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ANTIBIOTIC RESISTANCE PATTERNS OF *ESCHERICHIA COLI* ISOLATES FROM HUMAN, PET, LIVESTOCK AND POULTRY LIVING IN CLOSE CONTACT

O.B. SHITTU[1] C.A.K. NWAGBONIWE[1] AND O.O GEORGE [1]

[1]Dept. of Microbiology, University of Agriculture, Abeokuta, Nigeria

ABSTRACT

Antibiotic resistance patterns of *Escherichia coli* in human, pet, livestock and poultry living in close contact was carried out. A total of 130 fecal samples were obtained from human and animals. Out of 124 isolates with characteristics *E. coli* morphology obtained on Eosin methylene blue (EMB) agar, only forty (32.3%) isolates were identified as *E. coli.* Of all the *E. coli* isolates, 100% resistance to cotrimozaxole was obtained from cattle, poultry and goats while human, sheep and dog showed 92.3%, 81.8% and 58.3% resistance respectively. Isolates from dog showed no resistance to gentamicin, amoxicillin and augmentin, but exhibited more than 58.3% resistance to other antibiotics. Isolates from cattle, poultry, sheep and goats were generally resistant to all the antibiotics tested with percentage resistance ranging from 14.29% (augmentin) to 100% (cotrimozaxole). Notable is the increasing resistance of both the human and animal isolates to some antibiotics that are considered highly effective against resistant *E. coli* such as ofloxacin (31.8%), nalidixic acid (50.0%) and nitrofurantoin (76.1%).

Keywords: Antibiotics resistance, *Escherichia coli*, livestock, poultry, close contact.

INTRODUCTION

Escherichia coli is widely distributed in the intestine of humans and warm blooded animals and is the predominant facultative anaerobe in the bowel and part of the essential intestinal flora that maintains the physiology of the healthy host (Nataro and Kaper, 1998). The organism is acquired through contaminated food and water and once established, an *E coli* strain may persist for months or years. Even though, *E. coli* is a normal flora of the gastrointestinal system, and usually harmless to the host, a group of pathogenic strains has emerged that causes diarrhea diseases in humans and animals (Beutin, 1999; Puente and Finley, 2001) referred to as diarrheagenic *E. coli.*

The problem of antimicrobial resistance has increased rapidly in the last decade and is a major public health threat worldwide (Koplan, 2000). Antimicrobial resistance has been found in both pathogenic and non-pathogenic strains and antimicrobial resistant *E. coli* have shown the ability to transfer resistance to other strains of *E. coli* as well as other organisms within the gastrointestinal tract (Bettelheim, 1997) and to acquire resistance from other organism (Oppegaard *et al.,* 2001). *E. coli* being the most predominant species in the gut makes

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it ideal for studies in antimicrobial resistance, especially factors involved in the emergence of antibiotic resistance which primarily is the inappropriate use that exert selective pressure, one of which is feeding human antibiotics to farm animals to enhance their growth or threat infections (McEwen *et al*., 2002). Self medication with antibiotics (Okeke *et al.*, 1999) is also a risk factor in resistance selection. Since transmission of resistance from animal to man (Levy *et al*., 1976; Kariuki *et al*., 1999) and vice versa occur through various means, this study seeks to evaluate the incidence of antimicrobial resistance in *E. coli* isolated from human, livestock and poultry living in close contact.

MATERIALS AND METHODS

***Specimen collection and processing*:**
Thirty (30) fecal swabs sample from were collected from human, while a total of 100 were collected from cattle, sheep, chicken, goats and dogs (20 swab samples per animal). The samples were obtained from farms animals and workers and as well from household pet, children and adults living with the pet all within Abeokuta. The fecal swabs were inoculated onto the surface of MacConkey and Eosin Methylene Blue agars (Oxoid, Basingstoke, England). After incubation for 24 and 48hrs at 37^{o}C, three to six colonies with typical *E. coli* morphology were streaked on fresh tryptone soy agar and incubated at 37^{o}C for 24hrs. Each isolate was independently subjected to biochemical tests for identification using Cowan and Steels manual.

***Hemolysin testing*:**
All the confirmed *E. coli* isolates were screened for hemolytic activity on blood agar plates. The plates were incubated for 8hrs at 37^{o}C. A positive test showed either clear zones of hemolysis (α-hemolysis) or zones with green or brownish haloes (β-hemolysis).

Antibiotic susceptibility testing:
The susceptibility of the isolates to antimicrobial agents was tested by disk diffusion on Sensitivity test Agar (Oxoid, Basingstoke, England) according to the specifications of the National Committee for Clinical Laboratory Standards (NCCLS, 2001). Antibiotic disk containing: Tetracycline (30µg), Nalidixic acid (30µg), Cotrimoxazole (25µg), Gentamicin (10µg), Nitrofurantoin (300µg), Augmentin (30µg) and Amoxicillin (25µg) was used.

RESULTS

A total of 124 fecal bacteria were recovered from both human and animals as shown in Table 1. Forty (40) isolates were identified as *Escherichia coli* (32.3%), twenty three (23) as *Klebsiella pnuemoniae* (18.5%), seven (7) as *Shigella dysenteriae* (5.6%), sixteen (16) as *Salmonella typhi* (12.9%), twenty (20) as *Enterobacter aerogenes* (16.1%), fourteen (14) as *Proteus vulgaris* (11.3%) and four (4) as *Pseudomonas aeruginosa* (3.2%).

Out of the forty *E. coli* isolates subjected to hemolysis test, only 18 (45%) showed both α and β-hemolysis as shown in Table 2. None of the isolates from dog displayed either α and β-hemolysis.

Multiple resistance to all the antibiotics tested was demonstrated by all the isolates from human, cattle, poultry, sheep and goat while isolates from dog showed maximum percentage resistance of 50% to cotrimozaxole and nalidixic acid (fig. 1). Notable

is the 100% resistance of *E. coli* isolates from cattle, poultry and goat towards co-trimozaxole while human isolates showed 90% resistance. A total percentage resistance of 89.8, 76.1, 62.5, 50.0, 47.7, 45.5, 31.8 and 27.3% was given by the isolates to cotrimozaxole, nitrofurantoin, gentamicin, nalidixic acid, amoxicillin, tetracycline, ofloxacin, and augmentin, respectively (Table 3).

Table 1. Incidence of *E. coli* and other enteric pathogen from fecal samples

	Sources of isolates						
Organisms isolated	Human	Cattle	Poultry	Sheep	Goat	Dog	Total
Escherichia coli	13 (46.4)	6 (30.0)	7 (35.0)	4 (23.5)	5 (26.3)	5(25.0)	40(32.3)
Klebsiella pnuemoniae	7 (25.0)	3 (15.0)	3(15.0)	4 (23.5)	4 (21.1)	2(10.0)	23(18.5)
Shigella dysenteriae	0 (0.0)	1 (5.0)	2 (10.0)	1 (5.9)	1(5.3)	2(10.0)	7 (5.6)
Salmonella typhi	1(3.6)	4 (20.0)	2 (10.0)	2 (11.8)	2 (11.0)	5(25.0)	16 (12.9)
Enterobacter aerogenes	4 (14.3)	3 (15.0)	2 (10.0)	3 (17.6)	4(21.1)	4 (20.0)	20 (16.1)
Proteus vulgaris	1(3.6)	3 (15.0)	3 (15.0)	3 (17.6)	2 (11.0)	2 (10.0)	14 (11.3)
Pseudomonas aeruginosa	2 (7.1)	ND (0.0)	1 (5.0)	ND (0.0)	1(5.3)	ND (0.0)	4 (3.2)
n	28	20	20	17	19	20	124

ND= Non detected
n=Total number of isolates obtained from each source
Figures in bracket represent percentages of isolate from each animal species.

Table 2. Hemolytic test for *E. coli* isolates.

Sources of isolates	n	α- hemolysis	β-hemolysis	Total
Human	13	2 (15.4)	3 (23.0)	5 (38.5)
Cattle	6	2 (33.3)	1 (16.7)	3(50.0)
Poultry	7	1 (14.3)	3 (42.9)	4 (57.1)
Sheep	4	0 (0.0)	4 (100.0)	4 (100.0)
Goat	5	1 (20.0)	1 (20.0)	2 (40.0)
Dog	5	0 (0.0)	0 (0.0)	0 (0.0)
Total	40	10 (25.0)	8 (20.0)	18 (45.0)

n=Total number of isolates obtained from each source
Figures in bracket represent percentages of isolate from each animal species.

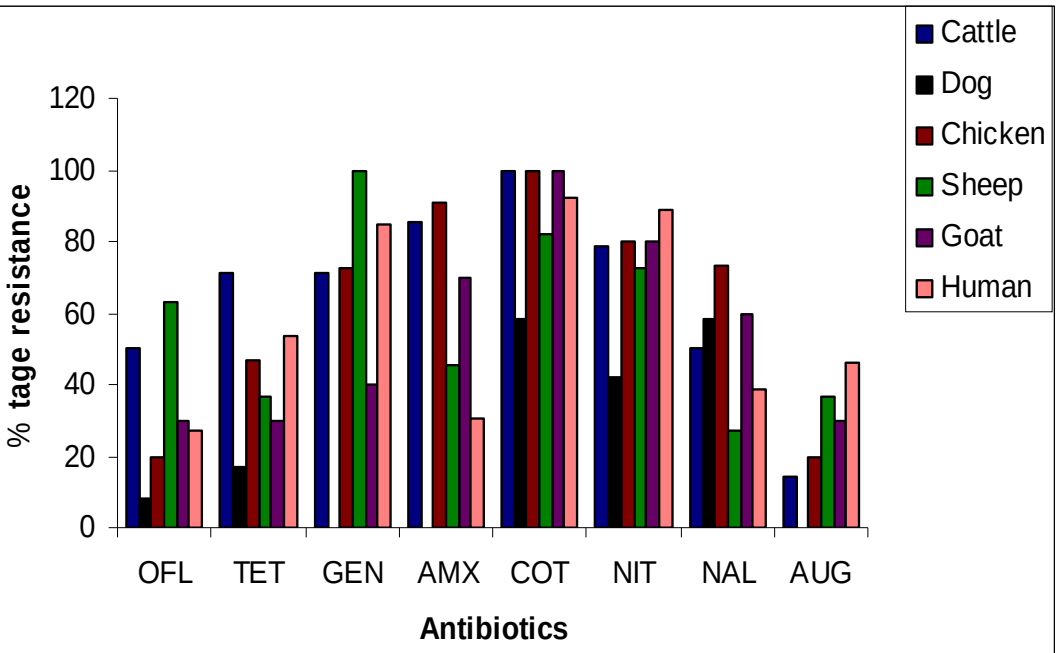

Fig.1. Antibiotic resistance of *E. coli* isolates

Table 3: Percentage resistance of *E. coli* isolates to the different antibiotics

Antibiotics	Percentage resistance (%)
Ofloxacin (OFL)	31.8
Tetracycline (TET)	45.5
Gentamicin (GEN)	62.5
Amoxycillin (AMX)	47.7
Cotrimoxazole (COT)	89.8
Nitrofurantoin (NIT)	76.1
Nalidixic acid (NAL)	50.0
Augmentin (AUG)	27.3

DISCUSSION

Antibiotic pressure exerted directly or indirectly selects for resistant strains. The practice of feeding livestock antibiotics that are used by humans almost certainly contributes to shared patterns of antibiotic resistance in the fecal flora of certain animals and humans (Graves *et al*., 2002). Antibiotic resistance patterns have been used to distinguish between *E. coli* strains from different sources (Scott *et al*., 2002).

The resistant patterns of *E. coli* to the different antibiotics observed in this study vary from one source to another. In this study, human isolates had antibiotic resistance of 53.8% to tetracycline and 38.5% to nalidixic acid much more different from that obtained in a study by Okeke *et al*. (2000), who observed that *E. coli* isolates from humans had a resistance of 100% to tetracycline and 3.2% to nalidixic acid. Considerable high resistance was also noted in this study for gentamicin (84.6%), nitrofurantoin (88.5%) and cotrimozaxole (92.3%) while ofloxacin, amoxicillin, and augmentin had 26.9, 30.8 and 46.2% resistance, respectively. This shows the impact of antibiotic pressure in selection of resistance strains in an environment. This implies that the more of antibiotic use and abuse, the more the resistance that will be observed from microbial isolates from that geographical location.

The antibiotic resistance patterns of *E. coli* isolates from cattle as observed in this study, showed 50 and 71.4%, respectively, to nalidixic acid and gentamicin is considerably higher than that observed by Bradford *et al*. (1999) with 12.5% resistance to nalidixic acid and 53.1% to gentamicin. *E. coli* isolates from cattle also showed 100% resistance towards cotrimozaxole while human isolates showed 90% resistance. This is a reflection of the over-the-counter availability and the misuse of the drug in Nigeria especially, treating cattle with antibiotics that are meant for human diseases.

E. coli isolates from human and chickens from this study showed similar antimicrobial resistance with the exception of augmentin, amoxicillin, and nalidixic acid.

E. coli isolates from sheep and goats were all resistant to the tested antibiotics with resistance ranging from 27.3 to 100%. This is of public health significance because an investigation for a pathogenic strain, verotoxin producing *E. coli* (VTEC) conducted by Beutin *et al*. (1993) on healthy animals (sheep, goats, cattle, pigs, chickens, dogs, and cats) resulted in the isolation of VTEC from 28.09% of the animals with sheep and goat accounting for 66 and 56.1%, respectively. While cattle had 21.1% VTEC, it was less frequent from pigs (7.5%), cats (13.8%), dogs (4.8%) and chicken (<0.7%). Nearly 60% of all VTEC O: H serotypes isolated in the study have been implicated as human pathogen.

Generally, the isolates from dogs had total susceptibility to gentamicin, amoxicillin and augmentin. This may be due to the fact that household dogs are only treated with antibiotics when suffering from infection, mainly given basic vaccines such as antirabies and not raised for commercial purpose with the need to fatten them up. The resistance of the isolates to ofloxacin (8.3%), tetracycline (16.7%), cotrimozaxole (58.3%), nalidixic acid (58.3%) and nitrofurantoin (41.7%) is alarming because

it is known that pets can be natural reservoirs of several organisms potentially able to cause diseases to humans, who in turn may also be carriers of countless infectious agents specific for animals (Rodriguez *et al*. 2004). Accordingly, children are central players in this cross-transfer game in view of their frequent non observance of proper hygiene as exemplified in a study by Rodriguez *et al*. (2004) who reported an observed similarity between *E. coli* isolates from a 3 year old healthy child and her 3month old diarrheic pet.

From this study, it has been shown that animals and human isolates have fairly similar antibiotic resistant patterns with a few exceptions; of noteworthy is the increasing resistance of the isolates to some antibiotics that are considered highly effective against resistant *E. coli* such as ofloxacin (31.8%), nalidixic acid (50.0%) and nitrofurantoin (76.1%).

CONCLUSION

Even though definitive links between antibiotic use in animal feeds and human health is yet to be shown, but the prevalence of antibiotic resistance bacteria has increased in recent years. Infection with enteric pathogen results from contamination through the fecal oral route, thus, any factor that can lead to increased risk of fecal contamination will also lead directly or indirectly into increased prevalence of antibiotic resistance. Improved water sources, watershed protection, sanitation, food handling and hygienic practices as well as proper antibiotic use, regulation and control are paramount factors that would go a long way in reducing the incidence of antimicrobial resistance in developing countries like Nigeria. The transferability of resistance genes between pathogenic and nonpathogenic intestinal flora remains a serious cause of public health concern especially with increasing prevalence in antimicrobial resistance to new drugs within a short period of time.

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