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PREVALENCE STUDY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AND ITS SCCMEC FEATURES IN HORSES AND HANDLERS IN ZARIA AND KADUNA, NIGERIA.

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SUMMARY

Methicillin resistant Staphylococcus aureus (MRSA) is a pathogen with public health implications being resistant to most used antibiotics. It has been associated with health facilities but has now become prevalent in community settings. The presence of MRSA in 240 apparently healthy horses and 65 horse handlers in Kaduna and Zaria was studied through nasal swabs collection by a one-stage cluster sampling and analysed using standard microbiological tests and genotyping methods. Questionnaires were also administered to assess for risk factors associated with MRSA carriage. Prevalence was 10% and 6.2% from the horses and horse handlers respectively. The S. aureus isolates showed highest resistance rates of 54.6% to penicillin and oxacillin. There were high resistance rates also to erythromycin, and tetracycline of 47.7% and 34.1% respectively. Resistance of 22.7% to amikacin and gentamicin, 6.8% to sulphamethoxazole+trimethoprim, 2.3% to ciprofloxacin, and 2.3% to chloramphenicol were also observed. Multidrug resistance (MDR) was found among 74.8% of the MRSA isolates. SCCmec typing showed types I, III, and IV in both the horses and the horse handlers while one horse was found to carry SCCmec V. Possible risk factors identified for MRSA carriage by horse handlers included being a veterinarian, exposure to antimicrobial agents, healthcare facility visitation, and personal hygiene. For the horses, risk factors from this study were allergy and wound management. MRSA nasal carriage in horses and horse handlers, as well as MDR (multidrug resistant) strains of S. aureus have been established from this study, this is of public health concern.

Keywords: MRSA, antibiotic resistance, horses, handlers.

INTRODUCTION

Staphylococci are one of the major groups of bacterial commensals isolated from skin, skin glands, and mucous membranes of mammals (Vengust et al., 2006), with Methicillin-resistant S. aureus, (MRSA) being one of several feared strains of *S. aureus* which are resistant to most antibiotics. MRSA is most often found associated with settings such as hospitals but has become increasingly prevalent in community-acquired infections. A variety of staphylococcal species are present in clinically normal individuals and are also opportunistic in nature, having become a leading cause of community-associated and hospitalassociated disease in humans and animals worldwide (O'Mahony et al., 2005; Weese et al., 2005a, 2006a). Some staphylococcal species (typically coagulase positive *Staphylococci*: CoPS) such as Staphylococcus aureus, Staphylococcus intermedius and Staphylococcus schleiferi ssp. coagulans are most known to be pathogenic causing disease (Cefai et al., 1994; O'Mahony et al., 2005; Weese et al., 2005a). While their role as hospital pathogens in human settings is important, their zoonotic potential and importance in veterinary medicine is still unclear (Boyle and Daum, 2007). The emergence and dissemination of antimicrobial resistance amongst staphylococci is an important problem in human and veterinary medicine (Archer, 1998). Methicillin-resistant S aureus (MRSA) is a great concern in human medicine worldwide and is an emerging problem in veterinary species (Weese, 2007). Methicillin resistance is mediated by the mecA gene: a gene that encodes a novel penicillin binding protein (PBP2a) (Vengust et al., 2006).

This penicillin binding protein mediates methicillin (oxacillin) resistance through a reduced affinity for beta-lactam antimicrobials (Chambers and Deleo, 2009). MRSA has been shown to be a cause of disease in several animal species including horses and dogs from different countries as well as transmission between humans and animals has been reported (Seguin et al., 1999; van Duijkeren et al., 2005; O'Mahony et al., 2005; Weese et al., 2005a, 2006b; Guardabassi et al., 2009). Colonization by methicillin-resistant Staphylococci (MRS) of any species may pose a risk for plasmid encoded transfer of antimicrobial resistance determinants between staphylococci and other bacterial organisms (Archer, 1998). Also, the presence of MRSA in animals in the community may pose a danger to veterinary care facilities because of the potential for adaptation of endemic MRS to animal species, and carries a substantial zoonotic potential (Vengust et al., 2006). Therefore, this study was designed to investigate the prevalence of MRSA in clinically normal horses and their handlers in the community.

MATERIALS AND METHODS

Study Population and Area

The study was conducted in Kaduna State, located in northwestern Nigeria, latitude 7°26′ 25″ E and longitude 10°31′23″ N, 704 m above sea level. A random sample involving a onestage cluster sampling (Hotchkiss et al., 2007) of clinically normal horses (n = 240) and their handlers (n = 65) was selected. Horses selected were housed in public establishments used for institutional purposes (80) by the army and police, recreational facilities in domestic settings used for durbar and casual/tourist rides (n = 80), and

performance/competition horses used for polo kept in horse clubs/schools (n = 80). Horses of various breeds and ages were all enrolled. Samples were collected from 19^{th} of April 2012 to 25^{th} September 2012. All swab samples were collected following informed consent. All samples were collected by a veterinarian. The study was conducted in Kaduna and Zaria towns of Kaduna State, Nigeria.

Sample Collection

Single nasal swabs were collected from each horse and horse handler as previously described (Vengust et al., 2006). Swabs were promptly placed in liquid Stuart's medium (Oxoid, Basingstoke, UK) and kept at 4°C until processing. Voluntary screening of Animal handling personnel was performed on all sampling locations of horse clusters of the community

Culture Techniques

Enrichment culture techniques were employed. Swabs were placed in the sterile 3ml BHI broth and incubated aerobically at 37°C for 24hours. Approximately 10.0µl of broth was then inoculated onto mannitol-salt agar surface and incubated aerobically at 37°C for 48h. Colonies consistent with staphylococci were sub-cultured onto blood agar for further identification via colony morphology, Gram stain appearance, ability to ferment sugars; mannitol, glucose, sucrose, maltose, inositol, catalase reaction, and tube coagulase test. Coagulase negative staphylococci were not speciated. (Weese et al., 2005; Vengust et al., 2006)

MRSA Identification and Typing

Screening for methicillin resistance in all S.

aureus was by growth on Mueller-Hinton agar with 4% NaCl and 6µg/mL oxacillin. Confirmation of methicillin resistance was by detection of PBP 2a by using the PBP 2a Latex agglutination test kit (Oxoid, Basingstoke UK) following manufacturer instructions. Antimicrobial susceptibility testing was performed by disk diffusion test following the Laboratory Standards Clinical Institute guidelines (CLSI, 2010). Isolates were typed after DNA extraction by SCCmec typing, as has been described, on all confirmed MRSA isolates of both colonized horses, and horse handlers (Shopsin et al., 1999: Boye et al., 2007). The staphylococcal cassette chromosome mec (SCCmec) types were determined by multiplex PCR for the mecA-IS431 gene, IS1272 gene, ccrC gene, and *ccrA2-B* gene, to confirm the identification and methicillin resistance of S. aureus. This was carried out as described by Boye et al., 2007. Four primer sets were used (Table I) to ensure amplification of two DNA targets from SCCmec type IV and two targets from SCCmec type V. PCR was performed in a total volume of 50µL containing 1*AmpliTaq PCR buffer, 1.5 mM MgCl, 200µM each dNTP and 1U of AmpliTaq DNA polymerase. Based on optimisation experiments, primer concentrations were as follows: primers b and a3, 0.2µM each; ccrCF and ccrCR, 0.25µM each; 1272F1 and 1272R1, 0.08µM each; and 5RmecA and 5R431, $0.1 \mu M$ each. Amplification comprised 4 min at 94°C, followed by 30 cycles of denaturation 30 s at 94°C, 30 s at 55°C and 60 s at 72°C, with a final extension for 4 min at 72°C. PCR products (10µL) were analyzed by electrophoresis on agarose 1.5% w/v gels, followed by staining with ethidium bromide.

The SCC*mec* type was determined based on the band pattern obtained after agar gel electrophoresis. Those isolates with no visible bands, or with a band pattern that was not in concordance with one of the five expected band patterns, were determined as non-typeable (NT).

Statistical Analysis

Assessment of Possible Risk factors associated with MRSA in horses and horse handlers.

A structured questionnaire pretested on a subset of the sample population was administered to all horse handlers whom either they or their horses were sampled. Written consent was sort for prior to administration. The questionnaire was administered through interview for non-literate individuals while for literate personnel they were given the questionnaire to fill themselves. Clinical information about the horses and handlers was recorded in a database – samples were coded to facilitate cross-reference between horses and handlers, but no person's name was used. Results were transferred to excel spread sheet, filtered for consistency, and finally put in SPSS version 16.0 for analysis.

Data Analysis

Variables were grouped into two categories expected outcomes/responses: based on binomial outcomes and multinomial outcomes/responses. Variables with binomial outcomes were assessed for risk of association with MRSA colonisation using Odds Ratio at 95% confidence interval, Chi square and pvalues were calculated to check for statistical significance. Variables with multinomial outcomes/ responses were assessed using Chisquare with p-values determined at 95% confidence interval to check for statistical significance.

Name	Primer sequence (5'3')	Amplicon	length	Target gene
		expected		
β	ATTGCCTTGATAATAGCCYTCTa	937 bp		ccrA2-B
αЗ	TAAAGGCATCAATGCACAAACACTa			
<i>ccrC</i> F	CGTCTATTACAAGATGTTAAGGATAATb	518 bp		ccrC
ccrCR	CCTTTATAGACTGGATTATTCAAAATATb			
1272F1	GCCACTCATAACATATGGAAc	415 bp		IS1272
1272R1	CATCCGAGTGAAACCCAAAc			
5RmecA	TATACCAAACCCGACAACTACc	359 bp		mecA–IS431
5R431	CGGCTACAGTGATAACATCCc			

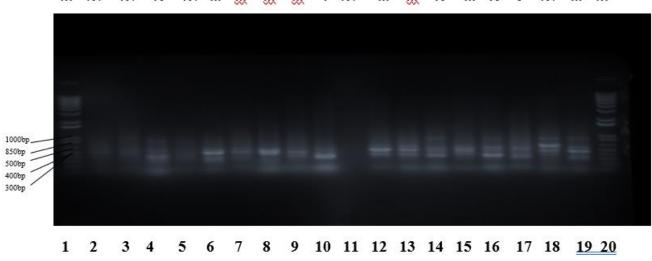
a. Ito *et al*.

b. Ito *et al*.

c. Boye et al.

RESULTS

In total, MRSA was isolated from 24 horses and 4 persons. Of the 24 equine isolates, 9 (11.3%) were from institutional horses used by the army and police mount troops, 9 (11.3%) were from performance horses used for polo, and 6 (7.5%) were from traditional/domestic housed horses used for durbar & tourist/casual rides. Twenty-two of the 24 (92%) horses were adults; the remaining 2 (8%) were less than 2 years of age. Clinical infections developed in 3 (13%) horses at 1 or more body sites. Lacerated wound infection (n = 1), pneumonia (n = 1), and vaginal abscess (n = 1) were identified. All three (100%) of the clinically affected horses had a history of contact with colonized persons. There was no history of known contact with colonized humans or horses with 11 (46%) of the clinically normal horses. One of the 3 isolates was from a vaginal abscess swab from a mare without any apparent clinical abnormalities or history of reproductive disease. Two were from purulent respiratory discharge swab with moist cough from a 7yr old mare and a lacerated wound infection from a young stallion following several failed antibiotic treatments. Whether MRSA was the cause in any of the cases was not investigated. The remaining 21 (87%) horses had subclinical infection and were nasal carriers.



м NT NT IV NT III Ш Ш Ш I NT ш III IV III IV III M v NT

Fig. 1. Gel electrophoresis of multiplex PCR analyses of 18 MRSA isolates, to identify their SCC*mec* type. Control strain with known *SCCmec* was also analysed. 100bp marker (first and last lanes) was used. Lanes 1, 2, 5, 11, 18 (SCC*mec* Non-Typeable), lane10 (SCC*mec* I) lanes 6, 7, 8, 9, 12, 13, 15, and 19 (SCC*mec* III), lanes 4, 14, 16 (SCC*mec* IV) and lane 17 (SCC*mec* V).

MIIINTIII III IIIIVINT

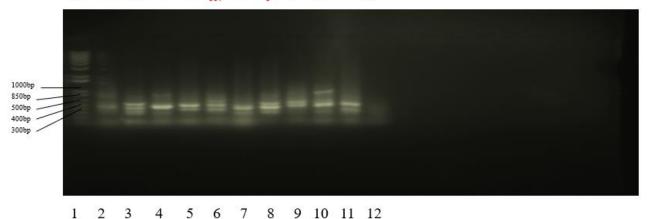


Fig. 2. Gel electrophoresis of multiplex PCR analyses of 11 MRSA isolates, to identify their SCC*mec* type. Control strain with known SCC*mec* was also analysed. 100bp marker was used (first lane). Lanes 3 and 12 (SCC*mec* Non-Typeable), lanes 4, 7, 8, 11 (SCC*mec* I), lanes 1, 5, 6, 9 (SCC*mec* III), lane 10 (SCC*mec* IV).

Molecular typing was performed on 27 MRSA isolates from both horses and horse's handlers while 1 of the MRSA isolates was lost during storage. Of the 27 tested isolates, including all 3 isolates from clinical infections, 13 isolates (46%) were identified as SCC*mec*III from eleven horses and two horse handlers (Figures 2 and 3). This strain is known to harbor the largest SCC*mec* element with the largest concentration of antibiotic resistance genes. Five isolates (18%) were identified as SCC*mec*I from 4 performance isolates from the traditional and performance horse settings, however, they were distinguishable in resistance profiles (Table II). horses and 1

institutional horse handler; SCCmecI is traditionally regarded together with SCCmecIII as hospital associated MRSA. All 3 tested isolates from horses with clinical infections contained SCCmecIII. Three isolates from clinically normal horses and 1 horse handler contained SCCmecIV. Five isolates from colonized horses were non-type able, while 1 horse isolate contained SCCmecV. All 11 isolates containing SCCmecIII were obtained from across the various horse clusters (4 from traditional setting, 4 institutional horses, and 3 performance horses) with the 2 horse handlers

Isolate	SCCmec type	Antibiogram	Source
HMT 05	Type III	PEN;ERY;OXA	Human
HRT 10	Type IV	AMI;PEN;TET;OXA	Horse
HRT 19	NT	AMI;PEN;GEN;OXA	Horse
HRT 35	Type III	CIP;SUL;PEN;ERY;TET;OXA	Horse

 TABLE II. SCCmec types and antibiogram properties of the MRSA isolates

HRT 64Type IIIPEN;ERYHorseHRT 80Type IIIVAN;PEN;ERY;OXAHorseHMIA 13Type IPEN;OXAHumanHRIA 16NTVAN;AMI;PEN;ERY;OXAHorseHRIA 22Type IIIPEN;TET;OXAHorseHRIA 24Type IIIAMI;PEN;TET;OXAHorseHMIP 05Type IVPEN;OXAHumanHRIP 01Type IIIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 26Type IIIPEN;OXAHorseHRS5 14Type IIIVAN;PEN;ERY;OXAHorseHRS5 52Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;CAHorseHRS5 57Type IIIVAN;PEN;ERY;CAHorseHRS5 57Type IIIVAN;PEN;ERY;CAAHorseHRS5 57Type IIIVAN;PEN;ERY;CAAHorseHRS5 57Type IIVAN;PEN;ERY;CAAHorseHRS5 57Type IIVAN;PEN;ERY;CAAHorseHRS5 57Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 582Type IIPEN;CXAHorseHRS5 84Type IVPEN;ERY;OXAHorseHRS5 84Type IIVAN;PEN;ERY;OXAHorseHRS5 87Type IIVAN;PEN;ERY;OXAHorse	HRT 57	Type III	VAN;PEN;ERY;OXA	Horse
HMIA 13Type IPEN;OXAHumanHRIA 16NTVAN;AMI;PEN;ERY;OXAHorseHRIA 22Type IIIPEN;TET;OXAHorseHRIA 24Type IIIAMI;PEN;TET;OXAHorseHMIP 05Type IVPEN;OXAHumanHRIP 01Type IIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRS5 14Type IIIVAN;PEN;ERY;OXAHorseHRS5 24NTAMI;PEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIVAN;PEN;ERY;OXAHorseHRS5 57Type IIPEN;CXAHorseHRS5 57Type IIPEN;ERY;OXAHorseHRS5 57Type IIPEN;ERY;OXAHorseHRS5 57Type IIPEN;ERY;OXAHorseHRS5 582Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRT 64	Type III	PEN;ERY	Horse
HRIA 16NTVAN;AMI;PEN;ERY;OXAHorseHRIA 22Type IIIPEN;TET;OXAHorseHRIA 24Type IIIAMI;PEN;TET;OXAHorseHMIP 05Type IVPEN;OXAHumanHRIP 01Type IIIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRS5 14Type IIIVAN;PEN;ERY;OXAHorseHRS5 24NTAMI;PEN;OXAHorseHRS5 57Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIIPEN;OXAHorseHRS5 57Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIIPEN;OXAHorseHRS5 57Type IIPEN;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRT 80	Type III	VAN;PEN;ERY;OXA	Horse
HRIA 22Type IIIPEN;TET;OXAHorseHRIA 24Type IIIAMI;PEN;TET;OXAHorseHMIP 05Type IVPEN;OXAHumanHRIP 01Type IIIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 76Type IIPEN;ERY;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HMIA 13	Type I	PEN;OXA	Human
HRIA 24Type IIIAMI;PEN;TET;OXAHorseHMIP 05Type IVPEN;OXAHumanHRIP 01Type IIIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRIP 26Type IIIPEN;OXAHorseHRS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 52Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;CAAHorseHRS5 67Type IIIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IPEN;CXAHorseHRS5 82Type IIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIA 16	NT	VAN;AMI;PEN;ERY;OXA	Horse
HMIP 05Type IVPEN;OXAHumanHRIP 01Type IIIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRIP 26Type IIIPEN;OXAHorseHMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 52Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIA 22	Type III	PEN;TET;OXA	Horse
HRIP 01Type IIIVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRIP 26Type IIIVAN;PEN;ERY;OXAHorseHMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 51Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIA 24	Type III	AMI;PEN;TET;OXA	Horse
HRIP 07Type IVVAN;SUL;PEN;ERY;TET;OXAHorseHRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRIP 26Type IIIVAN;PEN;ERY;OXAHorseHMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 51Type IIVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIVAN;PEN;ERY;CHL;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HMIP 05	Type IV	PEN;OXA	Human
HRIP 18Type VVAN;PEN;ERY;TET;OXAHorseHRIP 21NTPEN;OXAHorseHRIP 26Type IIIPEN;OXAHorseHMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 41Type IVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IVAN;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIP 01	Type III	VAN;SUL;PEN;ERY;TET;OXA	Horse
HRIP 21NTPEN;OXAHorseHRIP 26Type IIIPEN;OXAHorseHMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 41Type IVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIP 07	Type IV	VAN;SUL;PEN;ERY;TET;OXA	Horse
HRIP 26Type IIIPEN;OXAHorseHMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 41Type IVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IIVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIP 18	Type V	VAN;PEN;ERY;TET;OXA	Horse
HMS5 14Type IIIVAN;PEN;ERY;OXAHumanHRS5 24NTAMI;PEN;OXAHorseHRS5 41Type IVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IIPEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIP 21	NT	PEN;OXA	Horse
HRS5 24NTAMI;PEN;OXAHorseHRS5 41Type IVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRIP 26	Type III	PEN;OXA	Horse
HRS5 41Type IVAN;PEN;ERY;CHL;OXAHorseHRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HMS5 14	Type III	VAN;PEN;ERY;OXA	Human
HRS5 52Type IIIPEN;OXAHorseHRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRS5 24	NT	AMI;PEN;OXA	Horse
HRS5 57Type IIIVAN;PEN;ERY;OXAHorseHRS5 67Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRS5 41	Type I	VAN;PEN;ERY;CHL;OXA	Horse
HRS5 67Type IVAN;PEN;ERY;TET;OXAHorseHRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRS5 52	Type III	PEN;OXA	Horse
HRS5 76Type IAMI;PEN;ERY;TET;OXAHorseHRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRS5 57	Type III	VAN;PEN;ERY;OXA	Horse
HRS5 82Type IIIPEN;ERY;OXAHorseHRS5 84Type IVPEN;ERY;OXAHorse	HRS5 67	Type I	VAN;PEN;ERY;TET;OXA	Horse
HRS5 84Type IVPEN;ERY;OXAHorse	HRS5 76	Type I	AMI;PEN;ERY;TET;OXA	Horse
	HRS5 82	Type III	PEN;ERY;OXA	Horse
HRS5 87Type IVAN;PEN;ERY;OXAHorse	HRS5 84	Type IV	PEN;ERY;OXA	Horse
	HRS5 87	Type I	VAN;PEN;ERY;OXA	Horse

PEN; Penicillin OXA; Oxacillin VAN; Vancomycin AMI; Amikaxin ERY; Erythromycin TET; Tetracycline SUL; Sulphamethoxazole-Trimethoprim CIP; Ciprofloxacin GEN; Gentamicin CHL; Chloramphenicol

MRSA was isolated from 4 persons; 2 (3%) were veterinarians both in the institutional setting, 1 (1.5%) was a horse groom in the performance horses cluster, and 1(1.5%) was a stable cleaner in the traditional horses' cluster. The veterinarians were colonized with MRSA strain that contained SCCmecIV & SCCmecI, with the former strain isolated from 2 horses that had been under that person's care. All except 1 person (96%) had previous contact with 1 or more MRSA-positive horses; in 3 (75%) of 4 persons, recent contact with a horse infected with an indistinguishable subtype was identified. The colonized stable cleaner reported no contact with horses; however, isolate from this person was indistinguishable from an isolate recovered from a horse within that environment. Antimicrobial susceptibility testing

was performed on 37 of the horse isolates and all 7 human isolates both MRSA and MSSA strains. All MRSA isolates except 3 tested were susceptible to ciprofloxacin, chloramphenicol, & gentamicin. Isolates of both horses & horse handler related strains were found to be highly erythromycin (62.5%) & tetracycline (41.7%) resistant. The susceptibility test results are presented in the Table III Associated risk factors from the variables tested include being a veterinarian (OR= 9.0:

include being a veterinarian (OR= 9.0: p=0.019), wearing face mask (OR= 10.3: p=0.009), as well as hand gloves (OR= 19.0: p=0.001). Others were daily contact (OR= 3.9: p=0.000), and hospital visit irrespective of reason (OR= 2.09: p=0.000).

Antibiotic	MRSA from horses, N=24	MRSA from horse handlers,
		N=4
<u>β-lactams</u>		
Penicillin (5units)	0 (00)	0 (00)
Oxacillin (5µg)	0 (00)	0 (00)
<u>Aminoglycosides</u>		
Amikacin (30µg)	16/24 (66.7%)	4/4 (100%)
Gentamicin (10µg)	23/24 (95.8%)	4/4 (100%)
<u>Macrolide</u>		
Erythromycin (5μg)	9/24 (37.5%)	2/4 (50%)
<u>Tetracycline</u>		
Oxytetracycline (30µg)	14/24 (58.3%)	4/4 (100%)
<u>Sulphonamide</u>		
Sulphamethoxazole+trimethoprim (25µg)	21/24 (87.5%)	4/4 (100%)

TABLE III. MRSA isolates susceptibility levels to various classes of antimicrobials.

<u>Fluoroquinolone</u>			
Ciprofloxacin (5µg)	23/24 (95.8%)	4/4 (100%)	
<u>Phenicols</u>			
Chloramphenicol (30µg)	23/24 (95.8%)	4/4 (100%)	
<u>Polypeptide</u>			
*Vancomycin (30µg)	14/24 (58.3%)	3/4 (75.0%)	
Key: N=number of MRSA perc	entage of resistance in parer	thesis	

Key; N=number of MRSA, percentage of resistance in parenthesis. *Vancomycin resistance is only confirmed after MIC test.

DISCUSSION

The identification of MRSA in horses in the study area agrees to a report from Ontario, Canada and New York, USA, which found a MRSA prevalence of 4.7% in horses on local horse farms (Weese et al., 2005a) with the peculiarity of a particular MRSA clone, carried by horses and uncommon in humans in the same geographical area (Weese et al., 2005a). Similarly, a peculiarity for a particular MRSA clone to colonize horses was reported in Ireland (O'Mahony et al., 2005). In contrast, other studies showed no MRSA isolated from healthy horses in the Netherlands and Slovenia, although the sample populations were relatively small (Busscher et al., 2006). According to those studies, in Slovenia, the proportion of human MRSA isolates dropped significantly same as in Ireland and the Netherlands between 2000 and 2002 (Tiemersma et al., 2004). These countries all together form part of the European Antimicrobial Resistance Surveillance System (Tiemersma et al., 2004), thus data from other such countries may give greater insight into the relationship between human and animal MRSA colonization. Subtyping information provides evidence supporting both human-to-horse and horse-to-human transmission

(Weese et al., 2005a).

This study has identified a confirmed case of clinical MRSA infection in horses and nasal carriage by horse personnel concurrently of similar isolates. The prevalence of MRSA colonization in horses and horse handlers is relatively high; however, screening was performed only once. The prevalence of human colonization is of public health concern, particularly because of the likelihood of transmission between horses and humans on these settings. Similarly like the horses, the prevalence data must be interpreted with caution in the human handlers because of the nature of sampling. The emergence of MRSA in horses may be due to greater exposure of horses to MRSA-carrier persons, horses adapted special strain types, antimicrobial drugs pressure, or a combined effect of all these factors. Recent humans works in suggest that the fluoroquinolones may predispose patients to infection with or carriage of MRSA (Weber et al., 2003: Crowcroft et al., 1999). Whether fluoroquinolone use in horses has facilitated emergence of MRSA is yet unknown, as no current data is available on fluoroquinolone use in equine medicine (Weese et al., 2005a). Enrofloxacin is widely used in some parts of

The Kaduna horse population, particularly polo horses. Isolates in this study were also multidrug resistant, in contrast to results of several reports of community-associated MRSA in humans (Kenner et al., 2003). Therefore, determining the origin of these isolates is not straightforward and requires further study. Now, there is a lack of proven, safe, and acceptable options of eradication of nasal colonization in horses. To date isolation of infected horses and use of barrier precautions have been employed (Weese et al., 2005a), however, such methods may be challenging in horse clusters of our settings. The non-expression of the mecA gene in 2 out of the 27 MRSA isolates could not be readily explained in this study, however other researchers have reported such phenomenon and referred to such isolates as pre-MRSA (Brown et al., 2005). Antibiotic resistance appeared to be more pronounced among the S. aureus isolated from horses compared to the S. aureus isolated from horse handlers. This enhanced antibiotic resistance of the horse S. aureus strains might be caused by horizontal gene transfer under the pressure of an enhanced antibiotic treatment of the host. These results also indicate that S. aureus of human origin, at least the strains investigated in the present study seemed to be of minor importance. As previous studies from South-West Nigeria had shown (Ghebremedhin et al., 2009), it appears that there is a high proportion of S. aureus isolates resistant to erythromycin and tetracycline. This could be associated with the fact that tetracycline historically had wide clinical application, is inexpensive, orally administered, and available from diverse sources where they are sold with or without prescription in Nigeria. In this study, all the trimethoprim resistant S. aureus isolates were MDR and carried SCCmec III element which is large and can carry a lot of resistance genes (Deurenberg et al., 2007), but has

also been suggested that mutation of the dihydrofolate reductase (DHFR) enzyme of the bacteria is responsible for such resistance (Chongtrakool et al., 2006). Minor differences between human and equine isolates were found in the SCCmec composition of MRSA from this study such as identification of the site-specific recombinase gene (ccrAB2) in four equine same recombinase isolates. the system associated with most human MRSA strains (SCCmec IV): of which only one human isolate was identified in this study. This may reflect independent acquisition of allelic variants from coagulase-negative staphylococci equine strains or from human MRSA (Vengust et al., 2008). However, 12 MRSA isolates possessing the SCCmec type III element was alarming as this element is associated with HA-MRSA, thus reflecting the possible shift of MRSA from a nosocomial pathogen to a community associated pathogen and may even explain the high level of multidrug resistance encountered. This study has shown MRSA carriage as significant in horses, which agrees with earlier reports (Weese et al., 2005b: Cuny et al., 2010). MRSA infection can become a serious problem and endemic in the horse population of Kaduna, which is quite significant. This study identified several risk factors that were associated with MRSA colonization in horses of the area. However, it was surprising that residence on a farm where one or more horses had previously diagnosed MRSA been with carriage/colonization was not associated with MRSA carriage in the handlers, as identification of multiple colonized horses on a farm is not uncommon (Weese et al., 2006). In this study there was no association between MRSA and equipment sharing as reported in earlier studies (Weese,

2007). Antimicrobial therapy within the last 4months was associated with MRSA colonization in this study as has been reported previously (Mamoon et al., 2008). Antimicrobial therapy has also been reported as a risk factor in humans, yet there is increasing recognition of MRSA infection in humans without risk factors, such as antimicrobial use. Regardless, the association of antimicrobials with MRSA colonization highlights the need for prudent antimicrobial use in veterinary medicine. The finding that being a veterinarian or veterinary assistant was risk factor is understandable. However, it was surprising that protective clothing such as face mask and hand gloves were risk factors. This perhaps may not be unrelated to the fact that they can serve efficiently as fomites for the carriage and spread of MRSA. Interestingly, previous antimicrobial therapy was not a risk factor for horses in this study: in fact, the only identified risk factor for horses with statistical significance was allergy. In humans, contact with healthcare facilities is a commonly reported risk factor for CA-MRSA infection or colonization. (Cuny et al., 2006) as was also found in this study. Care must be taken in comparing human and equine data, because the nature of the equine population and equine healthcare is quite different from that of humans. In fact, some major subsets of the equine population, such as breeding farms, may mimic the human healthcare situation more closely than the human community situation because of the frequent contact of horses with veterinary personnel, frequent antimicrobial use, regular inter-farm movement of horses, and care by a constant group of farm personnel that may concurrently be managing sick horses. Also identified in this study as a risk factor was contact during restraint; this may be explained by the fact that restraint in horses is mainly of the head or limbs (prevent kicking) thus enhancing the

likelihood of transmission of MRSA from the nares, facial area, and hoof of the horse. Protective factors identified from this study include exercise contact, handling 4-5horses daily, and being a groom; these are without a real explanation. Other protective factors include feeding and watering contact which may be; since the horses are usually out in the paddocks and stables are cleaned when this activity is taking place thus minimizing contact between the handler and horses. Also included are washing hands with water only following contact with the horse, a tertiary education, and a fair knowledge of MRSA. Another interesting protective factor was sex (being female); this may however be because of sampling bias since only two females, both veterinarians were available for this study. This finding emphasizes the need to consider putative risk factors in concert others when with investigating relationships with any outcome.

CONCLUSION

Because of the extensive movement of horses, especially performance and traditional horses, between and within Kaduna and neighboring States, MRSA prevalence among horses may be more widespread than recognized. Finding MRSA in apparently healthy horses is of concern because they may serve as community reservoir and source of infection and reinfection for persons especially horse handlers such as veterinarians. In view of the size of the Northern Nigerian horse population and the frequent close contact between many persons and horses, this concern must not be ignored. Further studies are essential to clarifying the role of this pathogen in equine diseases and infection epidemiology between horses and humans.

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