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Full Length Research Paper

Antibiotic susceptibility profile of *Staphylococcus aureus* isolated from healthy chickens in poultry farms in Kano state, Nigeria

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***Staphylococcus aureus* infection in poultry birds have been linked to the cause of increased morbidity and mortality in poultry management and treatment due to antibiotic resistance. This aim of this study is to proffer better treatment option in the management of *S. aureus* infection from poultry farms in Kano State using standard microbiological methods. The result showed that out of the 1260 samples collected from 12 farms in Kano State, 600 were from cloacae, 600 from nostril and 60 were from poultry farm workers. Using conventional identification methods, 562 isolates showed presumptive characteristics of *S. aureus*. The use of STAPH Agglutination kit reduced the number of the isolates to 290 while further identification method using Microgen STAPH kit confirmed 33.8% (98) of the presumptive isolates to be *S. aureus.* 8.3% (5) of the confirmed *S. aureus* were from the nostrils of poultry farm works. The antibiotic susceptibility profile study showed that high percentage (71.4%) of the isolates was resistant to Oxytetracycline, Oxacillin and Ampicillin, 61.2% to Chloramphenicol, 55% to Erythromycin and 30.6% to Cefoxitin. Vancomycin showed high activity against 74.5% of the isolates, Augumentin 69.4%, Ciprofloxacin 64.3% and Gentamicin 60.2%. Three of the isolates were observed to be resistant to all the 12 antibiotics tested while only one isolate was susceptible to all the antibiotics tested. Statistical analysis showed that isolates from layers were more resistant than those from broiler and poultry farm workers at $p \leq 0.05$ level of significance. We conclude that the isolates from this study have varied antibiotic susceptibility pattern and that Vancomycin, Augumentin, Ciprofloxacin and Gentamicin could still be proficient in the management of *S. aureus* infection from poultry farm in Kano State, but surveillance and publicity should be carried out to curtail the wide spread of antibiotic resistance induced through irrational antibiotic misuse.**

Key words: Poultry farm, antibiotics susceptibility profile, *Staphylococcus aureus*, resistance.

INTRODUCTION

Staphylococcus aureus is one of the most world renowned pathogenic strain of Gram positive bacterial with significant resistance to different antibiotics used in its treatment (Verhegghe et al., 2013). Antibiotics resistance in *S. aureus* have been reported to vary from one geographical location to another, creating the need for periodic environmental evaluation of antibiotic susceptibility profile of *S. aureus* in different sub-regions of the world due a probability of increased options for empirical and directed therapy of infections caused by these strains (Chua et al., 2011). As emergence of pathogenic and multidrug resistance strains e.g. Methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *S. aureus*, which could cause resistance to virtually all β-lactam antibiotics continues to surge both in human and several livestock-derived products including

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pig, cattle and poultry foods (Persoons et al., 2009; Verhegghe et al., 2013), the need for continuous monitoring of global epidemiology and emerging drug resistance data is critical for the effective management of *S. aureus* infections. In animal, this strain has been found to colonise and infect a wide range of host species including livestock, wildlife, and companion animals, with bovine mastitis among dairy cattle, lameness in poultry, and severe and lethal infections in farmed rabbits (Gavin et al., 2014). *S. aureus* is resistant to low water activity, high salt content, osmotic stress, freezing and can survives well in food stored below -20°C (Stewart, 2003). *S. aureus* enterotoxins are extremely resistant to heating and can survive the process used to sterilize low acid canned foods (Stewart, 2003). This has indeed contributed largely to *S. aureus* infections in human and animals, ranging from food poisoning, minor skin infections to serious and life-threatening infections such as endocarditis, pneumonia, respiratory infections, wound sepsis, tenosynovitis, omphalitis, femoral head necrosis, infected hock and stifle joints secondary to coccidiosis and “bumblefoot” and toxic shock syndrome (Stewart, 2003; Gavin et al., 2014). It is therefore imperative to study the antibiotic susceptibility profile of *S. aureus* in Kano State as a form of surveillance strategy in order to proffer quick pharmaceutical response to infections associated with *S. aureus* in poultry farm in Kano State, Nigeria.

METHODOLOGY

Sample collection

One thousand two hundred and sixty (1260) poultry samples consisting of 600 cloacae samples, 600 nostril samples and 60 from poultry farm workers were collected aseptically in a clean sterile universal bottle from 12 poultry farms in Kano State and transported on an ice park to the laboratory for bacteriological examination.

Staphylococcus species isolation, microscopy and biochemical Identification

S. aureus isolation and microscopy was carried out using the method described by Onaolapo (2005) and Cheesbrough (2000). Preliminary identification of *S. aureus* was carried out using catalase, coagulase and deoxyribonuclease (DNase) tests as described by Cheesbrough (2000) while further confirmatory test was carried out using STAPH Agglutination kit and Microgen STAPH kit.

Antibiotic susceptibility test and multiple antibiotic resistance index (MARI) evaluation

The susceptibility profiles of the identified *S. aureus* was tested against 10 selected antibiotics (Oxytetracycline, Ampicillin, Ciprofloxacillin, Vancomycin, Gentamicin, Chloramphenicol, Erythromycin, Cefoxitin, Augumentin and Oxacillin) using disc diffusion method as described by Cheesbrough (2000) and the corresponding results interpreted using CLSI (2014). The multiple antibiotic resistant (MAR) index was determined for each isolate as described by Paul et al. (1997).

RESULTS

Out of the 1260 samples evaluated from the three senatorial districts in Kano State, 1010 showed the cocci-cluster shape of *Staphylococcus spp.* Under microscope, 981 were catalase positive, 774 were DNase positive while 622 coagulase (sheep plasma) positive. Using Oxoid Staph. agglutination kit, 290 of the isolates showed positive agglutination while using Microgene Kit only 7.78% (980) of the total sample were confirmed as coagulase positive *S. aureus.* Table 1 showed the occurrence of *S. aureus* from poultry farms in Kano State.

The antibiotic susceptibility profile study showed that high percentage (71.4%) of the isolates was resistant to Oxytetracycline, Oxacillin and Ampicillin, 61.2% to Chloramphenicol, 55% to Erythromycin and 30.6% to Cefoxitin. Vancomycin showed high activity against 74.5% of the isolates, Augumentin 69.4%, Ciprofloxacin 64.3% and Gentamicin 60.2% (Table 2). Three of the isolates were observed to be resistant to all the 12 antibiotics tested while only one isolate was susceptible to all the antibiotics tested (Table 2).

This result showed the antibiotics susceptibility profile of *S. aureus* from poultry farms in Kano State.

Comparison of antibiotic resistance profile of *S. aureus* isolates from broilers, layers and poultry farm workers showed statistically that isolates from broiler were more resistant than those from layers compared to poultry farm workers at $p \leq 0.05$ level of significance (Table 3 and Figure 1).

This result showed the comparison of antibiotics resistance among *S. aureus* from poultry farms in Kano State.

DISCUSSION

Processed poultry products have been noted as favorable environments for the survival and transmission of various commensal, spoilage, and potentially pathogenic bacteria in the human food chain, with *Staphylococci* spp. accounting for one of the most predominant bacteria groups (Huys et al., 2005). In view of this, isolation and antibiotics susceptibility profile of *S. aureus* from healthy chickens nostrils and cloacae, and farm workers were evaluated in Kano State. The isolation of *S. aureus* from poultry and human samples in this

Table 1. Sample Collection, Isolation and Identification of *Staphylococcus aureus* from Poultry Farms in Kano State.

S/N	Sample Collection				GS	Presumptive *Staphylococcus aureus* ID			Agglutination Test	Microgene Kit
	NSB	CSB	NSW			Catalase	DNase	Coagulase		
Kano central (Farms 1-4)										
1	50	50	5	NSB	175	175	168	120		
2	50	50	5	CSB	170	170	163	160		
3	50	50	5	NSW	11	11	9	8		
4	50	50	5	Total	365	356	240	288		
Kano North (Farm 5 – 8)										
5	50	50	5	NSB	160	160	145	100		
6	50	50	5	CSB	120	120	80	50	290	98
7	50	50	5	NSW	15	15	10	5		
8	50	50	5	Total	295	295	235	155		
Kano South (Farm 9 – 12)										
9	50	50	5	NSB	190	190	175	90		
10	50	50	5	CSB	150	130	115	80		
11	50	50	5	NSW	10	10	9	9		
12	50	50	5	Total	350	330	299	179		
Total	600	600	60	1260	1010	981	774	622	290	98

Keys: NSB = Nasal Swab of birds, CSB = Cloacae Swab of birds, NSW = Nasal Swab of Workers, GS = Gram staining, ID = Identification

Table 2. Antibiotics resistance profile of *Staphylococcus aureus* from poultry farms in Kano state.

Antibiotics	Potency(μg)	No. of *Staph. areus* (%)	
		Resistant	Sensitive
Ampicillin	10	70 (71.4)	28 (29.6)
Augmentin	30	30 (30.6)	68 (69.4)
Chloramphenicol	30	60 (61.2)	38 (38.8)
Ciprofloxacin	5	35 (25.7)	63 (64.3)
Cefoxitin	30	30 (30.6)	68 (69.4)
Erythromycin	15	54 (55)	44 (45)
Gentamicin	10	39 (39.8)	59 (60.2)
Neomycin	30	45 (45.9)	53 (54.1)
Oxacillin	1	70 (71.4)	28 (29.6)
Oxytetracycline	30	70 (71.4)	28 (29.6)
Trimemthoprin/sulfamethaxazole	25	50 (51)	48 (49)
Vanomycin	30	25 (25.5)	73 (74.5)

Table 3. Comparison of Antibiotic resistance of *Staphylococcus aureus* isolates from broilers, layers and poultry farm workers using One Way ANOVA.

Antibiotics	Poultry *(%)*		
	Broilers (n=66)	Layers (n=32)	Poultry farm Workers (%) (n =5)
Ampicillin	40 (60.6)	30 (93.8)	0 (0)
Augmentin	16 (24.2)	14 (43.8)	0 (0)
Chloramphenicol	35 (53.0)	25 (78.1)	0 (0)
Ciprofloxacin	15 (22.7)	20 (62.5)	0 (0)
Cefoxitin	25 (37.9)	4 (12.5)	1 (20)
Erythromycin	35 (53.0)	18 (56.3)	1 (20)
Gentamicin	11 (16.7)	28 (87.5)	0 (0)
Neomycin	30 (45.5)	13 (40.6)	2 (40)
Oxacillin	36 (54.5)	30 (93.8)	4 (80)
Oxytetracycline	38 (57.6)	32(100)	0 (0)
Vanomycin	10 (15.2)	15 (46.9)	0 (0)
Trimemthoprin/sulfamethaxazole	30 (45.5)	19 (59.4)	1 (20)

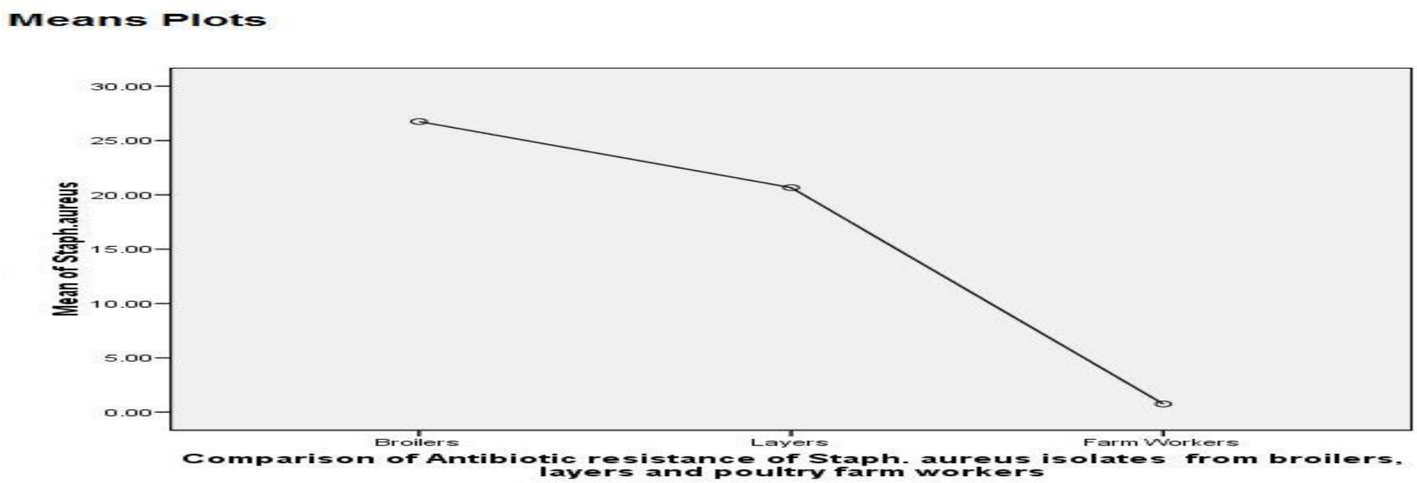

Figure 1. Statistical Comparism of antibiotics resistance of *S. aureus* Isolated from broilers, layers and poultry farm workers using one way ANOVA.

study further support the commensalism and ability of *S. aureus* to survive in various environment such as skin, neck skin of chicken carcasses and the possibility of zoonotic infections in human (Ellerbroek, 1997; Huys et al., 2005). The high percentage of *S. aureus* isolated in this study concurs with the report of Suleiman *et al.,* (2013) who reported 83% incidence of *S. aureus* from tracheal swabs of 100 apparently healthy chicken with 54% been coagulase positive in Maiduguri. These findings further buttress the need for food hygiene during processing, the need for good storage temperature and product discard policy, which should be carefully monitored to ensure that products are microbiologically stable with enhanced safety. Also it highlighted the need for consumers to be informed and follow the basic instructions regarding storage temperature, cooking to prevent contamination and cross-contamination (Olimpia et al., 2006). Antibiotics such as Oxytetracycline, Oxacillin and Ampicillin were observed to have low activity against the test isolates, with 71.4% of the isolates being resistant. Similar patterns of antimicrobial susceptibility have been reported in Zaria, Nigeria by Otalu et al., (2011), Italy (Pesavento et al., 2007), United States (Waters et al., 2011) and Ireland (Leonard and Markey, 2008). This might be as a result of extensive usage of these antimicrobial agents in animal husbandry over time, which has contributed to the selection of drug-resistant strains (Mostafa et al., 2008). The variation in the percentage of antibiotics resistance (Chloramphenicol (61%), Erythromycin (55%) and Cefoxitin (30.6%)) observed in this study concurs with the report of Mostafa et al. (2008), who reported 82.2% resistance in old *S. aureus* isolates and 91.7% in their new isolates when compared. Vancomycin showed high activity of 74.5% against the isolates, Augumentin 69.4%, Ciprofloxacin 64.3% and Gentamicin 60.2%. These findings concur with the report of Suleiman et al. (2013) who reported 100% susceptibility to Gentamicin and 66.7 to Augumentin. This might be as a result of non-availability/high cost of Vancomycin, while the combination therapy of betalactam and betalactamase inhibitor might be the major reason of the low rate of resistance observed against Augumentin in this study. Also, the broad spectrum activity of Ciprofloxacin and parenteral administration of Getamicin which involve specialized “hand” for injection, might have curtailed their abused. Three of the isolates were observed to be resistant to all the 12 antibiotics tested while only one isolate was susceptible to all the antibiotics tested. This suggest that such resistant isolates originated from a high risk source of contamination where antibiotics are often used or that a large proportion of the bacterial isolates have been pre-exposed to several antibiotics (Christopher et al., 2013). Also the use of antibiotics as prophylaxes, growth promoters or inaccurate dosages given to sick flocks by unqualified personnel may likely result in plasma concentrations that are inconsistent with the desired objectives which might possibly influence resistance (Suleiman et al., 2013). Statistical analysis showed that isolates from broilers were more resistant than those from layers and poultry farm workers at $p \leq 0.05$ level of significance. The treatment of broiler and layers infected with these isolates will thus be more difficult due to their resistance to multiple antimicrobials (Mostafa et al., 2008).

Conclusion and recommendations

Isolates from this study has varied antibiotic susceptibility pattern and antibiotics such as Vancomycin, Augumentin, Ciprofloxacin and Gentamicin could still be proficient in the management of *S. aureus* infection from poultry farm in Kano State, but surveillance and publicity should be carried out to curtail the wide spread of antibiotic resistance induced through irrational antibiotic misused in poultry. Also there is the need for periodic monitoring of global epidemiology and emerging drug resistance for effective management of *S. aureus* infections in poultry.

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