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Occurrence of Methicillin-Resistant *Staphylococcus aureus* in Camels Slaughtered at Kano Abattoir, Kano, Nigeria

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ABSTRACT

This study assess the occurrence of Methicilin Resistant *Staphylococcus aureus* among camels in Kano abattoir. A total of 300 nasal swabs were collected from camels at the lairage in Kano abbatoir, Nigeria to isolate and 'biochemically characterize *Staphylococcus aureus* and confirm Methicillin-resistant *Staphylococcus aureus* among isolates using oxacillin resistance screening agar basal medium (ORSAB), disc diffusion method and also through detection of penicillin binding protein 2' (PBP2'). Fourteen percent (42/300) suspected *Staphylococcus* spp isolates were confirmed using coagulase, DNase, hemolysis and sugar fermentation test. Of the 42 isolated *Staphylococcus aureus,* 35.7 % (15/42) were confirmed to be MRSA on ORSAB medium of which twelve were also resistant to oxacillin, using disc diffusion method. Five (33.3%) of the fifteen purtative MRSA were confirmed to produce penicillin- binding protein 2' by PBP2' latex agglutination test kit. The prevalence of *Staphylococcus aureus* and MRSA was higher in males than in females ($p > 0.05$). 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 statistical significance difference in antibiotic resistance between *Staphylococcus aureus* and MRSA to amikacin, ciprofloxacin, chloramphenicol, cloxacillin, erythromycin, gentamicin, penicillin,

tetracycline, sulphamethoxazole, vancomycin with *p*-value > 0.05 but there was statistical significance to oxacillin with *p*-value of 0.0001 and Odds Ratio of 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.

Keywords: Occurence, methicillin resistant, *staphylococcus aureus*, multidrug resistance

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. 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 major public health concern since the bacteria can easily circulate in the environment (Deresinki *et al*., 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 refered 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 popuation (Schwartz and 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 camels play when it comes to zoonotic disease transmission.

MATERIALS AND METHODS

Study Area

Kano state is the most populous State in Nigeria with an estimated population of 9,383,682 people (2006 census). One-humped camel

(*Camelus dromedarius)* is the breed usually slaughtered in Kano abbatoir 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 they were incubated for about 20 hrs at 37^{o}C (Lee *et al.*, 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), Trypticase Soy Broth, Mueller Hinton agar, Nutrient agar (Oxoid) and Oxacillin resistance screening agar base (Oxoid).

Bacterial Isolation and Identification

A loopful of each broth inoculum was streaked on Baird Parker agar and the culture plates incubated at 37^{o}C for 24-48 hrs and growth observed for typical 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 identified as positive growth. Gram staining was done to observe the 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 (manitol, sucrose, glucose, lactose and xylose.

Phenotypic identification of MRSA among *Staphylococcus* spp. isolates was done by culturing all *Staphylococcus aureus* on Oxacillin resistance screening basal medium (ORSAB) agar at 35^{o} C for 24 hours. All cultures showing bright blue coloured growth were taken as 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 profile of the MRSA isolates following Clinical Laboratory Standard Institute (CLSI) method.

All *S. aureus* isolates were subjected to in-vitro antimicrobial testing method on Muller-Hinton agar, using fresh trypticase soy broth culture and antibiotic discs according to performance standards of Clinical and Laboratory Standard Institute (CLSI, 2009). A panel of twelve antimicrobial discs were 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), chloxacillin (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 more than 3 antibiotics were classified as multidrug resistant *S. aureus*. (Hidron *et al*., 2007).

Putative MRSA were also subjected to latex agglutination test to detect the production of penicillin binding protein 2'.

Data Analysis

The data obtained from this research work were entered and stored in Microsoft Excel® 2010 where descriptive statistics was 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 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 (Thrusfield, 1997).

RESULTS

Results of Isolation and Identification of *S. aureus*

The total prevalence of *Staphylococcus aureus* was 14% and MRSA 5% (Table 1 and 2). The prevalence of *Staphylococcus aureus* was higher in males than in females, but this difference was found to be statistically insignificant

with a p value of 0.550 (Table 2). The sex prevalence of MRSA in male and female camels was found to be statistically insignificant with p value > 0.05 (Table 2).

Table 1: Main biochemical characteristics of 106 suspect *Staphylococcus* spp

Test	No (%) Positive
Catalase	73 (24.3)
Coagulase	59(19.7)
Dnase	71(23.7)
Haemolysis (α and β)	30(10)
Xylose	7(2.3)
Glucose	42(14)
Sucrose	42(14)
Lactose	42(14)

Table 2: Prevalence of *S. aureus* and MRSA in male and female camels sampled in Kano abbatoir, 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

Results from Oxacillin resistance screening agar base media culture of *S. aureus* isolates revealed 15 (5%) to be putative MRSA. The results of antibiotic resistant 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%), chloxacillin 32(76.2%), erythromycin 32(76.2%), gentamicin 3(7.1%), oxacillin 14(33.3%), penicillin 42(100%), tetracycline 29(69%), sulphamethoxazole 26(62%), and vancomycin 29(69%). This shows that isolates were 93% susceptible to Gentamicin and Ciprofloxacin and the highest susceptibility was to Amikacin 97.7% (Table 3 and Fig. 1).

Table 3: Resistance and susceptibility of MRSA(15) and MSSA(27) isolates to test antibiotics

	Susceptible		Resistant		
Antibiotics	**MSSA(%)**	**MRSA(%)**	**MSSA(%)**	**MRSA(%)**	**Total**
P	0	0	27(100)	15(100)	42
AMP	0	0	27(100)	15(100)	42
OB	7(25)	3(20)	20(75)	12(80)	42
OX	27(100)	3(20)	0(0)	12(80)	42
AK	27(100)	14(93)	0(0)	1(7)	42
TE	9(33)	4(26)	18(67)	11(74)	42
CN	25(92)	14(93)	2(80)	1(7)	42
E	8(29)	3(20)	19(71)	12(80)	42
RL	11(40)	5(33)	16(60)	10(67)	42
VA	9(33)	4(26)	18(67)	11(74)	42
C	11(40)	5(33)	16(60)	10(67)	42
CIP	26((96)	14(93)	1(4)	1(7)	42

KEY: P-Penicillin, OX- Oxacillin, CN- Gentamicin, VA- Vancomycin, AMP-Ampicillin, AK- Amikacin, E- Erythromycin, C- Chloramphenicol, OB-Chloxacillin, 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

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 (Table 4). Isolates had a high multiple antibiotic resistance indexes (MAR) which were greater than 0.2 (>0.2) (Table 5).

Out of the 15 MRSA tested by latex agglutinition, five (5) were positive which directly confirm the production of PBP2' protein and consequently the presence of *mec* A gene in positive MRSA Table 6.

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, Fatihu *et al*. (2010) recorded only 7% recovery from lung lesions of pneumonic camels in Nigeria.

Table 4: Table showing the 32 antibiotic resistance patterns (antibiograms) of the 42 *S. aureus* isolates

Isolates	No of Isolates	Resistance Profile
12F*	1	P, AMP, OB, OX, AK, TE, E, RL,VA, C
13M*	1	P, AMP, OB, OX, E, C
190M*	1	P, AMP, OB, OX, TE, E, VA, C
205M, 241M, F36*	3	P, AMP, OB, OX, TE, E, RL,VA
213M*	1	P, AMP, OB, OX, TE, E, RL,VA, C, CIP
33M, 40M, 207M, 248M*	4	P, AMP, OB, OX, TE, E, RL, VA, C
24M*	1	P, AMP, OB, OX, TE, E, RL
6M	1	P, AMP, AK, VA
9M	1	P, AMP, E, VA
10M, 10F	2	P, AMP, OB, TE, E, RL , VA, C
26M	1	P, AMP, OB, RL, VA, C
29M,	1	P, AMP, TE, E, RL, VA, C
36M, 45M	2	P, AMP, E
39M, 123M	2	P, AMP, OB, RL, VA
41M	1	P, AMP, OB, TE, E, RL, VA, C
42M	1	P, AMP, TE, E, RL, VA, C, CIP
60M	1	P, AMP, OB,TE, E, RL, VA
111M	1	P, AMP, OB, E, RL, VA, C
112M	1	P, AMP, TE, RL
131M	1	P, AMP, TE, E, RL
134M	1	P, AMP, OB, TE, E, CN, RL, VA, C

141M	1	P, AMP, TE, RL, VA, C
149M	1	P, AMP, E, RL
157M,179M	2	P, AMP, OB, TE, E, RL, VA, C
181M	1	P, AMP, OB, TE, RL, VA, C
182M	1	P, AMP, OB, TE, E, VA, C
214M,221M	2	P, AMP, OB, TE, E, VA
218M	1	P, AMP, TE, E, VA, C
2F	1	P, AMP, E, RL, VA
14F	1	P, AMP, E, CIP
16F	1	P, AMP, OB, TE, CN, E, RL, C, CIP
23F	1	P, AMP, OB, TE, E, RL, VA, C

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

*** -** MRSA isolates

All (100%) *S. aureus* isolates were coagulase positive in tube test method using plasma from rabbit. 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 which is lower than the 35.5% reported in Saudi Arabia by Alzohairy (2011). Slightly higher percentages of MRSA were detected in bovine milk by Idbeis (2010), Farzana *et al*. (2004) and Devriese *et al*. (1997), who recorded percentage MRSA of 10.52%, 10%, and 10% respectively.

All of the *S. aureus* isolated were resistant to penicillin and ampicillin and also a high percentage were resistant to erythromycin and tetracycline which is in agreement with report from similar work by 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, 40% to oxacillin by Al-Doughaym *et al*. (1999) where 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.

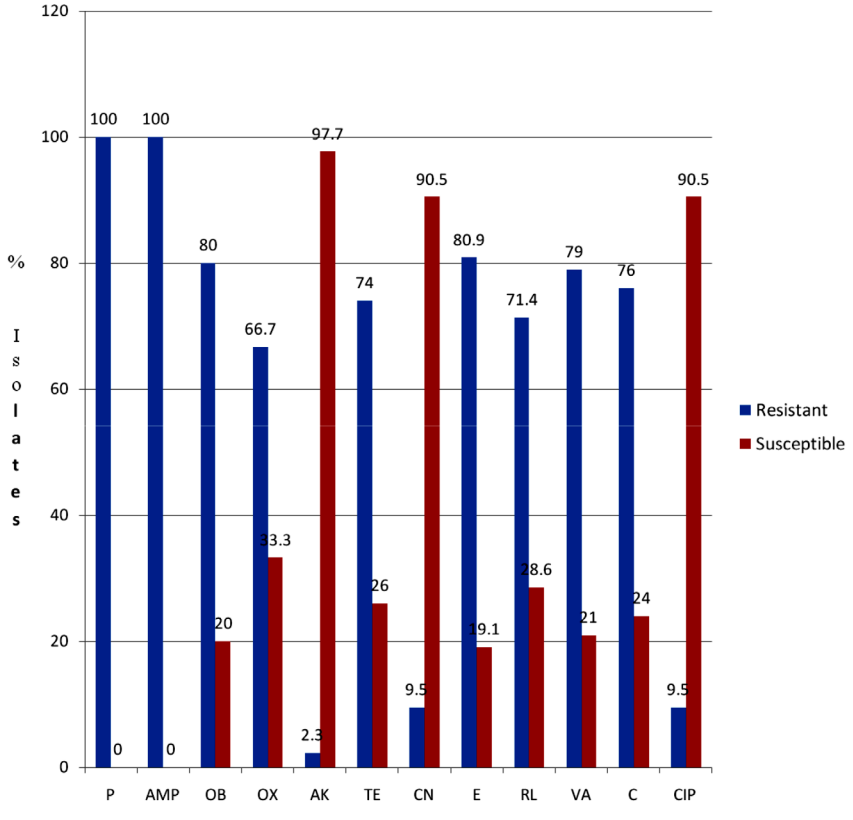

Fig. 1: Percentage distribution of resistance of *S. aureus* to antimicrobial agents

P-Penicillin; OX- Oxacillin; CN- Gentamicin; VA- Vancomycin; AM-Ampicillin; AK-Amikacin; E- Erythromycin; C- Chloramphenicol; OB-Chloxacillin; TE-Tetracycline; RL-Sulphamethoxazole; CIP- Ciprofloxacin.

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 than other animals he sampled from (41.1%).

Table 5: Antibiotic resistance pattern and Multiple Antibiotic Resistance (MAR) Index of 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-Chloxacillin; TE-Tetracycline; RL-Sulphamethoxazole; CIP- Ciprofloxacin.

Table 6: The Result of the Latex Agglutination of the fifteen putative MRSA

Result	Number of Sample	Percentage
Positive	5	33.3 %
Negative	10	66.7%
Total	15	100 %

MRSA and 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 humans, they have recently been isolated from various animal species hence animals have become important reservoirs (Seguin *et al*., 1999; Lee 2003; Van Duijkeren *et al*., 2004).

The in-vitro susceptibility of the 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 they may be less 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 profiles may suggest that similarities exist between these isolates, but further studies are required to prove these similarities in antibiotic profiles.

The presence of MRSA in camels as shown by this study raises public health issues as camels have become a common food animal in 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*., 2006a; 2006b; Olonitola *et al*., 2007). Also, several studies provided compelling epidemiological and microbiological evidence that humans living in close contact with animals are at risk of being colonized or infected with MRSA.

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 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, 28.6% in Kano, Nigeria (Nwanko *et al*., 2010) and various other reports. Hence these varied and high isolation rates of MRSA in Nigeria necessitate urgent action to prevent an epidemic.

Latex agglutination was able to confirm five of the fifteen isolates tested to be positive by detecting the penicillin binding protein 2 (PBP2). The PBP2 is the protein product of the resistance *mecA gene*, which functions as a surrogate transpeptidase in methicillin-resistant *S. aureus* (MRSA) strains (Pinho *et al*., 2001).

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