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ANTIBIOTIC RESISTANCE PATTERN OF PATHOGENIC BACTERIA ISOLATED FROM POULTRY DROPPINGS IN AKURE, NIGERIA.

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ABSTRACT
Antibiotic resistance bacteria pathogens especially in food animal is an emerging problem of public health concern, resistant pathogens is acquired by man through food chain. This study aimed at investigating antibiotic resistance pattern of pathogenic bacteria in poultry droppings. Samples of fresh poultry dung were obtained from free-range chicken and nine commercial chicken farms, in Akure, Ondo State, Nigeria. Samples were plated on selective and differential media. Isolated bacteria were identified by standard microbiological method. Pathogens isolated include both Gram positive and Gram negative bacteria, namely; *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella* spp. *Citrobacter* spp. *Salmonella* spp. *Serratia marcescens*, *Shigella dysenteriae*, *Proteus* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Micrococcus luteus.* Antibiotic susceptibility testing was carried out using the disk diffusion technique. Antibiotics used for gram negative pathogens were; ofloxacin, amoxycillin, tetracycline, gentamycin, augmentin, ceftriazone, nitrofuration, cotrimozazole, ciprofloxacin and chloramphenicol while cotrimoxazole, erythromycin, augumentin, chloramphenicol, tetracycline, cloxacilin, gentamycin, streptomycin were used for gram positive pathogens. The resistant pattern in gram negative pathogens revealed that more than 90% were resistant to Augmentin, Ceftriaxone, Nitrofuratoin, Amoxicillin and Cotrimoxazole, 80.15% resistant to tetracycline, 83.97% resistant to chloramphenicol, 35.11% resistant to gentamycin, 16.79% resistant to ciprofloxacin and 8.40% resistant to ofloxacin, gram positive pathogens were 100% sensitive to streptomycin and 100% resistant to cotrimozazole and augmentin, 83.33% resistant to tetracycline, 38.89% resistant to cloxacilin and 22.22% resistant to gentamycin, erythromycin and chloramphenicol. Conclusively, the conventional use of antibiotics in poultry has resulted to the emergence of antibiotic resistant pathogenic bacteria.
Key words: Pathogenic bacteria, antibiotic resistance pattern, poultry droppings

INTRODUCTION
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 main risk factor for an increase in bacterial resistance is an increased use of antibiotics. It is similar in humans and in animals. In animals, antimicrobial agents are not used only for therapy and prevention of bacterial infections but also as growth promoters. It is very important to monitor the resistance to antibiotics not only in human bacterial pathogens but also in pathogenic and commensal bacteria of animal origin (Kolář *et al.,* 2002).

The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the potential post-antibiotic era threatening present and future medical advances (Raghunath, 2008). The microorganisms that are mainly involved in the resistance process are the, so called the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*,

Pseudomonas aeruginosa, and enterobacteriaceae) emphasizing their capacity to "escape" from common antibacterial treatments (Matteo *et al.*, 2013).

In antibiotic charged environment, bacteria develop ways to fight off antibiotics by: preventing antibiotics from reaching their target cells (changing the permeability of cell walls or pumping the drugs out of the cells); changing the structure of target cells or entirely replacing them; or producing enzymes that destroy antibiotics. Also, bacteria may gain resistance by getting copies of resistant genes from other bacteria. Antibiotic resistant bacteria pass between humans, between animals and between humans and animals in both directions through the food chain. Copies of antibiotic-resistant genes can also move between bacteria, and this exchange can occur in the human gut, so in some cases the bacteria causing a human infection will not be of farm-animal origin, but the resistance will be. 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).

Development of antibiotic resistance among pathogenic bacteria is a major public health concern, it can cause significant danger and suffering to individuals, families and the entire community who have common infections that once were easily treatable with antibiotics. The emerging resistant bacterial strains will adversely affect the efficacy of antibiotic chemotherapy for those that acquired the new strains of infectious disease. Furthermore, it encourages the need for more expensive and toxic medications. Some resistant infections can cause death (Apata 2009). Thus, it became imperative to provide information on occurrence of human pathogenic bacteria and antibiotic resistance pattern of the bacteria pathogens from poultry droppings in Akure, Ondo State, Nigeria.

MATERIALS AND METHODS

Sample collection

Six hundred and eighty four (684) samples of fresh poultry dung (layers and broilers) were collected from commercial poultry farms in nine (9) different locations (FUTA, Aba, Apatapiti, Ijoka, Oritaobele, Ado road, Ondo road, Alagbaka, and Lafe), while that of free range chicken was collected from chicken feeding ground at different locations in Akure, Ondo State, Nigeria. One gram of poultry dropping was collected in sterilised Mac Cartney bottle that contained peptone water and transported to Microbiology Research Laboratory of Federal University of Technology Akure within one hour of collection for bacteriological analysis. The samples were collected between November 2015 and January 2016.

Isolation of Bacteria from poultry droppings

Bacteriological examinations were carried out using standard methods for aerobic bacteria (Brown, 2005). Sample collected in Mac Cartney bottle was gently shake and stirred with sterile glass rod until the dung mixed thoroughly, aliquot (1.0 ml) was transferred into the test tube containing 9.0 ml of sterile distilled water and diluted serially in one-tenth stepwise to 10^{-7} dilution factor and 1.0 ml each of dilution 10^{-5} , 10^{-6} and 10^{-7} was pure plated on Nutrient agar and some selective and differential media (Salmonella Shigella agar, Eosine Methyline Blue agar, MacConkey agar, Manitol salt agar and Cysteine Lactose Electrolyte Deficient agar), the plates were inverted and incubated aerobically at 37 ^{o}C for 24 hours after which the plates were examined for growth.

Biochemical characterization

Biochemical characterisation and presumptive identification of isolates were carried out as described by Cheesbrough, (2009).

Antibiotics susceptibility test

Antibiotics susceptibility test of all the isolates was determined by the disk diffusion method and interpreted as

susceptible, intermediate and resistant as described by CLSI, (2014). Gram negative pathogens were tested against the following antibiotics; Tetracycline (30µg), Ofloxacin (30µg), Gentamicin (20µg), Chloramphenicol (30µg), Augmentin (30 µg), Ceftriazone (30 µg), Nitrofuratoin (300 µg), Cotrimoxazole (25 µg), Ciprofloxacin (10 µg) and Amoxicillin (30µg) while gram positive isolate were tested against Cotrimoxazole (25 µg), Erythromycin (10µg), Gentamicin (20µg), Augmentin (30 µg),Streptomycin (10 µg), Cloxacilin (5µg) Tetracycline (30µg) and Chloramphenicol (30µg).

Quality control

Typed culture *(Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) was used as quality control for antimicrobial susceptibility testing as recommend by Clinical and Laboratory Standards Institute (CLSI, 2014)

Statistical analysis

Data was statistically analysed using SPSS version 20, the results obtained were statistically analysed using analysis of variance (ANOVA), and tests of significance carried out by New Duncan's multiple range test at $p \leq 0.05$

RESULTS

Total bacterial viable counts poultry dung

Mean total bacterial viable counts are shown in **Table 1**. The bacterial population isolated from droppings ranged from 9.35×10^7 cfu/g to 10.58×10^7cfu/g, 5.65×10^7 cfu/g to 6.80×10^7 cfu/g in broilers and layers respectively while 10.05×10^7 cfu/g was observed in free range chicken.

Table 1: Total bacteria count in layers and broilers droppings

	Broilers	Layers
Sample locations	Bacterial count (cfu/g) × 10^7	Bacterial count (cfu/g) × 10^7
A	$9.35^{a} \pm 1.73$	$6.23^{abc} \pm 1.40$
B	$9.40^{ab} \pm 1.26$	$6.13^{abc} \pm 1.32$
C	$9.90^{abc} \pm 1.22$	$6.25^{abc} \pm 1.21$
D	$10.15^{abc} \pm 1.59$	$5.65^{a} \pm 1.42$
E	$10.05^{abc} \pm 1.54$	$6.08^{abc} \pm 1.80$
F	$10.58^{c} \pm 1.32$	$6.80^{c} \pm 1.40$
G	$9.85^{abc} \pm 1.78$	$6.58^{c} \pm 1.41$
H	$10.20^{bc} \pm 1.71$	$5.78^{ab} \pm 1.69$
I	$9.80^{abc} \pm 1.52$	$6.53^{bc} \pm 1.48$
J	$10.05^{abc} \pm 1.95$	

Keys:
A - FUTA, **B** - Aba, **C** - Apatapiti, **D** - Ijoka, **E** - Oritaobele, **F** – Ado road, **G** - Ondo road, **H** - Alagbaka, **I** - Lafe, **J** – Free range chicken, **cfu/g** – colony forming unit per gram. Values are means of triplicates ± SE. Values in the same column carrying the same superscript are not significantly different according to Duncan's multiple range test at ($P < 0.05$).

Pathogenic bacteria isolated from poultry dung

Prevalence of bacteria pathogens is presented in **Figure 1.** Total number of one hundred and fifty seven (157) bacterial pathogens was isolated and identified. *Escherichia coli* 84 (53.50%) was the most prevalent while the least prevalent bacterial pathogens were *Enterobacter* spp. 3(1.91%), *Shigella* spp., 2(1.27%) *Citrobacter* spp. 3(1.91%), *Pseudomonas* sp. 3(1.91%), *Seratia* sp. 2(1.27%) and *Micrococcus luteus* 3(1.91%).

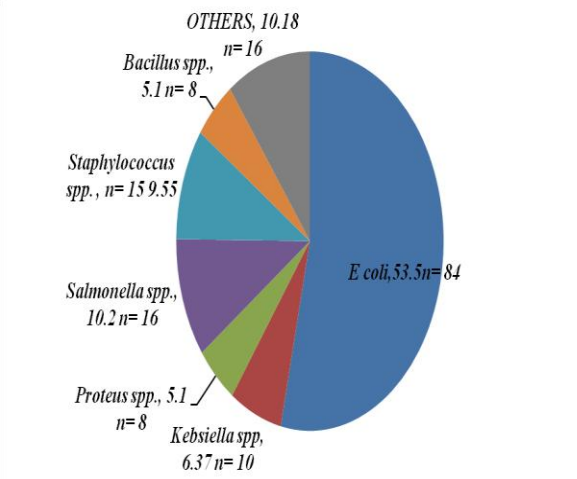

Figure 1: Percentage distribution of bacteria isolated from poultry droppings in different locations.
Key: Others*: Enterobacter* spp., *Shigella* spp., *Citrobacter* spp., *Pseudomonas* sp., *Seratia* sp. and *Micrococcus* spp.

Distribution of bacteria across different sample locations

The result of bacterial pathogens isolated from different poultry locations is presented in **Table 2.** From the result, one hundred and fifty seven bacteria were isolated from six hundred and eighty four (684) samples. Highest number of pathogen was isolated from Alagbaka (**H**) 27 (17.20%) and lowest was recorded in Free range chicken (**J**) 11 (7.01%).

Table 2: Distribution of bacteria across different sample locations

Sample Locations	Number samples collected	Total counts of bacteria isolated (%)
A	80	19 *(12.10)
B	50	14 (8.92)
C	80	15 (9.55)
D	70	16 (10.19)
E	80	14 (8.92)
F	71	12 (7.64)
G	65	16 (10.19)
H	80	27 (17.20)
I	68	13 (8.28)
J	40	11 (7.01)
Total	684	157 (100)

Keys: A - FUTA, **B** - Aba, **C** - Apatapiti, **D** - Ijoka, **E** - Oritaobele, **F** – Ado road, **G** – Ondo road, **H** - Alagbaka, **I** - Lafe, **J** – Free range chicken. ***(%)** – Percentage of total bacteria distribution.

Incidence of antibiotic resistance among bacteria isolated from Layers, Broilers and Local Chicken droppings.

Antibiotic resistance pattern of all the pathogenic bacteria isolated from Layers, Broilers and Local Chicken droppings is presented in **Figure 2,** the result showed high resistant profile to multiple antibiotics in Layers and Broilers, isolates from Local Chicken show high resistant to Gentamicin and Chloramphenicol than those from layers and broilers.

Resistance Pattern of gram negative bacteria to antibiotics.

Table 3 shows the resistance pattern of all the gram negative bacteria to tested antibiotics. The result reveled that all isolate were 100 % resistant to Amoxicillin, *Seratia* spp, *Citrobacter* spp., *Shigella* spp., *Enterobacter* spp. and *Proteus* spp. were not resistant to Ofloxacin. However, there was significant difference ($P \leq 0.05$) of percentage resistance to Ofloxacin in *E coli*, *Kebsiella* spp, *Salmonella* spp. and *Pseudomonas* spp. Overall resistance pattern reveled that there is no significant difference ($P \leq 0.05$) in the resistance pattern exhibited by isolates to Augmentin, Ceftriaxone, Nitrofuratoin, Amoxicillin, and Cotrimoxazole.

Resistance Pattern of gram positive bacteria to antibiotics.

Figure 3 shows the resistance pattern of all the gram positive bacteria to tested antibiotics. The result reveled that all isolate were 100 % resistant to Cotrimoxazole and Augumentin, *Micrococcus* sp. was found to be 3(100 %) resistant to Cloxacillin and significantly higher at ($P \leq 0.05$) than *Staphylococcus* spp. 4(26.67%). Overall resistant pattern reveled that all the isolate were sensitive to streptomycin and there was no significant difference ($P \leq 0.05$) in the resistant pattern of gentamicin, erythromycin and chloramphenicol 4 (22.22%). However resistance to Cotrimoxazole and Augumentin are significantly high 18 (100 %).

Susceptibility pattern of all the isolate to different antibiotics

The overall susceptibility pattern of all the isolates is presented in **Figure 4**. The result revealed that all the isolates were resistant to Amoxicillin and sensitive to Streptomycin. However, the highest intermediate was observed in Ciprofloxacin.

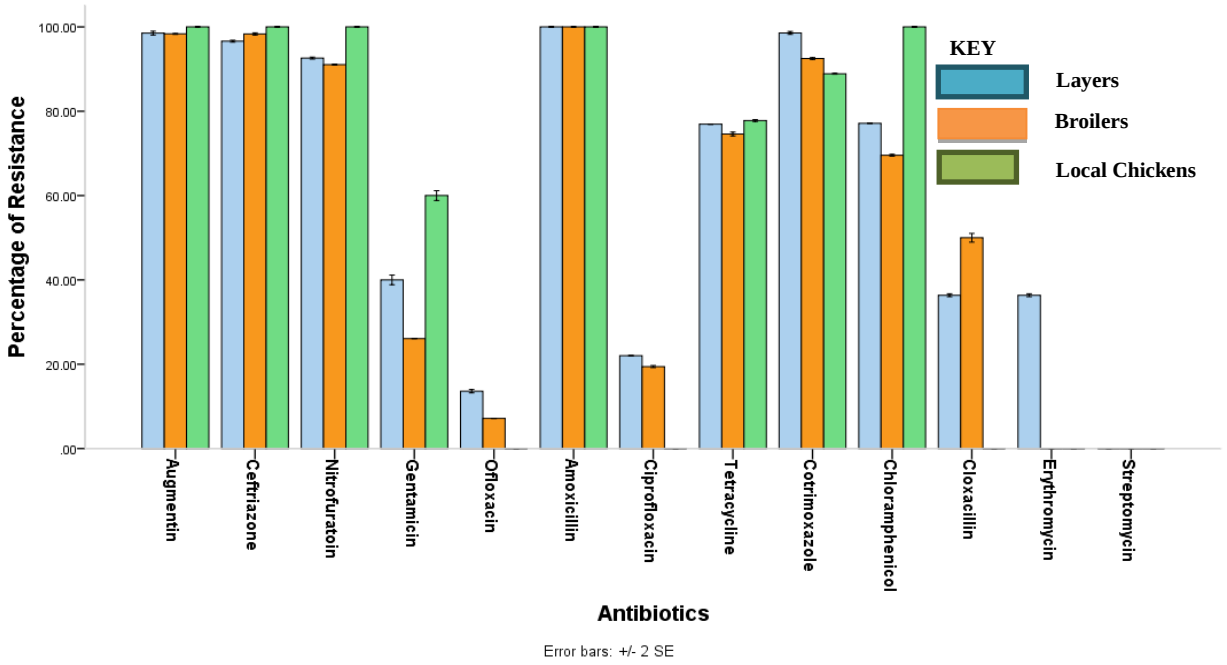

Figure 2: Incidence of antibiotic resistance among bacteria isolated from Layers, Broilers and Local Chicken droppings.

Table 3: Percentage Resistance Pattern of gram negative bacteria to antibiotics

Antibiotics	*E coli* n=84	*Kebsiella* spp. n= 10	*Proteus* spp. n= 8	*Enterobacter* spp. n= 3	*Salmonella* spp. n= 16	*Shigella* spp. n= 2	*Citrobacter* spp. n= 3	*Pseudomonas* sp n= 3	*Seratia* sp n= 2	Total = 131
Augmentin (30µg)	84(100^{b})	8(80.00^{a})	8(100^{b})	3(100^{b})	16(100^{b})	2(100^{b})	3(100^{b})	3(100^{b})	2(100^{b})	129(98.47)
Ceftriaxone (30µg)	81(96.43^{a})	10(100^{b})	8(100^{b})	3(100^{b})	16(100^{b})	2(100^{b})	3(100^{b})	3(100^{b})	2(100^{b})	128(97.71)
Nitrofuratoin (300µg)	77(91.67^{a})	10(100^{b})	8(100^{b})	3(100^{b})	16(100^{b})	2(100^{b})	3(100^{b})	3(100^{b})	2(100^{b})	124(94.66)
Gentamicin (10µg)	27 (32.14^{c})	1(10.00^{a})	4(50.0^{e})	1(33.29^{d})	5(31.25^{b})	1(50.00^{e})	3(100^{g})	2(66.66^{f})	2(100^{g})	46(35.11)
Ofloxacin (10µg)	7(8.33^{c})0	1(10.00^{d})	0(0.00^{a})	0(0.00^{a})	1(6.25^{b})	0(0.00^{a})	0(0.00^{a})	2(66.69^{e})	0(0.00^{a})	11(8.40)
Amoxicillin (30µg)	84(100^{a})	10(100^{a})	8(100^{a})	3(100^{a})	16(100^{a})	2(100^{a})	3(100^{a})	3(100^{a})	2(100^{a})	131(100)
Ciprofloxacin (10µg)	8(9.52^{b})	1(10.00^{c})	0(0.00^{a})	1(33.33^{d})	7(43.75^{e})	2(100^{g})	1(33.33^{d})	2(66.67^{f})	0(0.00^{a})	22(16.79)
Tetracycline (30µg)	62(73.81^{b})	10(100^{d})	8(100^{d})	3(100^{d})	13(81.25^{c})	1(50.00^{a})	3(100^{d})	3(100^{d})	2(100^{d})	105(80.15)
Cotrimoxazole (25µg)	84(100^{d})	8(80.00^{b})	5(62.50^{a})	3(100^{d})	13(81.25^{c})	2(100^{d})	3(100^{d})	3(100^{d})	2(100^{d})	123(93.89)
Chloramphenicol (30µg)	84(100^{d})	9(90.03^{c})	0(0.00^{a})	0(0.00^{a})	10(62.50^{b})	2(100^{d})	0(0.00^{a})	3(100^{d})	2(100^{d})	110(83.97)

Key: Values indicate number of resistant isolates (percentage of resistant isolate) to mentioned antibiotic. Values in the same row carrying the same superscript are not significantly different according to Duncan's multiple range test at ($P \leq 0.05$). n – Total number of isolate tested for antibiotic sensitivity.

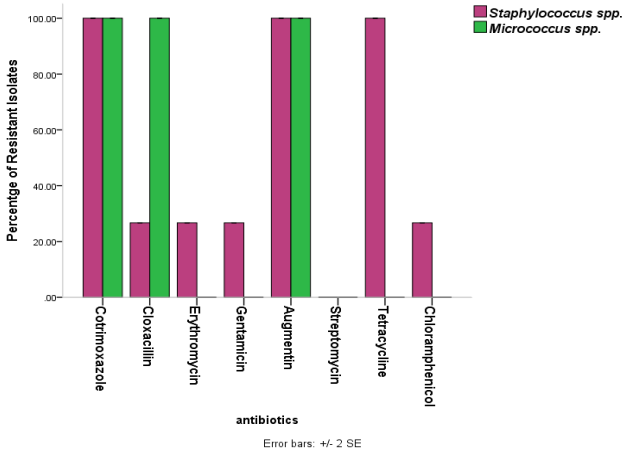

Fig 3: Percentage Resistance Pattern of gram positive bacteria to antibiotics

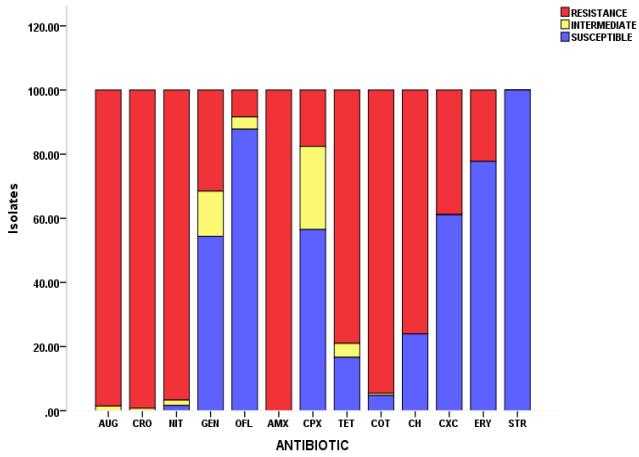

Figure 4: Susceptibility pattern of all the isolate to different antibiotics

Keys:

AUG - Augmentin, **CRO** - Ceftriaxone, **NIT** - Nitrofuratoin, **GEN** - Gentamicin, **OFL** - Ofloxacin, **AMX** - Amoxicillin, **CPX** - Ciprofloxacin, **TET** - Tetracycline, **COT** - Cotrimoxazole, **CH** - Chloramphenicol**, CXC** – Cloxacillin, **ERY** – Erythromycin, **STR** – Streptomycin

DISCUSSION

The rise in antibiotics resistance had been reported in the past two decades, and antibiotic resistance still remains a global problem today Hemen *et al.* (2012). 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 microfloral. In this research, bacterial pathogen isolated were mostly enteric bacteria and *Staphylococcus* sp. with *E coli* having highest prevalence and is in agreement with the report of Omojowo and Omojasola (2013) and Adegunloye 2006; 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 variation in the carriage of the organisms in both poultry birds and local birds. This could be due to a host of factors that are beyond the scope of this study but such variations may be due to the environmental settings in which the birds are raised, the nutritional status of the birds, and so on. Furthermore, the probiotic and physiological state of the gut of animals 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 and Egbebi (2011).

High resistance to the multidrugs Augmentin**,** Ceftriaxone, Nitrofuratoin, Amoxicillin, Tetracycline, Cotrimoxazole and Chloramphenicol were observed in all the isolate. This observation was consistent with previous reports Hemen *et al.* (2012) that multidrug-resistant bacteria were isolated in poultry litters. Also, high multidrug resistance was observed in isolates from local

chicken compared to those in in layers and broilers. Resistance to Gentamycin was significantly higher than those isolates from poultry. This is not unexpected because such birds have unhindered access to the environment, particularly agriculture-influenced and urban-influenced treated and untreated water. Such water samples have all been reported to harbour either bacteria, which carry (multiple) antibiotic resistance genes, or plasmids carrying these genes (Schwartz *et al*, 2003; Pei *et al.*, 2006; Amy *et al.*, 2006). Improper use and disposal of the antibiotics lead to residues of these being released to the environment.

CONCLUSION

The result of this study indicates that poultry dung is a carrier of pathogenic bacteria which is capable of transmitting bacterial pathogens to human. The most resistant isolates were *E coli* and *Pseudomonas aeruginosa* and were found to be resistant to the entire antibiotic tested 7(4.70%). These have very important implications on human health, as antibiotic resistant bacterial infections are difficult to treat and often require expensive antibiotics and long term therapy. This can increase the cost of treatment and even mortality. Antibiotic resistant bacteria should be closely monitored in our environment, further work is also necessary on the genetic basis of antibiotic resistant human pathogenic bacteria from poultry and the use of alternative therapy in poultry management.

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