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Antimicrobial Susceptibility Pattern of *S. aureus* and *Salmonella sp* Isolated from Poultry Feed Sold in Ile Ife, Nigeria

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Abstract

The emergence of antibiotic resistance among humans has prompted concerns about the public health implications of antibiotic use in agriculture. One hundred (100) Poultry feed samples were collected, serially diluted and cultured using pour plate method. Identification of the isolates was based on the morphological and biochemical characteristics using Bergeys Manual of Determinative Bacteriology. The antibiotics susceptibility testing was carried out using the disk diffusion method. The microbial load of each samples ranged between 2.2×10^5 to 6.5×10^6. The overall percentage occurrence of the isolates revealed Staphylococcus spp. 25 (62.5%) and Salmonella spp. 15 (37.55%). Antibiotic sensitivity patterns of the bacterial isolates were tested against six commonly used antibiotics viz., gentamycin (GEN), vancomycin (VAN), oxacillin (Ox), penicillin (P), chloramphenicol (C) streptomycin (S). There was a slight susceptibility to gentamycin and vancomycin by Salmonella sp and S. aureus and moderately resistant to chloramphenicol and streptomycin but 100% resistant to penicillin and oxacillin used. Improper antibacterial treatment and overuse of antibiotics for agricultural purposes which contributed to increase incidence of multiple antibiotic resistances in farm animals must be discouraged.

Keywords: Poultry feeds; Antibiotics resistant; *Salmonella sp*; *Staphylococcus aureus*; Microbial load

Introduction

Commercial feed and feed ingredients are usually sourced from various locations, they remain the environment major vehicles for the introduction of both commensal and pathogenic microbes to farm [1].

All four basic types of poultry feeds viz., starters, growers, finishers and layers. However, may potentially become contaminated with food borne pathogenic microbes during harvesting, processing, handling, and marketing of the bagged feeds [2]. Prominent bacterial species in the poultry feeds include *Bacillus, Escherichia, Salmonella, Enterococcus, Campylobacter, Clostridium, Staphylococci* and *Lactobacillus* that have been shown to be of critical importance in tropical countries [3].

Antibiotics have been broadly used in farm animals for the purpose of antimicrobial therapy, prophylax is and growth promotion [4,5]. This increasing handling of antibiotics has led to a worldwide problem in the development of antibiotic resistance among bacterial populations during recent decades [6].

Staphylococcal infections are frequently treated with antibiotics and consequently resistance to it and or acquired resistance develop [7]. Currently, medical attention focuses to both coagulase positive and coagulase-negative staphylococci because they represent a serious therapeutic problem.

Moreover, they may develop multi-antimicrobial resistance [8] Salmonellosis is endemic and a major threat to commercial poultry farming in Nigeria [9]. *Salmonella typhi*, *S. paratyphi* and *S. choleraesuis* are highly adapted to humans and cause severe diseases [10].

In poultry, *S. pullorum* and *S. gallinarium* commonly cause Pullorum disease and fowl typhoid. These infections can be ingested through feaces, fluff, litter and water (Tables 1-3).

Table 1 Percentage distribution of bacteria isolates.

Isolates	Number of Isolates	Percentage (%)
S. aureus	25	62.5
Salmonella sp	15	37.5
Total	40	100

Colonization of *Salmonella* covers humans and animals including livestock, poultry, rodents, reptiles and birds [11].

Salmonellosis occurs mainly by a faeca-oral route through the consumption of contaminated feed and water [12].

Table 2 Antibiotic susceptibility profile of the *S.aureus* isolated.

Isolates	Gentamycin (cn) (mm) 10 µg	Vancomycin (va) (mm) 10 µg	Penicillin p (mm) 30 µg	Oxacillin (ox) (mm) 1 µg	Chloramphenicol (c) (mm) 30 µg	Streptomycin (s) (mm) 10 µg
PF5	8 (R)	13(R)	7(R)	11(R)	16(I)	13(I)
PF 9	25 (S)	13(R)	7(R)	7(R)	19(S)	13(I)
PF25	15 (S)	15(S)	0(R)	0(R)	22(S)	11(R)
PF42	9(R)	13(R)	7(R)	8(R)	17(I)	13(I)
PF49	8(R)	12(R)	9(R)	6(R)	18(S)	12(I)
PF55	16(S)	16(S)	9(R)	0(R)	20(S)	18(S)
PF58	21(S)	20(S)	7(R)	8(R)	16(I)	21(S)
PF70	7(R)	13(R)	7(R)	6(R)	21(S)	12(I)
PF75	8(R)	13(R)	8(R)	7(R)	20(S)	13(I)
PF85	8(R)	14(R)	7(R)	8(R)	20(S)	13(I)
PF20	7(R)	10(R)	6(R)	6(R)	17(I)	12(I)
PF11	17(S)	14(R)	0(R)	0(R)	25(S)	10(R)
PF30	0(R)	9(R)	0(R)	0(R)	14(I)	10(R)
PF40	7(R)	10(R)	6(R)	6(R)	20(S)	12(I)
PF59	21(S)	13(R)	7(R)	0(R)	27(S)	16(S)
PF 1	10(R)	10(R)	13(R)	0(R)	22(S)	6(R)
PF89	17(S)	18(S)	7(R)	6(R)	20(S)	17(S)
PF62	8(R)	12(R)	7(R)	6(R)	20(S)	14(I)
PF65	8(R)	12(R)	7(R)	7(R)	18(S)	12(I)
PF82	8(R)	17(S)	9(R)	6(R)	18(S)	14(I)

Table 3 Antibiotic susceptibility profile of the *Salmonella sp.* Isolated.

Isolates	Gentamycin (CN) (mm) 10 µg	Vancomycin (VA) (mm) 10 µg	Penicillin (P) (mm) 30 µg	Oxacillin (Ox) (mm) 1 µg	Chloramphenicol (C) (mm) 30 µg	Streptomycin (S) (mm) 10 µg
Spf 20	16(S)	10(R)	11(R)	6(R)	10(R)	8(R)
Spf 07	14(I)	0(R)	0(R)	0(R)	15(I)	10(R)
Spf01	13(I)	21(S)	10(R)	0(R)	10(R)	0(R)
Spf12	18(S)	7(R)	10(R)	0(R)	9(R)	9(R)
Spf10	13(I)	8(R)	0(R)	0(R)	17(I)	12(I)
Spf11	17(S)	0(R)	0(R)	0(R)	0(R)	0(R)
Spf03	12(R)	0(R)	0(R)	0(R)	11(R)	0(R)
Spf19	13(I)	0(R)	0(R)	0(R)	10(R)	10(R)
Spf21	16(S)	7(R)	10(R)	10(R)	18(S)	13(I)
Spf22	16(S)	25(S)	0(R)	0(R)	18(S)	12(I)

Spf14	14(I)	7(R)	7(R)	0(R)	15(I)	10(R)
Spf17	17(S)	7(R)	7(R)	7(R)	11(R)	15(S)
Spf15	10(R)	30(S)	6(R)	6(R)	12(R)	12(I)
Spf16	13(I)	18(S)	0(R)	0(R)	9(R)	13(I)
Spf13	14(I)	7(R)	0(R)	0(R)	10(R)	9(R)

Sources of *Salmonella* infections into poultry farms include contaminated feed and feed ingredients, water, equipments, personnel, rodents and hatchery related unhygienic activities [13]. The improper antibacterial treatment and overuse of antibiotics for agricultural purposes have contributed to the increased incidence of multiple antibiotic resistances in farm animals [14,15].

The study is designed with the aim of determining the bacterial load and the antibiotics susceptibility of the isolates obtained from the poultry feeds sold in Ile- Ife, Southwestern Nigeria.

Materials and Methods

Sample collection

One hundred different samples of poultry feeds were collected from different sales points in Ile-Ife, the samples were immediately taken to the Department of Microbiology's laboratory for analysis.

Method of isolation

Using an aseptically cleaned pistil and mortal, small piece, each of the poultry feed samples were grinded into fine powdery form, one gram from each of the 100 samples was weighed and added aseptically into sterile test tubes which contain 9 ml of sterile distilled water each and shaken thoroughly for even distribution of organisms to make a stock. Ten-fold dilution was carried out by transferring 1 ml each of the mixture into sterile test tubes that contain 9 ml of sterile distilled water labelled 10^{-1} to make 10 ml, using a new sterile pipette 1 ml of the solution was pipetted from the test tube labelled 10^{-1} into another test tube labelled 10^{-2} containing 9 ml of distilled water, this same method was repeated until it get to 10^{-5} test tube using serial dilution method. 1 ml each was pipette from 10^{4} and 10^{5} into sterile Petri dishes already labeled in duplicate using pour plate method and molten nutrient agar were separately poured aseptically on it, swirled and allowed to set on horizontal surface. The nutrient agar plates were incubated at 37°C for 24 hours. After 24 hours of incubation some colonies were transferred to mannitol salt agar and MacConkey agar and incubated for 48 hours. The colonies that ferment the mannitol turned yellow and were further purified and stocked for biochemical identification. Colonies on MacConkey agar were transferred to *Salmonella Shigella* agar plates and incubated for 48 hours, colonies on the SSA plates were picked, purified and stocked on agar slants then refrigerated at 4°C for further identification.

Bacterial isolation and identification

Biochemical test were performed to identify microbes that could not be characterized morphologically. Biochemical tests applied were standard catalase test, DNAse, citrate utilization, oxidase, Voges Prokauer, indole production, motility, sucrose, maltose, lactose, nitrate reduction and mannitol.

Antimicrobial susceptibility test

The disk diffusion method as described by the National Committee for Clinical Laboratory Standard (2015) was employed to determine the drug susceptibility patterns of isolates. Commercially prepared paper discs of uniform size impregnated with specific concentrations of different antibiotics disc were used. The antibiotics disc used were gentamycin 10 µg, streptomycin 10 µg, chloramphenicol 30 µg, vancomycin 10 µg, penicillin 10 µg, oxacillin 1 µg. Isolates were suspended in test tubes containing sterile nutrient broth and incubated for 24 hours until turbidity corresponding to 0.5 McFarland standards was attained. The solidified Mueller Hinton agar plates were flooded with the broth culture and poured away aseptically. The antibiotics discs were then aseptically applied to the surface of the inoculated agar plates with a pair of sterile forceps. The plates were incubated in an inverted position at 37°C for 24 hours. Afterwards, diameter of each zone of inhibition was measured in millimeter using a calibrated transparent ruler and results obtained were compared with CLSI chart (2016) to score the susceptibility pattern of test isolates to the chemotherapeutic agents as resistant, intermediate and sensitive.

Results

Out of 100 different poultry feed samples obtained from different sales points and locations in Ile Ife, 25(62.5%) *Staphylococcus aureus* strains and 15(37.5%) *Salmonella* species were isolated altogether. The microbial load of the samples ranged from 2.2×10^{-5} CFU/ml to 6.5×10^{-5} CFU/ml.

Percentage susceptible, intermediate and resistance to the antibiotics among *Salmonella sp.* Isolates.

Percentage susceptible, intermediate and resistant to the antibiotics

The percentage Susceptibility of *S aureus* and *Salmonella sp* recovered from poultry feed, the Intermediate and Resistant to various antibiotics used are shown on **Figure 1** respectively. The two bacteria isolates were susceptible to gentamycin and vancomycin and 100% resistant to penicillin and oxacillin used.

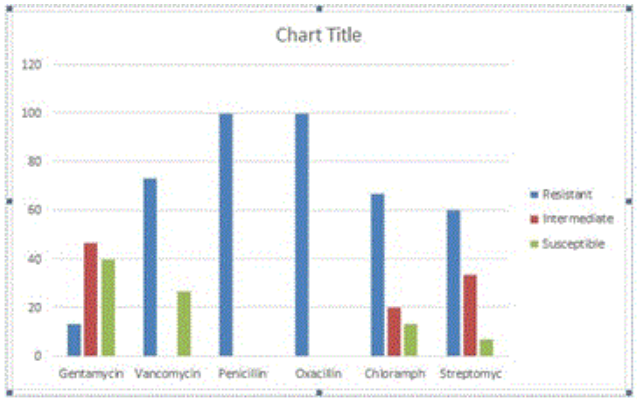

Figure 1 Percentage susceptible, intermediate and resistance to the antibiotics among *S. aureus* isolates.

Discussion

This study established that two bacterial genera were isolated in the feed samples analyzed. Recovery of bacteria species of such public health concern may indicate certain potential hazard to the animals. In our findings, the microbial load of feed sample PF25 was higher than what is recorded for other feed samples in some locations which corroborated the work of Uwaezuoke and Ogbulie, (2008) who reported the contamination of poultry feeds with Staphylococcus aureus and Salmonella sp which likely might have resulted from the manufacturer or the ingredients used in compounding the feed since good manufacturing practice often enhances good products.

The result of the bacterial load obtained in the feed samples used in this study was lower, 2.2×10^5 cfu/ml and 6.5×10^5 cfu/ml than what was reported by David and Ogulade (2013) who reported the microbial load ranging between 7.58×10^5 and 6.36×10^6 CFU/g for feed samples produced by local industries. *Salmonella sp* and *Staphylococcus aureus* are capable of producing acute and chronic infections in all or most types of birds and animals. In general the transmission of *Salmonella sp* through the environment has been shown to be cyclic, and poultry feeds had been reportedly viewed as important links for contamination in poultry [16-20].

The antibiotic susceptibility test carried out showed that some of the isolates have the ability to resist the common antibiotic. It was also observed that most isolates of *Salmonella sp* and *S. aureus* showed resistance to oxacillin. Sensitivity of the isolated organisms to vancomycin could be related to less frequent usage of these drugs for therapeutic purposes, therefore reducing the chance for resistance to develop. Antibiotic resistance pattern of Salmonella sp from poultry feed in ile ife exacerbated the global problem of antibiotic resistance and a serious health related implication for antibiotic use in poultry. The present study shows that commercial feeds and live poultry birds could be important vehicles for the introduction of multi-drug resistant (MDR) genes from *S. aureus* or *Salmonella sp* into humans through poultry. In addition, these pathogenic bacteria pose threat to health by food poisoning and infection to animals and humans. Presence of pathogenic bacteria in the poultry samples also implies that eggs and meat should not be consumed half-cooked or raw.

Conclusion

The bacteria load recovery in this study may indicate a potential hazard to both animals and humans. The high occurrence of bacteria species of public health concern may indicate obvious health hazard in terms of direct consumption of bacteriological contaminated feed or their toxins by farm animal and subsequent public health problem. The occurrence of Salmonella sp and Staphylococcus aureus could be as a result of their high pathogenicity trends. These organisms although on their own can cause several poultry and farm animal infections; they also produce toxins that are also of public health importance to both human and the farm animals. The socio-economic and health implication of these findings are enormous. Economically, the presence of these bacterial has been reported to overwhelmingly affect the viability of some animal husbandry undertaking and agriculture in general. With the high colonization of bacteria of public health concern in poultry feeds, good manufacturing practice, handling and retailing methods need to be improved to enhance the microbiological quality of these products.

Regular microbiological analysis should be carried out to determine the quality of poultry feeds in ensuring both human and animal safety.

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