ANTIBIOTICS PROFILE AND PUBLIC HEALTH IMPLICATION OF PATHOGENIC ENTERIC BACTERIA ASSOCIATED WITH POULTRY STOOL DROPPINGS

ABSTRACT

Poultry waste are not properly disposed and most rural farmers make use of it as manure therefore poultry wastes may serve as source of enteric organism capable of infecting humans. The antibiotic resistance from these bacteria can be transferred to natural microbial community as a result of involvement of antibiotics in poultry farming. This research was carried out to assess the multidrug resistant pattern of enteric bacteria in poultry stool dropping. samples of poultry droppings were obtained from a free-range commercial poultry farm in Otuoke, Bayelsa State, Nigeria. Samples were cultured on selective and differential media. 160 isolates of enteric bacteria was obtained from poultry stool droppings. The isolates identified were four genera. *Salmonella* species which is predominant 6(37%) followed by *Escerichia coli* 5(31%) *Proteus* species 3(19%) and *Kiebsiella* species 2(13%). Antibiotic susceptibility testing was carried out using the disk diffusion technique. Gram negative antibiotics including crioxacin cefprozil, nitrofuration, ceftazidime co-trimoxazole, genetamicin, cefuroximo. The resistant pattern in among the gram negative pathogens revealed that more than 98% were resistant to nitrofuratioin cefprozil, gentamicin and augumentin while 12.89% resistant to certazidime, 90.10% resistant to augmentin, 15.32% cefuroxime, 12.30% co-trimoxazole, 38.17% resistant to gentamicin, 15.20% resistant to cefprozil and 50.20% resistant to ofloxacin, Multidrug resistance species were isolated from poultry stool dropping and some of the bacterial isolates are potentially pathogenic to humans and animals and therefore poses a serious threat to public health.

Key words: Multidrug resistance, Poultry, Enteric bacteria, Stool dropping

Introduction

Sources of food from Poultry has played an important role in the food industries on a global scale and chicken is the most commonly farmed. Poultry droppings/litters is a waste containing a community of microorganisms according to Balan *et al* (2010), this waste is a mixture of stool, feed, beddings and feathers (Wilkinson *et al*, 2011). The microbial concentrations in poultry stool can reach 10^{10} colony forming unit per gram and Gram-positive bacteria, including *Clostridia, Actinomycetes,* and *Lacto bacilli* account for nearly 90% of the microbial diversity. Reports by Stern and Robech (2003), Ngodigha and Owen (2009) and Bolan *et al* (2010) reveals a variety of pathogens such as *Bordetalla, Clostridium, Escherichia coli, Mycobacterium, Antinobacillus, Staphylococcus, Salmonella* and *Sstreptococcuss* in poultry litter-base organic fertilizers. According to Wilkenson *et al* (2011) some of these bacteria such as *Campylobacter jejuni, Listeria monocytogens and Salmonella,*

can potentially contaminate the environment and for that reason are frequently associated with food borne outbreak. The frequent use of antimicrobials in animal and poultry is likely to accelerate the development of antibiotic resistance in bacteria. This can then result to difficulty in management, economic loses and a source of gene pool for transmission to human. According to WHO the resistance of bacteria to antibiotics is the ability of bacterial population to survive, the effect of inhibitory concentration of antimicrobial agent (Catry *et al*, 2003).

In general, when an antibiotic is used in any setting the susceptible bacterial strain is eliminated while those with traits that can resist the drugs remains. These bacteria resisting the drugs will then multiply and become the dominant population. These genes are then transferred to other bacteria which were previously susceptible (Madigan *et al*, 2014).

Bacteria resisting antibiotics can be transferred from poultry products to human through consumption or handling of meat contaminated with pathogens (Van den and Stobberingh, 2000). There are multidrug resistance bacterial strains present in animals and birds (Amara *et al* 1995). This multidrug resistance acquired creates widespread difficulties in poultry management and infections caused by the pathogen.

The infections caused by these multi drug resistant bacteria will result in increased healthcare cost or even death (Gupta *et al*, 2001). In effect once these multidrug resistance strains are present, they threaten future treatments of infection caused by such microorganism.

MATERIALS AND METHOD

Location of the Study

The sample was collected in a poultry farm in otuoke communities in Bayelsa State Nigeria. the community lies between latitude of $4^0$51'05.23" N, longitude $6^0$20'19.8"E. It is bounded to the North by Elebele community, to the East by emeyal 1 and to the South by Onuobum and Otuogiri and to the South by Otuaba and Ewoi communities. The community is hosting the federal university Otuoke.

Sample Collection

A total of one hundred sixty (160) chicken dropping were collected using a sterile universal bottle. The number of birds in farms where about 2000 in a free-range system. Antibiotics were used in the farm but the exact practice of how and when they give the birds was not disclosed.

Isolation and Identification of Bacteria

A sterilized spatula was used to introduce 1 gram of the faecal droppings into 9ml buffered peptone water and incubated at 37^{o}C for 24 hours. Thereafter 1ml of the cultures were transferred into a 9ml of Selenite Broth and incubated at 37^{o}C for 24 hours (Mitchel and Shane, 2000; Al-Abadi and Al-mayah, 2011). loopful of the overnight Selenite broth culture was streaked on Xylose lysine deoxycholates Agar (XLD) and the plates incubated overnight at 37^{o}C for 24 hours (Menghistu *et al*., 2011). Two presummed colonies on XLD were purified by sub culturing on nutrient agar and incubated at 37^{o}C for 24 hours for isolation of pure culture and subsequent biochemical confirmation. The presumptive isolates were identified using biochemical methods (Golden *et al*, 2021). The isolated strains were read and identified as described by (Pujiastuti *et al*., 2018 and Merchant *et al*., 2012).

Antibiotics Susceptibility Test

Antibiotics susceptibility test of isolates was determined using disk diffusion method and interpreted as susceptible and resistant as described by CLSI, (2014). enteric pathogens were tested against the following antibiotics; ofloxacin (30µg), gentamicin (10µg), augmentin (30 µg), cefprozil (30µg), nitrofuratoin (300µg), ceftazidime (25µg), cotrimoxamicin (10µg) and cefuroxime (30µg). Oxoid sensitivity test agar plates was swabbed with cells from the bacteria stock solution, pre-adjusted to 0.5 McFarland's turbidity standard. The discs was then placed, carefully on the agar using a sterile forceps. incubation at 37°C for 24 hours immediately after placing the disc. Zones of sensitivity was measured with a meter rule in millimeter.

RESULT AND DISCUSSION

Result

A total of 16 *enteric* bacteria were obtained from poultry stool droppings. The bacteria were identified by conventional biochemical test (table 1). The occurrence of the bacteria cell in poultry stool droppings include *Salmonella* Species which had 6 (37%) followed by *Escherichia coli* (31%), *Proteus* 3(19%) and the least observed is *Klebsiella* 2(13%) as shown in figure 1 below

List 1: Identified Isolates

Bacteria	Number of bacteria isolated
Proteus SP	3

Escherichia coli	5
Salmonella sp	6
Klebsiella sp	2
TOTAL	16

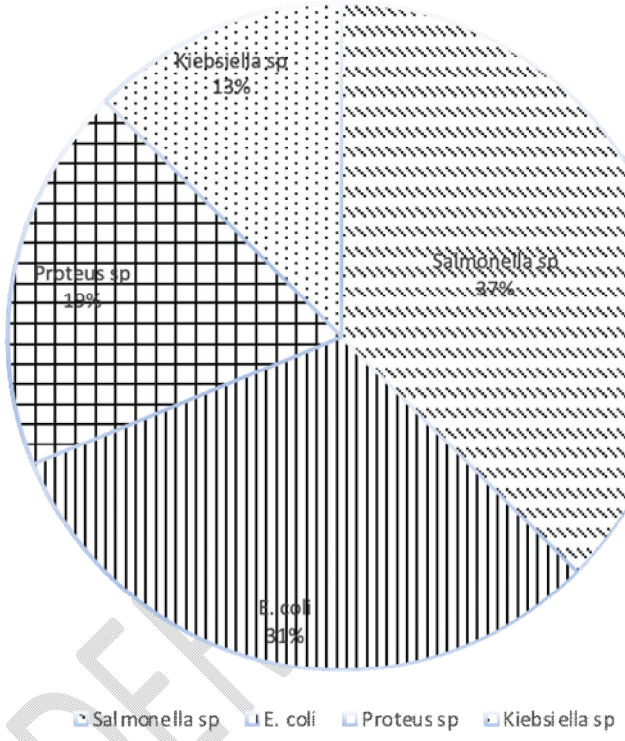

Figure 1: Pie chart showing the percent number of Isolates

The enteric bacteria strains observed for drug resistance and susceptibility shows all the antibiotics had varying degree. Resistance among *Salmonella* strains was significantly different from the other strains. The resistance includes *Proteus, Klebsiella, E. coli,* and *Salmonella*. It was observed that antibiotic resistance between *Proteus* and *Klebsiella* isolates was not different. *Salmonella, E. coli, Proteus* were most resistant to Nitrofuratioin 72.0%, 66.2%, 92.1%, and Ofloxacin 82.0%, 61%, 65.9% and 72.5%. These isolates were least resistant to cotrimoxazole 25.1%, 12.2%, 43.6%. the sensitivity of *Salmonella, E. coli* and *Proteus* isolates to Ceftazidime were relatively high while *Klebsiella* isolates were highly

sensitive to cotrimoxazole. The level of sensitivity among all antibiotic is as follows: *E. coli, Salmonella, Proteus* and *Klebsiella*. The bacteria sensitivity to drug was significantly different among the genera as revealed in figure 1 and table 2 below.

Table 1: Percentage Isolate Resistance to Antibiotics

Percentage Resistance (%)				
Antibiotic	*E. coli spp*	*Proteus Spp*	*Salmonella*	*Klebsiella*
Nitrofuratioin	72.0	66.2	92.1	27.2
Cefprozil	52.4	20.9	92.4	37.6
Cerftazidime	40	37.2	53.9	12.9
Cotrimoxazole	25.1	12.2	43.6	2.1
Gentamicin	50.9	59.1	55.1	40.2
Cefuroxime	53.6	40.5	62.5	16.0
Ofloxacin	82.0	61	65.9	53.4
Augmentin	52.9	52.7	72.5	16.4

Table 2: Percentage Isolate Sensitivity to Antibiotics

Percentage Resistance				
Antibiotic	*E. coli spp*	*Proteus Spp*	*Salmonella*	*Klebsiella*
Nitrofuratioin	6.3	4.1	19	2
Cefprozil	25.2	13.2	35.1	10.2
Cerfazidime	40.1	35.6	22.7	4
Cotrimoxazole	50.3	31.2	32.0	10
Gentamicin	19.2	0	0	13.9
Cefuroxime	12.3	14	15	6.4
Ofloxacin	30.8	19.0	29.0	32.1
Augmentin	42.1	13.1	20.5	25.6

Discussion

The waste from commercial poultry are not appropriately disposed and most rural farmers use these wastes as manure to enrich the soil, this practice is common in Nigeria. These poultry wastes may serve as a reservoir for enteric bacteria that will later infect man. The detection of *E. coli, Salmonella, Proteus* and *Klebsiella* agrees with the fact that the bacteria are part of the enteric flora of the poultry birds and thus in agreement with Bamidele *et al* (2022). However, the result obtained in this study reveals that there is variation in the carriage of the bacteria in poultry birds, which could be due to factors such as the environmental, nutritional status of the birds, probiotic and physiological state of the animal these factors could have influenced the distribution of the bacteria. *E. coli, Salmonella, Proteus,* and *Klebsiella* was revealed in this study from bacteria isolate. Figure 1 shows the percentage distribution of isolated enteric bacteria from poultry droppings. In List 1 the biochemical identification of enteric bacteria isolated from poultry dropping was revealed. the bacteria were *E. Coli* 5(31%), *Proteus* 3(19%), *Salmonella* 6(37%) and *Klebsiella* 2 (13%). All the enteric bacteria isolated in this study observed similar trend and cluster pattern between the bacterial species suggesting the concurrence of *Salmonella* spp., *E. coli, Klebsiella* spp., and *Pseudomonas* spp. The findings is similar to a studies carried out by Mansaray *et al*., 2022.

The variability of isolates resistant to antibiotic to common drug is overwhelming. The occurrence of *E. coli* reported in this study comparable with reports from the West Indies (98.5%), Nigeria (90%) and Senegal (84.5%) (Abdu., 2021, Amadi *et al*.,2015 and Vounba *et al*., 2018). Studies from Cameroon (Moffo *et al*., 2021) and North West Ethiopia (Eyasu *et al*., 2017) reported 45% in the prevalence of *E. coli.* The isolation of *E. coli* was as low as 11% in free range birds of Nigeria in a study carried out by Joseph *et al*., 2018. The variability in these findings could possibly be as a result of the differences in the type of poultry system, type of

sample used and the age of the birds. Other studies however used birds of 15–45 days old (Merchant *et al*., 2012, Vounba *et al*., 2018 and Adelowo *et al*., 2014), this study used excreta of birds aged between 12-21 weeks.

The rise in antibiotics resistance had led to an increasing incidence among strains of *Enterobacteriaceae* in the past decades which remains a global public health problem. Improper use of antibiotics has lead to increase in resistance in pathogenic bacteria strains forming a part of the endogenous floral of human and animals. Results from the antimicrobial test in this study is similar to findings by (Kilonzo-Ntheng *et al*., 2008) result from his study shows multidrug-resistance in the following antibiotics; ampicillin, ciprofloxacin, erythromycin, kanamycin, and nalidixic acid in *Campylobacter* spp. that were isolated from chickens and guinea fowl. Bacteria that are Multidrug resistant from zoonotic origin may spread into the human population by direct contacts and food. Antibiotics added to feeds and rodents finding their way into the farms, this possibility could be responsible for the spread of *Salmonella* specie in poultry farm other possibility for the spread may be contamination of feed and water used in the farm. It is also important to note that one of the features revealed that poultry dropping has *E. coli*, *Proteus* and *Klebsiella*. This is expected because these birds have hindered access to the environment but water supply is from untreated water such water sample have harbor potential pathogens, which carry antibiotic resistance genes.

Conclusion

The findings in this research work indicates that poultry droppings is a carrier of multidrug resistant pathogenic bacteria which is capable of been transmitted to human population and therefore worrisome as its possess serious public health concern. The resistance among the different strains has been attributed to the indiscriminate use of different antibiotics in the treatment of bacterial infection in poultry birds. Many instances exposure to these bacteria resistant to several antibiotics are from the environment as bacteria migrates to the environment via many sources. Bacteria resistant to several antibiotics have been found in poultry and poultry products including carcasses and these poses health hazard to handlers and consumers therefore a major threat to global public health. The above information calls for surveillance and monitoring of antibiotic usage in both animal husbandry and humans throughout the world.

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