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ANTIBIOTIC RESISTANT PATTERN OF BACTERIA ISOLATED FROM *GALLUS GALLUS DOMESTICUS*

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ABSTRACT

Antibiotic use in livestock rearing for growth promotion, therapeutic and preventative actions gives rise to resistance in bacteria which can be transmitted directly to humans. This study was aimed at investigating the antibiotic resistance pattern of bacteria isolated from the Caeca of *Gallus gallus domesticus* (Old layer and Broiler) from mile 3 market of Port Harcourt metropolis, Rivers state. Thirty samples were collected and analyzed using standard microbiological procedures. A significant difference was observed in the heterotrophic count between broiler and old layer at ($P \geq 0.05$). The mean total heterotrophic bacteria count was 7.02 ± 0.05^a and 6.99 ± 0.04^b for broiler and old layer respectively. However, coliform count did not show any significant difference as mean total coliform count for broiler and old layer were 4.92 ± 0.04^a and 4.87 ± 0.07^a respectively. Eight genera of bacteria were isolated: *Escherichia coli* (11.5%), *Enterobacter* (16.7%), *Klesiella* (10.4%,), *Salmonella* (7.2%), *Providencia* (14.6%) , *Shigella* (8.3%), *Vibrio* (16.7%) and *Staphylococcus* (14.6%). Isolates were subjected to antimicrobial susceptibility test by disc diffusion method. Susceptibility testing revealed varying levels of resistance amongst isolates. Isolates were highly resistant to at least three antibiotics with augmentin, gentamycin and streptomycin showing the highest resistance amongst isolates. However, Staphylococcus isolates showed resistance to ampicillin (50%), rocephin (50%) and erythromycin (50%).There are concerns about poultry as a conduit for transmission of resistant bacteria to humans. Adequate monitoring of antibiotic use, misuse and abuse in livestock raring should be encouraged to help moderate development and spread of antibiotic resistance in the populace.

KEYWORDS: *Gallus gallus domesticus*, gut, antibiotic resistance, multidrug resistance.

INTRODUCTION

There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Van *et al*.,2001). Recent reports have shown that different types of food and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-producing animals (Anderson *et al.,* 2003; Schroeder *et al.,* 2004). In practice, chickens are given antibiotics for either treatment or prophylaxis of infections, and for growth promotion to increase profits. These antibiotics belong to similar chemical categories to those used for treatment of microbial infections in humans. This raises concern on the possibility of human cross-infecting with chicken-infecting bacteria or the later transferring resistance traits to bacterial population that causes human infections (Khachatourians, 1998; Austin *et al.,* 1997). Antibiotics use in livestock rearing for growth promotion, therapeutic and preventative actions and in livestock feed at sub-therapeutic doses for feed efficiency, is one of the main contributors in the development of antibiotic resistant bacteria(JETCAR,1999).

The gut of chicken is replete with bacteria, mostly enteric, and often pathogenic. These bacteria fulfill various beneficial roles for the host, including helping to resist colonization by pathogens. It can also facilitate the conjugative transfer of multidrug resistance (MDR) plasmids between commensal and pathogenic bacteria which is a significant public and animal health concern as it may affect our ability to treat bacterial infections (Card *et.al,* 2017).Chickens are a source of human infection by zoonotic pathogens(ESFA, 2014 ; Hopkins *et al*.,2007).

When an antibiotic is applied is poultry farming the drug eliminates the sensitive bacteria strains leaving behind those variants with unusual traits that can resist it. These resistant bacteria then multiply and become the predominant microorganism in the population and such bacteria transmit their genetically defined resistant characteristics to subsequent progeny of the strain and to other bacteria species (Gould,2008). Antibiotic resistance in enteric bacteria in poultry has been an issue of major

concern because the presence of resistant bacteria has further implications of infecting humans via the food chain. Potential transfer of resistant bacteria from poultry product to human population may occur through consumption or handling contaminated meat (Van *et al*.,2000). Once acquired, the resistant bacteria can colonize the human intestine and the gene coding resistance to antibiotic can be transferred to other bacteria belonging to the endogenous flora of humans thereby jeopardizing the effective treatment of bacterial infection (DeLeener *et al*.,2005).

The development and transfer of multidrug resistant strains of several bacteria is a wide-ranging problem. The development of resistance to antibiotics in bacteria led to a discussion about the careful use of antimicrobial agents, especially in veterinary medicine, nutrition and agriculture (Caprioli *et al* .,2000).

It is now generally known that the widespread use of antibiotics is the main risk factor for an increase in the occurrence of bacterial resistant strains (Apata,2009). Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources (Obire *et al*.,2009).

MATERIALS AND METHODS

Sample Collection
Thirty samples of chicken caeca (Broiler and Old layer) were collected from three different slaughter points within the market. The chicken gut was collected immediately after butchering and the ceaca dissected from the gut. Dissecting instrument were cleaned with 70% ethanol after use on each birds. Samples were placed in a sterile bottle, the bottle were placed in a cool box containing ice-cubes and transported to the Microbiology laboratory within an hour of collection for bacteriological analysis**.**

Isolation and Enumeration of Organism
One gram of the chicken gut (caeca) was weighed with the electronic weighing balance (Denver Instrument Electronic weighing balance). A 10 fold serial dilution was carried out by transferring 1g of the caeca into 9ml

of normal saline and was agitated to dislodge the caeca content. An aliquot (0.1 ml) of the 10^{-2} and 10^{-4} dilutions were inoculated onto prepared MacConkey Agar, for the isolation of coliform, Thiocitrate bile salt-sucrose (TCBS) Agar for vibrio, *Salmonella-Shigella* Agar and Nutrient Agar for total heterotrophic bacteria. Inoculated plates were incubated at 37°C for 24 hours. After incubation, ensuing colonies which developed on the different media plates were counted and the counts were used to calculate the colony forming units. Distinct colonies that developed were sub-cultured by streaking onto freshly prepared nutrient agar media and stored as stock cultures.The pure cultures obtained were used for the biochemical screening and antibiotic sensitivity testing.

Identification of Bacterial Isolates
The colonies on the Nutrient Agar, MacConkey Agar (MAC), Salmonella Shigella Agar (SSA), Thiosulfate citrate bile salts-sucrose Agar,(TCBS) were observed . Biochemical characterisation and presumptive identification of isolates were carried out as described by Cheesbrough, 2006.Results from biochemical characterization were confirmed by comparison with known standard Bergys Manual of Systematic Bacteriology (2003).

Antimicrobial Susceptibility Testing
The disk diffusion assay was performed and results interpreted according to the method described by Clinical and Laboratory Standards Institute (CLSI,2015*)*. Gram negative isolates were tested with Amoxiacillin (30μg), Streptomycin (30μg), Septrin (30μg), Sparfloxacin (10μg), Chloramphenicol (30μg), Ciprofloxacin (10μg), Ampicillin (30μg) Augmentin (30μg), Gentamycin (10μg) and Pefloxacin (10μg), while the gram positive isolate was tested using, Ampicillin (30μg) Augmentin (30μg), Ampiclox (10μg), Ciprofloxacin(10μg), Erythromycin (10μg), Gentamycin (10μg), Pefloxacin (10μg), Rocephine (30μg), Streptomycin (30μg), Septrin (30μg) and Zinnacef (30μg).

RESULTS

Total Microbial Counts
The mean values of viable heterotrophic bacterial counts are shown in **Table1**. The bacterial population for broiler and old layer isolated from the ceaca ranged from 6.96 Log10 Cfu/g to 7.06 Log10 Cfu/g and 6.94 Log10 Cfu/g to 7.06 Log10 Cfu/g for broiler and old layer respectively.

Table 1: Total Viable Heterotrophic Count (Log$_{10}$ Cfu/g) for Broiler and Old Layer.

Location Type of Chicken	A		B		C	
Sampling	Broiler	Old Layer	Broiler	Old Layer	Broiler	Old Layer
1	7.04	7.01	7.05	6.97	7.01	7.06
2	6.99	6.92	7.03	6.99	7.07	7.00
3	6.96	6.94	7.04	7.02	6.09	7.00
4	7.04	6.99	6.98	7.05	7.07	6.95
5	7.08	6.98	7.06	6.96	7.05	7.03
Mean±SD	7.02±0.05	6.97±0.03	7.03±0.03	6.99±0.05	6.86±0.05	7.01±0.04

At P > 0.05 with the same superscript there is no significant difference.

Distribution of Isolates

Based on colony morphology isolate identified belong to 8 genera namely; *E.coli, Enterobacter, Klebsiella, Providencia, Salmonella, Shigella, Staphylococus and Vibrio*.

**Table 2: Distribution of Isolates from Samples.**

Isolate	A		B		C	
	Broiler	**Old layer**	**Broiler**	**Old layer**	**Broiler**	**Old layer**
E.coli	+	+	+	+	+	+
Enterobacter sp	+	+	+	+	+	+
Klebsiella sp	+	+	+	+	+	+
Providencia sp	+	+	+	+	+	+
Salmonella sp	-	+	-	+	-	+
Shigella sp	+	+	+	+	+	+
Staphylococus sp	+	+	+	+	+	+
Vibrio sp	+	+	+	+	+	+

Pathogenic Bacteria Isolated From Ceaca

Prevalence of bacteria pathogens is presented in **Figure 1.** Total number of ninety six bacterial pathogens was isolated and identified. *Enterobacter sp* 16(17%) and *Vibrio sp* 16(17%), were the most prevalent while the least prevalent bacterial pathogens were *Salmonella sp* 7(7%) and *Shigella sp* 8(8%).
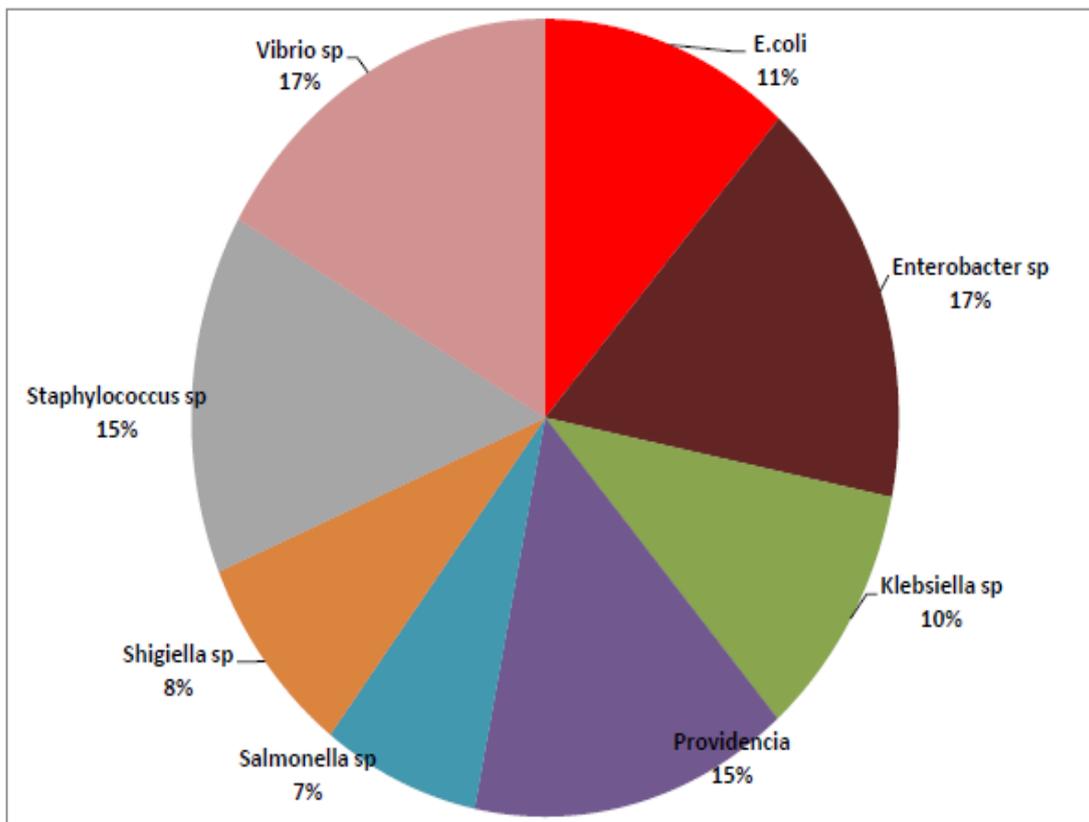
Fig. 1: Percentage Distribution of bacteria isolates.

The result of antimicrobial susceptibility test is shown in **Figures 2-9,** Varying levels of resistance to different antibiotics were shown amongst isolates from broiler and old layer. The result revealed that all isolates were highly resistant to augmentin followed by streptomycin and gentamycin, while *Salmonella* species isolated from broiler and *providencia* species isolated from old layer were sensitive to all antibiotics except augmentin. Overall resistance revealed that most isolates displayed multidrug resistance showing high resistance to at least three antibiotics used for the test.
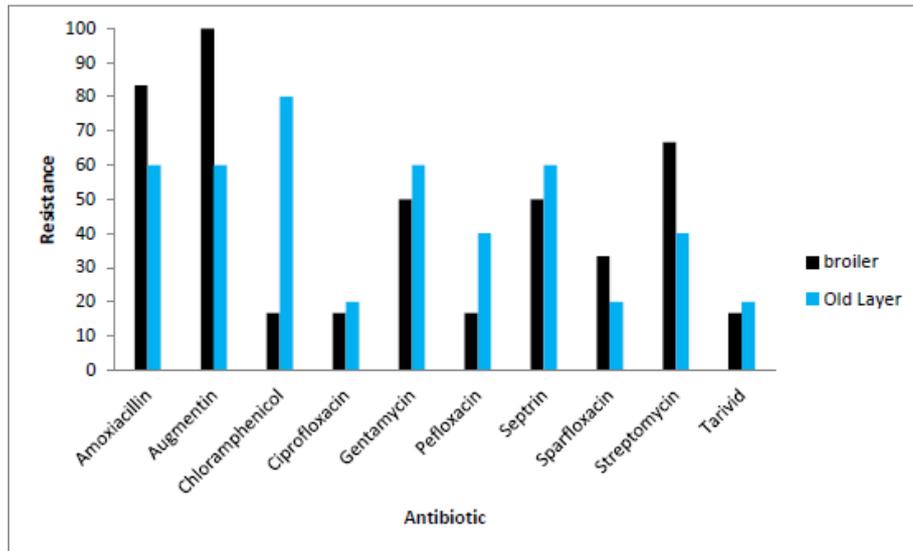
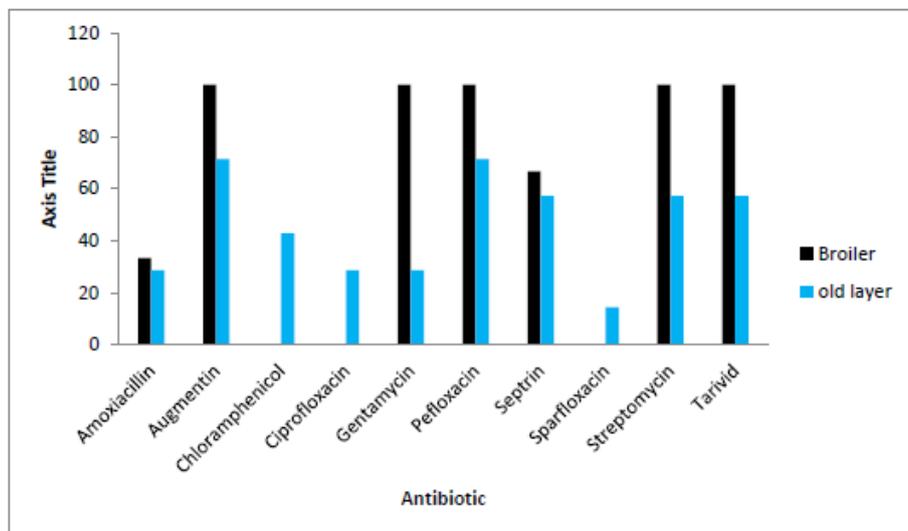**Fig. 2: Resistant pattern of *Escherichia coli.***
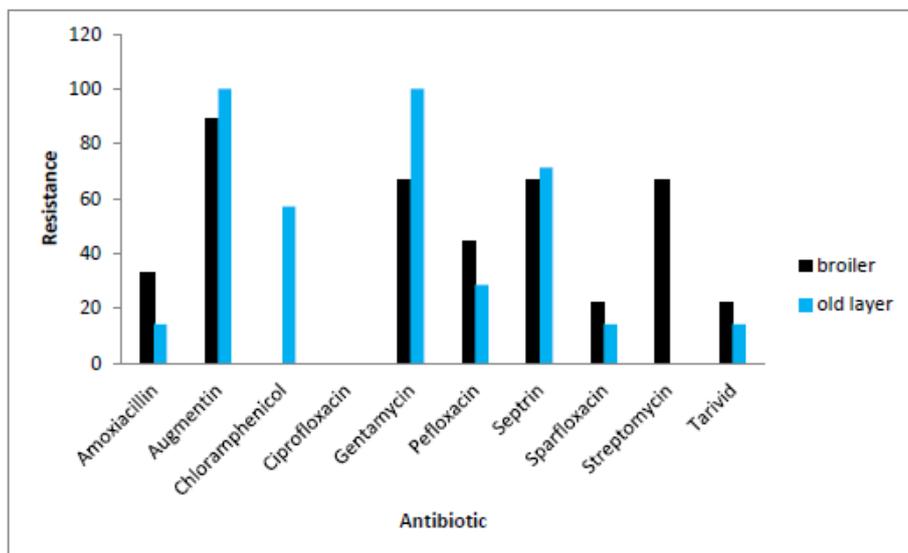

Fig. 3: Resistant pattern of *Klebsiella.*

Fig. 4: Resistant pattern of Enterobacter specie.
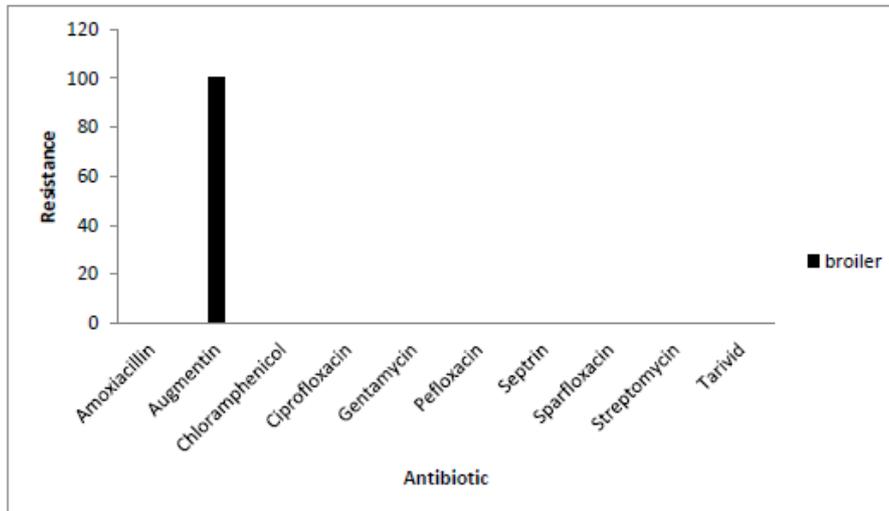
Fig. 5: Resistant pattern of *Salmonella* species (broiler).
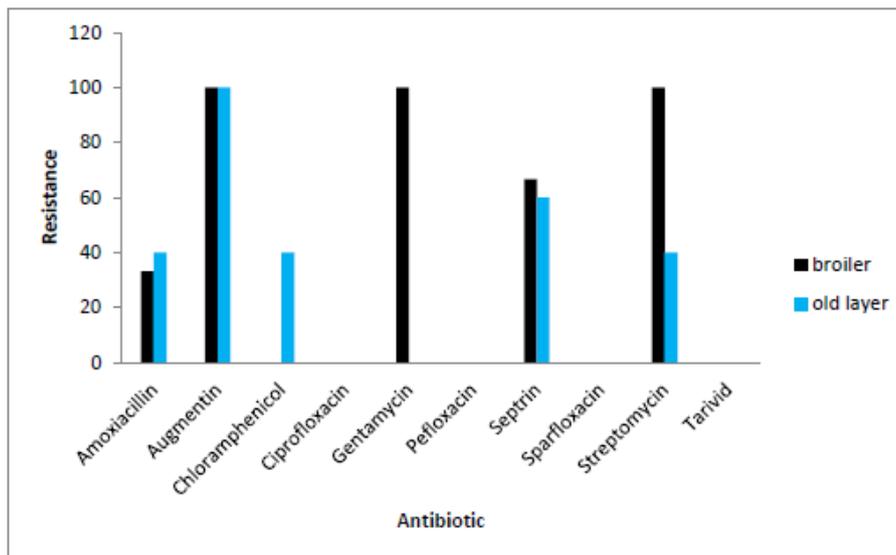

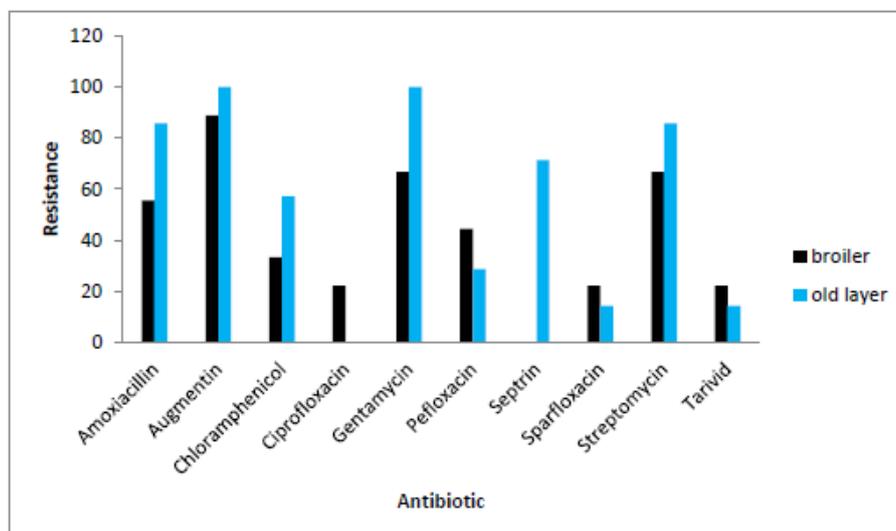**Fig. 6: Resistant pattern of *Shigella* species.**
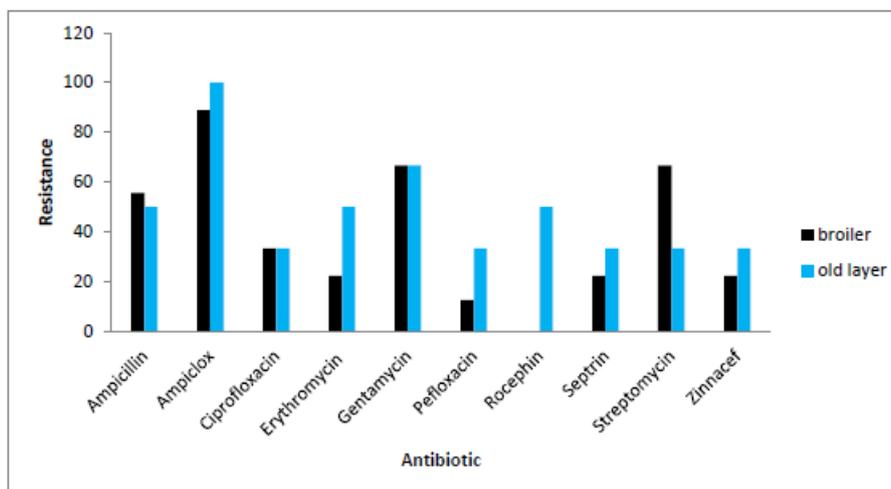

Fig. 7: Resistant pattern of *Vibrio* specie.

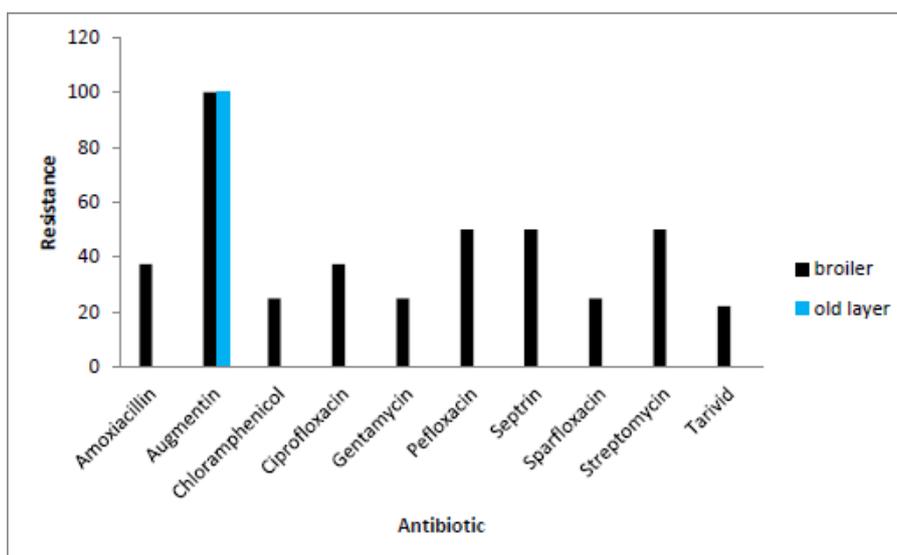**Fig. 8: Resistant pattern of *Staphylococcus* specie.**

Fig. 9: Resistant pattern of *Providencia* specie.

DISCUSSION

The rise in antibiotic resistance had been widely reported and antibiotic resistance still remains a global problem today (Heman *et al.,* 2012). Resistance to antibiotics is highly prevalent in bacteria isolates worldwide. Particularly for antibiotic therapy and resistance control (O'Brien, 1997). The rising frequency of bacterial resistance present a serious problem nowadays, application of antibiotics bring about an increase in resistance to antibiotics not only in pathogenic bacterial strains but also in strains forming a part of the endogenous floral of human and animals. Multidrug resistant bacterial of animal origin may spread into the human population by direct contacts and through food from animal source (Kolář *et al.,* 2002). These resistant bacteria can cause infection in man or colonise the human intestine and the gene coding for the antibiotic resistance can be transferred to natural micro floral.

In this research, potential bacterial pathogen isolated were mostly enteric bacteria with Enterobacter sp and Vibrio sp having highest prevalence, these pathogens are of public health importance. The detection of these organisms in this study agrees with the fact that the bacteria are part of the enteric flora of the poultry birds. However, it was observed from results obtained that there is a difference in the microbial diversity of broiler and old layer. The composition of the intestinal microbial community is influenced by a number of environmental factors, including feed, water, sanitary condition of the poultry and handlers, it is therefore likely to find differences between chickens from various production systems. (Bjerrum *et al.,* 2006). Several factors may be responsible for such variations including the probiotic and physiological state of the gut of animals which has been described as one of the factors that could influence the distribution, and ultimately the recovery rate of organisms from the gut of animals (Ajayi *et al.,*2011).

High resistance to multidrug Augmentin, Amoxicillin, Gentamycin, Septrin and Streptomycin were observed from the study. Results also showed that similar isolates from old layer and broiler showed variation in their sensitivity to antibiotics. Resistance to Augmentin was

significantly high in both species with most of the isolates showing 100% resistance to Augmentin. This report is consistent with previous reports, This finding is in consonance with other studies that have also confirmed the high incidence of antibiotic resistance among bacteria recovered from poultry birds (Akonde *et al.*, 2009; Wouafo *et al,.* 2010).

All isolates where highly resistant to at least three of the antibiotics used for the study except Salmonella which was isolated from only broiler chicken that showed 100% resistance to Augmentin like other species but also displayed 100% sensitivity to Chloramphenicol, Gentamycin, Pefloxacin, Septrin, Sparfloxacin and Tarivid. This current study has demonstrated the presence of antibiotic resistant bacteria in the guts of old layer and broiler poultry birds. These bacterial isolates are potential pathogens which belong to genera that have been implicated in gastro intestinal ailments as well as urinary tract infections and several other infections.

CONCLUSION

Antibiotics have been used widely in poultry industry in order to treat and prevent infectious bacterial diseases. They have also been used at low levels in feed as growth promoters. Such practice has improved poultry performance effectively and economically but an increase in numbers of antibiotic-resistant bacterial strains like Escherichia coli, Staphylococcus sp. and Enterococcus sp. did occur which can be transmitted from poultry to humans through the food chain with serious consequences on public health. The judicious use of antibiotics in animals should be enforced to ensure that the risk of development of resistant commensals and zoonotic pathogens in animals and their spread in the environment is reduced. Formulation of a robust surveillance system to monitor the use of antibiotics in livestock rearing to help track emergence and spread of resistance. This in turn will lessen the risk to human health as the result of handling of animals and through consumption.

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