



Antibiotic resistance of enteric bacteria isolated from duck droppings

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ABSTRACT

Objectives: Ducks are potential carriers of pathogenic bacteria which are capable of transmitting zoonotic diseases to humans as a result of the various interactions with man. Ducks droppings may contaminate the environment with antimicrobial-resistant bacteria. This work was set to identify organisms isolated from duck droppings that may be pathogenic to man.

Methodology and Results: In this study, fecal droppings were obtained from 60 ducks in two rural areas in Badagry, Nigeria. Isolation and identification of enteric bacteria done by using enrichment media, selective media, and biochemical tests. Antibiotic susceptibility testing by the disk diffusion method was conducted on the enteric bacteria using ofloxacin, amoxicillin, augmentin, tetracycline, cotrimoxazole, nitrofurantoin (furadantin), gentamicin and nalidixic acid.

Conclusions and application of findings: Prevalence of *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Proteus mirabilis* were respectively found to be 30%, 8.6%, 13.4%, 9.9% and 38.3%. Organisms were 100% sensitive to ofloxacin; 92.2% resistant to amoxicillin, 88.2% resistant to Augmentin, 98.1% resistant to Tetracycline, 54% resistant to cotrimoxazole, 88.2% resistant to nitrofurantoin while those resistant to gentamicin and nalidixic acid was 17% and 11% respectively. *Salmonella* sp. had 80% sensitivity to nalidixic acid. The risk posed by the droppings of these birds with organisms like *Salmonella* spp., and other zoonotic organisms capable of leading to life threatening diarrhea in man is emphasized.

Keywords: enteric bacteria, antibiotic resistance, duck droppings

INTRODUCTION

Animals are known to constitute a vast reservoir of enteric bacteria with the general problem of environmental contamination by organic waste in regard to human and animals. Enterobacteriaceae has acquired a new importance for the developed countries due to extended livestock farming. Human populations, animal populations, and the environment are all interconnected, and there is a blurring of the lines previously drawn that

distinguished human diseases from animal diseases (Chomel, 1998). Infections of humans and animals with antimicrobial resistant bacteria and contamination of food drink and the environment with resistant bacteria has become of significant concern. Antimicrobials used for the treatment or growth promotion in animals are used for disease control in humans (Poppe *et al.*, 2001). Increasing microbiological and clinical evidences

reveal that resistant bacteria or resistance determinants might be passed from animals to humans resulting in infections that are more difficult to treat. Livestock function as a reservoir of resistant bacteria for environmental contamination, particularly in cases where higher levels of resistance were seen in fecal isolates than in farm environment isolates (Sayah *et al.*, 2004). In addition to the consequences for human health, concerns have been raised about the contamination of surface water with resistant bacteria from livestock operations and septic output from humans. Resistant bacteria have been isolated from a variety of sources, including domestic sewage, drinking water, rivers, and lakes (Mulamattathil *et al.*, 2000; Sayah *et al.*, 2004). The levels of antimicrobial agent resistance that have been reported range from 72% up to 100 and 87% for fecal and nonfecal coliform, respectively (McKeon, *et al.*, 1995). A study found that livestock contributed more than humans to fecal coliform contamination of surface water and that reducing livestock access to surface water reduced the fecal Coliform levels by an average of 94% (Mulamattathil, 2000). Antimicrobial agent resistance has been recognized as an emerging worldwide problem in both human and veterinary medicine, and antimicrobial agent use is considered the most important factor for the emergence, selection, and dissemination of antimicrobial agent-resistant bacteria (Neu, 1992; Witte, 1998). The principle behind the development of resistance is that bacteria in the guts of humans and animals are subjected to different types, concentrations, and frequencies of antimicrobial agents. Over time, selective pressure selects resistant bacteria that have specific fingerprints for resistance to the antimicrobial agents that have been used (Prescott *et al.*, 2000, Troy *et al.*, 2002). Antibiotics have become commonplace in our environment (Col and O' Connor, 1987). They are widely used in medical therapy, animal husbandry and agriculture (Houndt and Ochaman, 2000; Vidaver, 2002). Microbes may develop resistance to antibiotics under selective pressure, or they may acquire antibiotic resistance determinants without direct exposure to antibiotics. The widespread use

of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria (Goossens *et al.*, 2005). They are often used in animals but also in other industries which at least in the case of agricultural use, lead to the spread of resistant strains to human populations. In some developing countries (e.g. Nigeria), antibiotics are sold over the counter without a prescription which compounds the problem. In human medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients (World Health Organization, 2002). Other practices contributing towards resistance include the addition of antibiotics to feeds of livestock (Mathew *et al.*, 2007). Also unsound practices in the pharmaceutical manufacturing industry such as production of counterfeit drugs can contribute towards the likelihood of creating antibiotic resistant strains (Larsson and Fick, 2009).

Antimicrobial-resistant organisms in domestic animals such as poultry, beef and swine are well documented (NARMS, 2002) and have been implicated as reservoirs for multidrug-resistant food borne pathogens. Interaction with waste materials from these livestock species may confer resistant pathogens and genetic elements to free-ranging wildlife, potentially creating an additional environmental reservoir of resistant organisms (Hudson *et al.*, 2000). In 2001, the Union of Concerned Scientists estimated that greater than 70% of the antibiotics used in the US are given to food animals (e.g. chickens, pigs and cattle) in the absence of disease. In 2000 the US Food and Drug Administration (FDA) announced their intention to revoke approval of fluoroquinolone use in poultry production because of substantial evidence linking it to the emergence of fluoroquinolone resistance *Campylobacter* infections in humans. The final decision to ban fluoroquinolone from use in poultry production was not made until five years later because of challenges from the food animal and pharmaceutical industries. (Nelson *et al.*, 2007). In bacteria, the potency of almost every known class of antibiotics is weakened by the defense

mechanism of some strains of bacteria and as such strains of pathogens resistant to antibiotics have begun to develop at an alarming rate. The goals of this study were to determine if enteric bacteria could be cultured from the faecal

droppings of ducks and to find out the frequency of this. It was also aimed at determining the antibiotic susceptibility of such organisms by the use of minimal inhibitory concentration (MIC) testing.

MATERIALS AND METHODS

Sample collection: Duck feces were collected randomly from two rural areas (Iragon and Ikoga) in Badagry town, Lagos State of Nigeria. A total of sixty samples were collected from 60 ducks raised at different homes, farms and those found on the streets. Ducks were watched as they defecated and faecal dropping were collected (within 5 min of defecation) from the ground with sterile swab sticks and packaged into alkaline peptone water. Thirty samples were collected from each location. Care was taken to collect only the fresh fecal samples, avoiding soil and grass contaminants.

Microbial enumeration and identification; All samples were processed within 24 hours. Isolation and identification were done using standard bacteriological methods. Isolation and identification of *enteric bacteria* were done by using enrichment media, selective media, and biochemical tests.

Antibiotic resistance testing: The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles of the enteric bacteria. The minimum inhibitory concentration were measured and recorded as whether the organism is susceptible (S), intermediately susceptible (I), or resistant (R) to the antibiotic. Commonly used antibiotics such as tetracycline, cotrimoxazole, amoxicillin in the treatment of food poisoning and diarrhea diseases in Nigeria environment were selected and used to test their effectiveness in suppressing these organisms. Isolated colonies of each species were picked and antibiotic susceptibility was determined. Using McFarland standard, suspension of isolates were prepared and spread evenly onto Mueller-Hinton agar, disks impregnated with various defined concentrations of different antibiotics are placed onto the surface of the agar. After incubation, a clear circular zone of no growth in the immediate vicinity of a disk indicates susceptibility to that antimicrobial. The zones

of inhibition were measured and the results were recorded based on World Health Organization Drug Information and National Committee for Clinical Laboratory Standard (NCCLS, 2006). Using reference tables the size of zones was related to the Minimum Inhibitory Concentration and results recorded as whether the organism is susceptible (S), intermediately susceptible (I), or resistant (R) to that antibiotic.

1. Each isolate was tested against ofloxacin OFX (30µg), amoxicillin AMX (25µg), augmentin-AUG (30µg), nalidixic acid NAL (30µg), cotrimoxazole COT (25µg),

furadantin nitrofurantoin NIT (30µg), gentamicin GEN (10µg) and tetracycline TET (30µg) (Abtek Biologicals Ltd). The zones of inhibition were measured and the results were recorded based on World Health Organization Drug Information and National Committee for Clinical Laboratory Standard (NCCLS, 2006). Isolates that showed resistances to multiple antibiotics were tested for their plasmid Multiple antibiotic resistance (MAR) values for each isolate were calculated by summing the number of antibiotics to which the isolate was resistant and dividing by the total number of antibiotics assayed (Kaspar *et al.*, 1990).

Plasmid Extraction (TENS Method): Isolation of plasmid DNA by 'TENS mini - prep' procedure was used. TENS is a composition of Tris HCl 25Mm, EDTA 10Mm, NaOH 0.1N and SDS 0.5%. This method was used to extract the plasmids of both gram negative and positive bacteria. (Zhou *et al.*, 1990). Data were analysed using SPSS Version 13 with frequencies, means.

RESULTS

There were eighty-two isolates in all, with enteric bacteria isolates being (62.19%) while *Proteus mirabilis* constituted 37.81%. The enteric bacteria consisted of

30.5% isolates of *Salmonella spp.* {*Salmonella enteritidis* (12.2%), *S. choleraesuis* (8.5%), *S.typhimurium* (9.8%)}, 13.8% *Klebsiella pneumonia*,

8.5% *Escherichia coli*, and 9.8% *Citrobacter freundii* while 37.8% were *Proteus mirabilis* (Table 1).

Minimum inhibitory concentration values for 8 antibiotics were determined for each of the 51 enteric bacterial isolates. Overall, there was a 100% resistance to tetracycline by all the isolates, 100% sensitivity to Ofloxacin and $\geq 75\%$ to Nalidixic acid. Antibiotic resistance was detected in all enteric bacteria isolated

from fecal samples collected. The most frequently encountered form of resistance in all the enteric bacteria isolated was resistance to amoxylin (89.85%), followed by resistance to augmetin (87.77%), tetracycline (83.23), cotrimoxazole (57.23%), furadantin (17.14%) and to nalidixic acid (5.83%). There was no resistance to ofloxacin

Table 1: Total number (and percentage) of identified *Proteus mirabilis* and enteric bacteria

Microorganisms	Number isolated	%
<i>Proteus mirabilis</i>	31	37.8
<i>Salmonella enteritidis</i>	10	12.2
<i>Salmonella cholerasuis</i>	7	8.5
<i>Salmonella typhimurium</i>	8	9.8
<i>Klebsiella pneumoniae</i>	11	13.8
<i>Escherichia coli</i>	7	8.5
<i>Citrobacter freundii</i>	8	9.8

The *Salmonella* species exhibited resistance to all antibiotic used except ofloxacin, while *E. coli* and *Citrobacter* showed resistance to three antibiotics (furadantin, nalidixic acid and ofloxacin) and *Klebsiella* showed resistance to four antibiotics (furadantin, gentamicin, nalidixic acid and ofloxacin). Resistance to amoxylin, augmentin, tetracycline and cotrimoxazole was present in all enteric bacteria isolated, while gentamycin resistance was found in all except one isolate. furadantin and nalidixic resistance were found in only two enteric bacteria. None of the enteric bacteria was resistance to ofloxacin. *Salmonella cholerasuis* had 100% susceptibility to ofloxacin, 85.71% to nalidixic acid and 71.43% to gentamycin. However the same isolates had 100% resistance to tetracycline, 85.71% resistance to gentamycin and 85.71% to amoxicillin (Fig 1). There was 100% susceptibility of *Salmonella enteritidis* to ofloxacin, 70% to nalidixic acid and gentamicin while it had 100% resistance to amoxycilin, cotrimoxazole, augmentin and tetracycline (Fig 2). Figure 3 that showed *Salmonella typhimurium* had 100% susceptibility to ofloxacin and 100% to nitrofuratoin, 75% to nalidixic acid and 75% to gentamycin while there was 100% resistance to amoxycilin and 75% resistance to augmentin and 75% to tetracycline. In figure 4, *Escherichia coli* was 100%,

100%, 85.71% susceptible to ofloxacin, nalidixic acid and gentamycin respectively while it was resistance to amoxycilin (100%), augmentin (100%) and tetracycline (75%). *Klebsiella pneumoniae* (Fig. 5) was 100% susceptible to ofloxacin, nalidixic acid and gentamicin, and 81.82% to furadantin. There was 90.91% resistance each to augmentin and amoxycilin. Fig 6 shows the effect of different antibiotics on *Citrobacter freundii*. The isolates were 100% susceptible to ofloxacin and nalidixic acid and 87.5% to gentamycin. Tetracycline and augmentin had 75% resistance on the isolates while amoxycillin had 62.5%. Figure 7 showed the overall effect of the antibiotics on all the isolates. The isolated enteric bacteria were least resistance to ofloxacin, nalidixic and gentamycin by 0%, 5.83% and 16.73% respectively however they had higher resistances for amoxycilin, augmentin and tetracycline by 89.85%, 88.77%, and 83.23% respectively.

All the isolates except three had plasmid. Table 2, shows the molecular weight of the samples which was deduced from the DNA Molecular Weight Marker II (0.12-23.1kbp). The result shows that the plasmid had a molecular weight of 23.4kbp. Minimum inhibitory concentration values for 19 drugs were determined for each of the 54 *Enterococcus* isolates.

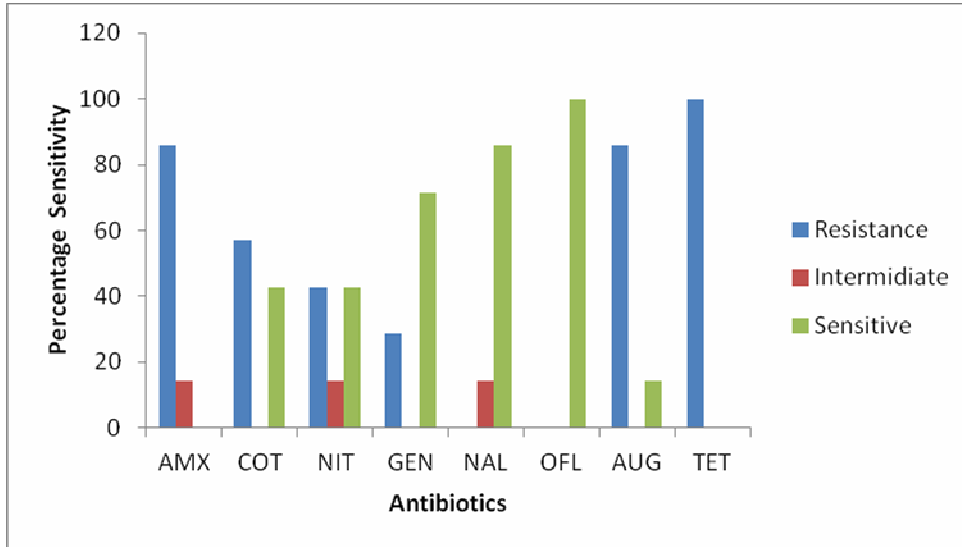


Figure 1: Effects of different antibiotics on *Salmonella choleraesuis*

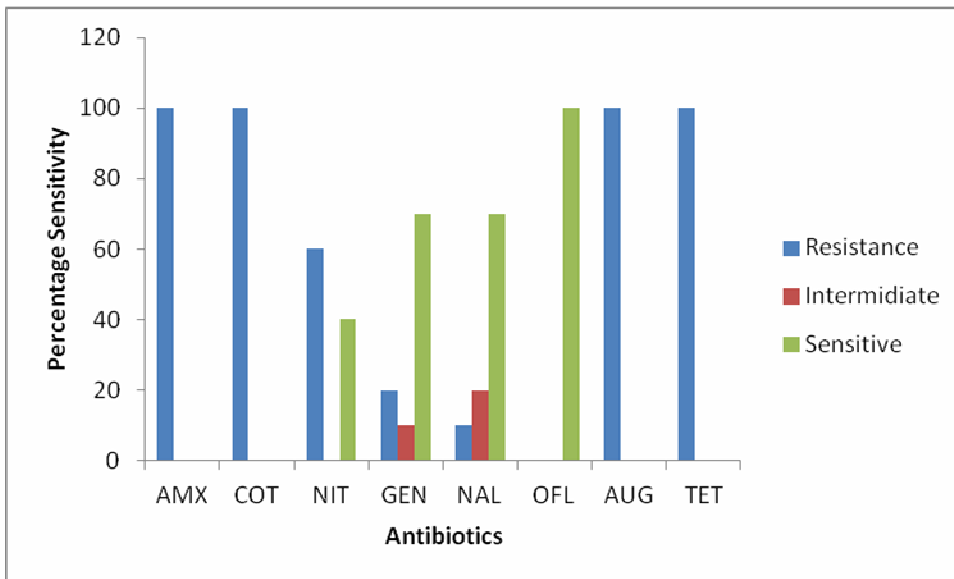


Figure 2: Sensitivity and resistance of *Salmonella enteritidis* to antibiotics

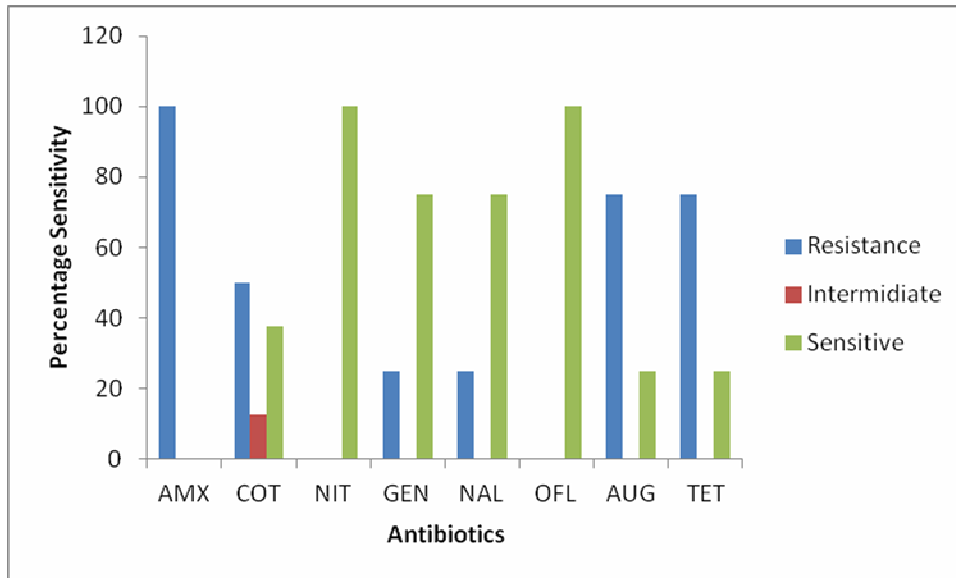


Figure 3: Effects of different antibiotics to *Salmonella typhimurium*

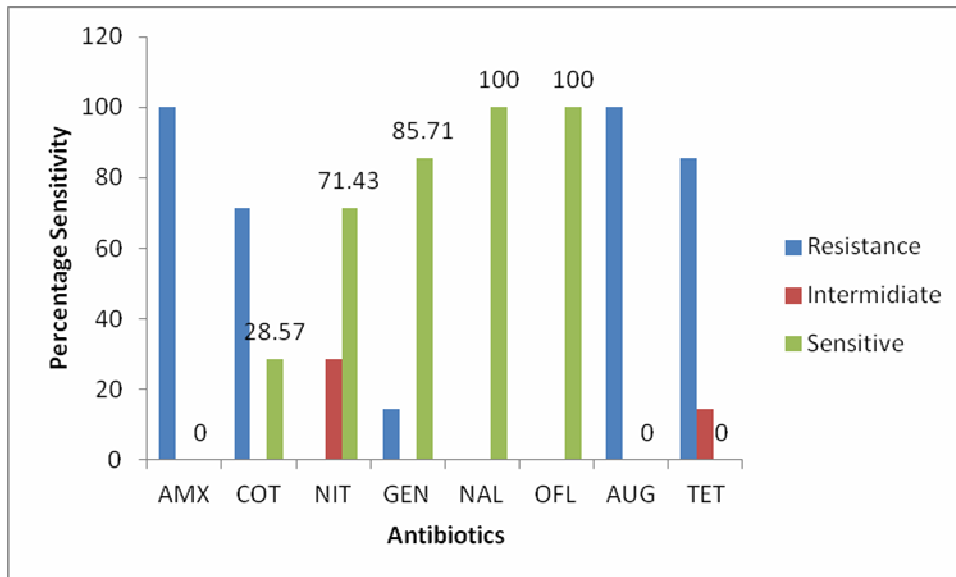


Figure 4: Effects of antibiotics on *Escherichia coli*.

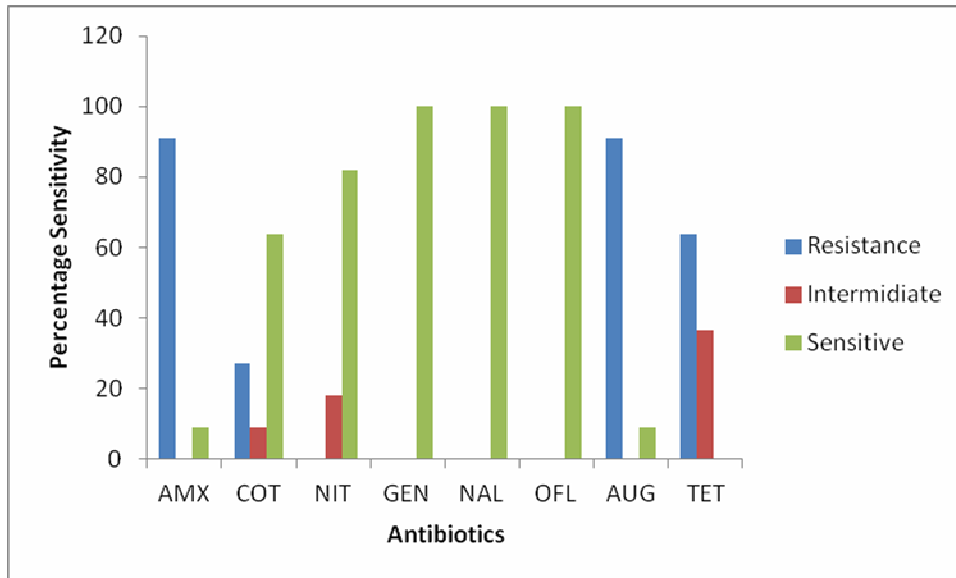


Figure 5: Effects of antibiotics on *Klebsiella pneumoniae*

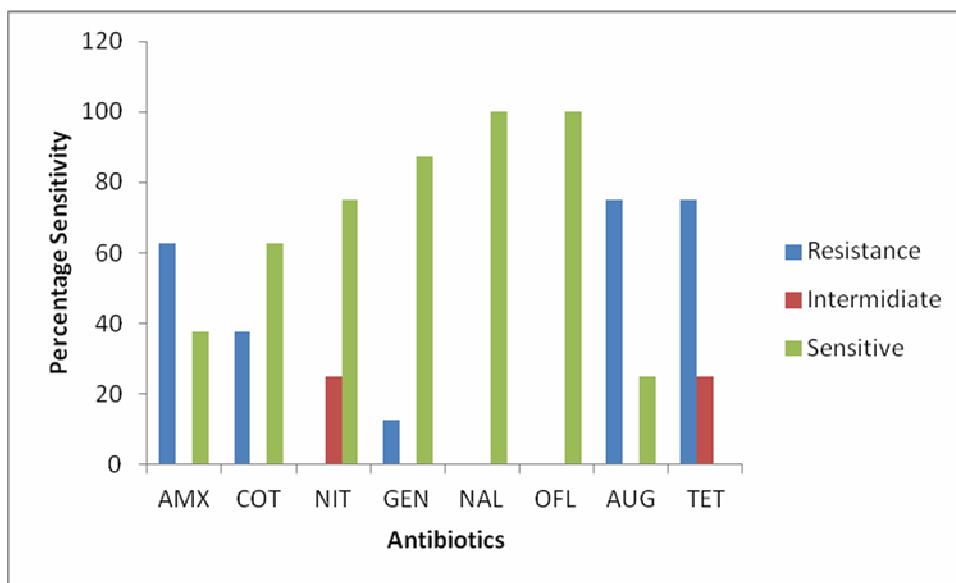


Figure 6: Effects of antibiotics on *Citrobacter freundii*

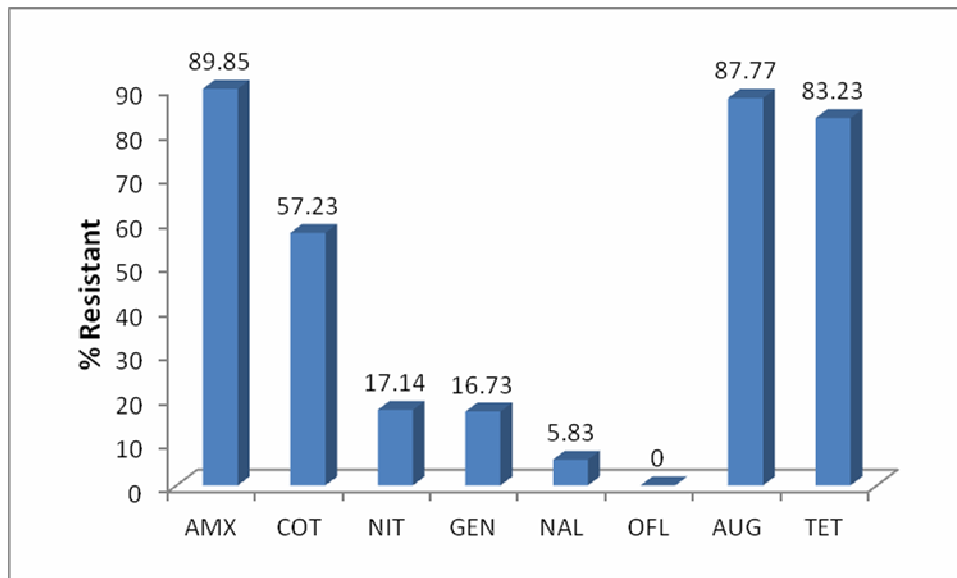


Figure 7: Antibiotic multiple resistant pattern of isolated enteric bacteria

Table 2: Molecular Weight of Isolated Enteric Bacteria

	Molecular Weight	Log of molecular weight	Distance moved
<i>Salmonella enteritidis</i>	23130	4.3	8.2
<i>Salmonella cholerasuis</i>	9416	3.97	7.7
<i>Salmonella typhimurium</i>	6557	3.82	7.4
<i>Klebsiella pneumoniae</i>	4361	3.64	7.0
<i>Escherichia coli</i>	2332	3.37	5.8
<i>Citrobacter freundii</i>	2027	3.31	5.3

Distance moved by samples= 8.3

8.2cm = 23130

8.3cm =X X = 8.3 x 23130/8.2 = 2314 base pair = 23.4kbp

DISCUSSION

The sensitivity pattern obtained from the antibiotic treatment of the bacteria from duck droppings in this study varied. It was found that a high incidence of multiple antimicrobial drug resistance especially of *Salmonella* strains, and a probable resistance transfer factor which could be as a result of the plasmid profile. Out of the 81 enteric bacteria isolated from duck droppings, 30% were *Salmonella* spp., 13.4% were *Klebsiella pneumoniae*, 8.6% *Escherichia coli*, and 9.9% were *Citrobacter freundii*. The rest were *Proteus mirabilis* (38.3%), an organism known to be an opportunistic bacterium causing urinary tract infections in humans. Resistant bacteria have been isolated from a variety of sources, including domestic sewage, drinking water, rivers, and lakes (Kasper *et al.*, 1990, Mckeon *et al.*, 1995, Mulamattathil *et al.*, 2000). One study found that livestock contributed more than humans to fecal coliform contamination of surface water and that reducing livestock access to surface

water reduced the fecal coliform levels by an average of 94% (Hagedorn *et al.*, 1999).

The result of the antibiotic susceptibility test showed that *Salmonella* spp. was resistance to amoxicillin, augmentin and tetracycline was 100%, 92% and 96% respectively. These antibiotics are very common and readily available to consumers in Nigeria and therefore should not be considered first line antibiotics in diarrhea diseases. The effect of using these antibiotics and delaying response to therapy should therefore be noted. *Salmonella* is an etiological agent of Salmonellosis in which case the patient develops diarrhea, fever and abdominal cramps 12 to 72 hours after infection {Ryan and Ray (2004)}. Severe morbidity and mortality usually accompany infection with this organism. Resistance of a single bacterial isolate to more than one antimicrobial drug is commonly reported. Multiple antimicrobial drug resistance profiles have been used to identify and differentiate *E. coli*

strains from different animal species (Krumperman *et al.*, 1983). This type of testing is simple, cost-effective, and suitable for surveillance (Troy *et al.*, 2002), and it has been used for *E. coli* strains collected from human and animal sources (Krumperman *et al.*, 1983). From this study it was observed that *Escherichia coli* (common causes had a 100% resistance to amoxicillin, augmentin and tetracycline. This is similar to what was observed by Aibinu *et al.*, (2004) who reported 100% resistance of their *E. coli* isolates to ampicillin and amoxicillin. Resistance to amoxicillin observed in this study was similar to what was observed in South Africa, Israel, (62% - 84%) and Hong Kong, Philippines (64% - 82%) by Stelling *et al.*, 2005. It was equally observed that the 100% susceptibility of *Escherichia coli* to both ofloxacin and nalidixic acid; 85.7% to gentamycin and 71.4% to furadantin was similar to the report of Egri-Okwaji (1996) who reported 100% susceptibility of *E. coli* isolates to ofloxacin, 85% sensitivity to gentamicin, 71.4% sensitivity to furadantin and cotrimoxazole. However there was just 29% susceptibility of *E. coli* to cotrimoxazole contrary to 71.4% observed in the report of Egri Okwaji (1996). Sayah *et al.*, 2004 also reported lowest levels of resistance of *E. coli* to ofloxacin and nalidixic acid. There was also a 100% susceptibility of *Klebsiella pneumoniae* to nalidixic acid, ofloxacin and gentamicin; 81% to furadantin, 63.3% to cotrimoxazole while 90.9% resistance to amoxicillin and augmentin was observed. All the isolates of *Klebsiella pneumoniae* were resistant to tetracycline. *Klebsiella pneumoniae* is an etiological agent of human nosocomial infections. *Citrobacter freundii*, had 100% sensitivity to ofloxacin and nalidixic acid, 81.5% sensitivity to gentamicin, 25% sensitivity to amoxicillin and augmentin. There was no sensitivity to tetracycline. Boehme (2004), in his study reported that *Citrobacter freundii* had resistance to tetracycline (43%) and gentamycin (4%). The result may indicate that faecal samples collected from the environment studied were sources of resistance factors though it has been demonstrated by Rysz and Alvarez, (2004) that bacteria in the soil can acquire resistance to tetracycline from environmental exposure, possibly creating a reservoir of resistance factors generated

CONCLUSION

The result suggested that livestock functioned as a reservoir of resistant bacteria for environmental contamination, in agreement with the report of Sayah *et al.*, 2004. Ducks are potential carriers of pathogenic bacteria which are capable of transmitting zoonotic diseases to human as a result of the various

outside host animals. This finding also suggests that, while collection of environmental samples may not be a valid means of assessing the prevalence and distribution of antimicrobial agent resistance patterns, it may be a more accurate measure of exposure to resistance factors. However, there is need for additional research in this area, by expanding the collection of samples to other potential host sources of resistant bacteria and comparing the genetic characteristics of bacteria isolated from different environment to the genetic characteristics of bacteria isolated from uncontaminated specimens. The MAR values are primarily useful for comparing the resistance patterns of bacterial isolates within a sample and for determining the range of antibiotic resistance determinants present within a sample population. The study found a wide range of MAR patterns in the enteric bacteria isolated from fecal droppings of ducks, indicating great diversity in the antibiotic susceptibility of enteric bacteria. The highest levels of resistance were observed for amoxylin, augmetin and tetracycline in all enteric bacterial isolates collected from all samples (Figure7). The patterns of resistance to the antimicrobial agents may be due to indiscriminate, widespread and lengthy use of amoxylin, augmetin and tetracycline. Tetracycline is a commonly used first-line antibiotic in the poultry and is often used before the antimicrobial agent resistance of a pathogen has been determined (Prescott *et al.*, 2000).

The highest levels of susceptibility to all enteric bacterial isolates found in this study were to ofloxacin and nalidixic acid (Figure 7) in line with the findings of Sayah *et al.*, 2004. It was observed that these antimicrobial agents are members of drug classes that have restricted uses. Engberg *et al.*, 2001 reported that the use of fluoroquinolones (ofloxacin and nalidixic acid) has been restricted since the 1990s, after the rapid emergence of resistance to fluoroquinolones after the introduction of ciprofloxacin into poultry production in Europe. The plasmid profiles of the isolates were similar and high which may suggest that the plasmid could have conferred the resistance on the isolates.

interactions man had with them since they are domesticated animals. From this study, enteric bacteria were isolated from duck droppings. The antibiotic sensitivity testing shows that all the organisms were 100% sensitive to ofloxacin. The most resistant antibiotics common to all the isolates were amoxicillin,

augmentin and tetracycline. The potential role of domestic animals and habitats as vectors of antimicrobial resistance in the environment should further be studied. The results showed that antimicrobial agent resistance was present in isolated enteric bacteria and that the resistance varied from one organism to the other. Isolated bacteria appeared to show higher levels of resistance or reduced susceptibility to some specific antimicrobial agents. This study revealed that surface water and the environment may easily be contaminated by domestic

animals, therefore it has become increasingly important to develop low-cost screening tools that can be used to identify the most probable source of fecal contamination. From the plasmid profile, it was discovered that all the isolates with multiple drug resistance had plasmid which is 23.4kbp. This may be responsible for the multiple resistances to the antibiotics. The implication of this on the choice of antibiotics in relation to zoonotic infections in our environment should be noted and effort should be made to stop indiscriminatory use of antibiotics.

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