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ANTIBIOTICS SUSCEPTIBILITY PATTERN OF STAPHYLOCOCCI ISOLATED FROM POULTRY AND POULTRY ENVIRONMENT IN OWERRI, NIGERIA

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ABSTRACT

Food borne diseases and poisoning have great public health concern worldwide. *Staphylococcus* is one of the foods borne pathogen responsible for causing food borne illness worldwide after ingestion of food contaminated with staphylococcal toxins. This study was aimed to evaluate the prevalence and antibiotic susceptibility pattern of *Staphylococci* isolated from poultry and poultry house environment in Ihiagwa, Imo State, Nigeria. A total of 140 samples which includes cloacal swabs, body swabs, footpad swabs, water and environment swabs were collected under aseptic precautions using a sterile swab sticks and cultured on mannitol salt agar. *Staphylococcus* was identified based on their conventional cultural characteristics, Gram staining reaction and standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) on Mueller-Hinton Agar. Out of 140 samples of poultry and poultry house environment, 90 (64.38%) yielded Staphylococcal isolates. The highest prevalence of Staphylococcal isolates in farm A was recorded in footpad (72%), while the least was observed in body (42.86%). In farm B, the highest prevalence was observed in water, environment, cloacal samples (100% of each), and least was body (71.43%). Most of the isolates were resistant to Nalidixic acid (62.50%) followed by Sulphamethoxazole-Trimethoprim (60.94%), Erythromycin (57.81%), Oxacillin (54.69%) and were sensitive to Amoxycillin/Clavulanic acid (68.75), Amoxycillin (62.50), Ampicillin (56.25%), and Norfloxacin (50%). This study highlighted high prevalence and antibiotic resistance; thus, the need to adopt new strategies in the control of antibiotic resistance cannot be overemphasized.

KEYWORDS

Staphylococcus, Antibiotics resistance and Poultry.

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INTRODUCTION

Staphylococcus aureus was first observed in pus by Robert Koch in 1878. Ogston in 1882 named the organism, "Staphylococcus" a name derived from the Greek word "Staphyle", meaning a bunch of grapes and "coccus" meaning a grain or berry. Rosenbach in 1884 was probably the first person to grow Staphylococci in pure culture and to study their characteristics in the laboratory (Baird-Parker,

1990)[1]. Most staphylococci are coagulase-negative, the only exceptions being *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus delphini, Staphylococcus schleiferi subsp*. coagulans and some strains of *Staphylococcus hyicus* (Kazmierczak *et. al.,* 2014)[2].

Staphylococci are commonly found in the breeder house, environment and can be isolated from the litter, dust and feathers of chickens. The bacteria are considered to be a normal resident of the chicken, located on the skin, feathers, respiratory and intestinal tracts. Their remarkable ability to acquire antibiotics resistance has contributed to their survival (Diekema *et. al.,* 2001)[3]. Multi-drug resistance strains of Staphylococci have been reported with increasing frequency worldwide, including isolates that are resistant to methicillin, aminoglycosides, fluoroqinolones, lincosamides, macrolides or combination of these antibiotics (Zargar. *et al.,* 2014)[4].

Extensive use of antibiotics in human and animals for therapeutic and preventive purpose is a major cause for the prevalence of drug resistance among food borne pathogens (Suleiman *et al.,* 2014)[5]. Different antimicrobial agents such as penicillin, erythromycin, and tetracycline are extensively used in poultry for treating staphylococcal and other infections, which leads to development of antimicrobial resistant strains of the pathogens (Osman *et al.,* 2015[6], Pesavento *et al.,* 2007[7], Plata *et al.,* 2009)[8]

A staphylococcus infection, or staphylococcosis, refers to a variety of diseases in poultry caused by staphylococci bacteria (Eric *et al.,* 2001)[9]. Approximately 20 species have been isolated but only *Staphylococcus aureus,* seems to be of veterinary importance in poultry breeders. Infections of *Staphylococcus aureus* are often difficult to treat because of frequency of multiple antibiotics resistance of strains (Al-Bahry *et. al.,* 2014)[10]. Staphylococcus spp. has been a food safety concern for poultry meat producers and food processors for decades because they are widely spread in the environment and often detected in air, dust, water, raw meat, other foods, and on environmental surfaces. They survive desiccation and tolerate high levels of salt.

The transmission of antibiotic resistance traits from food animals to humans through the food chain continued to be a major public health problem facing humanity. This study therefore, was aimed to determine the prevalence and antibiotic susceptibility pattern of Staphylococci isolated from two poultry farms in Owerri Nigeria. As poultry business is a source of economic development in Nigeria, the findings from this work will help to better understand and potentially predict trends in antibiotic-resistance patterns, as well as establish adequate infection control programs in poultry industry in the region; thus, therefore, ensure quality meat as well as to prevent losses in poultry industry due to Staphylococcal infections.

MATERIAL AND METHODS

Sample Collection, Cultivation and Isolation of Staphylococcus Spp

A total of one hundred and forty (140) poultry and poultry house environment samples which includes cloacal swabs (36 samples), body swabs (35 samples), footpad swabs (30 samples), water samples (27 samples) and litter sample (12 samples) were collected randomly from two different poultry farms in Owerri, Imo state, South-East Nigeria. The samples were cultured on mannitol salt agar and incubated overnight at 37°C. The tiny pin head golden yellow colonies that grew on mannitol salt agar were selected and identified as *Staphylococcus* by catalase test and Gram staining.

Antibiotic Susceptibility Testing

The antibiotic susceptibility tests were performed using the disk diffusion method. Briefly, a loop full of the inoculum was compared with 0.5McFarland standard and was swabbed on Muller-Hinton agar plates with sterile cotton swabs and left for a while. Then using an ethanol dipped and flamed forceps different antibiotic discs were placed on the agar surface at about two cm apart. The discs were slightly pressed with the forceps to make complete contact with the medium. The plates were incubated for 18-24 h at 37 °C. After the incubation, the diameter of inhibition zones was measured and interpreted using the European Committee on Antimicrobial Susceptibility Testing breakpoint

(EUCAST 2018). The isolates were tested against 11 antibiotics namely: Gentamycin (30µg), Nalidixic acid (30 µg), Erythromycin (15 µg), Oxacillin OX (1 µg), Ampicillin (10 µg), Streptomycin (10 µg), Norfloxacin 10 (µg), Ciprofloxacin (5µg), Sulphamethoxazole-Trimethoprim (25 µg), Amoxicillin/Clavulanic acid (30 µg), Amoxicillin (10 µg) (Oxoid UK).

RESULTS

Out of the 140 poultry and poultry house environment samples comprising of two different farms, 90 (64.28%) yielded *Staphylococci* isolates. The frequency of isolation increased with the number of samples (Table No.1). The sample with the highest prevalence was seen in Turkey (94.12%), followed by Broilers (88.89%), Layers (64.52%), then Local fowl (58.54%) (Table No.2). Considering the two farms sampled, the prevalence observed in farm A was as follows; footpad (72%), water (68.18), environment (60%), cloacal (53.57%) and body (42.86%). Then, in farm B the prevalence recorded was water, environment, cloacal (100% of each), footpad (80%) then body (71.43%) (Table No.3).

Antibiotics Susceptibility Test Result

All the 64 isolates of *Staphylococci* were tested for in vitro susceptibility towards 11 antibiotics and the result showed that most of the isolates were resistant to Nalidixic acid (62.50%) followed by Sulphamethoxazole-Trimethoprim (60.94%), Erythromycin (57.81%), Oxacillin (54.69%) and were sensitive to Amoxycillin/Clavulanic acid (68.75), Amoxycillin (62.50), Ampicillin (56.25%), and Norfloxacin (50%) (Table No.4). A total of 39 (60.9%) of the isolates were multidrug resistant (i.e. resistant to three or more antibiotic tested) (Table No.5).

DISCUSSION

Staphylococcus aureus is an important health care and community acquired infection in every region of the world (Hassan, *et al.,* 2013[11], CDC, 2011)[12]. Studies have shown that toxins produce by *S. aureus* in poultry possess a potential health hazard to consumers (Lee, 2003)[13] and isolation of such strains is useful as a parts of risk analysis of meat and poultry product (Zouharova and Rysanek, 2008)[14]. In this study, we observed a high prevalence (64.28%) of *Staphylococcus aureus* and other coagulase negative Staphylococci from poultry samples in Owerri and 60.9% of the isolates were multidrug resistant. This finding is of public health importance as these isolates may be as source of transmission of antibiotics resistance traits to human via the food chain.

Though high antibiotic resistance was observed in this study, however more isolates were considerably susceptible to ampicillin 36(56.25%) and in view of this, such antibiotic may be useful for treatment of Staphylococcal infections in the region. Pesavento *et al.* (2007)[7] detected 58.6% Ampicillin resistance in *S. aureus* isolates obtained from poultry. Similarly, Akbar and Anal (2013)[15] revealed antibiogram pattern of 38 S. *aureus* isolates. They found 55.2% resistance to the ampicillin. In the both study, resistance against ampicillin was higher than what we observed in this study. These results indicate that there was considerable variation in resistance pattern of the isolates towards ampicillin.

Our data showed that (54.79 %) of the isolates were resistant to Oxacillin; this is different from the finding of Abdalrahman *et al.* (2015)[16] who reported 47.6% resistance to Oxacillin. Similarly Attien *et al.* (2013)[17] recorded 58.0% resistance to Oxacillin which is similar to the present study. Increase in drug resistance we observed may be due to sub-therapeutic use of drugs or may be inaccurate dosages given to sick flocks by unqualified personnel which lead to development of antibiotics resistance by the Staphylococcal isolates.

The resistance of the isolates to Ciprofloxacin (51.56%) recorded in this study is not in accordance with the study of Citak and Duman (2011)[18] who reported 31.2 % resistance to Ciprofloxacin. However, lower resistance (15.4%) was reported by Otalu *et al.* (2011)[19]. While another author, Fan *et al.* (2015)[20] observed resistance (82.90%). Increase in resistance to Ciprofloxacin may be due to easily availability of drug in market and the drug may be widely used at poultry farms for prophylaxis.

With respect to Streptomycin, the 48.44% sensitivity recorded in this study is lower than Owuna *et al.* (2015)[21] who reported 79.3%

sensitivity to Streptomycin. Decrease in sensitivity of Staphylococcal isolates to Streptomycin may indicate that these antimicrobials were abused in the farm and may not be recommended for treatment of Staphylococcal infection at farm level.

The finding of the present study revealed 62.50% sensitivity of Staphylococcal isolates to Amoxycillin which was much higher than findings of Owuna *et al.* (2015)[21] who observed 13.8% of sensitivity in *S. aureus* isolated from poultry meat sold in Keffi Metropolis, Nigeria.

Gentamycin (48.44%) sensitivity to the isolates in this study was similar to the findings of Yeasmeen *et. al.* (2017), who recorded 50.0% sensitivity in poultry farms of Chiiagong City, Bangladesh. Owuna et al recorded a higher sensitivity (82.8%) which is in contrast to other two findings. In present study Amoxicillin/Clavulanic Acid, Amoxicillin and Ampicillin with values 68.75%, 62.50% and 56.25% respectively were considerably sensitive to Staphylococcal isolates and may be recommended for treatment of Staphylococcal infections in poultry.

In summary, the indiscriminate use of antibiotics/antimicrobials agents for prophylactic, therapeutic purpose and growth promoter in the food producing animals could be the reasons for increased antimicrobial resistance of Staphylococci which result in Methicillin resistant *Staphylococcus aureus* and Multidrug Resistant Staphylococcus aureus and threatening the human life as well as animal life. Based on this study undertaken on the occurrence of Staphylococci from poultry and poultry house environment, it was found that out of 140 samples 90 (64.28%) turned out to be positive for Staphylococci. The highest prevalence was detected from farm A in the order; footpad (72%), water (68.18), environment (60%), cloacal (53.57%) and body (42.86%). Then farm B recorded as it's highest in water, environment, cloacal (100% of each), footpad (80%) then body (71.43%). From this study it was however observed that the frequency of isolation of Staphylococci observed was high and isolates were more susceptible to Amoxycillin/Clavulanic acid and Amoxycillin and in view of this, such drugs may be useful for treatment of Staphylococcal infections in poultry industry in Owerri.

In conclusion, the findings of this study showed that both the prevalence and antibiotic resistance rate of *Staphylococci* isolates from poultry in two farms in Owerri is high and may be increasing day by day with the increasing trends of usage of antibiotics in the poultry industry. One major limitation of this study is that we didn't have enough resources to further identify the Staphylococci isolates to species level and carry out molecular characterization of strains which will provide more information on the isolates. Also, this study was based in two farms only; similar studies can be extended to other areas of Imo State, to determine the horizontal intensity of prevalence and frequency of antibiotics resistance. Further studies may also be done to determine the speed and rate of zoonotic transmission of Staphylococci infection which may help in assessing the risks posed by the infection to human health. The judicious use of antimicrobial agents is pivotal to the control of antimicrobial resistance in our environments. Thus, there is need for the development of antimicrobial policy that will guide the prescription, sale, and use of antibiotics through regular surveillance of resistant organisms in our environments.

Table No.1: The prevalence rate of *Staphylococci* isolated from the two farms sampled

S.No	Location (Farm)	No. Sampled	No. Positive (%) Frequency
1	Farm A	113	66 (58.41)
2	Farm B	27	24 (88.89)
3	**Total**	140	90 (64.28)

Table No.2: Total Number of positive and percentage frequency of S*taphylococci* isolates according to samples sources

S.No	Location	Sample Source	No. Sampled	No. Positive (%) Frequency
1	Farm A	Turkey	17	16 (94.12)
		Layers	31	20 (64.52)
		Broilers	24	6 (25)
		Local fowl	41	24 (58.54)
2	Farm B	Broilers	27	24(88.89)
3	**Total**		140	90 (64.28%)

Table No.3: Total number of samples positive (%) according to sample types

S.No	Sample type	Farm A		Farm B	
		No. of samples	No. Positive (%)	No. of samples	No. Positive (%)
1	Body	35	12 (42.86)	7	5 (71.43)
2	Water	22	15 (68.18)	5	5 (100)
3	Cloacal	28	15 (53.57)	8	8 (100)
4	Environment	10	6 (60)	2	2 (100)
5	Footpad	25	18 (72)	5	4 (80)
6	Total	113	66 (58.41)	27	24 (88.89)

Table No.4: Antibiotics susceptibility pattern of Staphylococci isolates from poultry samples in two farms in Owerri (n=64)

S.No	Antibiotics	No. of isolates sensitive (%)	No of isolates resistant (%)
1	Sulphamethoxazole-Trimethoprim	25 (39.06)	39 (60.94)
2	Nalidixic acid	24 (37.50)	40 (62.50)
3	Erythromycin	27 (42.19)	37 (57.81)
4	Amoxycillin/Clavulanic acid	44 (68.75)	20 (31.25)
5	Amoxycillin	40 (62.50)	24 (37.50)
6	Oxacillin	29 (45.31)	35 (54.69)
7	Gentamycin	31 (48.44)	33 (51.56)
8	Ciprofloxacin	31 (48.44)	33 (51.56)
9	Norfloxacin	32 (50)	32 (50)
10	Ampicillin	36 (56.25)	28 (43.75)
11	Streptomycin	31 (48.44)	33 (51.56)

Table No.5: Antibiotics resistant profile of *Staphylococci* isolates from poultry samples in two farms in Owerri (n=64)

No. of antibiotics	Antibiotics	No. of isolates	Percentage (%)
No Resistance		22	34.38
1	Amp Amc	1 2	4.70
3	SxtNaOx NaGenNor SxtENor EOxCip EGenAmp	1 1 1 1 1	7.81
4	NaAmlOxAmp SxtOxAmpS amcAmlOxAmp OxGenCipAmp EOxGenAmp AmcAmlCipS NaGenCipS AmcAmlGenAmp	1 1 1 1 1 1 1 1	12.50
5	AmcAmlOxGenS AmlOxGenCipNor EAmcOxGenS NaEOxNorS AmcAmlOxGenAmp NaAmcAmlOxGen NaAmcAmlGenAmp SxtNaEOxAmp NaAmlOxGenAmp EAmlGenNorAmp NaGenCipAmpS	1 1 1 1 2 1 1 1 1 1 1	20.31
6	NaEAmcAmlCipAmp SxtEAmcOxGenAmp SxtEOxGenAmpS EAmlOxGenCipAmp NaAmcAmlOxGenAmp NaEAmlGenCipNor	1 1 1 1 1 1	9.37
7	SxtAmcOxGenCipNorAmp NaAmcAmlOxGenCipAml SxtNaOxGenCipNorS	1 1 1	4.69
8	EAmlOxGenCipNorAmpS NaAmcAmlOxGenCipNorAmp	1 1	3.12
9	SxtEAmcAmlOxGenNorAmpS SxtNaAmcAmlOxGenCipNorAmp	1 1	3.12
Total		64	100

Key: Gen=Gentamycin (30µg), Na=Nalidixic acid (30 µg), E=Erythromycin (15 µg), Ox=Oxacillin (1 µg), Amp=Ampicillin (10 µg), S=Streptomycin (10 µg), Nor=Norfloxacin 10 µg (µg), Cip=Ciprofloxacin (5µg), Sxt=Sulphamethoxazole-Trimethoprim (25 µg), Amc=Amoxicillin/Clavulanic acid (30 µg), Aml= Amoxicillin

CONCLUSION

In conclusion, the findings of this study showed that both the prevalence and antibiotic resistance rate of Staphylococci isolates from poultry in two farms in Owerri is high and may be increasing day by day with the increasing trends of usage of antibiotics in the poultry industry. One major limitation of this study is that we didn't have enough resources to further identify the Staphylococci isolates to species level and carry out molecular characterization of strains which will provide more information on the isolates. Also, this study was based in two farms only; similar studies can be extended to other areas of Imo State, to determine the horizontal intensity of prevalence and frequency of antibiotics resistance. Further studies may also be done to determine the speed and rate of zoonotic transmission of Staphylococci infection which may help in assessing the risks posed by the infection to human health. The judicious use of antimicrobial agents is pivotal to the control of antimicrobial resistance in our environments. Thus, there is need for the development of antimicrobial policy that will guide the prescription, sale, and use of antibiotics through regular surveillance of resistant organisms in our environments.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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