Methicillin-Resistant *Staphylococcus aureus* Colonization in Egg – laying Chickens and Poultry Workers in Selected Farms in Odeda Local Government Area, Ogun-state, Nigeria

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a growing public health challenge. Although MRSA has been detected in pigs and other livestock in Nigeria, there are few reports on the prevalence and public health implications of MRSA in chickens. This study was conducted to determine the prevalence and antibiotic resistance patterns of Methicillin-resistant *Staphylococcus aureus* in selected poultry farms in Odeda local government area of Ogun state. Nasal and cloacal swab samples were collected from one hundred and fifty egg-laying birds from ten poultry farms within the local government area. Swab samples were also collected from nasal and hands of twenty-five poultry workers. All samples were screened for the presence of MRSA. Antibiotic susceptibility patterns of the MRSA isolates was determined using disc diffusion method. *Staphylococcus aureus* was isolated from 86 (57.3%) of the birds, and 19 (76%) of the farm workers. The prevalence of MRSA in layers and workers were 16.0% and 20.0% respectively. The prevalence of MRSA in the layers in different farms ranged from 0% to 33.3%. The MRSA isolates showed high level of resistance to oxacillin (100%), cefuroxime (79%), streptomycin (72%), erythromycin (72%), amoxicillin (72%), gentamycin (65%) and tetracycline (62%). The study further revealed the irrational usage of antimicrobials and poor hygienic practices in most of the poultry farms. The presence of multi-drug resistant MRSA in poultry farms therefore poses a risk not only to the birds, but also the farm workers, consumers and community.
Keywords: Antibiotic resistance, egg-laying birds, MRSA, poultry workers, prevalence

INTRODUCTION

Staphylococcus aureus is a versatile and opportunistic pathogen commonly found in people, community and health care settings (Michael *et al*., 2016). This pathogen causes a wide range of diseases in humans from minor skin infections to severe illness such as toxic shock and septicaemia (Paterson *et al*., 2014). This organism has the remarkable ability to acquire antibiotic resistance determinants, leading to the rapid emergence of Methicillin-resistant *Staphylococcus aureus* strains.

Methicillin-resistant *S. aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to methicillin or to virtually all available beta-lactam antimicrobials. It is a leading cause of nosocomial infection, causing a variety of life-threatening syndromes such as bacteremia, endocarditis, wound infections and pneumonia (Chambers, 2001). For many years, MRSA infections were acquired in hospitals and other healthcare facilities; however, more recently new MRSA strains have emerged in the community, causing infections in patients without previous healthcare contact (David and Daum, 2010). These strains, designated as community acquired (CA)-MRSA, are mainly responsible for skin and soft tissue infections, although deep-seated infections such as necrotizing pneumonia, sepsis and meningitis, have also been reported (Deleo *et al*., 2010; Monaco *et al*., 2013).

Although *Staphylococcus aureus* is a commensal of several mammalian species, the emergence of MRSA clone colonizing pigs and other farm animals (cattle and poultry) has been reported in some countries and there are concerns about the risks of animal populations as potential reservoirs of zoonotic MRSA infections (Voss *et al*., 2005; Hasman *et al*., 2010; Monaco *et al*., 2013; Smith *et al*., 2013; Nworie *et al*., 2017). These strains, known as livestock-associated (LA)-MRSA have been found in different countries worldwide including the US, Germany, Denmark and Netherlands (Hetem *et al*., 2013; Monaco *et al*., 2013), causing deadly infections in poultry and several other animals; and therefore, it is globally considered as a serious health concern (Goetghebeur *et al*., 2007; Zaheer *et al*., 2017). These strains have also been reported to be able to colonize persons working in close contact with pigs, such as farmers and veterinarians.

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Infections caused by LA-MRSA include endocarditis, osteomyelitis and ventilator-associated pneumonia (Hetem *et al*., 2013).

Epidemiological studies in several countries indicated the spread of LA-MRSA into hospitals particularly in individuals with frequent animal contact (Paterson *et al*., 2012). Voss *et al*. (2005) reported 23% of pig farmers from a pig farm in the Netherlands were colonized with MRSA while VanRijen *et al*. (2008) found 32% of farm workers were colonized with MRSA. Similarly, Stein (2009) conducted a study among pig farmers in North America and found colonization rates of 20% while Smith *et al*. (2013) obtained 20.9% among farm workers in swine farms in the USA. Similar epidemiological study conducted by Huang *et al*. (2014) on the LA-MRSA colonization among swine workers in Romania reported the prevalence of 6.8% while Mascaro *et al.* (2018) found 7.3% of swine workers in Italy were colonized with LA-MRSA, and therefore, alleged that LA-MRSA is strongly animal-exposure related. These results revealed the chances of animals becoming reservoirs of human MRSA infections regardless of location (Feingold *et al*., 2012).

Previous studies on the prevalence of MRSA in Nigeria have focused largely on clinical environment and large livestock animals. Poultry farming remains one of the largest and growing livestock farming in Nigeria with many small-scale producers in close proximity to households, thus raising concerns about poultry and human health. This study was undertaken to investigate the prevalence of MRSA colonization in egg-laying chickens and farm workers in selected poultry farms in Odeda Local Government Area, Ogun State, Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in Odeda Local Government Area (coordinates: 7º9'39"N 3º20'54"), Ogun state, Southwest Nigeria. Ten commercial poultry farms, situated at different locations, within this local government area were randomly selected. Official consents were obtained from the Directors of the poultry farms and informed consents from the poultry workers willing to participate. Information on the demographic characteristics and hygiene of the workers was collected through semi-structured questionnaires.

Collection of Samples

Using sterile swabs (Sterilin Ltd, UK) moistened in sterile peptone water, samples from nasal region and cloacae were collected from fifteen egg-laying birds in each farm. To collect the swab samples from the cloacae, the entire head of the swab was inserted into the cloaca of each bird, rotated gently for three times and then removed. The swab was shaken off to remove pieces of faeces. Swab samples were also collected from nostrils and hands of the poultry workers. Each swab was inoculated into 5.0 ml sterile nutrient broth supplemented with 6.5% Sodium chloride, transported in an ice-pack to the laboratory and incubated for 24 hours at 35ºC.

Isolation and identification of *Staphylococcus aureus*

After incubation, a loopful of the nutrient broth was streaked onto Mannitol salt agar plates and incubated at 35ºC for 48 hours. The bacterial isolates that produced colonies exhibiting deep yellow colouration were selected and characterized as *S. aureus* by Gram staining, and biochemical tests (tube coagulase test, DNAse test, urease test, methyl red test, oxidase test, catalase test and sugars fermentation test). The hemolytic activities of the isolates were determined on standard blood agar plates.

Identification of Methicillin-Resistant *Staphylococcus aureus*

The *S. aureus* isolates were further screened for their methicillin resistance capability by Kirby Bauer disc diffusion method on Mueller-Hinton agar using 1.0 µg oxacillin discs (Oxoid, UK) as described by Petinaki *et al*. (2001) and El-Gayar *et al*. (2014) with little modifications. Briefly, an overnight culture of each *S. aureus* isolate grown in nutrient broth at 35^{o}C was standardized to a turbidity equivalent to 0.5 McFarland standard (1.5×10^{8} cfu/ml). Then, 10.0 µl was spread on Mueller-Hinton agar plates containing 4% sodium chloride. Oxacillin discs were placed on seeded plates and the plates were incubated at 35^{o}C for 24 hours. Zones of inhibition were measured after 24 hours of incubation and a diameter of ≤ 10 mm was considered as resistant for oxacillin according to the Clinical and Laboratory Standards Institute (CLSI, 2017). The MRSA isolates were further confirmed on Mannitol salt agar supplemented with 2.0 mg/L of oxacillin. The isolates that exhibited resistance to oxacillin were considered as MRSA.

Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility of the MRSA isolates was determined by the Kirby-Bauer disc diffusion method. An overnight broth culture of each isolate grown in nutrient broth at 37ºC was standardized to 0.5 McFarland's turbidity. The standardized broth cultures were inoculated onto sterile Mueller-Hinton agar plates using sterile swab sticks. Antibiotic-impregnated discs were placed on seeded plates and the plates were incubated at 35ºC for 24 hours. The antibacterial agents tested included: Streptomycin (15µg), Gentamycin (10µg), Kanamycin (30µg), Cefuroxime (30µg), Ceftriaxone (30µg), Levofloxacin (5µg), Amoxycillin (30µg), Erythromycin (15µg), Tetracycline (30µg), Ciprofloxacin (5µg) and Oxacillin (1µg). Zones of inhibition were measured in millilitre (mm) and recorded after 24 hours of incubation. The isolates were classified as resistant (R), intermediate-susceptible (I) or susceptible (S) using standard recommendations of Clinical and Laboratory Standards Institute (CLSI, 2017).

RESULTS

Demographic and occupational characteristics of poultry workers

Table 1 shows demographic and occupational characteristics of participating poultry workers. Twenty-five poultry workers participated in this study. The average age of the participating workers was 33 years (range: 20–50 years). Majority of the participants were male (80%) and 60% are married. All the participating workers had received a level of formal education, 60% had tertiary education, 20% had secondary education while the remaining 20% had primary education.

Prevalence of *Staphylococcus aureus* and MRSA in egg-laying chickens and poultry workers

The results obtained showed that 24 of the 150 egg-laying birds and 5 of the 25 poultry workers harboured oxacillin-resistant *Staphylococcus aureus* revealing an MRSA prevalence of 16.0% and 20.0% respectively (Table 2). From a total of 150 layers, *Staphylococcus aureus* was obtained from the cloacae and nostrils of 86 (57.3%) birds. Out of these birds, 41.9% (36/86) were positive only in the nostrils, 37.2% (32/86) of the birds were positive only in the cloacae while 20.9% (18/86) were positive in both the cloacae and nostrils (Table 3). Similarly, from a total of 25 poultry workers, *Staphylococcus aureus* was found in the nostrils and hands of 19 (76%) workers. *S. aureus* was obtained in both the hands and nostrils of 9 (47.4%) of the *S. aureus*-colonized workers, 6 (31.6%) of the colonized workers were positive only in the hands and 4 (21.0%) of the colonized workers were positive only in the nostrils (Table 3). In addition, the study also showed that the percentage prevalence of *S. aureus* and MRSA among the layers varied between farms. The prevalence of *S. aureus* varied from 33.3% to 86.7% and from 0% to 33.3% for MRSA (Table 4). MRSA was not isolated in any egg-laying bird samples in one of the farms studied which indicated that MRSA is absent or present only in low numbers in that farm. Moreover, the high prevalence of *Staphylococcus aureus* and MRSA observed in this study could be attributed to the hygienic practices employed in these poultry farms as shown in Table 5.

Hygiene Practices among Farm Workers

Sixty percent (60%) of the farm workers change their work coat daily. In addition, high prevalence of MRSA was found among farm workers who never use protective mask and hand gloves (Table 6). Hence, the results showed that working in a poultry farm without protective mask and hand gloves could be the greatest risk factor for MRSA colonization.

Table 1: Demographic and occupational characteristics of participating poultry workers

Parameters	Frequency (%) n=25
Age (years)	
<20	0 (0)
20-30	10 (40)
31-40	10 (40)
41-50	5 (20)
>50	0 (0)
Sex	
Male	20 (80)
Female	5 (20)
Marital status	
Single	10 (40)
Married	15 (60)
Divorced	0 (0)
Widowed	0 (0)
Educational level	
Primary	5 (20)
Secondary	5 (20)
Tertiary	15 (60)

Table 2: Percentage prevalence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* among egg-laying chickens and poultry workers

Sample	No. of *S. aureus* (%)	No. of MRSA (%)	No. of MSSA (%)
Egg-laying chickens (n = 150)	86 (57.3)	24 (16.0)	62 (41.3)
Poultry workers (n = 25)	19 (76.0)	5 (20.0)	14 (56.0)
Total (%)	**105**	**29 (27.6)**	**76 (72.4)**

Key: MRSA: Methicillin-resistant *S. aureus* MSSA: Methicillin-sensitive *S. aureus*

Table 3: Percentage prevalence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* colonization among egg-laying chickens and poultry workers

Sample		Frequency (%) of *S. aureus* colonization	Frequency (%) of MRSA colonization	Frequency (%) of MSSA colonization
Egg-laying chickens (n = 150)	Nostrils	36	5	31
	Cloacae	32	10	22
	Nostrils + Cloacae	18	9	9
		86 (57.3)	24 (16.0)	62 (41.3)
Workers (n = 25)	Hands	6	1	5
	Nostrils	9	3	6
	Hands + Nostrils	4	1	3
		19 (76.0)	5 (20.0)	14 (56.0)
Total (%)		**105**	**29 (27.6)**	**76 (72.4)**

Table 4: Percentage prevalence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* in selected poultry farms in Odeda local government area, Abeokuta

Farm (n=15 per farm)	No. of *S. aureus*-positive layers (%)	No. of MRSA-positive layers (%)
A	6 (40.0)	2 (13.3)
B	13 (86.7)	5 (33.3)
C	12 (80.0)	4 (26.7)
D	9 (60.0)	3 (20.0)
E	8 (53.3)	2 (13.3)
F	6 (40.0)	2 (13.3)
G	11 (73.3)	2 (13.3)
H	5 (33.3)	0 (0.0)
I	7 (46.7)	2 (13.3)
J	9 (60.0)	2 (13.3)
Total (%)	**86 (57.3)**	**24 (16.0)**

Table 5: Hygienic practices in selected poultry farms

Farm	Hygienic practices employed				
	Entry rate of non-farm workers	**Fumigation practices**	**Sanitary agents**	**Antibiotics usage**	**Litter disposal**
A	Never	Weekly	Disinfectants	Once in 3 months	Weekly
B	Weekly	Once in 6 months	Soap	Monthly	Monthly
C	Monthly	Once in 6 months	Soap	Monthly	Monthly
D	Weekly	Once in 6 months	Soap	Once in 3 months	Monthly
E	Weekly	Monthly	Disinfectants	Monthly	Monthly
F	Monthly	Weekly	Disinfectants	Monthly	Weekly
G	Weekly	Once in 6 months	Soap	Monthly	Monthly
H	Never	Weekly	Disinfectants	Once in 3 months	Weekly
I	Monthly	Weekly	Disinfectants	Once in 3 months	Weekly
J	Monthly	Monthly	Soap	Monthly	Monthly

Table 6: Hygiene Practices among Farm Workers

Parameters	Frequency (%) n=25	MRSA positive (%) n = 5
Use of hand gloves		
Always	3 (12)	0 (0)
Sometimes	7 (28)	1 (20)
Never	15 (60)	4 (80)
Use of protective mask		
Always	5 (20)	0 (0)
Sometimes	8 (32)	1 (20)
Never	12 (48)	4 (80)
Change of work coat		
Daily	15 (60)	0 (0)
Weekly	10 (40)	5 (100)
Monthly	0 (0)	0 (0)

Antibiotic resistance pattern of the MRSA

The resistance pattern of MRSA isolates to the tested antibiotics is shown in Figure 1. The results indicated that most of the MRSA isolates showed high level of resistance to oxacillin (100%), cefuroxime (79%), streptomycin (72%), erythromycin (72%), amoxicillin (72%), gentamycin (65%) and tetracycline (62%).

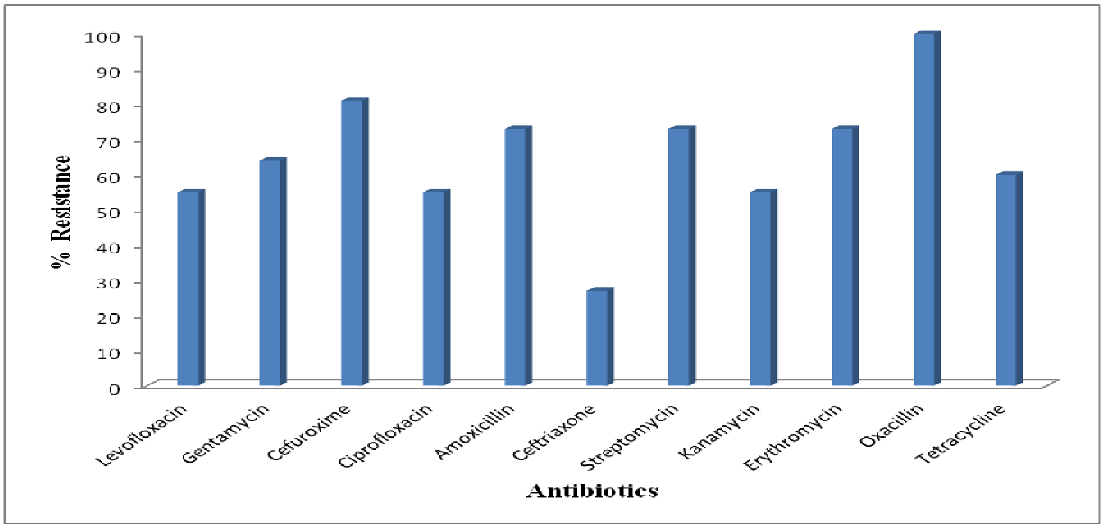

Figure 1: Antibiotics resistance profile of MRSA isolates

DISCUSSION

In the present study, high prevalence of MRSA (16.0%) was obtained among the egg-laying poultry birds in 90% of the farms sampled in Odeda local government area of Ogun state, predisposing the poultry workers in these farms to MRSA colonization. These MRSA isolates were obtained from the nostrils and cloacae of the layers, indicating that the cloacae and nostrils of poultry birds are important sites for the colonization of *Staphylococcus aureus* and MRSA. In a study of 900 layers from 9 poultry farms in Ebonyi state, Nigeria; Nworie *et al*. (2017) obtained a relatively lower prevalence of MRSA (0.4%). Lower prevalence of MRSA in poultry farms have also been reported by Persoons *et al*. (2009) and Friese *et al*. (2013). In the MRSA-positive farms, the number of MRSA-positive layers varied between 13.3% and 26.7%. Higher MRSA prevalence obtained in these farms could be attributed to the poor hygienic practices of these farms coupled with frequent use of antibiotic and farming operations employed in these farms as reported by Dahms *et al*. (2014). Antibiotic use in livestock farms may be a risk factor for MRSA colonization of animals. In addition, MRSA was not isolated from any egg-laying bird samples in farm H and this may indicate that MRSA is absent or present only in low numbers in layers in this farm, possibly because of the limited use of antimicrobial drugs and good hygienic practices employed in this farm. Similar observations were reported by Persoons *et al*. (2009).

In the same vein, the study revealed that a total of 5 of 25 poultry workers (20%) were positive for MRSA and these workers worked on poultry farms where MRSA was detected among the sampled layers. Similar MRSA prevalence (20%) was observed among swine farm workers in Ontario by Khanna *et al*. (2008) and 20.9% in the USA by Smith *et al*. (2013). Similarly, there was also a significant association between MRSA carriage among the poultry workers and the occupational characteristics of the workers as majority of the workers where MRSA was found had neither worn protective mask nor hand gloves. These workers also changed their work coat weekly. Hence, working in a poultry farm with MRSA-positive poultry birds without protective mask and hand gloves could be the greatest risk factor for worker's colonization. The study also demonstrated that people working in close contact with animals colonized with MRSA or their faecal materials have a high risk of MRSA colonization. Similar observations were reported by Oke and Oke (2013) and Smith *et al*. (2013). This observation was earlier reported by Johnson (2011) who stated that there was an increasing transmission of MRSA from colonized pigs to farm workers, abattoir workers and veterinarians who are in close contact with such animals in Europe and that the isolation of MRSA amongst these occupational groups constitutes a serious threat to public health and healthcare system.

Antibiotic susceptibility testing of the MRSA isolates revealed that the isolates exhibited varying degrees of resistance to the tested All the MRSA isolates were resistant to oxacillin, 72% of the MRSA isolates were resistant to erythromycin, streptomycin and amoxicillin while 62% were resistant to tetracycline. Tetracycline and other antibiotics like erythromycin, chloramphenicol and quinolones are commonly used as food supplements, growth promoters and prophylactics in most poultry farms in Nigeria without prescription which could contribute to the high levels of resistance observed in this study. The results corroborate the previous studies of Persoons *et al*. (2009) and Nworie *et al*. (2017) who reported high degrees of antibiotic resistance among MRSA strains isolated from poultry birds.

Finally, in order to avoid MRSA colonization and transmission in poultry farms, rational usage of antimicrobials and good hygienic practices should be adopted in the farms. Also, poultry workers should be properly educated on the handling of the chickens and wearing of protective masks and gloves. The infected poultry chickens should also be segregated and their litters should be properly disposed.

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antibiotics.

CONCLUSION

The study confirmed egg-laying chickens as reservoirs of Methicillin-resistant *S. aureus* in some poultry farms in Odeda local government area of Ogun state, which could be spread to the workers having physical contacts with the chickens and their litters. Inappropriate use of antimicrobial feed additives and antibiotics as well as hygienic practices employed in these farms could be among the factors which contribute to the colonization of MRSA on these chickens. Therefore, preventive measures need to be adopted in poultry farms in order to prevent and control the spread of zoonotic MRSA infections.

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