and these can impact health and disease conditions. This study aimed to isolate and identify preputial bacterial flora from stallions in Maiduguri metropolis of Borno State, Nigeria. Swabs were obtained from the prepuces of 40 randomly selected healthy stallions of three years and above. Using standard bacteriological identification techniques, the following bacteria were identified: *Bacillus* (23.0%), *Klebsiella* (20.6%), *Escherichia coli* (16.7%), *Salmonella* (16.7%), *Enterobacter* (13.5%), and *Proteus* (9.5%). *Bacillus, E. coli, Salmonella, Klebsiella,* and *Enterobacter* were susceptible to amoxicillin and ciprofloxacin, while *Proteus* was resistant. Those susceptible to gentamycin were *Bacillus, E. coli, Salmonella,* and *Enterobacter,* but *Bacillus, E. coli,* and *Enterobacter* were susceptible to streptomycin. However, *Klebsiella* and *Proteus* species showed resistance to ampiclox and chloramphenicol. The results of this study showed that some bacteria colonize the prepuces of stallions, indicating a need for preputial washing before breeding to reduce the bacterial load and potential transmission of these bacteria during mating. Amoxicillin, ampiclox, ciprofloxacin, gentamycin, and streptomycin could be used as therapeutic agents for diseases caused by the bacteria as mentioned earlier in stallions.

Keywords: Antimicrobial; Bacteria; Maiduguri; Preputial; Stallion

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INTRODUCTION

Horses, *Equus caballus,* are mammals which are domesticated globally for ceremonial exhibitions, sports, transport, draft, research, warfare and food (Bush and Marczak, 2005: Lee *et al*., 2007). In Nigeria there are different breeds of horses ranging from mixed Arewa breeds and their crosses with Arabian, Dongola and Sudanese breeds which are found mostly in the northern states where stallions of the Arewa breeds are kept and maintained for

ceremonial purposes (Yahuza, 2005; Hendricks and Dent, 2007; Garba *et al*., 2011).

In Nigeria, horses are one of the most valuable animals (Musa, 2013). They are used during ceremonial processions, polo, racing among others (Mshelia, 2013; Musa, 2013). They are kept by the police and army for defense and security operations (RIM, 1992) as well as the way of long-distance journeys (Mshelia, 2013). The total population of horses in Nigeria is estimated to be 340,000 (RIM, 1992) and more than 90% of this population are found in northern Nigeria. Stallions are seasonal breeders that produce sperm in April/May than other months. Factors such as age, season, testes size and number of times used in breeding or semen collection may influence sperm production. Most stallions attain sexual maturity between 212 to 408 days (Brown-Douglas *et al*., 2010).

Microflora are categorized on the basis of the microorganism's location in a body cavity (Hao and Lee, 2004). A community of beneficial bacteria that resides within the body is termed as microflora (Natividad *et al.,* 2015).

Most genital infections in stallions are caused opportunistic secondary invaders, such as *Klebsiella* which has been frequently isolated from majority of stallion (Natividad *et al.,* 2015). The reproductive tract is also invaded by opportunistic coliforms. These opportunistic bacteria under stressful conditions may cause genital infections leading to reduced reproductive performance in stallions (Shallali *et al.,* 2001; Mshelia *et al.,* 2014). These microorganisms caused diseases due to stress and reduction of the immunity of the reproductive system (Mavrogianni *et al*., 2007). Thus, the importance of studying such commensal flora is related to disease caused by these microorganisms due to reduction of the immunity of the reproductive system (Yaseen *et al*., 2019). The aim of this study was to isolate and identify bacteria in the preputial cavity of stallions and determine their antibacterial susceptibility profile.

MATERIALS AND METHOD

Study Animals

Apparently healthy stallions were used for this study from within Maiduguri metropolis, between the months of August and September 2023. The animals were kept in stables and were mostly used for ceremonial purposes. All animals sampled for this study were of 3 years and above.

Collection of Samples

Preputial swab samples were collected from forty stallions using sterile swab sticks. Each swab stick was first moistened by dipping them in distilled water before gently inserting into the preputial cavity of an already restrained stallion. Thereafter, the samples were immediately transported in an icebox for analysis at Bacteriology Laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri.

Preparation of Culture Media

The following media were prepared according to manufacturers' instruction before samples were brought from stables for identification: Nutrient broth (NB), Nutrient agar (NA), Mannitol Salt agar (MSA), MacConkey agar (MAC), Eosin Methylene Blue agar (EMB), and *Salmonella-Shigella* agar (SSA).

Isolation and Identification of Bacteria

Prior to inoculation of samples into Nutrient broth (NB), the bottles were labeled and inoculation was done into corresponding labeled medium by stirring the swab stick in the bottles. The swab stick was cut about 2cm from inoculum end and dipped into the NB and incubated at 37^0C for 24h. Thereafter, the broth was streak-plated onto solid media including NA, MSA, MAC, Eosin EMB, and SSA and incubated at 37^0C for 24h. Pure cultures were obtained and stored on agar slants at 4^0C for further analysis. The pure isolates were Gram stained and subjected to biochemical characterization including urease, citrate utilization, indole, motility, catalase and oxidase test; as well as sugar fermentation in Triple Sugar Iron (TSI) agar.

Antibiotic Sensitivity Test

Muller-Hinton Agar **(**Oxoid Limited, Basingstoke Hampshire, England**)** was prepared to conduct the antibiotic sensitivity test using isolated bacteria in this study. Briefly, 38g of the agar powder was suspended in 1 000mL of distilled water and was heated with gentle stirring until the powder has completely dissolved. This was then autoclaved at 121^0C for 15 minutes. After cooling the agar was poured into sterile petri dishes, allowed to solidify and then subsequently stored at 4^0C until required. The susceptibility pattern of the isolates against antibiotics was determined using the Kirby Bauer disc diffusion method (Bauer *et al.*, 1966) in Mŭller-Hinton Agar as prepared above. The antibiotic susceptibility test for the identified bacteria was performed with multi discs containing Amoxicillin (30µg), Ampiclox (30µg), Augmentin (10µg), Chloramphenicol (30µg), Ciprofloxacin (10µg), Erythromycin (10µg), Gentamycin (30µg), Septrin (30µg), Pefloxacin (30µg), Sparfloxacin (30µg), Steptomycin (30µg), Tarivid (10µg) and Zinnacef (20µg).

Data Analysis

Data obtained from the study were expressed as means± standard deviation and the results were presented in frequency distribution tables.

RESULTS

Age distribution of stallions

Table 1 below shows the age distribution of the stallions sampled in this study. Majority of the stallions were 6 years old (22.5%); followed by 20% each for those that were 3 and 4 years old. There was equally frequency of 15% each for stallions of 5 and 7 years; while those of 8 years were the least frequent (7.5%).

Table 1: Age Distribution of Stallions used for this Study

Age (years)	Frequency (%) n=40
3	8 (20.0)
4	8 (20.0)
5	6 (15.0)
6	9 (22.5)
7	6 (15.0)
8	3 (7.5)
Total	**40 (100)**

Distribution of bacterial isolates from the preputial cavity

Table 2 below presents the type of bacteria that were isolated from stallions sampled in this study. A total of 126 bacteria belonging to six genera were isolated from the forty stallions sampled in this study. Some stallions had multiple bacteria. *Bacillus* species appear to be most frequent bacteria that were isolated (23.0%). *Klebsiella* species (20.6%) are the second frequent bacteria that were isolated. Other commonly isolated bacteria were *Escherichia coli* (16.7%), *Salmonella* species (16.7%), *Enterobacter* specie (13.5%) and *Proteus* (9.5%).

Table 2: Distribution of bacterial isolates from the preputial cavity of apparently healthy stallions at Maiduguri metropolis Borno state

Isolated Bacteria	Frequency (%) N = 40
Bacillus	29 (23.0)
Klebsiella	26 (20.6)
Escherichia coli	21 (16.7)
Salmonella	21 (16.7)
Enterobacter	17 (13.5)
Proteus	12 (9.5)
Total	**126 (100)**

Gram staining characteristics and biochemical test

Gram staining properties of the isolates presumptive of *Bacillus* (n=29) showed they were gram positive and appeared as rod-shaped. They were positive for catalase test and negative for citrate and urease tests. Isolates presumptive of *Klebsiella* species (n=26) were gram negative and appeared as rod-shape. They were positive for catalase, citrate, urease, H_2S and gas tests. Isolates presumptive of *Escherichia coli* (n=21) were gram negative and appeared as bacilli. They were positive for catalase, H_2S and gas tests. Isolates presumptive of *Salmonella* (n=21) were gram negative and appeared as rod-shape. They were positive for citrate, H_2S and gas tests. They were negative for catalase and urease tests. Isolates presumptive of *Enterobacter* (n=17) were gram negative and appeared as rod-shape. They were positive citrate test and negative for catalase, urease, H_2S and gas tests. Isolates presumptive of *Proteus* species (n=12) were gram positive and appeared rod-shape. They were positive for citrate, urease, H_2S and gas tests. They were however negative for catalase test.

Table 3: Result of gram staining characteristics and biochemical test of bacterial isolates from prepuce of stallions in Maiduguri

Bacterial isolates	Tested isolates	Gram Stain (+/-)	Appearance	Catalase	Citrate	Urease	H_2S	Gas
Bacillus	29	+	rod shape	+	-	-		
Klebsiella	26	-	rod shape	+	+	+	+	+
Escherichia coli	21	-	bacilli	+			+	+
Salmonella	21	-	rod shape	-	+	-	+	+
Enterobacter	17	-	rod shape	-	+	-	-	-
Proteus	12	+	rod shape	-	+	+	+	+

Antibiotic Susceptibility profiles

The result of the antibiotic sensitivity test for bacterial isolates from preputial cavity of apparently healthy Stallions is presented in table 4 to 9. Table 4 shows the antimicrobial susceptibility pattern of 29 *Bacillus* isolates in this study, revealing 100% resistance to septrin and 100% susceptibility to chloramphenicol. Notable resistance rates were observed for gentamycin (62%) and sparfloxacin (40%), while high susceptibility rates were seen with augmentin (90%) and ciprofloxacin (86%).Table 5showsthe antimicrobial susceptibility pattern of *Proteus species* in this study. The isolates showed resistance rates of 42% to septrin, 25% to pefloxacin, and 17% to multiple antibiotics. However, high susceptibility rates were observed for augmentin (92%), tarivid (92%), and erythromycin (92%).Table 6 presents the antimicrobial susceptibility pattern of *E. coli* in this study. The isolates exhibited resistance rates of 62% to septrin, 57% to zinnacef, and 29% to pefloxacin and steptomycin. Nevertheless, high susceptibility rates were seen with augmentin (86%) and gentamycin (86%).Table 7 shows the antimicrobial susceptibility pattern of *Salmonella* in this study. The isolates displayed resistance rates of 57% to septrin, 52% to tarivid, and 48% to ciprofloxacin. However, high susceptibility rates were observed for augmentin (81%) and gentamycin (71%).Table 8 presents the antimicrobial susceptibility pattern of *Klebsiella* in this study. The isolates exhibited resistance rates of 50% to septrin, 46% to steptomycin, and 39% to tarivid. Nevertheless, high susceptibility rates were seen with augmentin (85%) and zinnacef (854%).Table 9 shows the antimicrobial susceptibility pattern of *Enterobacter* in this study. The isolates showed resistance rates of 47% to steptomycin and 18% to septrin and tarivid. However, high susceptibility rates were observed for augmentin (94%) and zinnacef (94%).

Table 4: Antimicrobial susceptibility pattern of *Bacillus* species from prepuce of stallions

Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Septrin (30 µg)	29 (100)	0 (0)	0 (0)
Chloramphenicol (30 µg)	0 (0)	0 (0)	29 (100)
Sparfloxacin (30 µg)	40	20	0 (0)
(Ciprofloxacin (10 µg)	4 (14)	0 (0)	25 (86)
Amoxicillin (30 µg)	8 (27)	0 (0)	21 (73)
Augmentin (10 µg)	3 (10)	0 (0)	26 (90)
Gentamycin (30 µg)	18 (62)	0 (0)	11 (38)
Pefloxacin(30 µg)	3 (10)	6 (20)	20 (70)
Tarivid (10 µg)	0 (0)	9 (30)	20 (70)
Steptomycin(30 µg)	8 (27)	0 (0)	21 (73)
Ampiclox (30 µg)	15 (52)	0 (0)	14 (48)
Zinnacef (20 µg)	9 (30)	0 (0)	20 (70)
Erythromycin (10 µg)	9 (30)	0 (0)	20 (70)

Table 5: Antimicrobial susceptibility pattern of *Proteus* species from prepuce of stallions

Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Septrin (30µg)	5 (42)	0 (0)	7 (58)
Chloramphenicol (30µg)	2 (17)	0 (0)	10 (83)
Sparfloxacin (30µg)	2 (17)	4 (33)	6 (50)
Ciprofloxacin (10µg)	2 (17)	2 (17)	8 (67)
Amoxicillin (30µg)	0 (0)	4 (33)	8 (67)
Augmentin (10µg)	0 (0)	1 (8)	11 (92)
Gentamycin (30µg)	2 (17)	0 (0)	10 (83)
Pefloxacin(30µg)	3 (25)	0 (0)	9 (75)
Tarivid (10 µg)	1 (8)	0 (0)	11 (92)
Steptomycin(30 µg)	2 (17)	0 (0)	10 (83)
Ampiclox(30 µg)	2 (17)	4 (33)	6 (50)
Zinnacef(20 µg)	2 (17)	0 (0)	10 (83)
Erythromycin(10 µg)	0 (0)	1 (8)	11 (92)

Table 6: Antimicrobial susceptibility pattern of *E. coli* from prepuce of stallions

Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Septrin (30 µg)	13 (612)	0 (0)	8 (38)
Chloramphenicol (30 µg)	5 (24)	5 (24)	11 (52)
Ciprofloxacin (10 µg)	4 (19)	0 (0)	17 (81)
Sparfloxacin (30 µg)	5 (24)	4 (19)	12 (57)
Amoxicillin (30 µg)	4 (19)	0 (0)	17 (81)
Augmentin (10 µg)	2 (10)	1 (5)	18 (86)
Gentamycin (30 µg)	3 (14)	0 (0)	18 (86)
Pefloxacin (30 µg)	6 (29)	0 (0)	15 (71)
Tarivid (10 µg)	5 (24)	0 (0)	16 (76)
Steptomycin (30 µg)	6 (29)	2 (10)	13 (62)
Ampiclox (30 µg)	1 (5)	6 (29)	14 (67)
Zinnacef (20 µg)	12 (57)	0 (0)	11 (52)
Erythromycin (10 µg)	4 (19)	5 (24)	12 (57)

Table 7: Antimicrobial susceptibility pattern of *Salmonella* species from prepuce of stallions

Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Septrin (30 µg)	12 (57)	0 (0)	9 (43)
Chloramphenicol (30 µg)	8 (38)	2 (10)	11 (52)
Ciprofloxacin (10 µg)	10 (48)	3 (14)	8 (38)
Sparfloxacin (30 µg)	3 (14)	7 (33)	11 (52)
Amoxicillin (30 µg)	6 (29)	5 (24)	10 (48)
Augmentin (10 µg)	4 (19)	0 (0)	17 (81)
Gentamycin (30 µg)	4 (19)	2 (10)	15 (71)
Pefloxacin (30 µg)	8 (38)	4 (19)	9 (43)
Tarivid (10 µg)	11 (52)	0 (0)	10 (48)
Steptomycin (30 µg)	7 (33)	3 (14)	11 (52)
Ampiclox (30 µg)	3 (14)	3 (14)	15 (71)
Zinnacef (20 µg)	5 (24)	2 (10)	14 (67)
Erythromycin (10 µg)	6 (29)	6 (29)	9 (43)

Table 8: Antimicrobial susceptibility pattern of *Klebsiella* species from prepuce of stallions

Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Septrin (30 µg)	13 (50)	0 (0)	13 (50)
Chloramphenicol (30 µg)	5 (19)	6 (23)	15 (58)
Sparfloxacin (30 µg)	4 (15)	6 (23)	16 (62)
Ciprofloxacin (10 µg)	5 (19)	6 (23)	15 (58)
Amoxicillin (30 µg)	3 (12)	3 (12)	20 (77)
Augmentin (10 µg)	2 (8)	2 (8)	22 (85)
Gentamycin (30 µg)	5 (19)	5 (19)	16 (2)
Pefloxacin (30 µg)	5 (19)	6 (23)	15 (58)
Tarivid (10 µg)	10 (39)	3 (12)	13 (50)
Steptomycin (30 µg)	12 (46)	4 (15)	10 (39)
Ampiclox (30 µg)	5 (19)	5 (19)	16 (62)
Zinnacef (20 µg)	3 (12)	3 (12)	20 (77)
Erythromycin (10 µg)	3 (12)	2 (8)	21 (81)

Table 9: Antimicrobial susceptibility pattern of *Enterobacter* species from prepuce of stallions

Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Septrin (30 µg)	3 (18)	5 (29)	9 (53)
Chloramphenicol (30 µg)	2 (12)	2 (12)	13 (77)
Sparfloxacin (30 µg)	1 (6)	2 (12)	14 (82)
Ciprofloxacin (10 µg)	1 (6)	1 (6)	15 (88)
Amoxicillin (30 µg)	2 (12)	1 (6)	14 (82)
Augmentin (10 µg)	1 (6)	0 (0)	16 (94)
Gentamycin (30 µg)	1 (6)	1 (6)	15 (88)
Pefloxacin(30 µg)	2 (12)	1 (6)	14 (82)
Tarivid (10µg)	3 (18)	0 (0)	14 (82)
Steptomycin (30 µg)	8 (47)	0 (0)	9 (53)
Ampiclox (30µg)	1 (6)	2 (12)	14 (83)
Zinnacef (20 µg)	1 (6)	0 (0)	16 (94)
Erythromycin (10 µg)	1 (6)	2 (12)	14 (82)

DISCUSSION

This study was conducted to identify bacterial flora in preputial cavity of apparently healthy stallions in Maiduguri metropolis, Borno state, Nigeria. A total of 126 bacterial isolates from six genera where isolated from 40 apparently healthy stallions. The bacteria include *Bacillus* species, *Klebsiella* species, *Escherichia coli, Salmonella* species, *Enterobacter* species, and *Proteus* species. These bacteria were isolated from healthy stallions and could therefore be considered as part of the normal flora of the stallion preputial bacteria. Gross preputial abnormality was not observed in any of the animal sampled for this study. This is expected as only apparently healthy stallions were selected for this study. However, the result of this studies agreed with a similar study by (Ferris *et al*., 2017) with isolation of similar bacterial species from the prepuce of stallions in Sweden. The source of preputial contamination is usually ascending rather than blood-borne (Hoffner and Källenius (1988) and are usually caused by bacteria within the soil which are picked when the animal rest on the ground or are obtained from the female genital tract during mating (Wickware *et al.*, 2020). These microorganisms usually gain entry through the preputial orifice (Paray *et al,* 2018). The preputial cavity in most cases is therefore a vital source of microorganisms that are capable of establishing and maintaining venereal diseases or source of microbial spread through use of infected semen during artificial insemination (Parikh *et al.,* 2020).

A high microbial load in preputial cavity is a reflection of microbial contamination of the prepuce and has an effect on motility and morphology of sperm cells and other semen quality parameters (Najee *et al.,* 2012). These effects may be due to direct effect or competition for nutrients between aerobic bacteria and sperm cells in collected semen. Some opportunistic pathogenic organisms may even lead to reproductive disorders when they passively get into female animals through mating and cause lower conception rate, increase in embryonic mortality, abortions and other unwanted obstetric complications.

Several types of bacteria have been isolated from frozen semen (Mozo-Martin *et al*., 2010) which leads to the production of phagocytic cells (macrophages and PMNs) and these cells give rise to reactive oxygen species that compromise the sperm function and reduces sperm's fertilization capability (Morrell, 2006).

Antimicrobial agents have been used in the management of reproductive diseases in farm animals (Drillich 2006). Antimicrobial susceptibility tests are used to determine which specific antibiotic a particular bacterium is sensitive to and can guide choice of medicine and dosage for difficult to treat infections. Since susceptibility patterns of bacteria are constantly changing, it is thus, essential to determine the antibiogram of bacterial isolates before institution of antibacterial therapy during acute or chronic infections. Antimicrobial susceptibility patterns of bacterial isolates in this study revealed a worrying trend. *Bacillus* isolates, which accounted for 23% of the total isolates, exhibited a concerning 100% resistance to septrin. However, they were highly susceptible to chloramphenicol (100%) and augmentin (90%). Similarly, *Proteus* isolates (10% of total isolates) showed a significant 42% resistance to septrin, but were highly susceptible to augmentin (92%) and tarivid (91%). *Escherichia coli* isolates (17% of total isolates) exhibited 62% resistance to septrin and 57% resistance to zinnacef. Nevertheless, they were highly susceptible to augmentin (86%) and Gentamycin (86%). *Salmonella* isolates (17% of total isolates) showed a significant 57% resistance to septrin and

52% resistance to tarivid, but were highly susceptible to augmentin (81%) and ampiclox (71%). *Klebsiella* isolates (21% of total isolates) exhibited a worrying 50% resistance to Septrin, 46% resistance to steptomycin, and 39% resistance to tarivid.

Despite the high resistance observed, findings in this study suggest that some antibiotics may still be effective treatment options. *Enterobacter* isolates (14% of total isolates) showed a significant 47% resistance to steptomycin, but were highly susceptible to augmentin (94%) and zinnacef (94%). Overall, the study highlights the need for prudent antibiotic use and regular monitoring of antimicrobial susceptibility patterns to ensure effective treatment of bacterial infections in stallions and prevent the spread of antimicrobial resistance.

CONCLUSION

This study showed that multiple bacteria species colonize the prepuce of stallions. Bacteria such as *Bacillus, Klebsiella, Escherichia coli, Salmonella, Enterobacter* and *Proteus* were frequently isolated from apparently healthy stallions in Maiduguri. These bacteria have the potential of causing and maintaining venereal diseases in horse population in Maiduguri. It is therefore recommended that preputial washing for horses be done prior to natural mating or semen collection to reduce bacterial contamination of semen. Amoxicillin, Ampiclox, Ciprofloxacin, Gentamycin and Streptomycin could be used as therapeutic agents for diseases that could be caused by the aforementioned bacteria in stallions.

Conflict of Interest

The authors have no conflict of interest to declare.

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