

Nasal Colonization of Pigs and Farm attendants by *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Kebbi, Northwestern Nigeria

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Abstract

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial, community and livestock-associated colonization and infection worldwide. This study aimed at investigating the nasal colonization of pigs and farm attendants by *S. aureus* and MRSA in Kebbi, North-Western Nigeria. A cross-sectional study was conducted in Kebbi Northwestern Nigeria using simple random sampling. A total of 212 nasal swabs were collected from two farms consisting of 100 samples each from pigs and 12 samples from farm attendants. A well-structured questionnaire was administered for risk factor analysis. Nasal swabs were examined using routine bacteriological culture and identification. Presumptive MRSA was confirmed by PCR assay. Antimicrobial resistance (AMR) profiles of MRSA isolates were evaluated using the disk diffusion method. Among the 212 samples examined, 19.4 % (41/212) of pigs tested positive for MRSA and 1.4% (3/212) of farm attendants were MRSA positive. All the isolates were susceptible to vancomycin, with an AMR index >0.3. The findings of this study indicated nasal colonization of pigs and humans by *S. aureus* and MRSA, thus suggesting that individuals in close contact with animals are at risk of being colonized.

Keywords: MRSA, Nasal Colonization, Kebbi, Pigs

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Introduction

Staphylococcus aureus is an important bacterial pathogen that is also emerging as a potential zoonotic risk. It is a resident flora of the skin and mucous membrane of the nares of humans and animals (Kwoji et al., 2017). The pathogen is responsible for widespread disease conditions ranging from asymptomatic colonization, food poisoning and mastitis, to life-threatening illness such as endocarditis and necrotic pneumonia in humans and animals (Boost et al., 2013). The pathogenicity of these bacteria is linked to a combination of genetic factors mediating virulence, invasiveness, ability to evade the immune system and antibiotic resistance (Chua et al., 2014). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading nosocomial pathogens causing hospital-associated infections in humans worldwide. The ability of these pathogens to thrive in the hospital setting is due to the acquisition of a mobile genetic element that carries resistance genes (*mecA*). This in turn, leads to reduced treatment options, increased healthcare costs, prolonged hospital stays and mortality (Dantes et al., 2013; Bitrus et al., 2016). Development of methicillin resistance occurs via the acquisition of a mobile genetic element carrying the *mecA* or *mecC* gene that codes for an alternative penicillin binding protein with reduced susceptibility to methicillin or oxacillin.

Methicillin-resistant *S. aureus* (MRSA), including the livestock-associated methicillin-resistant strain (LAMRSA), has been isolated from pigs in the Canada and the Netherlands (Khanna et al., 2008; Broens et al., 2011). The risk of Livestock-associated MRSA is most commonly seen in individuals in close contact with livestock rather than the general community. Even though it was previously thought to be restricted to the livestock population, LAMRSA has been reported to be causing infection in the hospital setting and among individuals in the community without prior contact with livestock (Liu et al., 2015; Larsen et al., 2016), suggesting a possible adaptation to infect humans and transmission through the food chain. The emergence of several MRSA clones, coupled with the organism's rapidly changing epidemiology on a major scale has profound clinical implications for the patient, animal husbandry, veterinary and health care workers as new clones harboring different resistance and virulence determinants (Bitrus et al., 2016). There is a dearth of information with regards to the occurrence of MRSA in pigs in Kebbi and its environs and to the best of our knowledge, this is the first report of nasal colonization of pigs and farms attendants by *S. aureus* and MRSA in the northern western part of Nigeria which consists of seven states or provinces including Kebbi, Sokoto, Jigawa, Kano, Zamfara, Katsina and Kaduna.

Materials and Methods

Study Area: Zuru is a Local Government Area in Kebbi State, Nigeria. It lies between 11° 26' 6.79" N latitude and 5° 14' 5.78" E longitude. It is also the Headquarters of Zuru Emirate. Based on the 2006 census, the city has a population of 24,338 people; the predominant tribe includes, the C'lela people, Hausa and Yoruba with Islam and Christianity as the two major religions.

Zuru Local Government Area is an agrarian community. It is endowed with highly fertile soil, vast agricultural land and economically viable rivers sheltered by a fine tropical climate. Agriculture has remained the major source of revenue and indeed the backbone of the economy of the State (Federal Republic of Nigeria, Official gazette, 2009).

Determination of Sample Size: A total of 212 nasal samples were randomly collected from two different farms in Zuru using proportionate sample allocation. The sample size was determined using an estimated prevalence of 46% as reported by Reynaga et al., (2016) in the formula described by Thrusfield, (2010): $N = t^2 * P_{exp} / (1 - P_{exp}) / d^2$.

$$N = 1.96^2 * 0.46 * (1 - 0.46) / 0.025$$

$$N = 200.14$$

Study Design and Sample Collection: A cross-sectional study was conducted using nasal swabs collected from each pig. Information about potential risk factors such as age, sex and antibiotic use were collected. A total of two hundred pigs were randomly sampled from two piggeries in the study area (Farm A=100, Farm B=100). Each of the 100 samples comprised 50 each from both sexes and within each sex 25 were from the young and 25 from adults.

Ethical Clearance: All the sampling procedures were done in accordance with the guidelines of the Animal Research Ethics Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/03/2019).

Bacterial isolation: Nasal swabs were cultured directly on blood agar containing 5% horse blood and incubated aerobically at 37 °C for 24h. Presumptive colonies that characteristically appeared like *Staphylococcus* spp. were preliminarily identified using colony morphology and Gram staining for cellular morphology. Colonies that appeared relatively small, circular, convex, smooth and grayish to white were picked and streaked on a freshly prepared Mannitol Salt agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24h. Two to three presumptive colonies of the *Staphylococcus* species that appeared small, smooth, golden, shiny, convex and with golden yellow zones were picked and sub-cultured in nutrient agar (Oxoid) slant and incubated at 37 °C for 18-24h. *Staphylococcus* isolates were further confirmed biochemically using catalase and coagulase tests as described by Ochei and Kohlhatkar, (2000) and Cheesbrough, (2000). Confirmation of *S. aureus* isolates was done using PCR amplification of the endonuclease (*nuc*) gene fragments specific for *S. aureus*.

Phenotypic characterization of MRSA: Overnight fresh cultures of *S. aureus* were inoculated onto a freshly prepared Oxacillin Resistance Screening Agar Base using an appropriate supplement (ORSAB; Oxoid) to determine phenotypic methicillin resistance. Presumptive colonies on ORSAB that appeared intense

blue in color on a colorless background are phenotypically considered as MRSA.

Antimicrobial susceptibility test: Antimicrobial susceptibility of the isolates to the following antimicrobials erythromycin (E 15 µg), oxytetracycline (OXT 30µg), neomycin (N 5µg), penicillin (P 30 µg), sulphonamide (SUL 23.75 µg), gentamicin (CN 10µg) and vancomycin (VAN 30µg) was determined using the Kirby- Bauer disk diffusion method. Zones of inhibition were recorded and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2013).

Genotypic characterization of MRSA

Genomic DNA extraction: Genomic DNA was extracted using the traditional boiling method as described by Chen *et al.*, (2009) with slight modifications. Briefly, a loop-full suspension of overnight grown cultures on nutrient agar plate was transferred into a 1.5 µL micro-centrifuge tube containing 100 µL of sterile distilled water. The suspension was first incubated at room temperature for 5 mins, and then heat treated to 96 °C for 10 mins. This was then followed by centrifugation at 12,000 X g for 5 mins. The supernatant (containing the DNA) was then collected in a new 1.5 µL tube using a micropipette and kept at 4°C until use.

PCR detection of the *mecA* gene: DNA templates of all the positive ORSAB *S. aureus* isolates were subjected to PCR assay for the detection of the 163 bp fragment of the *mecA* gene using the following primers; *MecA1* 5'-AAAATCGATGGTAAAGTTGGC-3' (forward), *MecA2* 5'AGTTCGTCAGTACCGGATTTGC-3' (reverse) as described by Mehrotra, (2000). The PCR assay was performed in a 25 µL reaction mixture containing 3 µL of nuclease free water, 5 µL (0.5µg) of the DNA template, 1 µL (0.2µM) of each forward and reverse primer, 2.5 µL of coral load and 12.5 µL (100µM) of the master mix (Qiagen). The amplification protocol consisted of 35 cycles of amplification with an initial denaturation of 94°C for 5 mins, denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min and then final extension at 72°C for 7

mins. The PCR products were visualized after electrophoresis for 45 mins at 90 volts in a 1% agarose gel. The amplicons were then viewed under a UV trans-illuminator.

Data analysis: The results obtained are presented in tables and percentages were computed using Microsoft Excel Programme version 2010. Chi-squared statistics was performed using Invivostat v 4.1 to determine the possible association between MRSA infection and some potential risk factors.

Results

Nasal colonization of *S. aureus* and MRSA from pigs and farm attendants: Of the 200 pig nasal swabs examined, 51.5% (103/200) were positive for *Staphylococcus aureus* based on culture, biochemical and molecular characterisation. Farm B had the highest prevalence with 61% (61/100), while Farm A had 42% (42/100). There is a statistically significant association ($P=0.01$) between the recovery rate of *S. aureus* and Farms, with farm B more likely to have *S. aureus* colonization than farm A. Based on age, piglets were likely to be more colonized with *S. aureus* 71% (71/100) than adult pigs 32% (32/100) and the results were statistically significant ($P<0.001$). Similarly, a strong association ($P<0.001$) between sex and colonization with *S. aureus* was observed; sows were found to be more likely to be colonized with *S. aureus* 64% (64/100) than boars 39% (39/100) (Table 1). PCR detection of the *mecA* gene showed that 20.8% (44/ 212) of MRSA isolates from pigs and farm attendants were positive (Figure 1). This comprised 19.4% (41/212) nasal swabs from pigs and 1.4% (3/212) from farm attendants. Farm B had the highest occurrence rate with 26% (26/100) of the pigs being colonized by MRSA, while only 15% (15/100) of the pigs were colonized in Farm A. There was no statistically significant association ($P=0.08$) between the recovery rate of MRSA and farm type (Table 1). The prevalence of MRSA in both farms based on age and sex was 14% (14/100) adult/males and 27% (27/100) young/females respectively (Table 1).

Table 1 Proportion of MRSA from selected pig farms in Zuru, Kebbi

Variables	No. Sampled	Proportion of MRSA	P-Value
Sample Source			
Pigs	200	0.19	
Farm attendants	12	0.014	
Farm type			
A	100	0.15	0.0798
B	100	0.26	
Age			
Young	100	0.14	0.0001
Adult	100	0.27	
Sex			
Female	100	0.14	0.0356
Male	100	0.27	

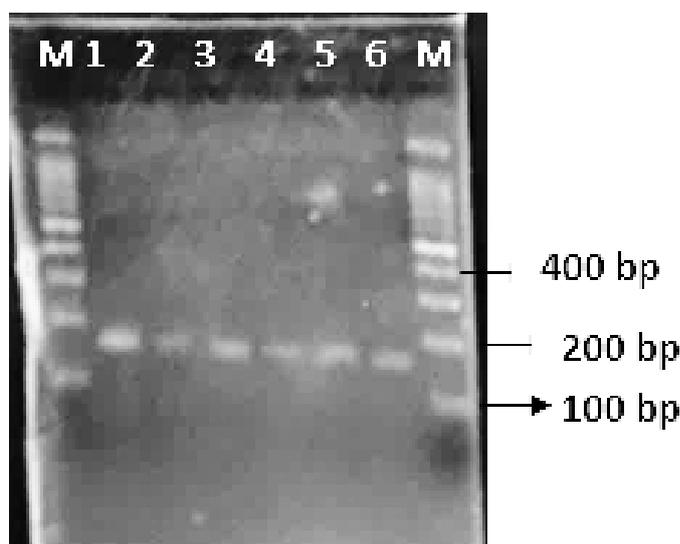


Figure 1 Gel image of 163bp gene fragment of the *mecA* gene using 100bp molecular weight DNA ladder

Multiple Antimicrobial resistance profiles of *S. aureus* and MRSA from pigs and farm attendants:

Antimicrobial susceptibility tests revealed that the ORSAB positive isolates exhibited varying levels of resistance against the antimicrobials tested. Of the 44 MRSA isolates recovered from pig farms, 52% (23/44) were resistant to erythromycin, oxytetracycline, neomycin, sulfonamides and gentamycin, while 55%

(24/44) were resistant to penicillin. All isolates were susceptible to vancomycin (Table 2). A total of 9 isolates were found to show multidrug resistance (MDR) with ERY-OXY-NEO-PEN-SUL-GEN being the most common pattern and the multiple antibiotic resistance index (MARI) showed an index of >0.2 (Table 2).

Table 2 Antibiotic resistant pattern for MRSA isolates from pig farms in Kebbi State.

Antibiotic resistant pattern	Number of isolates	Multiple Antibiotic resistance index
ERY-OXY-PEN-SUL	1	0.60
ERY-OXY-NEO-PEN	1	0.60
ERY-OXY-NEO-SUL-GEN	1	0.71
OXY-NEO-PEN-SUL-GEN	1	0.71
ERY-PEN-SUL-GEN	2	0.60
ERY-OXY-NEO-PEN-GEN	2	0.71
OXY-NEO-PEN-SUL	2	0.60
ERY-OXY-NEO-PEN-SUL	6	0.71
ERY-OXY-NEO-PEN-SUL-GEN	9	0.86

Discussion

Farm animals have been reported to serve as potential sources for the transmission of livestock-associated MRSA to humans (Broens *et al.*, 2011). In this study, nasal carriage of *S. aureus* from pigs was 51.5% (103/200). In other parts of Nigeria, varying levels of nasal carriage of *S. aureus* in pigs have been reported in Enugu (2.8%), Oyo (49.3%) and Imo (59.6%) respectively (Okunlola and Ayandele., 2015; Nsofor *et al.*, 2018; Okorie-Kanu *et al.*, 2020). This can be attributed to the difference in sensitivity of the test methods used and possibly due to unhygienic practice or poor sanitary conditions on the farms. Interestingly, the nasal carriage of MRSA in pigs and humans reported in this study was 19.4%. This is significantly less than the 31.5% and 40% carriage reported in Nsukka and Imo respectively (Ugwu *et al.*, 2015; Nsofor *et al.*, 2018). In contrast, Okorie-kanu *et al.*, (2020) reported a 0.9% nasal carriage of MRSA carriage

in pigs in Enugu. These could also be due to difference in the sensitivity of the detection method, sample size and source and the indiscriminate use of antimicrobials in pig production. Nasal carriage of MRSA based on sex and age, showed that young and female pigs were more colonized than adult and male pigs.

Antibiotic susceptibility testing revealed that all MRSA isolates were susceptible to vancomycin, a glycopeptide. This finding is not surprising because vancomycin is rarely used in the treatment of diseases in livestock in the study area (unpublished data). Suleiman *et al.*, (2012a) and Rodrigues *et al.*, (2017), reported high susceptibility to vancomycin in communities where the drug is not used in food animal production either for routine treatment or chemoprophylaxis.

Resistance to penicillin was also observed in 55% (24/44) of the isolates. Penicillin is the drug of choice in the study area and significant levels of resistance observed in this study was a likely finding. In

agreement with the findings of this study, Anueyiagu and Isiyaku, (2015) and Jahan *et al.*, (2015) reported both 100% resistant *S. aureus* from livestock and raw milk respectively. Also, resistance to oxytetracycline 52% (23/44) was observed. Tetracycline is commonly used as a feed additive and for prophylaxis in pig production in Nigeria (Olatoye, 2010). Resistance to tetracycline is a major problem in veterinary practices in Nigeria and Ethiopia, because significant levels of tetracycline resistance have been reported in MRSA strains from livestock (Usman and Mustapha, 2016; Tessema, 2016). Fifty two percent resistance to erythromycin and gentamycin has also been observed. This could possibly be a reflection of its frequent use in livestock in the study area. Mirzae *et al.*, (2012) and Anueyiagu and Isiyaku, (2015), reported significant levels of resistance to erythromycin and gentamycin in Iran (76%) and Nigeria (85.7%) respectively. Neomycin and sulphonamide resistance were also observed in 52% (23/44) of the isolates. This is baffling because the drug is not routinely used in veterinary practice in Nigeria. Possibly, it was the result of cross-infection between the farm attendants and pigs with resistant pathogens.

Multi-drug resistance is defined as the resistance of an isolate to at least one antimicrobial agent in three or more antimicrobial categories (Olayinka *et al.*, 2010). All 44 MRSA strains isolated from this study showed multidrug resistance. The isolates were resistant to a combination of the four, five and six antibiotics tested. Multi-drug resistance in *S. aureus* may be attributed partly, to the acquisition of resistance determinants domiciled in mobile genetic elements (Bitrus *et al.*, 2018). All Methicillin Resistant *S. aureus* examined in this study had an MAR index of 0.6 and above. The MAR index gives an indirect indication of the probable source of an organism. An organism originates from an environment with high levels of antibiotic use when its MAR index is greater than 0.2 (Furtula *et al.*, 2013). The public health significance of this finding is that antibiotic-resistant strains of *S. aureus* from pigs may be transmitted to humans through occupational exposure or contact.

To date, the correlation between MRSA phenotype and genotype must be considered from the surrogate test for oxacillin and *mecA* gene existence. In this study, all 44 MRSA strains examined carried the *mecA* gene, indicating that the phenotypic resistance exhibited by the strains on ORSAB was due to the possession of the *mecA* gene. This finding differed from the report of Umaru *et al.*, (2013), who detected the *mecA* gene in only four out of the 18 MRSA strains isolated from livestock in Zaria. Our findings also disagreed with the results obtained by Suleiman *et al.*, (2012b), who also reported the presence of the *mecA* gene in only 2 out of the 26 MRSA strains isolated from livestock in Plateau State. Phenotypic expression of resistance to methicillin in MRSA varies and each strain has a characteristic profile of the proportion of bacterial cells that grow at specific concentrations of methicillin (Plata *et al.*, 2013). In conclusion, a positive correlation was found between the phenotypic resistance to methicillin and PCR detection of *mecA*. The antibiotic susceptibility profile of the MRSA strains isolated from pigs showed significant levels of resistance to

penicillin, erythromycin, gentamycin and tetracycline. This finding is of great public health concern because these antibiotics are commonly used in Nigeria either therapeutically in human and veterinary practices or as growth promoters and for prophylaxis in animal production. The findings of this study also supported the assertion that pigs are one of the major vehicles for the transmission of MRSA to man and can serve as baseline data for further studies.

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