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Antibiotic Susceptibility Pattern of *Escherichia coli* Isolates from Human and Animal Specimens in Owerri, Nigeria

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ABSTRACT
Antibiotic susceptibility patterns of Escherichia coli isolates from human and domestic livestock in Owerri, Nigeria was carried out. A total of 252 fecal specimens obtained from humans, poultry and cattle were cultured on Eosin methylene blue (EMB) agar and incubated overnight at 37°C. Distinct colonies showing green metallic sheen on EMB were identified as E. coli and later confirmed by various biochemical tests. The antibiotic susceptibility testing of isolates was determined by the disc diffusion method on Müller-Hinton agar. The total rate of isolating E. coli from human specimens was 37.5% while 56.7% of animal specimens were positive for E. coli. Susceptibility of isolates to antibiotics varied greatly. The highest resistance was recorded against tetracycline (100%) in both human and animal specimens; and chloramphenicol (90%) in human specimens and 60% in animal specimens respectively; nitrofurantoin was the most active drug tested. Statistical analysis showed that average number of resistance phenotypes per isolate was not significantly different in any of the sample sources (P<0.05). The incidence of antibiotic resistance in E. coli isolates from apparently healthy humans and domestic life stock is high in the study area, hence, indiscriminate use of antibiotics in both human and animal medicine should be discouraged.
Key Word: *Escherichia coli, Antibiotic Resistance, Nigeria.*

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INTRODUCTION

Escherichia coli is well recognized as a commensal inhabitant of the gastrointestinal tract 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 [1]. However, it is also associated with diarrhoea and a range of extra-intestinal diseases in both man and animals. *Escherichia coli* are also the most frequently encountered microorganism in the food industry. Various disease outbreaks have been reported to be due to ingestion of food contaminated with pathogenic *E. coli* strains [2]. Pathogenic strains of *E. coli* acquire virulence genes with which they cause diseases especially diarrhea and extra-intestinal infections in humans and animals [3]. In the debilitated or immune-suppressed host or when gastrointestinal barriers are violated, normal non-pathogenic strains of *E. coli* can cause infection.

The problem of antimicrobial resistance has increased rapidly in the last decade and is a major public health threat worldwide [4]. 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 [5] and to acquire resistance from other organisms [6]. *E. coli,* being the most predominant species in the gut makes 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 [7]. Self-medication with antibiotics [8] is also a risk factor in resistance selection. Since transmission of resistance from animal to man [9] and vice versa occur through various means, this study seeks to evaluate the incidence of antimicrobial resistance in *E. coli* isolated from human and domestic livestock in Owerri, South East Nigeria.

MATERIAL AND METHOD

Sample collection, cultivation and identification of *E. coli*

Fresh chicken and cattle droppings were collected by using a plastic vial and taken immediately to the Laboratory for culturing. Human stool specimens were collected using sterile wide mouth bottle. A sterile inoculation loop was used to stab fecal specimens and inoculated directly by streaking on MacConkey agar (Oxoid). The plates were incubated overnight at 37ºC. A pink colony was picked and sub-cultured on EMB agar (Oxoid). Colonies with metallic green sheen on EMB (characteristic of *E. coli*) were later confirmed by biochemical tests [10]. Only isolates confirmed to be *E. coli* were selected for antibiotics susceptibility testing.

Antibiotics susceptibility testing

The susceptibility pattern of *E. coli* against the five antibiotics Viz: chloramphenicol (30µg), erythromycin (5µg), tetracycline (30µg), gentamicin (10µg) and nitrofurantoin (100µg) (Oxoid, England) were evaluated. The bacterium inoculum was inoculated from the standardized culture by using sterile cotton wool stick. The culture was standardized using 0.5McFarland standard. The antibiotics disks were then picked with a sterile pin and placed on the inoculated plates, 25mm apart. The inoculated plates were incubated at 37ºC for 16-18 hours, the inhibition zone diameter (IZD) was measured and interpreted based on Clinical Laboratory Standard Institute breakpoints [11]. *E. coli* (ATCC 25922) was used as control strain.

Statistical analysis

Comparative resistance rates for *E. coli* strains from the different sample sources were statistically analyzed by T-test and results were considered significant at 95% confidence level

RESULTS

The results show that a total of 72 isolates of *E. coli* (37.5%) was isolated from human specimens while 34 (56.7%) was isolated from animal specimens (Table 1). Multiple resistance to all the antibiotics tested was demonstrated by all the isolates from human and animal specimens (Table 2). The highest resistance was recorded against tetracycline (100%) in both human and animal specimens; and chloramphenicol (90%) in human specimens and 60% in animal specimens respectively. A greater percentage of *E. coli* isolates from human specimens were susceptible to nitorfurantoin, (70%) at St David hospital, (60%) at Federal Government Girl's Collage, (65%) at Imo State University, Owerri and (50%) at General hospital Umuguma while isolates from animal specimens were mostly susceptible to gentamycin 75% and 70% respectively. Statistical analysis showed that average number of resistance phenotypes per isolate was not significantly different in any of the sample sources (P<0.05).

Table 1. The rate of isolation of *E. coli* according to sample sources

Sample source	M A	L E S	F E M	A L E S
	No. Sampled	No. Positive %	No. Sampled	No. Positive %
St. David Hospital, Owerri	30	09 (30)	30	21(70)
General Hospital Umuguma, Owerri	18	08(44.4)	12	02(16.7)
Federal Government Girl's Collage	-	-	30	16(53.3)
Imo State University Students, Owerri	12	06(37.1)	18	10(62.5)
Total	60	23(38.3)	132	49(37.1)

Table 2. The antibiotics susceptibility pattern of *E. coli* isolated from various sources

Sample Source	A	N	T	I	B	I	O	T	I	C S
	T		G		E		C		F	
	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R
St. David Hospital, Owerri	00	100	20	80	00	100	10	90	70	30
General Hospital Umuguma, Owerri	10	90	20	80	40	60	50	50	60	40
Federal Government Girl's Collage	20	80	30	70	25	75	10	90	60	40
Imo State University Students, Owerri	25	75	35	65	20	80	20	80	65	35
Obinze Cattle Market, Owerri	15	85	70	30	45	55	40	60	70	30
Imo State University Farm, Owerri	00	100	75	25	25	75	56	44	69	31

Key: T=Tetracycline, C=Chloramphenicol, E=Erythromycin, F=Nitrofurantoin, G=Gentamycin.

DISCUSSION

Antibiotic resistance has been recognized as an emerging worldwide problem in human and veterinary medicine [12, 13] both in developed and developing countries. It is also well documented that widespread

use of antibiotics in agriculture and medicine is accepted as a major selective force in the high incidence of antibiotic resistance among gram-negative bacteria [14]. A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production [15]. Antibiotic resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections, a common cause of both community and hospital-acquired bacteraemia Salvadori *et al.*, [16] as well as a cause of diarrhea [1]. In addition, resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract and to acquire resistance from other organisms [17].

In this study, we surveyed antibiotic resistance in *E. coli* isolates from human and animal specimens in Owerri, southeast Nigeria. Highest resistance was observed in tetracycline from both animal and human sources; this confirms the increasing numbers of reports detailing circulation and amplification of antimicrobial resistance genes especially tetracycline resistance in the environment [18, 19, 13, 20, 21]; which could facilitate the emergency and spread of antibiotic resistance in bacteria. Rysz and Alvarez, [21] demonstrated that bacteria in the soil could acquire resistance to tetracycline from environmental exposure, possibly creating a reservoir of resistance factors generated outside animal host. The pattern of tetracycline resistance has been attributed in part to widespread and lengthy use of tetracycline in farm animal industry [22, 23, 24]. Since tetracycline is a natural derived compound, bacteria can be exposed to these agents in nature outside any human use for disease treatment, for prophylaxis, or for livestock growth promotion. Tetracycline is a commonly used first line antibiotic in both human and veterinary medicine and is often used before the antibiotic resistance profile of a pathogen has been determined. Resistance to tetracycline is plasmid mediated, with a wide variety of genetic determinants [25, 26].

High-level resistance was observed in chloramphenicol, erythromycin, gentamicin and nitrofurantoin in isolates from both animal and human sources, this calls for an important consideration since fluoroquinolones and aminoglycosides are used to treat range of *E. coli* infections in humans. This finding concurs with previous reports [24, 27], and underscores the need to monitor quinolone and aminoglycoside resistant bacteria in animal production, as their emergency is an important health concern in food safety. Furthermore, multi-drug resistance (resistance to at least three to four antibiotics) was found in *E. coli* from both animal and human specimens. When this multi-drug resistance was compared it was found to occur on common antibiotics in both *E. coli* sources. Little or no differences was observed in terms of rate of resistance within the two groups, this can be explained in terms of the interactions of organisms (associated with the host) and potential horizontal gene transfer in their respective environment.

In conclusion, this study has shown high rate of antibiotic resistance against the drugs tested and this is a matter of public health concern. Since there is a reservoir of antibiotic resistant genes within the community, and that the resistance genes and plasmid-encoded virulent genes are easily transferable to other strains, pathogen cycling through food chain is very common and might pose a potential health risk to the consumer. Therefore, cautions are necessary to decrease the incidence of antibiotic resistant strains of *E. coli* in human and animals. In order to achieve this, good hygienic practices are necessary from the farm to the family table especially in the abattoirs to prevent contamination of cattle and poultry products and abattoir environment with intestinal content. Health authorities should focus on implementing the legislation that forbids irrigation with untreated sewage water of both root and leafy vegetables. Finally, there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials in agriculture and medicine. In addition, regular antimicrobial susceptibility surveillance is essential.

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