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Occurrence of Methicillin Resistant *Staphylococcus aureus* among Horses and Horse Handlers in Kano Metropolis, Northwestern, Nigeria

Sanda, M. I*[1]., Kwaga, J. K. P[2]., Suleiman, A. B[2]., Idris, A. M[3].

[1]Department of Animal Sciences,
Faculty of Agricultural Sciences,
Bayero University, Kano, Nigeria

[2]Department of Veterinary Public Health and Preventive Medicine,
Faculty of Veterinary Medicine,
Ahmadu Bello University, Zaria,
Kaduna, Nigeria

[3]Department of Medical Microbiology and Parasitology,
Faculty of Clinical Sciences,
College of Health Science,
Bayero University, Kano, Nigeria

Email: amikibiya@gmail.com

Abstract
Methicillin-resistant *S. aureus* (MRSA) is one of the great public health concerns. The organisms become dangerous pathogen for humans and animals which are often very difficult to treat due to their resistance to broad-spectrum antibiotics and sophisticated immune evasion tactics. MRSA having transited from a nosocomial to a community-associated infection and now has been associated with livestock and companion animals. The present study aimed to determine the occurrence of MRSA among horses and horse-handlers in Kano metropolis, Nigeria. A total of 160 horses and 90 horse handlers were recruited based on the availability and consented participants. Structured questionnaires were administered to 90 horse handlers to assess for risk factors associated with MRSA infection in horses and horse handlers. Nasal swabs were collected from each horse and horse handler and cultured and identified using standard bacteriological procedure according to Clinical Laboratory Standard Institute (CLSI). MRSA was screened using the disk diffusion method with Cefoxitin (30µg) and Oxacillin (1µg). The overall MRSA prevalence rate of 6.3% and 21.1% were obtained from horses and horse handlers respectively. The highest incidence 11.3% and 35.0% were obtained among traditional horses and their handlers. Therefore, it is important to increase the handlers' awareness of possible risk factors of MRSA infection to control cross-infection between horse and their handlers.

Keywords: *Staphylococcus aureus*, MRSA, Cefoxitin, Horse

**Author for Correspondence*

INTRODUCTION

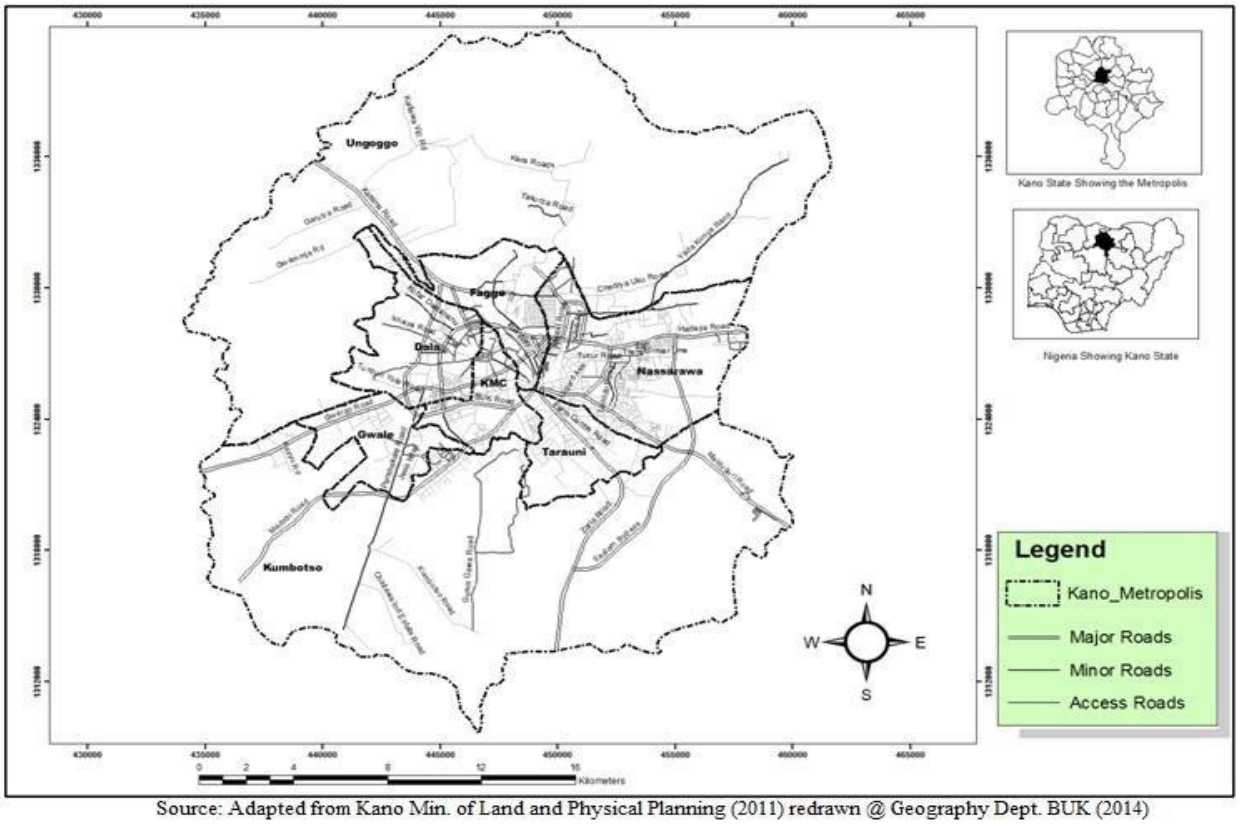
Staphylococcus aureus is an important pathogen of both humans and animals that implicated in a wide variety of infections, from mild to serious and invasive infections (Abdulkadir, 2014 and Idris *et al*., 2019). The emergence of antibiotic-resistant *S. aureus*, particularly Methicillin resistance in S. aureus, (MRSA) is recognized as a very serious issue due to the difficulty in combating infection by the strains (Idris *et al*., 2019). MRSA is conferred by the acquisition of the mec A gene in the Staphylococcal chromosome cassette mec (SCCmec) elements which encoding a penicillin-binding protein (PBP2a) that has reduced affinity for β-lactam antibiotics (Cikman *et al*., 2019). There is evidence of increasing the spread of MRSA from nosocomial to a community-associated infection involving productive livestock, pet animals, and horses in the community setup (Cuny *et al.,* 2010). WHO (2019) reported that people who carried MRSA are estimated to be 64.0% more likely to develop fatal infections and lead to death than in people with a non-resistant form of the infection. Thus, reliable identification of MRSA is very important to choose appropriate therapy, to prevent misuse of antibiotics, and to take necessary measures for infection control (WHO, 2019).

In Nigeria, there is increased contact between human beings and horses, for recreational, traditional, sports, and breeding purposes over the years (Abdulkadir, 2014). Prevention of the spread of community-acquired MRSA 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 MRSA strains in both handlers and their horses are required for efficient control of the infection. Presently, there is inadequate information on community-acquired MRSA infection in horses and their handlers in Nigeria. Therefore, assessing the nature of MRSA infection in both horses and handlers and the route of transmission will highlight the magnitude of the problem and aid in the successful control of the infection. This study was aimed at evaluating the occurrence of MRSA in traditional, performance, and institutional horses and their handlers in Kano Metropolis, Nigeria

MATERIALS AND METHODS

Study Site

The study was carried out at Kano metropolis, Nigeria. The state is located in northwestern Nigeria on latitude 8° 30′ E and longitude 11° 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 (Figure 1).



Source: Adapted from Kano Min. of Land and Physical Planning (2011) redrawn @ Geography Dept. BUK (2014)

Figure 1 Map of the Kano Metropolis

Study Design and Study Population

The study was a cross-sectional study using a convenience sampling technique among clinically normal horses selected from the study area. The horses selected were in three categories; performance horses (polo, racing and institutional 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 technologist.

Sample Size Determination

The sample size of the study was defined using standard epidemiological formula by Lwanga and Lemeshow (1991) thus:-

$$n = \frac{Z^2 Pq}{d^2}$$

Where: n= number of samples
Z = statistic for level of confidence at 95% = 1.96
P = prevalence from recent past study
d = allowable error of 5%, (0.05)
q = 1 – P
The previous prevalence was adopted from work done by Abdulkadir (2014), in Kaduna among horses and horse handlers were MRSA infection was 10% and 6.2% respectively.

The sample size for horses

$$n = \frac{1.96^2 \times 0.10(1-0.10)}{0.05^2} = 138.$$

However, in order to improve accuracy the sample size was rounded up to 160 for horses.

The sample size for horse handlers

$n = \frac{1.96^2 \times 0.062(1-0.062)}{0.05^2} = 89.$

However, to improve accuracy the sample size was rounded to 100 horse handlers.

Data Collection

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

Sample Collection

Sterile swab sticks were used to collect the nasopharyngeal sample from each 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 4°C in a Coleman box and transported to the laboratory for processing. Each of the samples collected was appropriately labeled.

All the samples were taken to Microbiology Laboratory and processed according to standard microbiological procedures (Cheesbrough, 2010).

Sample Processing

All the samples collected in liquid Stuart's medium were cultured onto blood agar and mannitol salt agar and incubated at 37°C for 18 hours. Blood agar plate was incubated at -5% CO_2 atmosphere condition (in a candle jar). The suspected Staphylococci isolates were confirmed using the standard bacteriological procedure, which includes Gram reaction, catalase reaction, coagulase test, and mannitol fermentation (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).

Screening for Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA screening was performed using both (Oxoid, UK) Cefoxitin (30µg) and Oxacillin (1µg) disk for phenotypic detection of MRSA (CLSI, 2019).

Briefly, a bacterial suspension adjusting to 0.5 McFarland was inoculated onto Muller–Hinton agar (Lib Biotech). A filter paper disk containing Cefoxitin (30µg) and Oxacillin (1µg) was placed on the inoculated Muller–Hinton agar. All the plates were incubated at 37°C overnight, after which the plate was held a few inches above a black, non-reflective surface illuminated with reflected light, and a ruler was used to measure each zone with the unaided eye while viewing the back of the Petri dish. The result was recorded and was compared with the zone diameter interpretive standard of the CLSI (2019).

Data Analysis

The data generated in this study was analyzed using the Statistical Package for Social Sciences (SPSS version 25.0) used for statistical analysis and data interpretation. The values were expressed as means and percentages.

Results

A total of 160 horses and 90 horses were sampled. Most of the handlers were males 80(88.9%) and 10(11.1%) were females. About 32 (35.6%) of the handlers had tertiary education, 45(50%) attended secondary school while 13(14.4%) attended primary school. Thirty-five (38.9%) of the handlers are owners, 23(25.6%) were both jockey and grooms and 9(10%) are veterinary specialists. Majority of the horses 80(50.0%) are traditional, 41(25.6%) racing, 37 (23.1%) polo and 2 (1.3) are institutional (Table 1).

The prevalence rate of *S. aureus* was 125 (78.1%) and 33 (36.7%) among horses and horse handlers respectively. Furthermore, the prevalence of *MRSA* based on the disc diffusion method was 10 (8.0%) in horses and 19 (57.9%) in handlers (Table 2).
The highest prevalence rate of both *S. aureus* and MRSA was obtained in traditional horse handlers 19 (47.5%) and 14 (73.7%) respectively. Furthermore, 71 (88.8%) *S. aureus* were obtained from the horse, and 9 (12.7%) MRSA, zero (0.0%) MRSA were found from both institutional performances (Table 3 and 4)

The highest resistance was observed with Sulphamethoxazole-Trimethoprim followed by Ampicillin and least resistance was observed with Gentamicin and Erythromycin in MRSA isolated from horses, while Chloramphenicol has the least resistance in horse handlers (Table 5).

Table 1 Demographical Distribution of the Participants

Demographical Parameters	Frequency	Percentages
Age (Years)		
15 – 24	21	23.3
25 – 34	47	52.2
35 – 44	11	12.2
45 – 54	8	8.9
55 – 64	3	3.3
Gender		
Male	80	88.9
Female	10	11.1
Educational Status		
Primary	13	14.4
Secondary	45	50.0
Tertiary	32	35.6
Horse Handlers		
Owner	35	38.9
Vet Specialist	9	10.0
Grooms	46	51.1
Total	90	100.0
Horse Performance		
Traditional	80	50.0
Racing	41	25.6
Polo	37	23.1
Institution	2	1.3
Total	160	100.0

Table 2 **Prevalence of MRSA Using Disk diffusion method in both horses and handlers**

Sample Source	No. Examined	*S. aureus* (%)	MRSA (%)
Horses	160	125 (78.1)	10 (8.0)
Handlers	90	33 (36.7)	19 (57.6)

Table 3 MRSA Distribution among Horse Handlers

Performance	No. Examined	*S. aureus* (%)	MRSA (%)
Traditional	40	19 (47.5)	14 (73.7)
Racing	22	6 (27.3)	2 (33.3)
Polo	25	7 (28.0)	3 (42.9)
Institution	3	1 (33.3)	0 (0.0)
Total	90	33 (36.7)	19 (57.6)

Table 4 MRSA Distribution among Horse

Performance	No. Examined	*S. aureus* (%)	MRSA (%)
Traditional	80	71 (88.8)	9 (12.7)
Racing	41	32 (78.1)	1 (3.1)
Polo	37	20 (54.1)	0 (0.0)
Institution	2	2 (100.0)	0 (0.0)
Total	160	125 (78.1)	10 (8.0)

Table 5 Antibiotic Resistance Pattern among MRSA Isolated from Horses and Horse Handlers

Antibiotics (µg)	**MRSA in horses 10 (%)**	**MRSA in horse handlers 19 (%)**
Ampicillin	9 (90.0)	18 (94.7)
Gentamicin	0 (0.0)	3 (15.8)
Amikacin	6 (60.0)	3 (15.8)
Erythromycin	0 (0.0)	3 (15.8)
Tetracycline	8 (80.0)	8 (42.1)
Sulphamethoxazole-Trimethoprim	10 (100.0)	18 (94.7)
Ciprofloxacin	1 (10.0)	2 (10.5)
Chloramphenicol	2 (20.0)	1 (5.3)
Vancomycin	6 (60.0)	4 (21.1)
Clindamycin	1 (10.0)	3 (15.8)

Discussion

In this study, the prevalence of MRSA among horses is 8.0%. This prevalence is higher than 4.7% reported from horses in equine farms by Weese *et al.* (2005a), 1.2% in home farms by Val den *et al.* (2013), and 7.0% that was found among recreational horses in farmhouses, racecourse, and slaughterhouse by Parisi *et al.* (2017). Higher prevalence of 10.0% and 16.0% have also been reported by Abdulkadir *et al.* (2014) and Weese *et al.* (2005b) respectively.

The prevalence of MRSA among human-horse handlers in this study is 57.6%. This prevalence is on the high side when compared with the outcome of many studies. Lower prevalence of MRSA among horse handlers of 2.4%, 4%, 6.8%, 7%, and 13% have been reported by Van den *et al.* (2013), Weese *et al.* (2005b), Abdulkadir *et al.* (2014), Weese *et al.* (2017) and Weese *et al.* (2005a) respectively.

This study found that the prevalence of MRSA was higher in handlers than in horses. The high prevalence of MRSA among horse handlers may not be unconnected with the handlers living near horses in a typical traditional way. Traditionally, there is a possibility of having community-acquired MRSA when handlers take care of a relative in the hospital or use the

traditional method of wound management for the horses or keep pets like a cat. Similar studies have also reported a higher prevalence of MRSA infection among handlers when compared with the horses (Weese *et al.*, 2005a and Van den *et al.*, 2013). The possible reason for the similarity in findings between this study and that of Weese *et al.* (2005a) and Van den *et al.* (2013) could be because of the proximity in the living environment between horses and handlers in both studies. Other studies have on the other hand reported a higher prevalence of MRSA among the horses when compared with the handlers (Abdulkadir *et al.*, 2014 and Weese *et al.*, 2005b) probably because animals enrolled from Veterinary teaching hospital have higher chances of acquiring MRSA infection.

In this study, MRSA in handlers and horses is highly resistant to Ampicillin, Sulphamethoxozole + Trimethoprim, and Cefoxitin. The high resistance of MRSA to Ampicillin and Sulphamethoxozole may be due to the indiscriminate use of these drugs in horses and handlers. These drugs are commonly available as over-the-counter drugs and self-prescribed. The study also found that MRSA was more resistant in horses to Amikacin, Tetracycline, and Vancomycin. Tetracycline is one of the antibiotics that have a history of being used for many years in equine practice in Nigeria. This may have been the reason for high MRSA resistance into Tetracycline in horses.

CONCLUSION AND RECOMMENDATION

A high MRSA prevalence rate of 8.0% and 57.6% were reported from horses and handlers respectively. Therefore, there is a need to increase the handlers' awareness of the incidence of MRSA to take proper precautions of MRSA cross-infection between horse and their handlers.

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