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ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM ANIMAL AND HUMAN CLINICAL SAMPLE.

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Antimicrobial usage is considered the most important factor promoting the emergency, selection, and dissemination of antimicrobial-resistant microorganisms in both veterinary and human medicine. Antibiotics resistance of twenty-eight isolates of *E. coli* isolated from human clinical samples (urine=18) and intestine of healthy animals (cow=10) were studied. Samples collected from humans and animals were screened and characterized for the presence of *E. coli* using standard microbiology techniques. Antibiotics resistance patterns of *E. coli* isolated from human clinical samples and animal samples were determined using a disc diffusion method following the Clinical Laboratory Standard Institute (CLSI) protocol against the following antibiotics: ciprofloxacin, ofloxacin, ampicillin, cefotaxime, ceftazidime, amikacin, gentamicin, tobramycin, sulphamethoxazole/trimethoprim, trimethoprim, and tetracycline. The susceptibility pattern of these isolates showed that *E. coli* from animal intestine were highly susceptible to ciprofloxacin (92%) and amikacin (96%), but was resistant to ampicillin (72%), cefotaxime (79%), ceftazidime (88%), gentamicin (81%), sulphamethoxazole/trimethoprim (76%), trimethoprim (80%), tobramycin (93%), ofloxacin (73%) and tetracycline (90%). *E. coli* from human clinical sample was susceptible to ciprofloxacin (92%), trimethoprim (82%), amikacin (81%) and cefotaxime (90%) respectively while high-level resistance was observed with ampicillin (82%) ceftazidime (91%), gentamicin (86%), sulphamethoxazole/trimethoprim (89%), tobramycin (78%), ofloxacin (87%) and tetracycline (93%). Multi-drug resistance was observed in *E. coli* from both animal and human sources but was higher in frequency and proportion in *E. coli* isolated from human clinical samples. In conclusion, our findings suggest that resistant strains of *E. coli* are disseminated in animal population and warrant further investigation of the possibility of animal sources acting as reservoirs of spread.

Keywords: *Escherichia coli,* antibiotics resistance, clinical sample, cow intestine.

INTRODUCTION

Antibiotics have been critical in the fight against infectious diseases and antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the twentieth century (WHO, 2002). With the constant use of antibiotics over a period of time, bacteria resist not only single but also multiple antibiotics making some diseases particularly troublesome to treat. The worldwide emergency of antibiotic-resistant bacteria threatens to undo the dramatic advances in human health that were ushered in with the discovery of these drugs in the mid 1900s. Today, resistance has rendered most of the original antibiotics obsolete for many infections, mandating an increased reliance on synthetic drugs (Cirz *et al*., 2003). In animal production antibiotics are widely used as growth promoter and in treatment of infectious diseases (Wolfgang, 1998). The use of antibiotics in poultry production industries for promotion of growth largely contributes to the high resistance to antimicrobial agents in normal flora of poultry (Allan *et al*., 1993; Aronson *et al*., 1975) and pathogenic microorganism (Amara *et al*., 1995). At slaughter, resistant strains from the gut may contaminate poultry carcasses and as a result poultry meats are often associated with multi-

resistant *E. coli* (Chaslus-Dancia and Lafont, 1985; Jayaratne *et al*., 1990; Turtura *et al*., 1990). However, the mechanism of spread of antibiotic resistance from food animals to humans remains controversial. Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers (Linton *et al*., 1977) and there is historical evidence that animals are a reservoir for *E. coli* found in humans (Cooke *et al*., 1971; Levy *et al*., 1976). 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. Resistance may be transferred vertically among bacteria of different genera and families or horizontally among different bacteria species within the same genus or family (Adhikari *et al*., 2000; Nikolich *et al*., 1994).

E. coli is one of the main causes of nosocomial infections in humans, it is also a common inhabitant of the human and animal guts and is considered an indicator of fecal contamination in food. This microorganism is of clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections such as urinary tract infections, blood stream infections, wounds, otitis media and other complications in humans (Gebre-Sellassie, 2007; Khan *et al*., 2002). Occurrence and susceptibility profiles of *E. coli* show substantial geographic variations worldwide (Erb *et al*., 2007). These organisms specially posses the genetic ability to acquire and transfer antibiotics resistance through plasmids and transposons and they often exist as commensal components of the flora associated with food producing animals that may also have impact on consumers (Murray, 1990). The aim of this study was to investigate the prevalence of resistant strains of *E. coli* isolated from healthy animals' intestines and compare it with isolates obtained from human clinical samples.

MATERIALS AND METHODS

Collection of human clinical samples: A total of eighty-six human urine samples were collected from male (35) and female (51) patients attending Ebonyi State University Teaching Hospital (EBSUTH) Abakaliki from September to December 2011. These urine samples were inoculated on the surface of a MacConkey and CLED agar plate, incubated at 37^0C for 18-24 hrs. After incubation, the morphology of the colonies was observed. Colonies suspected to be *E. coli* was subjected to Gram staining, sugar fermentation test, and indole test. (Chessbrough, 2000).

Collection of intestine samples: Pieces of 20 cm of intestine collected from 75 different slaughtered cows ready for selling in Abakaliki Abakpa Market were taken to the Applied Microbiology Department laboratory to screen for the presence of *E. coli*. The collected intestine was washed with sterile water and a sterile swab stick was used to make a swab of the internal portion. This was inoculated on MacConkey and CLED agar plates and incubated at 37^0C for 18-24 hrs. After incubation, morphology of the colonies was observed. Colonies suspected to be *E. coli* were subjected to Gram staining, sugar fermentation test, and indole test. (Chessbrough, 2000).

ANTIBIOTICS SUSCEPTIBILITY STUDIES

Antibiotic susceptibility studies were performed by disc diffusion method as described by the CLSI 2008a&b using the following antibiotics: ofloxacin, ciprofloxacin, cefotaxime, ceftazidime, ampicillin, amikacin, gentamicin, tobramycin, sulphamethoxazole/trimethoprim, trimethoprim, and tetracycline (Oxoid UK). Quality control was performed as recommended using *E. coli* strain ATCC 25922.

RESULTS

Antibiotics resistance prevalence of *E. coli* isolated from animal and human clinical samples were compared in the present study. *E. coli* isolation was more frequent in human clinical samples than from animal. Of the 86 urine samples collected from patients attending EBSUTH within a period of four months (September-December 2011), *E. coli* was isolated from 18 (20.9%) while out of 75 cow intestine collected, *E. coli* was isolated from 10 (13.3%) Table 1. There was a trend towards higher resistance frequency in *E. coli* isolated from animal intestine across different classes of antibiotics although susceptibility was observed with ciprofloxacin (92%) and amikacin (96%). A greater percentage of *E. coli* isolated from human were susceptible to ciprofloxacin (96%), trimethoprim (82%), amikacin (81%) and cefotaxime (90%). All animal samples were resistant to the beta lactams (cefotaxime 79%, ceftazidime 88%, ampicillin 72%), aminoglycoside (gentamicin 81% and tobramycin 93%), tetracycline (90%), sulphamethoxazole/trimethoprim (76%), trimethoprim (80%) and ofloxacin (73%) Table 2. Majority of *E. coli* isolated from human clinical samples were resistant to ampicillin (82%), ceftazidime (91%), gentamicin (86%), sulphamethoxazole/trimethoprim (89%), tobramycin 78%, ofloxacin 87% and tetracycline (93%) Table 3. Prevalence of multiple resistant patterns among *E. coli* isolated from animal and human clinical samples are given in Table 4. *E. coli* strains from animal samples were resistant to four or more antimicrobials while human isolates were resistant to two or three antimicrobials. The most frequent resistance phenotype in animal isolates was with tobramycin (93%) and tetracycline (90%) while in human it is with ceftazidime (91%) and tetracycline (93%).

Table 1: Frequency of *E.coli* occurrence in animal and human clinical samples.

Total number of Human clinical isolates (urine) screened for the presence of *E. coli*	83
Total number of animal intestine (cow) screened for the presence of *E. coli*	75
No of *E. coli* isolated from human clinical sample (urine)	18
No of *E. coli* isolated from animal intestine (cow)	10

Table 2: Overall antimicrobial susceptibility pattern of *E. coli* isolate from human clinical sample

Antimicrobials	Total number of isolates tested	Percentage (%) of resistant strains
Ampicillin	18	82
Cefotaxime	18	10
Ceftazidime	18	91
Ciprofloxacin	18	3
Ofloxacin	18	87
Gentamicin	18	86
Amikacin	18	13
Tobramycin	18	78
Trimethoprim	18	78
Sulphamethoxazole/ trimethoprim	18	89
Tetracycline	18	93

Table 3: Overall antimicrobial susceptibility pattern of *E. coli* isolate from animal intestine (cow)

Antimicrobials	Total number of isolates tested	Percentage (%) of resistant strains
Ampicillin	10	72
Cefotaxime	10	79
Ceftazidime	10	88
Ciprofloxacin	10	8
Ofloxacin	10	73
Gentamicin	10	81
Amikacin	10	4
Tobramycin	10	93
Trimethoprim	10	93
Sulphamethoxazole/ trimethoprim	10	76
Tetracycline	10	90

DISCUSSIONS

In this study we surveyed antimicrobial resistance in *E. coli* isolated from human clinical samples and cow intestines sold at Abakpa market in Abakaliki, Ebonyi State, Nigeria. We chose *E. coli* as study organism because of its ubiquitous nature both in the environment and in the intestine/colon of both animal and human. Most of the cows that are sold in Abakaliki are brought from the Northern part of Nigeria and their health statues are not determined before slaughter; also the sanitary standard of the environment where they are slaughtered is to be questioned hence this study is necessary. Because of geographic sampling of animal, this surveillance provides a representative sample of the resistant trends in cow meat sold in Abakaliki abbatoir/meat industry. Compared with other antimicrobial agents used in this study, animal isolates were only susceptible to ciprofloxacin and amikacin. The finding that *E. coli* isolates from cow intestine were highly

susceptible to ciprofloxacin is in agreement with a previous study where more than 80% of *E. coli* was found to be susceptible to ciprofloxacin (Quinolone) (Daniella *et al*., 2011). High resistance was observed with 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 (Adhikari *et al*., 2000; Cohen, 2000; Sayah *et al.,* 2005; Rysz and Alvarez 2004), which could facilitate the emergency and spread of antibiotic resistance in bacteria. Rysz and Alvarez, 2004 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 (Hinton *et al*., 1982; Piddock, 1996; Van den Bogaard and Stobberingh, 1999). 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 for many domestic animals 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 (Prescott *et al*., 2000). High-level resistance was observed for ofloxacin, gentamicin and tobramycin in *E. coli* from both animal and human sources, this calls for an important consideration since fluoroquinolones and aminoglycosides are used to treat a range of *E. coli* infection in humans. This finding concurs with previous reports (Van den Bogaard *et al*., 2001; Hofacre *et al*., 2000), 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 community. Quinolone resistance involves chromosomal mutations that reduce membrane permeability and decrease drug accumulation or alter DNA topoisomerases, resistance to fluoroquinolones is mostly associated with mutations in DNA gyrase (Webber and Piddick, 2001). *E. coli* resistance to aminoglycoside most often occurs by aminoglycosides-modifying enzymes (Galimand et al., 2003) encoded on transferable plasmids (Gonzalez et al., 2005).

Multi-drug resistance (to at least three to four antimicrobials) was found in *E. coli* from both animal and human clinical samples (Table 4). The frequency and proportion was higher in *E. coli* isolated from human than animal samples. 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.

Table 4: Multiple antimicrobial resistance patterns of *E. coli* isolates from human clinical and animal sources

Sample source	R1	R2	R3	R4
Human clinical sample (Human; n=18)	-	2	6	10
Animal intestine (Cow: N= 10)	2	1	3	4

Keys: R1, R2, R3 and R4- resistance to one, two, three and four antibiotics.

In conclusion, cow meat which can serve as a source of protein can also be a source of transfer of antibiotic resistant bacteria to man based on our findings. The overall results of this study provides evidence of significant antimicrobial resistance in *E. coli* isolated from cow intestine raised on farms without record of antimicrobial use. We noted also the presence of multi-drug resistant strains of *E. coli* from both human and animal sources.

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