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Phenotypic occurrence of methicillin-resistant *Staphylococcus aureus* in camels slaughtered at Kano abattoir, Kano, Nigeria

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Abstract

To assess the occurrence of MRSA among camels in Kano abattoir, a total of 300 nasal swabs were collected from camels at the lairage in Kano abattoir, Kano state, Nigeria to isolate and biochemically characterize *Staphylococcus aureus* and confirm methicillin-resistant *Staphylococcus aureus* among isolates using oxacillin resistance screening agar basal medium, disc diffusion method, PCR and also through detection of penicillin binding protein 2'. Samples were collected into universal sample bottles containing trypticase soy broth with 6.5% Nacl. Samples were incubated at 37^{0}C for 24 hrs and sub-cultured on Baird Parker agar (Oxoid Ltd, Basingstoke, UK). Suspect *Staphylococcus* spp isolates were confirmed using coagulase, DNAse, haemolysis and sugar fermentation tests (mannitol, sucrose, lactose, mannose and xylose). Fifteen of the 42 isolated *Staphylococcus aureus* were confirmed to be MRSA on oxacillin resistance screening agar basal medium of which 12 were also resistant to oxacillin using disc diffusion method. Five (33.3%) of the 15 isolates were confirmed to be MRSA using the PBP2 latex agglutination test kit. The isolates were however negative for *mec* A by PCR. The prevalence of *Staphylococcus aureus* and MRSA was higher in males than in females, but this difference was found to be statistically insignificant ($p > 0.05$). Multidrug resistance was displayed by all *Staphylococcus aureus* isolates with 100% resistance to ampicillin and penicillin, but 97.6% of the isolates were susceptible to amikacin and 90% to ciprofloxacin and gentamicin. There was no statistically significant difference in antibiotic resistance between *Staphylococcus aureus* and MRSA to amikacin, ciprofloxacin, chloramphenicol, cloxacillin, erythromycin, gentamicin, penicillin, tetracycline, sulphamethoxazole, vancomycin (p-value > 0.0), but there was statistical significance to oxacillin ($p = 0.0001$; OR = 0.7143). MRSA strains were found in 5% of camels and thus may play a potential role in disseminating the pathogen between animals and humans as well as within the community.

Keywords: Camels, Kano abattoir, Occurrence, Methicillin resistant *Staphylococcus aureus*, Multidrug resistance.
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Introduction

Staphylococcus aureus is one of the most frequently encountered bacterial pathogens in humans. It causes skin infections, osteoarthritis and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals (Didier *et al*., 2004). In this regard, the bacterial pathogen *Staphylococcus aureus* is one of the most important bacteria, particularly the methicillin-resistant strains.

methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of the increasingly prevalent, difficult-to treat nosocomial infections worldwide. Methicillin-resistance in staphylococci constitutes resistance to all of the β-lactam antibiotics and their derivatives (CLSI, 2009). The major mechanism is the acquisition of the *mecA* gene that codes for additional penicillin-binding protein 2a (PBP2a) (Mukarami *et al.*, 1991). Antibiotic resistant

staphylococci are of major public health concern environment (Deresinski, 2005).

The dromedary camel (*Camelus dromedarius*, one-humped) is a multipurpose animal, formerly used strictly for transport, as beast of burden and used as draught animal for agriculture popularly referred to as "the desert ship", but today it is changing status to food animal as it is used for milk, meat and hides with increase in their population (Schwartz & Dioli, 1992; Kadim *et al*., 2008). Camels are reared in close contact with humans and hence could serve as an important source of MRSA to humans.

There is minimal research work on camels and very little information can be found on the role that camels play when it comes to zoonotic disease transmission.

Materials and Methods

Study area

The study was carried out in Kano, Kano state. The state is the most populous state in Nigeria with an estimated population of 9,383,682 people (NPC, 2006). Kano is located at latitude $11^0$20′ to 12047′ North and Longitude $8^0$22′East to $8^0$39′. One-humped camel (*Camelus dromedarius)* breed are usually slaughtered in Kano abattoir and therefore were used for this study.

Sample collection

A total of 300 nasal swabs were collected over a period of 3 months from 300 camels in Kano abattoir, Kano state. All samples (n=300) were taken from live camels in the lairage. Commercial sterile swabs were used for nasal swabbing by rubbing against the mucosal surface for approximately 5-10 seconds, about 5cm – 10cm into the anterior nares. This was placed into 5ml trypticase soy broth supplemented with 6.5% NaCl and transported to the Department of Veterinary Public Health and Preventive Medicine Laboratory, Ahmadu Bello University, Zaria where the test tubes were incubated for 20 hrs at 37^0C as previously recommended (Lee, 2003).

All the media used for bacterial culture were prepared in the laboratory according to the manufacturers' instructions and these included Baird Parker agar (Oxoid Ltd, Basingstoke, UK), Trypticase Soy Broth, Mueller Hinton agar, Nutrient agar and Oxacillin resistance screening agar base (ORSAB).

Bacterial Isolation and Identification

A loopful of each broth inoculum was streaked on Baird Parker agar and the culture plates incubated at 37^0C for 24-48 hrs and growth observed for typical since the bacteria can easily circulate in the colonial morphology of *S. aureus. C*olonies that appeared as grey-black, shiny, convex with characteristic white edge surrounded by clear zones and rings around them were considered as positive growth. Gram staining was done to observe for Gram positive cocci in clusters and positive samples were stored on nutrient agar slants for further analyses.

Standard biochemical tests were used to identify *Staphylococcus aureus* among suspect isolates using conventional methods. Tests conducted include: catalase, coagulase, DNAse test, haemolysis (5% sheep blood) and fermentation of mannitol, sucrose, glucose, lactose and xylose.

Phenotypic identification of MRSA among *Staphylococcus* spp. isolates was carried out by culturing all isolates on ORSAB agar at 35^0C for 24 hours. All cultures showing bright blue coloured growth were considered to be presumptive MRSA positive strains, while all others were regarded as methicillin susceptible *Staphylococcus aureus* (MSSA) and a disk diffusion method using Mueller Hinton agar was used to determine the antibiotic susceptibility profiles of the MRSA isolates following Clinical and Laboratory Standards Institute (CLSI, 2009) method.

All *S. aureus* isolates were subjected to *in-vitro* antimicrobial testing method on Mueller-Hinton agar, using fresh trypticase soy broth culture and antibiotic discs according to performance standards of Clinical and Laboratory Standards Institute (CLSI, 2009). Briefly, the organisms were grown overnight in tryptic soy broth and adjusted to 0.5 Macfarland standard and spread on Mueller Hinton agar plates, which were incubated at 37^0C for 18 hours. A panel of twelve antimicrobial discs was used. The zones of inhibition around the discs were measured and interpreted as sensitive, intermediate, and resistant using the interpretation chart recommended by Clinical and Laboratory Standard Institute (CLSI, 2009). The antibiotics tested were Penicillin (10i.u), ampicillin (10µg), cloxacillin (10µg), oxacillin (1µg), amikacin (10µg), tetracycline (30µg), gentamicin (10µg), erythromycin (10µg), sulphamethoxazole (25µg), vancomycin (30µg), chloramphenicol (10µg) and ciprofloxacin (10µg).

Any isolates that showed resistance to 3 or more antibiotics were classified as multidrug resistant *S. aureus* (Hidron *et al*., 2007).

The two pairs of primers widely used for amplification of *mec*A gene used were MR1 and MR2 primers (primer set 1) and MR3 and MR4 (primer set

2) to generate 154 and 533 bp fragments, protocol including composition of reaction mixture and cycling parameters were done as previously documented (Yusuf, 2014).

Putative MRSA were also subjected to latex agglutination test to detect the production of penicillin binding protein 2' according to the recommendations of the manufacturer (OXOID Ltd, Basingstoke, U.K). In brief the protein was extracted as recommended from overnight culture on Mueller Hinton agar using extraction reagents provided. The supernatant of each culture (50µl) was mixed with test latex along with necessary controls on the test card, mixed, rocked and observed for agglutination.

Data analysis

The data obtained from this research work were entered and stored in Microsoft Excel® 2010 where descriptive statistics were used and analysed using SPSS® version 16.0 2007. Categorical variables were evaluated using Chi square test to check for association, Odds Ratio at 95% confidence interval respectively (Mukarami *et al*., 1991). The PCR was used to measure strength of association between variables and prevalence of *Staphylococcus aureus* and MRSA. Values of $p < 0.05$ were considered significant. Prevalence was calculated using the formula of Thrusfield (1997).

Results

Results of Isolation and Identification of S. aureus

The total prevalence of *Staphylococcus aureus* and of MRSA were 14% and 5% respectively (Table 1). The sex prevalence of MRSA in male and female camels was also not found to be statistically significant ($p > 0.05$) (Table 1).

Results from oxacillin resistance screening agar base media culture of *S. aureus* isolates revealed that 15 isolates (5%) were found to be putative MRSA. The results of antibiotic resistance profiles of the 42 *S. aureus* isolates to various antibiotics are as follows: amikacin 1(2.3%), ampicillin 42(100%), ciprofloxacin 3(7.1%), chloramphenicol 26(61.9%), cloxacillin 32(76.2%), erythromycin 32(76.2%), gentamicin

Table 1: Prevalence of *S. aureus* and MRSA in male and female camels sampled in Kano abattoir, Nigeria

	Total no. Sampled	No. positive for *S. aureus* (%) *	No. positive for MRSA (%) **
Males	251	36 (14.34)	13 (5.17)
Females	49	6 (12.24)	2 (4.08)
Total	300	42 (14)	15 (5)

MRSA= Methicilin resistant *S. aureus* * $\chi^2 = 0.824$, p value = 0.544, ** Fishers Exact Test p value = 0.550

Table 2: Resistance and susceptibilities of MRSA(15) isolates to test antibiotics

	Susceptible	Resistant
Antibiotics	MRSA(%)	MRSA(%)
P	0	15(100)
AMP	0	15(100)
OB	3(20)	12(80)
OX	3(20)	12(80)
AK	14(93)	1(7)
TE	4(26)	11(74)
CN	14(93)	1(7)
E	3(20)	12(80)
RL	5(33)	10(67)
VA	4(26)	11(74)
C	5(33)	10(67)
CIP	14(93)	1(7)

Key: P-Penicillin OX- Oxacillin CN- Gentamicin VA- Vancomycin
AMP-Ampicillin AK- Amikacin E- Erythromycin C- Chloramphenicol
OB-Cloxacillin TE-Tetracycline RL-Sulphamethoxazole CIP- Ciprofloxacin

MRSA- Methicillin-resistant *Staphylococcus aureus*
MSSA- Methicillin- susceptible *Staphylococcus aureus*
%- Percentage of the absolute Figure of susceptibility and resistance for the MRSA AND MSSA

Table 3: Antibiotic resistance pattern and multiple antibiotic resistance (MAR) index of 12 MRSA isolates

No of Isolates	Antibiotic Resistance Pattern	Multiple Antibiotic Resistance Index (MAR)
1	P, AMP, OB, OX, E	0.42
1	P, AMP, OB, OX ,TE, E, RL	0.58
4	P, AMP, OB, OX, TE, E, RL, VA, C	0.75
1	P, OB,OX, TE, E, VA, C	0.58
3	P, AMP, OB,OX, TE, E, RL,VA	0.67
1	P, AMP, OB,OX, VA, TE, E, RL,VA, C, CIP	0.92
1	P, AMP, OB,OX, AM, TE, E, RL, VA, C	0.83

P-Penicillin	OX- Oxacillin	CN- Gentamicin	VA- Vancomycin
AMP-Ampicillin	AK- Amikacin	E- Erythromycin	C- Chloramphenicol
OB-Cloxacillin	TE-Tetracycline	RL-Sulphamethoxazole	CIP- Ciprofloxacin

3(7.1%), oxacillin 14(33.3%), penicillin 42(100%), tetracycline 29(69%), sulphamethoxazole 26(62%), and vancomycin 29(69%). This shows that 93% of the isolates were susceptible to gentamicin and ciprofloxacin and the highest susceptibility was to amikacin 97.7% (Table 2).

Multidrug resistance was shown by all MRSA and MSSA tested with 3 of the 42 *S. aureus* isolates being resistant to 3 test antibiotics, 4 isolates resistant to 4 test antibiotics and 35 were resistant to more than 4 test antibiotics. Isolates had high multiple antibiotic resistance indices (MAR) i.e. >0.2 (Table 3). Altogether there were 32 patterns of resistance, most of which were resistance to one antibiotic. The most common antibiotics among those resistant to multiple agents were penicillin, ampicillin, oxacillin and cloxacillin.

All 15 isolates of *Staphylococcus aureus* phenotypically identified as MRSA based on their growth characteristics on ORSAB agar tested negative for *mec* A by PCR; however, five of them were positive for the production of PBP2' protein. Fourteen of the 15 isolates were also found to be resistant to oxacillin, which is one of the antibiotics routinely used as a surrogate for methicillin resistance.

Discussion

The prevalence of *S. aureus* in camels in this study was 14%. A higher prevalence of 56% has been reported by Alzohairy (2011) from camels in Saudi Arabia. The recovery of *S. aureus* in this study was also lower than the report of Al -Doughaym *et al*. (1999) who obtained 34.1% *S. aureus* from nasal swabs from pneumonic camel lungs. However, Abubakar *et al*. (2010) recorded only 7% recovery from lung lesions of pneumonic camels in Nigeria.

All (100%) *S. aureus* isolates were coagulase positive by the tube method using rabbit plasma. Coagulase production is one of the important properties of *S. aureus* and is used along with some other properties to identify this organism in most laboratories. A 5% prevalence for MRSA was observed in this study based on latex agglutination results for PBP2a, which is lower than the 35.5% reported in Saudi Arabia by Alzohairy (2011).

All of the *S. aureus* isolated were resistant to penicillin and ampicillin and also displayed high percentage resistance to erythromycin and tetracycline which is in agreement with the work of Tahnkiwale *et al*. (2002) who reported a high frequency of resistance by MRSA strains isolated from cattle to penicillin and oxacillin followed by erythromycin, Co-trimoxazole, gentamicin, and cephalothin. Most of the isolates were highly sensitive to amikacin, gentamicin and ciprofloxacin.

MRSA isolates also showed high resistance to vancomycin (73.3%) and oxacillin (80%) which is in line with a report of 100% resistance to penicillin, 93.33% to ampicillin, 53.34% to vancomycin, and 40% to oxacillin by Al-Doughaym *et al*. (1999) in which isolates were also obtained from nasal swab samples of pneumonic camels. Kataria (2008) found similar results for isolates of clinical cattle mastitis origin who reported that isolates were 100% resistant to penicillin. In the study by Onanuga *et al*. (2006b) 70% of the MRSA isolates were susceptible to ofloxacin, ciprofloxacin, sparfloxacin and gentamicin and resistant to ampicillin, cephalexin and clindamycin.

The 15 MRSA isolates exhibited 100% multiple drug resistance, as all were resistant to more than three antibiotics. Alzohairy (2011) has reported the highest rate of multidrug resistant MRSA from camels (41.1%) than other animals.

The high number of antibiotic resistance patterns shown by both MRSA and MSSA isolates in this study is quite alarming. Although MRSA exhibiting multiple

resistance have been generally isolated from various animal species, hence animals may become important reservoirs (Seguin *et al*., 1999; Lee 2003; Van Duijkeren *et al*., 2004).

The *in vitro* susceptibility of most isolates to amikacin (97.7%), ciprofloxacin and gentamicin (93%) is suggestive of the potential efficacy of these drugs in treating MRSA infections and may also reflect the fact that these antibiotics may not have been misused or abused in the study environment.

Resistance of all the isolates to penicillin is in accordance with the known natural resistance of *Staphylococcus* to β-lactams. Penicillin resistance is sometimes plasmid-borne, and therefore it spreads out very quickly to several other strains, with the result that in the 1980s approximately 90% of *S. aureus* had become resistant to the drug. In another research in Nigeria, a high level of resistance to tetracycline was established which was attributed to the excessive use of the drug in Nigeria (Kabir *et al*., 2004). Even though further genetic analysis was not done to compare the isolates in this study, similarities in the antibiotic resistance profiles suggest that similarities exist between these isolates, but further studies are required to prove this possibility.The presence of MRSA in camels as shown by this study raises public health issues as camels have become a common food animal in some northern parts of the country since camel milk and meat are now widely consumed. Of equal importance is the fact that dissemination of MRSA across low-income regions including Nigeria could have major implications for the cost of antibiotic treatment and poor outcome of treatment of serious *S. aureus* diseases.

Herdsmen travel with camels and are continuously in contact with them during grazing, feeding and other activities, hence the MRSA isolated from camels in this study could have originated from humans considering that the rate of methicillin resistance among human *S. aureus* isolates in Nigeria have been found to be high (Onanuga *et al*., 2006 a; 2006b; Olonitola *et al*., 2007).

Despite the fact that scientific literature and information may be lacking on the prevalence of MRSA in foods of animal origin in general in the study area, and Nigeria as a whole, MRSA colonization and infection has been reported in both healthy and ill humans. Onanuga *et al*. (2006b) observed a prevalence rate of 76.7% and 68.5% of MRSA in Abuja and Zaria among healthy women, while Olonitola *et al*. (2007) found a prevalence of humans, they have recently been isolated from 20% among healthy adults from non-hospital sources in Zaria.

Because of the ability of the staphylococci to change resistance pattern over time, MRSA may continue to be a problem in the future. In Africa, relatively high prevalence rates of MRSA have been reported especially in Nigeria, Kenya and Cameroon (20-76%) and below 10% in Tunisia and Algeria (Nwanko *et al*., 2010).

Several studies have documented the presence and infection by MRSA from human sources in Nigeria. For instance, a prevalence rate of 43% was reported at Jos University Teaching Hospital (Ikeh, 2003) and 28.6% in Kano, Nigeria (Nwanko *et al*., 2010). Hence these varied and high isolation rates of MRSA in Nigeria necessitate urgent action to prevent an epidemic in humans.

The MRSA isolates were found to be negative for *mec* A by PCR, which is a major limitation of the present study, but latex agglutination was able to confirm five of the twelve isolates tested to be positive. The definitive identification of MRSA is be hinged on either detection of *mec*A, PBP2a or minimum inhibition concentration of ≥2µg/ml for methicillin. Depending on the assay, some reasons that have been adduced for false negative PCR results may include missing or altered *spa*, altered *nuc*, altered *orf*X, as well as *mec*A ‘‘drop outs’’ or ‘‘empty cassette’’ variants (Kotsakis *et al*., 2011).

Mechanisms of reduced susceptibility and outright resistance to β-lactam antibiotics in staphylococci not related to *mec*A and PBP2a also exist. These include hyper-production of β-lactamase with significant methicillinase activity, which has been associated with borderline susceptibility/resistance (Bannerjee *et al*., 2010). False positive PCR tests may also occur and are usually the result of assays targeting the *orf*X/SCC*mec* junction fragment in the presence of ‘‘empty cassettes’’ resulting from specific loss of *mec*A. Thus, target sites of the assay are present, but the gene encoding resistance is not (Chlebowicz *et al*., 2010).

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