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Nasopharyngeal Carriage of *Staphylococcus aureus* among Horses and Horse Handlers in Kano Metropolis, Nigeria

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Abstract

Staphylococcal species are important opportunistic bacterial pathogens that can asymptomatically colonize both human and animals bodies. The presence of nasopharyngeal carriage *Staphylococci* has been an increased risk factor of acquiring an infection with this pathogen. The present study aimed at determining the nasopharyngeal carriage of *Staphylococcal aureus* in horses and horse handlers in Kano metropolis, Nigeria. One hundred and sixty (160) non-duplicated nasopharyngeal samples were collected using sterile swab stick from each horse and 100 from consented horse handlers. All the samples were analyzed using a standard bacteriological procedure. Antibiotics susceptibility testing to eight (8) most commonly used antibiotics was carried out using a modified Kirby Bauer method. One hundred and forty-two (88.8%) *staphylococcal* isolates were recovered from horses and 84 (84.0%) from horse handlers. Among all the isolated staphylococci high sensitivity was observed in response to Gentamycin and Ciprofloxacin antibiotics, while Tetracycline was found to have the least activity. The report of this study showed a high prevalence of *Staphylococci* among horses and horse handlers. Moreover, it confirmed the tendency of *Staphylococcal* isolates cross-transmission between horses and handlers in the study site. This study indicated the importance of increase in handler's awareness of possible risk factors of staphylococcal colonization that can lead to invasive infection.

Keywords: Antibiotics, Horses, Horse Handlers, Nasopharyngeal, *Staphylococci*

INTRODUCTION

Staphylococcus aureus is an important opportunistic bacterial pathogen that can asymptomatically colonize in both human and diverse animals (Saei and Safari, 2019). Among the human, *S. aureus* is a common colonizer of the skin, nasal cavity, and other mucosal membranes (Wertheim *et al.*, 2005; Agabou *et al.*, 2017). In animal domains, *S. aureus* has commonly inhabited the nostrils, nares, mouth, and perineum (Iverson *et al.*, 2015; Agabou *et al.*, 2017).

Nasal carriage Staphylococci play a major role in the pathogenicity of the *Staphylococcal* infections, in hospitalized patients especially among those who undergo delicate procedures such as surgery, dialysis, and patients in an intensive care unit (ICU) that have higher infection risks in persistent carriers (Sakr *et al.*, 2018). A study done by Van Belkum *et al.* (2009) reported that carriers of staphylococci are between 3 – 6 times more likely to develop a chronic staphylococcal infection than non-carriers (Van Belkum *et al.*, 2009; Malley *et al.*, 2015). Moreover, about 80.0% of the invasive staphylococcal infections have resulted from colonizer of the host normal flora (O'Malley *et al.*, 2015).

Staphylococcus aureus becomes one of the major public health problems worldwide, due to its high morbidity and mortality rates and its ability to resist different classes of antibiotics which were initially recommended for their treatment (Gaddafi *et al.*, 2020). Antibiotics resistance in staphylococci has developed through spontaneous mutations, enzymatic inactivation of the drugs, and target site alteration which reduces binding affinity (Pantosti *et al.*, 2007). There is increasing evidence that shifts the spread of resistance staphylococcal infection from hospital-associated infection to a community-associated infection as a result of increased contact between humans and productive livestock, pet animals, and horses inside and outside the hospital settings (Cuny *et al.*, 2006). Klous *et al.* (2016) in their study reported that zoonotic infections account for an estimated 60.0% of all human infections (Klous *et al.*, 2016).

In Nigeria, there has been increased contact between human beings and horses, for recreational, traditional, sports, and breeding purposes over the years (Abdulkadir, 2014).

Prevention of the spread of staphylococcal infection among the people requires accurate evaluation of the nature and type of resistance in both the horses and their handlers and by gaining insight into the transmission routes. Since horses can serve as sources of infection and/or re-infection of humans, with physical contact between humans and horses being unavoidable, adequate and routine assessments of staphylococcal colonization in both handlers and their horses are required for efficient control of the infection. The present study aimed at determining the nasopharyngeal carriage of *Staphylococcalaureus* in horses and horse handlers in Kano metropolis, Nigeria.

MATERIALS AND METHODS

Study Area

The study area was carried out at Kano metropolis, Nigeria. The state is located in Northwestern Nigeria on latitude 8o 30' E and longitude 11o 30' N, 402m above sea level (Kano Google satellite map, 2016). It has distinct wet and dry seasons within the guinea and part of the Sahel savannah zones of Nigeria. Kano is the commercial center of the horse business in Nigeria. Most of the imported breeds from Argentina, South Africa, Sudan, Cameroon, and Niger are domiciled in Kano for distribution to other states. Kano is the home of Durbar, leisure riding, polo, and racing and all horse activities take place throughout the year. It is near two neighboring international horse markets of Mai'Adua and Maigatari from where different breeds of horses usually find their way to the Kano metropolis (NBS, 2018).

Study Design

This was a cross-sectional study and a convenience sampling of clinically normal horses selected from the study area. The horses selected were in three categories; performance horses (polo and racing horses), traditional horses that are kept locally for recreation and cultural activities. Animals were sampled only after owners' consent was given. All samples were collected by a trained technician.

Microbiological Analysis

Data Collection

Self-administered structured questionnaires were given to each participant after consent.

Sample Collection

A total of 160 non duplicated nasopharyngeal samples were collected using sterile swab sticks from each horse and 100 from a human participant. A moistened cotton-tipped swab stick was inserted approximately 2cm for humans and 10cm for horses in one nasal passage, rotated for about 10 seconds and withdrawn with the swab in contact with the nasal mucosa. The swab was promptly placed in liquid Stuart‟s medium. The swab was kept at 4oC in a Coleman box and transported to the Microbiology Laboratory and processed according to standard microbiological procedures (Cheesbrough, 2010).

Media Preparation

All the media were prepared according to the manufacturer's instructions.

Gram Staining

Gram staining of the isolated colonies was carried out to identify the Gram reaction of the isolates as described by (Cheesbrough, 2010).

Biochemical Tests

Suspected Staphylococcal isolates were identified and confirmed using standard bacteriological procedures.

Catalase Test

This test is used to differentiate those bacteria that produce Catalase enzymes (e.g Staphylococci) from non-Catalase-producing bacteria (eg. Streptococci) (Cheesbrough, 2010).

Coagulase Test

This test is used to differentiate members of the genus staphylococcus such as *Staphylococcus aureus* that produce coagulase enzymes and other non-coagulase negative species (Cheesbrough, 2010).

Hemolysis Activities

Hemolysis activity of the member of the genus staphylococci was detected using blood agar. A blood agar base (Oxoid, UK) was prepared according to the manufacture's guidelines. About 20ml volume of the prepared blood agar was poured into Petri dishes and dried off surface moisture. A colony of the Staphylococci under test was touched with an inoculating loop, streak on the plate, and incubated at 37^{o}C for 24 hours in -5% CO2. The plate was checked for the presence of hemolysis activity (Cheesbrough, 2010).

Mannitol Fermentation

Mannitol salt agar was prepared according to the manufactures direction. The isolate was subcultured on the medium incubated for 24 hours at 37^{o}C. The plate was observed for mannitol fermentation by the isolates after the incubation (Cheesbrough, 2010).

Preparation of McFarland Turbidity Standard

Preparation of 0.5 McFarland standard (turbidity standard) Sulfuric sulfate (1% v/v) standard suspension was used as turbidity standard which was prepared following the procedure explained by Cheesbrough (2010).

Antibiotic sensitivity testing

Antimicrobial susceptibility testing against 8 most common used antibiotics was done using the modified Kirby-Bauer disk diffusion method on Mueller Hinton agar based on the Clinical and Laboratory Standards Institute (CLSI, 2019).

The antibiotics (Oxoid, UK) used include Erythromycin (ERY 5µg), Ciprofloxacin (CIP 10µg), Chloramphenicol (CH 30µg), Clindamycin (DA 2µg), Gentamycin (CN 10µg), Cefoxitin (FOX 30µg), Oxacillin (OX 1µg), Vancomycin (VA 30µg) and Tetracycline (TET 30µg) (CLSI, 2019).

Data Analysis

The data generated in this study were analyzed using the Statistical Package for Social Sciences (SPSS) for windows version 20.0 used for statistical analysis and data interpretation. The values were expressed as means and percentages. Comparison of variables was done using Chi-square test and values with $p \leq 0.05$ were considered significant.

RESULTS

A total of 160 horses and 100 horse handlers were enrolled in the study, the higher number of the participants is 80 (50.0%) horses and 47 (52.2%) from the category traditional horse activities (*Durba*). Institutional horses and their handlers have the least number of participants 2 (1.3%) and 3 (3.3%) respectively (Table 1). Among the samples collected from horses, 142 (88.8%) yield positive cultures and 84 (84.0%) were culture positive from horse handlers. This gives the overall nasopharyngeal colonization of 88.8% among horses and 84.0% among humans handling the horses in the study site (Table 2).

All the 142 (100.0%) isolates obtained from horses were Gram-positive cocci in pair and cluster and Catalase positive. Out of the 142 isolates, 125 (88.0%) were positive for slide and tube coagulase test, 47 (33.1%) showed beta-hemolytic, and 129 (90.9%) ferment mannitol when growing on mannitol salt agar. From 84 positive cultures found in horse handlers, 84 (100.0%) were both Gram-positive cocci in pair and cluster and Catalase positive. Thirty-three (39.3%) coagulase-positive, 20 (23.8%) showed beta-hemolytic, and 33 (39.3%) ferment mannitol (Table 3).

Table 4 shows the rate of occurrence of Staphylococcal nasopharyngeal carriage with regards to horse activity. The highest occurrence was obtained among institute horses 2 (100.0%) and horse handlers 3 (100.0%) and also 20 (100.0%) racing horse handlers, followed by polo horses 36 (97.3%) in horses and 43 (91.5%) horse handlers in traditional horse activities. Statistically in all the two groups, no significant relationship was obtained at a 95% confidence interval as p-value >0.05.

Antibiotics susceptibility profile of the isolated staphylococci obtained from both horses and horse handlers using conventional antibiotics shows high activity to Gentamycin (10µg) and Ciprofloxacin (10µg) and least activity was observed in Tetracycline (30µg) (Table 5).

Table 1: Classification of Horses and Horse handlers according to Category of Horse Activities

Horse Activity	Horses (%)	Horse Handlers (%)
Institution	2(1.3)	3(3.3)
Polo	37(23.1)	20(22.2)
Racing	41(25.6)	20(22.2)
Traditional	80(50.0)	47(52.2)
Total	160(100.0)	90(100.0)

Table 2: Prevalence of Staphylococci Colonizer in Horses and Horse Handlers in Kano Metropolis

	No. Examined	No. Positive (%)	No. Negative (%)
Horse	160	142 (88.8)	18 (11.3)
Horse Handlers	100	84(84.0)	16 (16.0)

Table 3: Phenotypic Characteristics of the *Staphylococcal* Isolates

Tests	Horse (n=142)	Horse Handlers (n=84)
	No. Positive (%)	No. Positive (%)
Gram's Reaction	142 (100.0)	84(100.0)
Catalase Test	142 (100.0)	84(100.0)
Coagulase Test	125 (88.0)	33(39.3)
Haemolysis Activity	47 (33.1)	20(23.8)
Mannitol Fermentation	129 (90.9)	33(39.3)

Table 4: Distribution of *Staphylococcal* Nasopharyngeal Carriage among Horses and Horse Handlers

Horse Activity	Horse		Horse Handlers	
Horse Activity	No. Examined	Positive (%)	No. Examined	Positive (%)
Institution	2	2(100.0)	3	3(100.0)
Polo	37	36(97.3)	20	18(90.0)
Racing	41	37(90.2)	20	20(100.0)
Traditional	80	67(83.8)	47	43(91.5)
Total	160	142(88.8)	90	84(93.3)
P-value	*0.961		**0.978	

*x2 = 0.297 df = 3 *P-value*= 0.961
**x2 = 0.200 df = 3 *P-value*= 0.978

Table 5: Antibiotics Susceptibility Pattern of *Staphylococcal* Isolates

Antibiotics(µg)	Horse N = 142 (%)			Horse Handlers N = 84 (%)		
	S	I	R	S	I	R
ERY (5)	116 (81.7)	9 (6.3)	17 (12.0)	71 (84.5)	7 (8.3)	6 (7.1)
GEN (10)	141(99.3)	1 (0.7)	0 (0.0)	81 (96.4)	0 (0.0)	3 (3.6)
CH (30)	123(86.6)	10 (7.0)	9 (6.3)	77 (91.7)	1 (1.2)	6 (7.1)
TET (30)	51 (35.9)	18 (12.7)	73 (51.4)	50 (59.5)	8 (9.5)	26 (31.0)
CIP (10)	138 (97.2)	2 (1.4)	2 (1.4)	82 (97.6)	0 (0.0)	2 (2.4)
DA (2)	102 (71.8)	38 (26.8)	2 (1.4)	76 (90.5)	5 (6.0)	3 (3.6)
VA (30)	102 (71.8)	28 (19.7)	12 (8.5)	68 (81.0)	10 (11.9)	6 (7.1)
FOX (30)	133 (93.7)	0 (0.0)	9 (6.3)	66 (78.6)	0 (0.0)	18 (21.4)

Key: S = Sensitivity, I = Intermediate, R = Resistance, ERY = Erythromycin, GEN = Gentamycin, CH = Chloramphenicol, TET = Tetracycline, CIP = Ciprofloxacin, DA = Clindamycin, VA = Vancomycin, FOX = Cefoxitin, OX = Oxacillin

DISCUSSION

In this study, the overall prevalence rate of nasopharyngeal carriage staphylococci among horses was 88.8%. The prevalence rate reported in this study was much higher than the 20.3% recorded by Saei and Safari (2019) in Iran, 15.2% by Agbou *et al*. (2017) in Algeria, 13.5% by Islam *et al*. (2017) in Denmark, 15.4% recorded by Abdulkadir (2014) in Zaria, Nigeria and 50.0% by Zunita *et al*. (2008) in Malaysia. These differences may be due to environmental factors, animal husbandry practices and hygienic status. The outcome of the present study indicated that horses in this study area can serve as a reservoir of staphylococcus species that can lead to zoonotic transmission to the horse handlers.Similar studies by Ansari *et al*. (2017); Selva *et al*. (2015) reported that nasopharyngeal colonization by *S. aureus* serves as an important source of staphylococci infection in humans as well as an increased risk factor for developing an invasive infection. Wertheim *et al*. (2005) earlier reported that nasopharyngeal carriage of staphylococci has been recognized as an increased risk factor of acquiring an infection with this pathogen.

The study findings revealed that he overall prevalence rate of nasopharyngeal carriage of staphylococci among horse handlers was 84.0%, and was found to be higher than the 10.8% carriage rate among horse handlersin a similar study in Zaria, Kaduna State, by Abdulkadir (2014). It is equally higher than the report of another study in Denmark, which recorded the prevalence rate of 50.0% among humans handling the horses (Islam *et al*., 2017).

In this study, the antibiotics susceptibility pattern of staphylococci isolates obtained from horses showed that 99.3% were sensitive to Gentamycin while 97.2% and 93.7% were susceptible to Ciprofloxacin and Cefoxitin, respectively. The isolates were less sensitive to Tetracycline (35.9%). This result is in agreement with the finding of Agabou *et al*. (2017) that recorded high sensitivity of Gentamycin (100.0%) and Ciprofloxacin (100.0%), and that of Abdulkadir (2014) that recorded the sensitivity of 93.8% to Gentamycin and Ciprofloxacin. This study further agrees with the work done by Adegoke and Okoh (2014) that recorded the high resistant of the isolates to Tetracycline (83.3%).

The staphylococcal isolates obtained from horse handlers showed more susceptibility to Ciprofloxacin (97.6%) followed by Gentamycin (96.4%). They agreed with the study reported by Agabou *et al*. (2017) and Abdulkadir (2014) that recorded high sensitivity of 100.0% to Ciprofloxacin and Gentamycin.

CONCLUSION AND RECOMMENDATIONS

This study reports a high prevalence of staphylococci carriage among horses and horse handlers. The study identifies the possibility of staphylococcal isolates to cross-transmission between horses and handlers in the study site. The study reported high susceptibility of the staphylococcal isolates to Ciprofloxacin and Gentamycin and high resistance to tetracycline. The study indicated the importance of increasing the handler's awareness of possible risk factors of staphylococcal colonization that can lead to invasive infection.

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