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Prevalence and antimicrobial susceptibility of Methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs in selected farms in Ilora, South Western Nigeria

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is an important human pathogen that causes serious infections both in hospitals and communities globally due to its multidrug resistance tendency. This study was undertaken to determine the prevalence of methicillin-resistant S. aureus and antibiotic sensitivity pattern among pigs in Ilora.Nigeria. Two hundred Nasal swab samples were collected from two hundred pigs in eleven farms in Ilora. The samples were subjected to standard microbiological techniques to identify S. aureus. Resistance to Methicillin was obtained by using Oxacillin. A total of 95 isolates of Staphylococcus spp. were recovered representing 47.5% (95/200) of total isolates, 43.2% (41/95) were identified to be S. aureus while MRSA carriage of 43.9% (18/41) was obtained. Male pigs had the highest prevalence of 55.5% (10/18) MRSA isolates and female pigs had prevalence of 44.5% (8/18). The antibiotic susceptibility profiles of the isolates to the commonly used drugs show high resistance to Cloxacillin (100%), Cephalexin (94.4%), Floxapen (88.9%), Augmentin (70%) and Gentamycin (70%). All isolates were susceptible to Ofloxacin and Ciprofloxacin. The prevalence of 43.9% of MRSA amongst pigs in this region calls for urgent intervention because pigs can serve as reservoir through which this multidrug resistant organism can spread to other animals, humans and community at large. Therefore, proper hygiene practices, control of indiscriminate use of antibiotics, and frequent screening of this population for MRSA, are hereby recommended both for prevention and control of livestock acquired MRSA infections.

Keywords: Methicillin-resistance *Staphylococcus aureus* (MRSA), Pigs, antimicrobial, susceptibility, Ilora.

INTRODUCTION

Antibiotic-resistant infections have become explosive issues globally [1]. *Staphylococcus aureus* is a well-adapted opportunistic pathogen often carried asymptomatically with a wide spectrum of diseases in both humans and animals, both in health care system and in the community [2; 3; 4]. Infections caused by this microorganism especially the antibiotic-resistant strains have reached epidemic level globally [2]. This organism has been implicated in diseases like minor skin infections, such as Furunculosis and carbunculosis to severe and highly debilitating diseases such as pneumonia, endocarditis and bacteremia [5; 6]. Ever since antimicrobial therapy was introduced, certain clones of this bacterium have shown ability to gain resistance against almost all classes of antimicrobial agent to which they are exposed [7]. The concern now is Methicillin-resistant *S. aureus* which is a strain of *S. aureus* that is resistant to methicillin or to virtually all available beta-lactam antimicrobials and other antibiotics, very difficult to treat and susceptible only to glycopeptides antibiotics such as Vancomycin and

Teicoplanin but these drugs are also losing their potency these days, while new drugs like Tigecycline, Daptomycin e. t. c. are effective but they are very expensive [8].

This resistance is due to acquisition of the *Mec*A gene [9]. *Mec* A gene encodes penicillin-binding protein 2a which is membrane-bound enzymes that has ability to catalyse the trans-peptidation reaction layer [10]. Methicillin was introduced in 1959 to treat infections caused by Penicillin-resistance *S. aureus* [11] and resistance to methicillin emerged in some strains of *Staphylococcus aureus* in 1960 [10]. MRSA has become a serious matter and MRSA infections has increased greatly in the last three decades and strains defined healthcare-associated MRSA (HA-MRSA) have become endemic in industrialized countries [12]. In the mid-1990s, a major change in epidemiology of MRSA was noticed, with the appearance of cases in the community affecting people having no epidemiological connection with hospital and this is now called community-acquired MRSA (CA-MRSA) [3].The CA-MRSA became not only a threat in the community but also, occasionally in hospital environment globally [13].

CA-MRSA differs from their HA-MRSA counterparts because they have a different accessory genome, which carry different staphylococcal cassette chromosome *mec* (SCCmec) elememts, affect different populations, and cause other clinical symptoms [14; 15and 16]. HA-MRSA strains carry a relatively large staphylococcal chromosomal cassette *mec* (SCCmec) belonging to type I, II, III and VIII. They all contain *mecA* gene signatures. HA-MRSA strains seldom carry the genes for the Panton-valentine leukocidin (PVL). But CA-MRSA strains carry smaller SCCmec elements with type IV, V, VI and VII. They also carry *mecA* gene signature but they are small compare with HA-MRSA and are more mobile [17]. They frequently carry PVL gene [15] but do not carry multiple antibiotic resistance genes [2]. But, it has now becomes even more difficult to distinguish HA-MRSA from CA-MRSA [18], because strains with hospital acquired genetic background enter the community and strains with community-acquired genetic background enter the hospital too [18].

Recently, MRSA has emerged as a frequent colonizer of animal populations which is possibly favoured by the large antibiotic use in animals [12].The first case of MRSA in animal was first reported in 1972, from a case of bovine mastitis, with isolates that were believed to be from human origin [3]. Cases of MRSA have also been reported in pets and horses [19]. An MRSA case was also reported in 2005 for the first time in a pig farmer [20]. MRSA, especially of CC398 lineage was first identified as a zoonosis in the Netherland [21]. Since then, MRSA CC398 has been identified in a number of countries in Europe, North America [22] as well as among pigs in Singapore [23]. MRSA cases have also been reported in African nations like: Tunisia, Libya, South Africa, Botswana, Nigeria and the likes [24; 25; 26; 27; 28; 29; 30 and 31]. Other MRSA lineages can also be found in animal and can be shared between animals and people in closed contact. Most strains in pets seem to be of human origin [4]. There is a potential health risk when people get exposed to animals that have been infected and colonized with this organism. Higher prevalence has been reported among veterinarians and workers associated with animals [32].

No population-based prevalence study has been carried out to determine MRSA carriage among pigs in Ilora, Oyo State, Southwest Nigeria. Therefore, this work was designed to determine the prevalence and nasal carriage of MRSA among pigs in Ilora and their *in vitro* susceptibility pattern to various conventional antibiotics used in the study region.

MATERIALS AND METHODS

This study was carried out in Ilora, Oyo State, Southwest Nigeria. The town is located within the co-ordinates $7^0$48'N and $3^0$54'E in Nigeria.

Sample collection

Two hundred (200) nasal swab samples were collected from pigs from eleven (11) different farms by swabbing both anterior nares with sterile swab sticks. 50 samples were collected from first farm, 13 samples from second, 15 from third, 22 from fourth, 15 from fifth, 9 from sixth, 15 from seventh, 11 from eight, 12 from ninth, 27 from tenth and 11 from eleventh farm. All these samples were obtained at Ilora, Oyo, Southwest Nigeria. The samples were taken to laboratory immediately for culturing.

Isolation of *Staphylococcus aureus*

Samples were cultured inside the test-tubes containing 10ml of 6.5% sodium chloride (NaCl) broth for 24 hours at 37^oC. 0.1ml of 24 hours old culture of 6.5% sodium chloride broth was inoculated into another test tube of nutrient broth containing Cefoxitine (4µg/ml) and Aztreonam (75µg/ml) and incubated for 24 hours at 37^oC. A loopful from this culture was streak on the set plates of Manitol salt agar (MSA) and incubated for 24hours at 37^0C. The characteristic isolates were aseptically isolated, characterized, and identified as *Staphylococcus aureus* by

established microbiological procedures and conventional tests which include: colony morphology (size and pigment), Gram staining, catalase, and coagulase test and Manitol fermenting [33].

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility testing of *Staphylococcus aureus* isolates to Oxacillin

The Oxacillin discs (1µg) used was purchased from Oxoid, UK. Kirby-Bauer disc diffusion technique [33] was used to determine antimicrobial susceptibility profile of all the isolates. Sterile wire loop was used to touch 3-5 well isolated colonies of the test organism and emulsify in 3-4 ml of sterile normal saline. The turbidity of the suspensions were compared with the standard (0.5McFarland standard solution) using a printed card or sheet of paper as background [33]. Sterile swab sticks were dipped into the suspension to pick the isolate. Excess fluid was removed by pressing and rotating the swab against the side of the test tube above the level of the suspension. The surface of Mueller Hinton agar plates were streak with the swab containing the isolates in three directions, rotating the plate approximately 60^0 to ensure even distribution. This was done for all the isolates in two (2) replicates. With the Petri-dishes lid in place, the inoculated plates were allowed to stand for 3-5 minutes for the surface of the agar to dry [33]. Sterile forceps was later used to place the Oxacillin discs on the inoculated plates. The discs were 30mm spaced from each other and 15mm away from the edge of the plate, each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Within 30 minutes of the application of the discs, the plates were inverted and incubated at 35^0C. After 18-24 hours of incubation, the plates were examined, and the diameters of zones of inhibition were measured in millimeter [33]. The diameter of zone of inhibition for Oxacillin was then translated into susceptible, intermediate and resistant categories according to (34).

Susceptibility testing of MRSA isolates to other conventional antibiotics

Multiple disc antibiotics containing; Ofloxacin (5µg), Erythromycin (10µg), Ciprofloxacin (5µg), Gentamycin (10µg), Clindamycin (10µg), Cephalexin (30µg), Cotrimoxazole (50µg), Ampicillin / Cloxacillin (30µg), Floxapen (30µg) and Augmentin (30µg) purchased from FONDOZ Laboratories, Lagos Nigeria were used to determine susceptibility profile of all the MRSA isolates to other antibiotics used in the study area. The same Kirby-Bauer disc diffusion technique [33] used for Oxacillin was also used to determine antimicrobial susceptibility profile of all the isolates. After 18-24 hours of incubation, the plates were examined, and the diameters of zones of inhibition were measured in millimeter. The diameter of zone of inhibition for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to (34).

RESULTS AND DISCUSSION

Out of the two hundred Nasal swab samples collected from two hundred pigs in eleven farms in Ilora, a total of 95 isolates of *Staphylococcus* spp. were recovered representing 47.5% (95/200). 43.2% (41/95) were identified to be *Staphylococcus aureus*. 43.9% (18/41) of the isolated *S. aureus* were MRSA, 51.2% (21/41) were methicillin sensitive *S. aureus* (MSSA) while 4.9% (2/41) were methicillin intermediate *S. aureus* (MISA) (Figure1). Male pigs had the highest prevalence of 55.5% (10/18) MRSA isolates and female pigs had prevalence of 44.5% (8/18) (Figure 2). The antibiotic susceptibility profiles of the isolates to the commonly used antibiotics show high resistance to Cloxacillin (100%), Cephalexin (94.4%), Floxapen (88.9%), Augmentin (70%) and Gentamycin (70%) and all the isolates were susceptible to Ofloxacin and Ciprofloxacin (Table 1and Figure3). Table 2 shows the prevalence of MRSA isolates by sources with highest prevalence (23.1%) at PGF2 while no occurrence observed from the samples collected from PGF7, 8, 9 &11 representing 0%. Also, multidrug resistance to commonly used antibiotics was observed from all the MRSA isolates with isolate PGF5-112 showing the least (Table 3).

S. aureus is an important opportunistic pathogen, colonizing humans and animals. MRSA has been reported in various animals and livestock farmers [31]. The prevalence of MRSA among pigs in this study was 43.9% which was lower than results obtained in Netherlands (80%) in 2005; Spain (83%) in 2008 and 85.7% in 2010 in Spain [35]. The MRSA carriage among pigs in this research is similar to [36] and [37] who reported carriage of MRSA to be 46% and 40% in Demark and Thailand respectively. Lower MRSA carriage has been reported among pigs in Switzerland (2%) [38]; also in Darker (1.3%) and USA (Connecticut) (3%) [39; 40]. This difference could be as a result of antibiotics being used, poor hygiene and exposure of the pig to the reservoirs e.g. rodents. The source of acquisition of MRSA in this study was not known but it might be due to contact with animal carrier or humans. The isolation of MRSA among these pigs may constitute a serious threat to public health and healthcare system. According to (41), colonization of animals and man constitute a reservoir and potential source of MRSA infection. Since molecular analysis of the MRSA isolates in the study was not carried out, it is difficult to establish the origin of the colonizing type which would have given insight as to whether it was livestock associated MRSA or the human type and therefore the likely route of transmission.

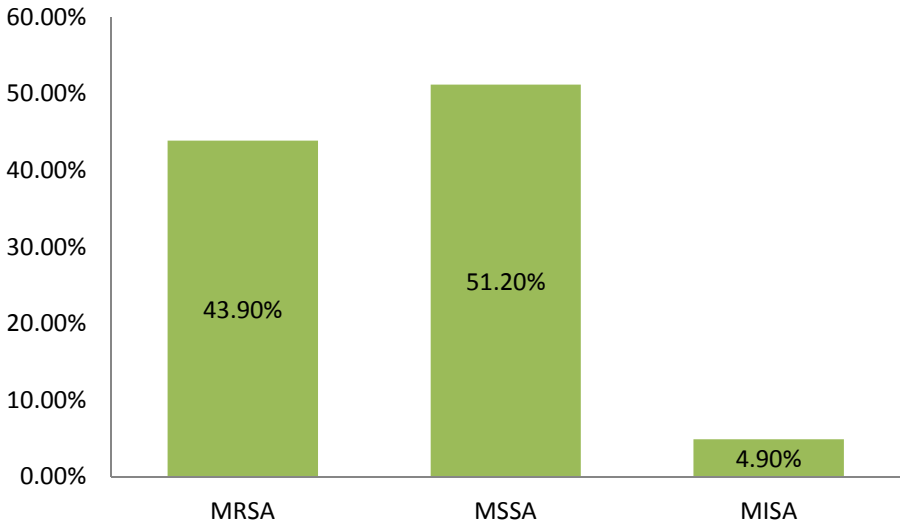

Figure 1: Prevalence of MRSA, MSSA and MISA of nasal samples of Pig in Ilora, Oyo State.
MRSA= Methiciline resistance S. aureus. MSSA= Methiciline sensitive S. aureus.
MISA= Methiciline intermediate S. aureus.

Another notable finding is that from pig population sampled, higher prevalence 10/18 (55.5%) was discovered among male pigs compare with 5/18 (44.5%) discovered among female swine, though the possible reasons for this are not known

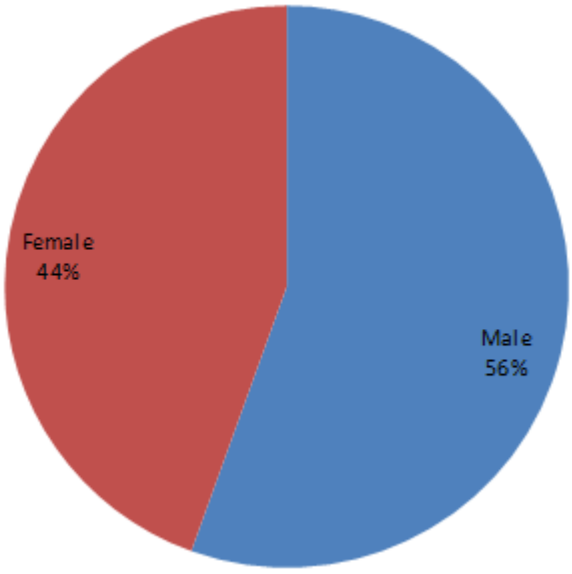

Figure 2: Distribution of isolated MRSA by sex of pigs samples from Ilora, Oyo State

The antimicrobial susceptibility profile of the isolates to commonly used antibiotics show varying resistance. The highest resistance was recorded against Ampicillin/Cloxacillin (100%), Cephalexin (94.4%) and Floxapen (89%). There was no resistance against Ofloxacin and Ciprofloxacin. The high sensitivity of Ofloxacin (100%) and Ciprofloxacin (100%) in this study is closely related to the finding of [8] who reported 78.6% for Ofloxacin and 88.1% for Ciprofloxacin. The high sensitivity of the isolates to Ofloxacin, and Ciprofloxacin indicate that, they are good antibiotics for the treatment of MRSA infection in this environment. Antibiotics used in animals for therapeutics, food production and diseases prevention promote antibiotic resistance [42] and this may be the reason for multidrug resistance observed in all the isolates in this study.

Herd size has also been reported to be associated with the prevalence of MRSA [35]. Though the results obtained in this study showed varying prevalence among the pigs farms used in this study. But PGF1 where the largest samples (50) were collected gave second highest prevalence result of 14%. Larger herds appeared more likely to be MRSA – positive compared to smaller herds, due to a higher probability of persistence in larger herds (within-herd dynamic) [35]. Apart from direct contact as mode of transmission, airborne transmission is also a possible transmission route

of MRSA between humans and has been reported in the hospitals [43]. Therefore, it is likely to occur between pigs within a farm. Moreover, airborne transmission might play a role in dissemination of MRSA between herds in close proximity to each other. Antimicrobial resistant *S. aureus* has been recovered outside pig facilities to at least 150m downwind [44]. This finding is also observed in this study from three of the farms used, sources PGF1, PGF2, and PGF3 are very close, in fact the proximity is less than 100m apart and the three farms have prevalence of 14.0%, 23.1%, and 13.3% of MRSA cases respectively.

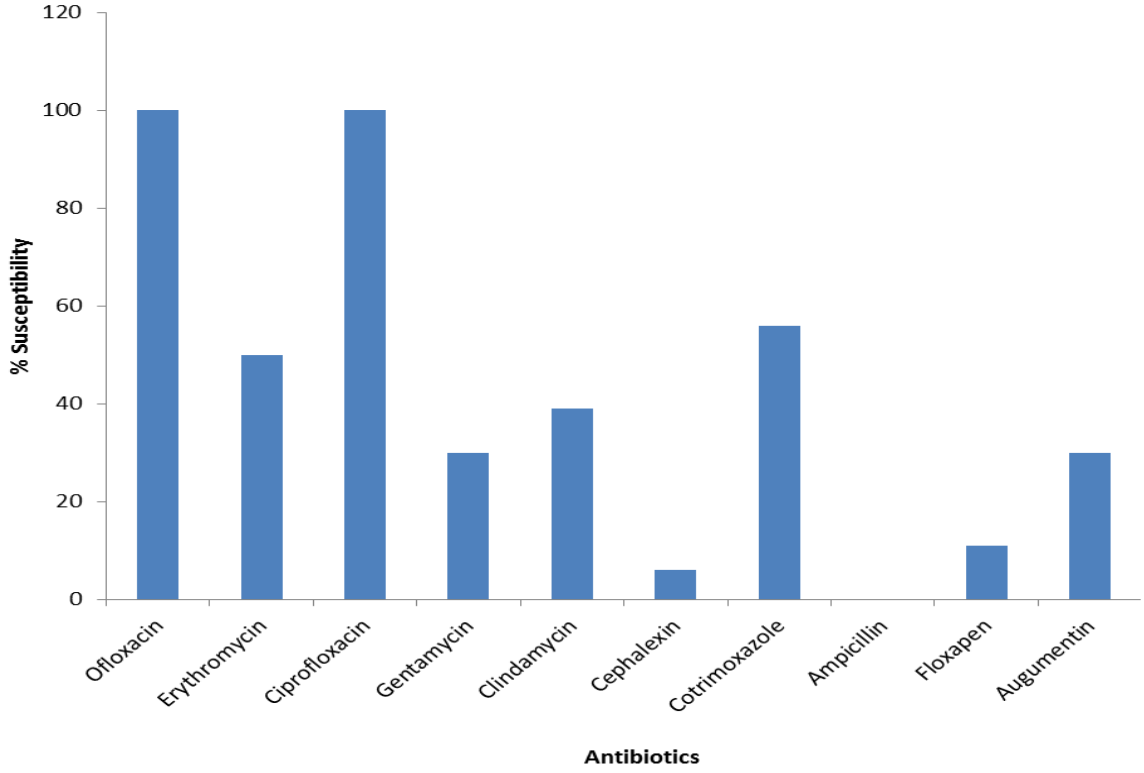

Figure 3: Antibiotic susceptibility pattern of isolated MRSA among pigs from Ilora, Oyo State

Table 1: Antibiotic susceptibility profiles of MRSA isolates

Drugs	Disc Potency (µg)	No of Resistant Strain (%)
Ofloxacin (OF)	5	0 (0)
Erythromycin (E)	10	9 (50)
Ciprofloxacin (CIP)	5	0 (0)
Gentamycin (GN)	10	14 (78)
Clindamycin (CD)	10	11 (61)
Cephalexin (CX)	30	17 (94)
Cotrimoxazole (CO)	50	8 (44)
Ampicillin/Cloxacillin (AP)	30	18 (100)
Floxapen (FX)	30	16 (89)
Augumentin (AU)	30	14 (78)

Table 2: Distribution of MRSA isolates by sources

Source	MRSA No (%)	Cumulative Frequency
PGF1	7/50 (14.0%)	50
PGF2	3/13 (23.1%)	63
PGF3	2/15 (13.3%)	78
PGF4	2/22 (9.1%)	100
PGF5	1/15 (6.7%)	115
PGF6	1/9 (11.1%)	124
PGF7	0/15 (0.0%)	139
PGF8	0/11 (0.0%)	150
PGF9	0/12 (0.0%)	162
PGF10	2/27 (7.4%)	189
PGF11	0/11 (0.0%)	200

PGF Means Pig farm.

Table 3: Phenotypic pattern of drug resistance among the MRSA isolates

Isolate	Resistance Pattern
PGF3-73	Ery Gen Clin Ceph Cot Amp/Clo Flo Aug
PGF4-88	Ery Gen Clin Ceph Cot Amp/Clo Flo Aug
PGF1-18	Ery Gen Ceph Cot Amp/Clo Flo Aug
PGF2-55	Ery Gen Clin Ceph Cot Amp/Clo Flo
PGF10-172	Ery Gen Clin Ceph Amp/Clo Flo Aug
PGF1-16	Gen Ceph Cot Amp/Clo Flo Aug
PGF1-17	Gen Ceph Cot Amp/Clo Flo Aug
PGF1-26	Ery Gen Clin Ceph Amp/Clo Aug
PGF1-50	Gen Clin Ceph Cot Amp/Clo Flo
PGF2-60	Ery Gen Clin Ceph Amp/Clo Flo
PGF2-62	Ery Gen Clin Ceph Amp/Clo Flo
PGF3-72	Ery Gen Clin Ceph Amp/Clo Flo
PGF10-177	Gen Ceph Cot Amp/Clo Flo Aug
PGF6-119	Ceph Cot Amp/Clo Flo Aug
PGF1-22	Ceph Amp/Clo Flo Aug
PGF1-29	Ceph Amp/Clo Flo Aug
PGF4-81	Ery Gen Clin Amp/Clo
PGF5-112	Ceph Amp/Clo Flo

Ery Erythromycin, Gen Gentamycin, Clin Clindamycin, Ceph Cephalexin, Cot Cotimoxazole, Amp/Clo Ampicilin/Cloxacillin, Flo Floxapen, Aug Augumentin

CONCLUSION

The data from this study showed that MRSA is present in Ilora and pigs can serve as reservoir of this multidrug resistance organism. Therefore, this study is a preamble to enable epidemiologists to understand the nature of MRSA isolates in this part of Nigeria. The rate of spread of this pathogen and its unique ability to acquire and transfer antibiotic resistance calls for urgent and well-coordinated surveillance programme to combat this situation.

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