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Antibiotic Resistance Inescherichia Coli Isolate From Healthy Food Animals in Ngor Okpala, Imo State, Nigeria

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Abstract: *Antibiotics resistance in faecalE. coli isolates were retrospectively analysed from April to July 2014. Isolates originated from clinically healthy food animals like goat, local fowls and broiler birds. Serial dilution was done on the faecal samples and plated on Eosin Methylene Blue (EMB) agar and incubated overnight.The isolates were identified and characterized using standard microbiological methods. The isolated E. coli was screened for antimicrobial resistance profile using disc diffusion method on Muller Hinton agar. The antibiotics tested were ampicillin (10mcg), gentamycin (10mcg), streptomycin (30mcg), oxytetracycline (30mcg), cephalothin (10mcg) and ciprofloxacin (10mcg). Six (6) different antibiotics resistance profiles were observed, with each isolate showing resistance rate of 57.1% ampicillin, 41.7% to gentamycin and streptomycin, 25.0% to cephalothin, 16.7% to oxytetracylin and 3.6% to ciprofloxacin. The frequency of resistance showed that the E.coilisolates was higher in local fowland goat compared to broiler. The significant public health concern observed in this study is that multidrug resistant commensalE.coilare found in food animals and can be transmitted to man through food chain.*

Key Words: *antibiotics resistance, Escherichia coli, food animals, public health concern and faecal samples.*

I. Introduction

Antibiotic usage is possibly the most important factors that promote the emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Daniels et al., 2009).

This acquired resistance occurs not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans). In intensively reared food animals antibiotics may be administered to whole flocks rather than individual animals, and antimicrobial agents may be continuously fed to food animals such as poultry, goats and cattle as growth promoter. Therefore the antibiotic selection pressure for bacteria to drug resistance in the animal is high and invariably their faecal flora contains a relatively high proportion of resistance bacteria (Amaechi, 2014[1]). The mechanism for spreading antibiotic resistance from animals to humans and verse vice remains controversial. Colonization of the intestinal tract with resistant E.colifrom chicken have been shown in human volunteers and there is historical evidence that animals are reservoir for E.colifound in humans (Akwaret al ;2008). Furthermore, spread of antibiotic resistance plasmids in E.colifrom chickens to human handlers or of antibiotics-resistance microorganisms from animals to human in various countries has been reported (Fang et al; 2008).

Resistance has been found in organisms common to both humans and animals such as E.coil (Davis et al; 2009). Due to the intricate balance of micro flora of different habitats within the ecosystem, the transfer of resistance genes among bacteria occupying different habitats has the potential to occur frequently.

Since transmission of resistance from animals to human (Kariukiet al; 1999) occur through various means, this study seeks to evaluate the incidence of antimicrobial resistance in E.coli isolated from livestock and poultry living in close contact. However, even in the absence of heavy use of antibiotic it is important to identify and monitor susceptibility profiles of bacterial isolates, particular of commensally organisms.This necessitates the need to evaluate the antibiotic resistance using an indicator bacterium like Escherichia coli isolates from clinically healthy food animals.

II. Materials And Methods

Materials Used

Materials used for this researches include; faecal samples from healthy broilers, local fowls and goats ,EMB(Eosin Methylene Blue)agar, nutrient agar, normal saline,glass slide,bursen burner, crystal violet,iodine,alcohol, safranin,immersion oil,microscope,tryptophan broth,chloroform,kovac's reagent, applicator stick, hydrogen peroxide, sodium citrate,simmon's citrate agar, tetra methyl-p=phenylenediaminedihydrochloride (oxidase reagent),filter paper,Pasteur pipette, wire-loop, MullerHinton

agar, antibiotic disc containing the following drugs, Ampicillin (10µg),Gentamycin(10µg), Streptomycin(30µg), oxytetracyclin(30µg),Cephalothin10µg),Ciprofloxacin (10µg).

Sample Collection

Faecal samples of healthy food animals such as broilers, goat and local fowl (not showing any sign of ill health) were collected. The samples were collected from three different locations. The faecal samples were collected between the period of 8.00am and 9.00am during the time of removal of overnight faecal droppings from April to July 2014. The faecal samples were collected with the aid of sterile scalpel into a sterile plastic rubber. Aseptic conditions were employed in the process of sample collection. The samples were labelled, covered very tight and sent to the laboratory in ice box for microbiological analysis within 2-3 hours after collections.

Preparation of Media

Media used in this work includes Eosin Methylene Blue (EMB), nutrient agar, tryptophan broth, Simmons citrate agar, Muller Hinton agar. They were prepared according to the manufacturers specifications. They were sterilized by autoclaving at 121°c for 15mins. They were allowed to cool up to 45^0c and poured into sterile Petri dishes for sample inoculation.

Sample Cultivation

One gram of each animal's faeces was homogenized in 9ml of sterile saline solution; the volume of the homogenate was made up to 10ml with saline solution. The content was mixed thoroughly and 10-fold of sterile saline solution was added to eachof the 10 test tube. 1ml from the stock was transferred into the 1st test tube (10^1), about 1ml was transferred again into the 2nd test tube(10^2),from the 1st test tube, this was done repeatedly till the 10th dilution (10^{10}).

This serial dilution was carried out to reduce the microbial concentration of the sample. From the suitable dilution specifically from the 2nd test tube,the 3rd test tube and the 4th test tube 0.1ml was plated on already prepared Eosin methylene blue (EMB) agar. The inoculatedplates were incubated overnight at 37^0c for 24hours in the incubator. After the expiration of the incubation period,the plates were checked for growth of E.coli.E.coli was selected from an individual samples for further characterization (Cheesbrough, 2000)

Characterization and Identification of Bacterial Isolates

Characterization and identification of the isolates was carried out using standard microbiological methods. Size,shapes, colour, elevation and margins of the bacterial colonies were observed following the growth of culturesby incubations for 24hours at 37°c. The shape and arrangement of the cells were observed by the microscope after staining. The colonies on the EMB were re-inoculated into nutrient agar for biochemical identification.

E.coli was fully identified using conventional microbiological test (Gram staining, oxidase test, indole test and citrate utilization test) (Cheesbrough, 2000).

Antibiotic Susceptibility Test

The isolated E.coliwas screened for antimicrobial resistance profile using the disc diffusion method (Cheesbrough, 2000); Clinical Laboratory Standard Institute (CLSI, 2004). Wire loop was used to pick up a colony from the re-inoculated culture (nutrient agar media) and streak on the surface of already prepared Muller Hinton agar. Using the forceps, the antibiotic discs were picked and placed on the surfaces of the plate and incubated for 24hours at 37^0c. The antibiotics disc contained the followingdrugs, Ampicillin(10mcg), Gentamycin(10mcg),Streptomycin(30mcg)oxytetracyclin(30mcg),Cephalothin(10mcg),Ciprofloxacin (10mcg).E. coliATCC 25922 and ATCC 35218 were used as quality control organism.

III. Results

The total number of E. coli isolates from the three different locations was 50. There were 28 E.coli isolates from location 1, 12 E. coli isolates from location 2 and 3 E. coli isolates from location 3.

The 50 E.coli isolates showed resistance rates of 57.1% to Ampicillin, 41.7% to Gentamycin and Streptomycin, 25.0%to Cephalothin 16.7% to Oxytetracyclin and 3.6% to Ciprofloxacin. Six (6) different antibiotics resistance profiles were observed with all the isolates showing resistance to at least two or more of the drugs tested (Table 1).

Table 1. Antimicrobial Resistance of E-coli Isolates from Livestock in Ngor-Okpala, Imo state

Site of Location	No of E-coli isolates tested	No(%) of isolate resistance	No (%) of isolate resistance to:-					
			AMP	GEN	STREP	CF	OXT	CIP
Location 1	28	20 (71.4)	16 (57.1)	10 (35.7)	7 (25.0)	6 (21.4)	3 (10.7)	1 (3.6)
Location 2	12	8 (66.7)	6 (50.0)	5 (41.7)	5 (41.7)	3 (25.0)	2 (16.7)	0 (0.00)
Location 3	10	6 (60.0)	3 (30.0)	3 (30.0)	2 (20.0)	1 (10.0)	0 (0.00)	0 (0.00)
Total	**50**	**34 (68.0)**	**25 (50.0)**	**18 (36.0)**	**14 (28.0)**	**9 (18.0)**	**5 (10.0)**	**I (2.0)**

KEYS: AMP- Ampicillin, GEN-Gentamycin, STREP-Streptomycin,
OXT - Oxytetracyclin, CF-Cephalothin. CIP- Ciprofloxacin.

The frequency resistance of the E.coli isolates from the different locations to antimicrobial agents by sources of samples, the highest degree of resistance to almost all the antibiotics was detected in the local fowls, followed by goat and the lowest is broilers.

The number in percentage (%) of E.coli isolates that were resistance are (86.7%) for goat, (84.0%) for local fowl and (60.0%) for broilers. Comparing resistance rates according to sources of samples, approximately 66.7% of the isolates from goats exhibited resistance to Ampicillin ,46.7% to Gentamycin and Streptomycin, 33.3% to Cephalothin, 26.7% to Oxytetracyclin and 20.0% to Ciprofloxacin (Table 2).

Table 2.Frequency of Resistance of E. coli. Isolate from Different locations to Antimicrobial Agents by Source of Sample.

			No (%) of isolates resistant to:-					
Sources	No of E-coilisolates tested	No (%)of isolates resistant	AMP	GEN	STREP	CF	OXT	CIP
Goat	15	13 (86.7)	10 (66.7)	7 (46.7)	7 (46.7)	5 (33.3)	4 (26.7)	3 (20.0)
Local fowl	25	21 (84.0)	19 (76.0)	17 (68.0)	17 (68.0)	10 (40.0)	6 (24.0)	2 (8.00)
Broilers	10	6 (60.0)	5 (50.0)	5 (50.0)	4 (40.0)	3 (30.0)	1 (10.0)	0 (0.00)
Total	**50**	**40 (80.0)**	**34 (68.0)**	**29 (58.0)**	**28 (56.0)**	**18 (36.0)**	**11 (22.0)**	**5 (10.0)**

Keys: AMP-Ampicillin, GEN-Gentamycin, STREO-Streptomycin, CF-Cephalothin, OXT-Oxytetracyclin, CIP-Ciprofloxacin.

In local fowl 76.0% exhibited resistance to Ampicillin, Ciprofloxacin was considered the lowest Resistance drug 8.00% observed among the isolates.

The isolates from broilers showed 50.0% resistance to Ampicillin and Gentamycin respectively and 40% and 30% Resistance to Streptomycin and Cephalothin. The lowest resistance of 0.00% to Ciprofloxacin observed in this study was seen among the isolates from Broilers.

Table 3.Antibiotic Resistance profiles ofE.coliisolated from all sources

ANTIBIOTICS RESISTANCE PROFILE	No of Isolates (%)
AMP, GEN, STREP	10 (20.0)
AMP, GEN, STREP, OXT, CF	0 (18.0)
AMP, STREP OXT, CF	5 (10.0)
AMP, CF	4 (8.0)
AMP, CIP	1 (2.0)
AMP, GEN, CF, OXT	1 (2.0)
OXT, CF, CIP	1 (2.0)
GEN, STREP, OXT, CF	1 (2.0)
OTHERS	2 (4.0)
NO RESISTANCE	16 (32.0)
TOTAL	**50**

This value is gotten by dividing the no (%) of isolates by the total no ofE-coli isolates tested multiply by 100.

$$\frac{10}{50} \times \frac{100}{1}$$

AMP-Ampicillin, GEN-Geentamycin, STREP-Streptomycin,CIP- ciprofloxacin, OXT-Oxytetracycline, CF-Cephalothin, No Resistance - isolates that are sensitive to the Antibiotics Resistance profiles.

Table 4.Reaction of E.coli, based on the Biochemical Test.

Sources	E.coil isolates	Indole test	Catalyze test	Gram staining	Oxidase test	Citrate utilization test
Goat	15	Positive	Negative	Rod-like	Negative	Positive
Local Fowl	25	Positive	Negative	Rod-like	Negative	Positive
Broilers	10	Positive	Negative	Rod-like	Negative	Positive
Total	**50**					

IV. Discussion

The possibility for transfer of antibiotics resistance genes among livestock in the environment is a direct threat to public health (Van den boggard and Stobberingh, 1999).

Animal faeces are potential sources of antibiotics resistant bacteria. If released into the environment, resistant strains may contaminate water and food sources and can be a potential threat to human health (Aminor and Mackie 2007).Not all antibiotic resistance genes are located in plasmids, some are located on the bacterial chromosomes (Amaechiet al; 2015). Multidrug resistantE.coli produces extended spectrum beta-lactamases (ESBLS) such as the CTX-M enzymes (Mora et al., 2005).

Antibiotics have helped in reducing diseases in animal husbandry; however, there is a growing awareness of public health concern associated with the use of antibiotics in animal husbandry (Amaechi, 2014[1]). Exposure to antimicrobial agents is a major factor with regard to development of antimicrobial resistant E. coli (Amaechiet al., 2015).The E.coli strains isolates exhibited high rates of resistance against the following antibiotics: Streptomycin, Oxytetracycline, CephalithinAmpicillin,Ciprofloxacin and Gentamycin.

Acar and Moulin(2006) reported a 79.8% frequency for resistance to antimicrobial against E.coli isolates recorded from livestock sampled at a veterinary clinic using similar antimicrobial agents, which is comparable.It has been postulated that general resistance to other beta-lactam antimicrobials results from use of beta-lactams such as Ampicillin which are commonly used in veterinary and human medicine (Schroeder et al;2002). This may explain the resistance of E. colito Cephalothin, which although not used in treatment protocols, is beta-lactam antimicrobial. Resistant phenotypes are due to acquisition of external genes that provides resistance to an entire class of antimicrobials (Whit and McDermott, 2001).

The antimicrobial resistance recorded despite of the fact that the farms under study do not have histories of supplementing animal feed with antibiotics as growth promoters showed that probably other factors and pressures have brought about this resistance in the absence of antibiotic exposure. Specifically, E.coli isolates from animals that rarely receive antibiotics recorded resistance against Ampicillin (57.1%),Gentamycin (41.7%),streptomycin (41.7%), oxtetracyline (16.7%), andcephalothin(25.0%). The figures presented here may not be out of place but a reflection of resistance events in other hosts (humans) sharing the same environment with these animals, which was not observed in this research.Earlier studies by Amaechiet al.,(2015) showed high resistance frequencies in non – pathogenic E. coli from poultry birds and pigs in Nigeria. Although, the food animals sampled in this study do not receive any modern veterinary attention they may have maintained close contact through a myriad of routes (such as water) with humans in their environment that had been previously exposed to various antibiotics. Taking resistance according to sources of samples, there is strong evidence that the use of antimicrobial agents in livestock can lead to the emergence and dissemination of E.coli (Van danboggard,2001; Schroederetal; 2002; Amaechi, 2014[2]): which can then be passed into people via food or through direct contact with the poultry (Schwarz and Chaslus –Dancla, 2001).

However, Forsbergetal. (2012) reported that farm environmental isolates showed reduced susceptibility (as measured by disc diffusion zone diameter) the major factors selecting for antimicrobial resistance in bacteria are antibiotic use, crowding and poor sanitation. These three factors are typical intensive livestock farming and explain the high prevalence and degree of resistance in fecalE.coli, of livestock in this research.

In conclusion, this study has showed that resistance traits are distributed across hosts in different environment rather than being host or environment-specific.A significant public health concern is that multidrug resistance commensally E.coli strains may constitute a potential bacteria genes that could be transferred to pathogenic bacteria, the high prevalence of multi-drug-resistance E.coli observed in this research suggests there is a need for improved education and communication on the issue of antibiotic use in human and livestock.The high prevalence of resistance to Ampicillin and Gentamycin agent amongst E.coli isolates may have therapeutic and zoonotic implications. The relatively high prevalence of resistance to streptomycin and ox tetracycline suggests that there must be more prudent use of antimicrobial agents by farmers.

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.colistrains from different domestic livestock.This type of testing is simple, effective and suitable for surveillance, and it has been used for E.coli strains collected from domestic healthy livestock.(NCCLS,1999).

References

[1]. Acar, J.F and Moulin, G (2006).Antimicrobial resistance at farm level. Revue Scientifiqueet Technique (International office of Epizootics). 26(2): 775- 792.

[2]. Akwar H.T, Poppe C.J, Wilson R.J, Josh W, Dayue S, Scott A.M (2008).Prevalence and Patterns of Antimicrobial Resistance of fecalEscherichia coli among pig on 47 farrow-to-Finishes Farm with Different In-feed Medication Policies in Ontario Columbia. Can. J. Vet. Res., 72 (20: 195-201.

[3]. Amaechi, N (2014[1]). A survey on antibiotic usage pigs and poultry birds in Abia State, Nigeria. Journal of Medical Research: C. Microbiology and Pathology.vol. 14. Issue 5 version1.0: 11 – 17.

[4]. Amaechi, N (2014[2]). Correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria in poultry and pig farms.Global Journal of Biology, Agriculture and Health Sciences. Vol. 3(3): 50- 54.

[5]. Amaechi, N., Abbey, S.D and Achi, O.K (2015). Plasmid profile and antimicrobial resistance ratings of Escherichia coli isolates from pigs and poultry birds in Abia State, Nigeria. International Journal of Current Microbiology and Applied Sciences. 4(2): 834 – 842.

[6]. Aminor, R.I and Mackie, R.I(2007). Evolution and ecologyof antibiotics resistance genes.FEEMS microbial.Microbial letter. 271: 146 – 161.

[7]. Cheesbrough M (2000) District Laboratory Practice in Tropical Countries, part 2. Cambridge University Press, Cambridge, UK, p. 434.

[8]. Daniels JB, Call DR, Hancock DD, Sischo WM, Baker KNK, Besser TE (2009). The role of CeftiofurI the Selection and Dissemination of blacmy-2-mediated Cephalosporin Resistance in salmonella enteric ans commensal Escherichia coli isolated from cattle. Appl. Environ. Microbiol.,75: 3648-3655.

[9]. Davis MA, Baker KNK, Call DR, Warnick LD, Soyer Y, Wiedmann M Grohn Y, McDonough PL, Hancock DD, BesserTE (2009) Multiple Locus Variable Number of Tandem Repeats typing Method for Salmonella Enteric SerovarNewport J.Clin. Microbial., 17: 1934-1938.

[10]. Fang H. ferda A. Goran H, DornbuschK (2008) Molecular Epidemiology of Extended-spectrum.

[11]. Forsberg, K.J. Reyes, A. Wang, B. Selleck, E.M. Sommer, M.O.A and Dantas, G (2012).The shared antibiotic resistance of soil bacteria and pathogens.Science. 337: 1107- 1111.

[12]. Kariuki, S., Gilks, C., Kimari, J., Obanda, A., Muyondi, J., Waiyaki, P. and hart, C. A.(1999): GenotopicAnalysis of Escherichia coliIsolates Isolated from Children and Chickens living in Close ContactAppl. Environm. Microbial.65: 472-476,.

[13]. Mora, A. Blanco, J.E and Blanco, M (2005), Antimicrobial resistance of Shiga toxin (verptoxin)- producing Escherichia coli0157: H7 and non-0157 strains isolated from humans, cattle, sheep and food in Spain. Research in Microbiolgy. Vol156. No7 pp 793806.

[14]. NCCLS, (1999).National Committee for Clinical Laboratory Standards, Performance standard for Antimicrobial Susceptibility testing, sixth edition.Approved Standard. NCCLS document M2-A6, supplement NCCLS Document M31-S1, vol. 24, No. 17 National Committee for clinical Laboratory standards, Wayne, Pennsylvania, USA.

[15]. CLSI,(2004). National Committee For Clinical Laboratory Standards, Performance Standards for Antimicrobial disc and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Informational Supplement NCCLS Document M31-S1, vol. 24, No. 17 National committee for Clinical Laboratory Wayne Pennsylvania, USA.

[16]. Schroeder, C., J. Meng, S. Zhao, C. Debroy, J. Torcolini, C. Zhao, P.F. Mcdermott, D. D. Wagner, R. D. Walker, D. G. White (2002): Antimicrobial Resistance of Escherichia coli O26, O103, O111, O128 and O145 from animals and humans. Emergence infect. Dis. 8, 1409- 1414.

[17]. Schwarz, S. and Chaslus-Dancla, E.(2001): Use of Antimicrobials in Veterinary Medicine and Mechanisms of Resistance. Vet. Res. 32:201-225.

[18]. Van den Boggaard, A.E (2001). Antimicrobial Resistance Relation to Human and Animal Exposure to Antibiotics.Journal of Antimicrobial chemotherapy 40, 453-4.

[19]. Van den boggaard, A.E. and Stobberingh, E.E.(1999). Antibiotic Usage in Animals- Impacts on Bacterial Resistance and Public Health. Drugs 58, 589-607.

[20]. White, D.,Mcdermott, P(2001).Emergence and Transfer of Antibacterial Resistance .J. Dairy Science.84, E151-155.