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MULTI-DRUG RESISTANT STAPHYLOCOCCUS *AUREUS* FROM POULTRY FARMS IN EBONYI STATE, NIGERIA

Amos Nworie[1], Michael O. Elom[1], Ibiam A. Gideon[1], Simon O. Azi[1], Simon I. Okekpa[1], Boniface N. Ukwah[1], Victor U. Usanga[1], Umoh N. Okon[1], Ezeruigbo Chinwe[2], Busayo O. Olayinka[3], Josiah A. Onaolapo[3] and Joseph O. Ehinmidu[3].

1. Department of Medical Laboratory Science, Faculty of Health Sciences, Ebony State University Abakaliki, Nigeria.
2. Department of Nursing Science, Faculty of Health Sciences, Ebonyi State University.
3. Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Author Name: Amos Nworie. Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria.nworieamos@yahoo.com.

ABSTRACT: *Over the years, methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a nosocomial and community pathogen worldwide, causing a plethora of diseases. A distinctive type of MRSA has also emerged in livestock and companion animals. Its isolation in chicken has been reported in some countries and its propensity for zoonotic transmission potentially represents a serious risk-factor for poultry farm workers and the general population. Nasal and cloacae swabs of chickens selected at random from 9 poultry farms and clinical isolates of staphylococci from Ebonyi State, Nigeria, were collected and screened for S. aureus using standard microbiological procedures. Antibiotic susceptibility pattern of the S. aureus to a panel of 14 commonly used and regulated antibiotics in the area were determined using the Kirby-Bauer disc agar diffusion (DAD) method according to the Clinical Laboratory Standards Institute (CLSI). Out of a total of 325 S. aureus isolated, 76% (247/325) were from poultry, while 24% (78/325) were from the clinics. The prevalence rates of S. aureus in broilers and layers were 49% and 51% respectively. The percentage carriage of MRSA in poultry was 6.1% and 15.3% in the clinics. The percentage of isolates showing multi antibiotic resistance index (MARI) of 0.3 and above was 13.97% displaying 46 antibiotic resistance patterns. All the methicillin-resistant S. aureus (MRSA) were multidrug resistant. This underscores the need for discretion in the application of antibiotics in animal feeds and its empirical use in the hospitals.*

KEYWORDS: Staphylococcus Aureus, Mrsa, Multidrug-Resistance-Index, Poultry, Antibiotic Resistance, Isolates.

INTRODUCTION

The emergence of pathogenic bacteria resistant to virtually all known antibiotics has become a global problem. The introduction of antibiotics into clinical medicine many decades ago, marked a new era in preventive and curative medicine. However, after the introduction of methicillin in the late 50s, a resistant strain of *Staphylococcus aureus* to this antibiotic emerged (Grundmann *et al.*, 2002) which was also found to be resistant to other antibiotics. This strain of *S. aureus*, the methicillin-resistant *Staphylococcus aureus* (MRSA) soon spread to different settings and became endemic in both hospital and community environment (Freney *et al*., 1999; Souli *et al*., 2010; Lupo *et al*., 2012). Globally, MRSA infections are major cause of morbidity and mortality (Zetola *et al*., 2005; Van Loo *et al*., 2007; Nabera, 2009). MRSA is said to be multidrug resistant because of its non-susceptibility to at least one antimicrobial agent in three or more categories and whose resistance to oxacillin or cefoxitin predicts non-susceptibility to most categories of β-lactam antimicrobials (Magiorakos *et al*., 2012).

Freshwater and hospital environments, animal husbandry and indiscriminate use of antimicrobial agents remain major contributing factors to microbial resistance ((Davis and Davis, 2010; Lupo *et al*., 2012). Variations in DNA sequences, genes long in soil and horizontal transfer of these genes, account for dissemination of antibiotic resistance among *Staphylococcus* isolates (Kayser, 1993; Davis and Davis, 2010; Chan *et al*., 2011).

Several mobile genetic elements (MGEs) that encode virulence and resistance to antibiotics in methicillin-resistant *Staphylococcus aureus* have been reported (Malachowa and Deleo. 2010). The production of an aberrant penicillin binding protein PBP2a with decreased affinity to beta-lactam antibiotics in MRSA is encoded by *mecA* (Berger-Barchi, 1995; Hunter *et al*., 2011) which carry insertion sites for mobile genetic elements that facilitate acquisition of resistance determinants to other antibiotics (Chambers, 2001; Ito *et al*., 2001).

The predominance of *S. aureus* in most developed countries where they occur as multi-drug resistant pathogen in healthcare environment serve as potential sources for outbreaks in these settings (Enright *et al*., 2000; Henderson, 2006) and have remained a major public health risk.

MRSA have been reported in poultry worldwide. The first case of livestock associated methicillin resistant *Staphylococcus aureus* strains ST398 emerging in healthy poultry in Europe was reported in Belgium in 2008 (Nemati *et al*., 2008). MRSA ST398*spa* type t1456 has been reported in poultry especially in broiler chickens in Belgium (Persoons *et al*., 2009). In the Netherlands, Mulders *et al*., (2010) reported both ST398 and ST9 in broiler chickens and slaughter house personnel. Other studies in addition have reported ST5 as dominant strain in poultry in the Netherlands and elsewhere (Lowder *et al*., 2009; Hasman *et al*., 2010) and in Germany (kock *et al*., 2013).

The development of multiple mechanisms of resistance for each and every antibiotic introduced into clinical and agricultural practice (Davies and Davies, 2010) has become a major public health issue. This study was carried out to determine the antibiotic resistance profile of *Staphylococcus aureus* and MRSA recovered from poultry and hospitals in Ebonyi State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Culture: A total of 3600 nasal and cloacae swabs obtained from 1800 poultry birds (nares and cloaca of each bird) selected at random and 150 clinical isolates of staphylococci obtained from 3 hospitals in the State were tested for the presence of *S. aureus* using standard microbiological techniques as described elsewhere (Boerlin, 2010).

Biochemical Testing: Further identification was based on catalase, slide and tube coagulase test reactions. Those that were positive for all these tests were confirmed using Staph Latex Agglutination test kit.

Antibiotic Susceptibility Testing (AST): Susceptibility of chicken and clinical isolates of *S. aureus* to a panel of 14 antibiotics; Gentamicin (10 µg), Rifampin (5 µg), Cefuroxime (30 µg), Trimethoprim/Sulphamethoxazole (1.25/23.75 µg), Linezolid (30 µg), Chloramphenicol (30 µg), Dalfopristin (15 µg), Doxcycline (30 µg), tetracycline (30µg), Erythromycin (15 µg), Levofloxacin (5 µg), Augmentin (30 µg), Vancomycin (30 µg) and Cefoxitin (30 µg) (all from Oxoid Co. UK), was determined on Mueller-Hinton agar using the Kirby-Bauer-CLSI modified disc agar diffusion (DAD) method as described elsewhere (Isenberg, 1998; Grundmann *et al*., 2002).

RESULTS

A total of 325 *Staphylococcus aureus* were isolated from poultry and the clinics; poultry 76% and hospitals, 24%.

Antibiotic Susceptibility Testing (AST): Antibiotic susceptibility testing (AST) was carried out to obtain antibiotic susceptibility profiles and pattern of all the *S. aureus* isolated from the poultry and hospitals against a panel of 14 antibiotics which cut across 11 categories with varying strengths. Only antibiotics with *S. aureus* resistance were shown in table1. There were no resistance against Linezolid, Quinupristin-dalfopristin, Rifampin and Vancomycin.

Table 1: Antibiotic resistance profiles of the *S. aureus* isolates.

Antibiotic	Strength	No. of *S. aureus*(%)
Augmentin®	30µg	10 (3.0%)
Cefoxitin	30µg	27 (8.3%)
Cefuroxime	30µg	22 (6.8%)
Chloramphenicol	30µg	63 (19.0%)
Doxcycline	30µg	26 (8%)
Erythromycin	15µg	60 (18.5%)
Gentamycin	10µg	19 (5.8%)
Levofloxacin	5µg	9 (2.8%)
Tetracycline	30µg	155 (47.7%)
Trimethoprim-sulfamethoxazole	1.25/23.75µg	139 (42.8%)

Antibiotic sensitivity of poultry and clinical isolates were as shown in figure 1 and 2. Clinical isolates were more resistant to each of the antibiotics than the poultry isolates except for Erythromycin where reverse was the case. The resistance to Augmentin by poultry isolates was 0.4%.

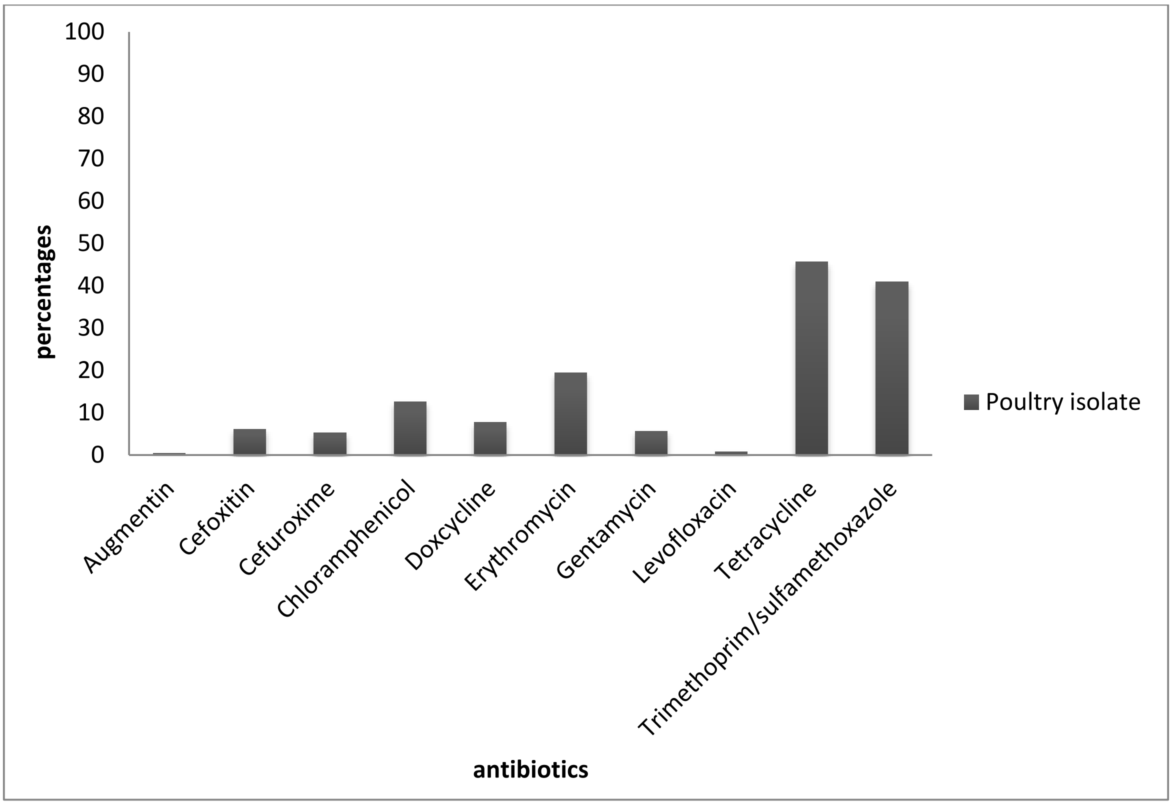

Figure 1: Antibiotic resistance profiles of poultry isolates of *S. aureus.*

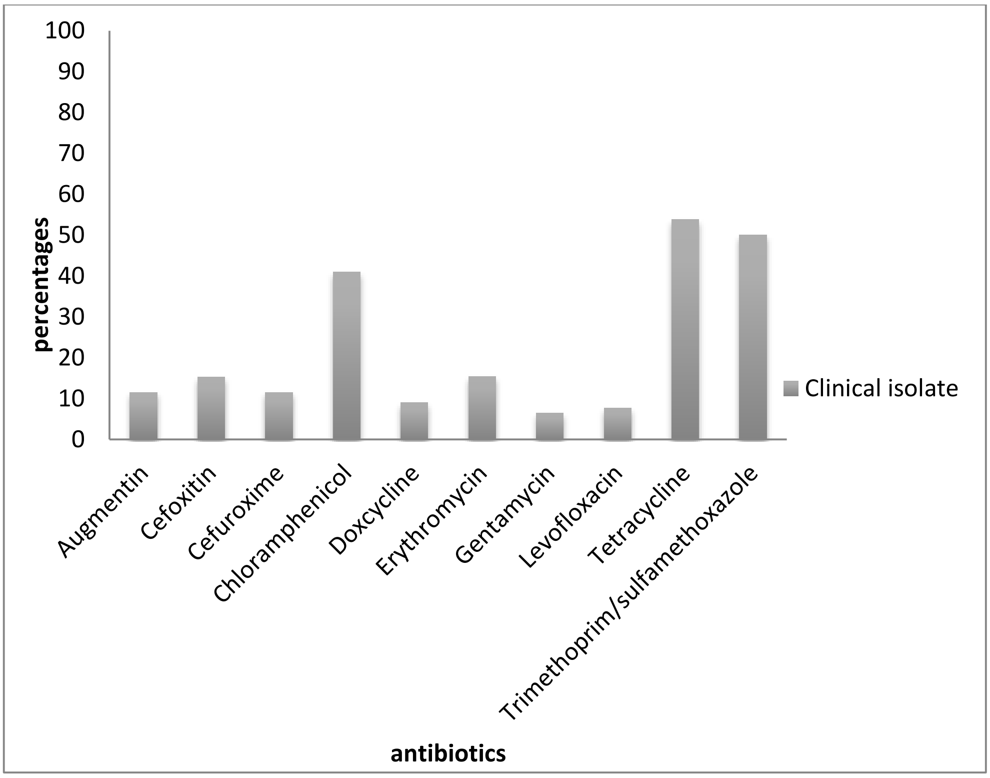

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Figure 2: Antibiotic resistance profiles of clinical isolates of *S. aureus*.

Table 2: *Staphylococcus aureus* and percentage MRSA from layers and broilers

Bird	birds sampled	birds positive for *S. aureus*	MRSA
layers	900	125	3% (4/125)
broilers	900	122	19% (11/122)

Multidrug resistance: Multidrug resistance (MDR) of all the 325 isolates of *S. aureus* was determined. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos, *et al*., 2012). The multi antibiotic resistance index (MARI) was used to determine the multi-antibiotic resistance index of all the isolates (table 3). The multidrug resistance pattern was as shown in (table 4).

Table 3: Antibiotic resistance index of *S. aureus*

MAR Index	Resistant isolates	%MARI
0	108	33.23
0.1	127	39.07
0.2	46	14.15
0.3	28	8.61
0.4	17	5.23

Table 4: Multidrug Resistance (MDR) Pattern of the isolates with MARI of 0.3 and above.

Antibiotics Resistances pattern	Number of Isolate	Percentage (%)	
Aug, Cef, Levo, Tetra	1	2.2	
Aug, Cef, Cefx, Tetra	1	2.2	
Aug, Cef, ,Chl, Trim/Sulf	1	2.2	
Chl, Levo, Tetra, Trim/Sulf	1	2.2	
Cef, Cefx, Genta, Tetra	1	2.2	
Chl, Ery, Tetra, Trim/Sulf	7	15.2	
Doxy, Genta, Tetra, Trim/Sulf	2	4.0	13.84% (Multidrug Resistant)
Cefx, Chl, Genta, Tetra	1	2.2	
Cefx, Chl, Doxy, Trim/Sulf	1	2.2	
Cefx, Chl, Tetra, Trim/Sulf	1	2.2	
Doxy, Ery, Tetra, Trim/Sulf	3	6.5	
Cef, Chl, Tetra, Trim/Sulf	3	6.5	
Cef, Cefx, Genta, Trim/Sulf	1	2.2	
Ery, Genta, Tetra, Trim/Sulf	1	2.2	
Cef, Cefx, Tetra, Trim/Sulf	1	2.2	
Chl, Genta, Tetra, Trim/Sulf	1	2.2	
Chl, Doxy, Ery, Trim/Sulf	1	2.2	
Cef, Doxy, Genta, Levo	1	2.2	

Aug, Cef, Ery, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Cefx, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Chl, Ery, Tetra	1	2.2
Chl, Ery, Levo, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Chl, Tetra, Trim/Sulf	1	2.2
Cefx, Chl, Doxy, Tetra, Trim/Sulf	2	4.0
Cef, Cefx, Doxy, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Cefx, Genta, Tetra	1	2.2
Cefx, Ery, Genta, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Ery, Genta, Trim/Sulf	1	2.2
Cef, Doxy, Genta, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Ery, Levo, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Chl, Genta, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Cefx, Doxy, Levo, Tetra	1	2.2
Aug, Cef, Chl, Ery, Tetra, Trim/Sulf	1	2.2
Total	45	100

The percentage occurrence of these patterns ranged from 15.2% > 6.5% > 4.3% > 2.1%

Key: Cef-Cefoxitin, Cefx-Cefuroxime, Chl-Chloramphenicol, Doxy- Doxycycline, Ery-Erythromycin, Genta-Gentamycin, Levo-Levofloxacin, Tetra-Tetracycline, Trim/sul-Trimethoprim/sufamethoxazole.

DISCUSSION

Staphylococcus aureus was recovered from 247 chickens in 9 farms spread across Ebonyi State. Several studies have reported *Staphylococcus aureus* in animals (Cuny *et al*., 2010; Umaru *et al*., 2011) especially in their nares (Persoon *et al*., 2009; Szabó *et al*., 2012) and in chicken droppings (Olayinka *et al*., 2010; Nworie *et al*., 2014). All the farms screened harbored *S. aureus*. In most of the chicken found to harbor *S. aureus*, recovery of the bacterium was from both cloacae and nostrils. A chicken was termed positive if any of the sites screened yielded growth of *S. aureus.* This was consistent with the reports of Williams (1963) and Basset *et al*, (2011) that *S. aureus* resides asymptomatically on the skin and in the nose of animals.

Antibiotic susceptibility testing (AST) was carried out to obtain antibiotic resistance profiles of the *S. aureus* isolates. Out of the 325 isolates that were screened for methicillin resistance, 27 of them were methicillin resistant, representing a prevalence of 8.3% in this environment. This finding was consistent with report elsewhere (Liu *et al*., 2012). Varying MRSA prevalence has been reported both in the clinics and in farms (van Cleef *et al*., 2010; Rafee *et al*., 2012). Data obtained in this study showed that most of the MRSA were resistant to four or more antibiotics. Cefoxitin resistance varied from hospital to hospital and between broilers and layers. The overall percentage prevalence of MRSA in the hospitals in this study was 15% while it was 6% in poultry. Shittu *et al*. (2011) had documented 16% oxacillin resistance amongst *S. aureus* from clinics and healthy individuals in Southwest Nigeria. Higher prevalence has been reported in many other populations and amongst clinical isolates of *S. aureus* in Nigeria. In Southwest Nigeria, a study in Ibadan involving 188 cases of wound infection in University College Hospital Ibadan between December 1994 and April 1995 showed that 41.5% (78/188) of them were methicillin resistant (Okesola *et al*., 1999). Similarly, Adegoke and Komolafe (2009) reported 50% methicillin resistance amongst isolates of *Staphylococcus aureus* in Ile-Ife. Ghebremedhin *et al*. (2009), reported MRSA prevalence of 20.2% in Southwest Nigeria. In Benin, South-South Nigeria, Sani *et al*. (2011) reported 48.78% methicillin resistance amongst *S. aureus* isolates from clincal samples (pus, urine, sperm, genital, catheter and blood) of hospitalized and extra hospitalized patients.

All the *S. aureus* displayed varying percentages of resistance to each of the antibiotics used. Resistance recorded were augmentin, 3.0%; cefoxitin, 8.3%, cefuroxime, 6.8%; chloramphenicol, 19.%; doxycycline, 8%; erythromycin, 18.5%; gentamycin, 5.8%; levofloxacin 2.8%; tetracycline, 47.7%; trimethoprim/sulfamethoxazole 42.8%. However, unlike the poultry isolates, the clinical isolates displayed higher percentage of resistance to most of the antibiotics. This might be as a result of routine use of these antibiotics in clinical therapy resulting in the generation of high level of resistance; a result consistent with findings of Shittu *et al*. (2011) who reported high antibiotic resistance amongst clinical isolates of *S. aureus*. All the *S. aureus* were found to be susceptible to Quinupristin/dalfopristin, Linezolid, Rifampicin and Vancomycin; a result also similar to reports elsewhere (Adwan *et al*., 2012) and consistent with earlier reports in the same environment (Nworie *et al*., 2013a; Nworie *et al*., 2013b; Nworie *et al*., 2014). Vancomycin has remained the last line of treatment for MRSA infections although cases of vancomycin resistance has been reported in many places (Sievert *et al*., 2008; Olayinka *et al*., 2010; Howden *et al*., 2011; Orji *et al*., 2012). The susceptibility of these isolates to vancomycin in this environment might be due to rare prescription of the drug in this environment. Above all, the complete susceptibility of all the isolates to quinupristin/dalfopristin, linezolid, rifampicin and vancomycin suggest that these antibiotics could hold great potential in the treatment of staphylococcal infections in Ebonyi State.

All the MRSA were multidrug resistant with higher multi-antibiotic resistance index than the *S. aureus*. *Staphylococcus aureus* with multi-drug resistance has been reported in poultry farms in China with prevalence that varied from farm to farm in relation to severity of use of antibiotics (Liu *et al.*, 2012). The percentage of isolates showing multi antibiotic resistance index (MARI) of 0.3 and above was 13.84% which suggests that the *S. aureus* with these resistance patterns were from environment where these antibiotics were routinely used (Krumperman, (1983). The percentage of isolates resistant to one antibiotic was 19% while 19.8% isolates were resistant to 2 antibiotics. Furthermore, while 14% and 9% of the isolates were resistant to 3 and 4 antibiotics respectively, 4% and 1.2% of the isolates were resistant to 5 and 6 antibiotics respectively. These level of multi-antibiotic resistance was not surprising as the area where this study was carried out was cosmopolitan with lots of anthropogenic activities including waste water which is a known source of antibiotic resistance genes as earlier reported elsewhere (Lupo *et al*., 2012). The number of multi-antibiotic resistant patterns displayed by the 46 multi-resistant isolates in this study was 34. The highest prevalent multidrug resistance pattern of 15.2% was a combined resistance to chloramphenicol, erythromycin, tetracycline and trimethoprim/sulfamethoxazole (Chl, Ery, Tetra, Trim/Sulf).

In conclusion, the high incidence of *Staphylococcus aureus* carriage among chicken in this study could mean an extensive distribution of this organism amongst poultry. It does indicate that poultry farm workers might be at high risk of infection and could be potential sources of community outbreaks. The high antibiotic resistance observed amongst poultry and clinical isolates of *S. aureus* against most of the antibiotics calls for caution in the use of these antibiotics in poultry and in the clinics. Further studies involving molecular studies are recommended to identify tetracycline resistant genes responsible for the high tetracycline resistance observed and the circulating *spa* and sequence types of *S. aureus* in Ebonyi State.

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