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Prevalence of Antibiotic Resistant *Escherichia coli* in Healthy Male and Female Students in Yaba College of Technology Lagos Nigeria

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Abstract
A study was conducted at the Yaba College of Technology, Lagos State, Nigeria to determine the prevalence of antibiotic resistant strains of selected microorganisms in healthy male and female students. 75 stool samples were collected and investigated for the presence of *Escherichia coli* using standard microbiological methods. 35 *E.coli* isolates were obtained. 18(51.4%) were from females while 17(48.6%) were from males. The *E.coli* isolates were subjected to antimicrobial susceptibility test. Results obtained showed that, marked resistance was observed for Septrin, Chloramphenicol, Ciprofloxacin, Augmentin, Amoxacillin and Gentamicin respectively. Multiple Antibiotic Resistance (MAR) index indicated that two of the isolates obtained from the male population were resistant to all the test antibiotics used. The prevalence of antimicrobial resistant *E.coli* in the female population was significantly lower than that obtained in the male population.
Keywords: Antibiotic resistance, *Escherichia coli,* healthy population, multiple antibiotic resistance index

1. Introduction

Enterobacteriaceae are widely distributed in nature occurring in the intestinal tract of man and animals as well as in water and soil, *Escherichia coli* is a prominent member of this group. The organism is defined as an important commensal pathogen that inhabits the gastrointestinal tract of man and other animals. It is regarded as an important source of antimicrobial determinant for other human and animal pathogens, where it plays an important role as a reservoir of resistance genes (Chikwendu *et al.*, 2008). Hence it is often exploited as a sensitive indicator in the surveillance of antimicrobial resistance (Lee *et al.*, 2006).

Bacterial resistance to a particular antibiotic can be a natural property of the bacteria or a secondary acquired mechanism. Acquired bacterial resistance is common in isolates from healthy individuals and from patients with community acquired infections, more especially in developing countries where the need for antibiotics is driven by the high incidences of infectious diseases (Okeke *et al*., 1999).

The environment is replete with drug resistance genes which once acquired are not easily lost but become a relatively stable part of the genome. Additional resistance determinants may join those already prevailing, thus broadening the multidrug resistance phenotype and further diminishing treatment options (Manges *et al*., 2001). An increasing number of bacterial isolates is resistant to practically all available therapeutic agents.

Multidrug resistance has been demonstrated in *Escherichia coli (*Dzidic and Bedekovic, 2003; Summers, 2006*)*. *Escherichia coli* is an important cause of community and hospital acquired infections such as, complicated urinary tract infections, pyelonephritis, and hospital bacteremia.

Factors that influence the development and spread of resistant organisms in healthy individuals include the use, misuse and outright abuse of antibiotics, poverty, which may interfere with the patient's compliance, and the continued use of antibiotics in the field of veterinary medicine. All these make antibiotic resistant bacteria a major threat to public health (Komolafe, 2003).

The effect of antibiotic resistance ranges from the failure of an individual patient to respond to therapy, the need for expensive and or toxic alternative drugs, higher morbidity and mortality rates, longer durations of hospitalization, increased health cost and the need for changes in empirical therapy (Essack, 2006). This growing threat from resistant organisms calls for concerted actions to prevent the emergence of new resistant strains and the spread of existing ones (Dzidic *et al.*, 2008).

2. Materials and Methods

Sample collection: A total of 75 stool samples were collected from healthy female and male students in Yaba College of Technology Yaba Lagos, Nigeria. Each student that participated in the investigation was given a sterile swab stick to bring freshly voided stool samples. The samples were then transported to the laboratory where they were processed within six (6) hours.

Culture: Each swab stick collected from each participant was dipped into 2ml sterile Normal Saline solution and a tenfold serial dilution was done. Using a standardized wire loop, a loopful of the second diluent was streaked

on MacConkey agar plates and incubated for 24 hours at 37°C. Distinct colonies on the MacConkey agar plates with *E.coli* morphology and appearance were aseptically streaked on Eosin Methylene Blue (EMB) agar plates. Colonies with green metallic sheen were tentatively identified as *Escherichia coli*. Further confirmatory tests were done by subjecting these colonies to gram staining and the following biochemical tests; catalase, citrate, indole and methyl red and Voges Proskaur. The confirmed colonies were then preserved on agar slants and refrigerated.

Antibiotic Susceptibility: The antibiotic susceptibility pattern of all the isolates obtained were determined using ten standard antibiotic discs; 30µg each of Streptomycin, Septrin, Chloramphenicol, Amoxacillin, Augmentin and Perfloxacin and 10µg each of Sparflomycin, Ciprofloxacin, Ofloxacin and Gentamicin.

Overnight culture of each isolate was used to seed the surface of the Nutrient agar plates. The antibiotic discs were then aseptically placed at equidistance on the seeded agar plates and were incubated at 37° C for 24 hours. The diameter of zones of inhibition were measured and interpreted as sensitive (S) or resistant (R) using the standard Interpretation chart (Cheesebrough, 2000). Multiple antibiotic resistance index (MAR) for each test isolates was calculated as recommended by (Krumperman, 1983).

3. Result

A total of 35 *Escherichia coli* isolates were obtained from the stool samples screened. 18(51.4%) of the *Escherichia coli* isolates were from the female students while 17(48.6%) were from the male students (Table 1). Antibiotic susceptibility test indicated that the isolates from the females were more sensitive to the antibiotics used compared to the males (Table 2).

Table 2 also shows that 82.4% each of isolates from the male population were highly resistant to each of the following antibiotics; Septrin, Chloramphenicol, Ciprofloxacillin and Augmentin. While in the female population 50% were resistant to Septrin, 11.1% to Chloramphenicol, 16.7% to Ciprofloxacillin and 66.7% to Augmentin.

Resistance to Gentamicin were significantly lower in the female population (16.7%) compared to the males (64.7%). However 72.2% of isolates obtained from the female population showed more resistance to Amoxacillin compared to that of the male population.

Calculated Multiple Antibiotic Resistance index (MRA) suggests that all the isolates exhibited significant MRA (Table 3). The MAR pattern for isolates obtained from the male population indicates that two (2) of the isolates were resistant to all the antibiotics. However in the female population none of the isolates showed resistance to all of the antibiotics used.

Table 1: Distribution Pattern of Isolates Obtained from Stool Samples from Male and Female Students

Sex	*E.coli* Isolates	Non *E.coli* isolates	**Total**
Male	17(42.5)	23(57.5)	**40**
Female	18(51.4)	17(48.6)	**35**
Total	35	40	**75**

Table 2: Antibiotic Sensitivity Profile of Isolated *Escherichia coli*

	Sensitive Isolates		Resistant Isolates	
Antibiotics	Male	**Female**	Male	**Female**
Streptomycin(S)	9(52.9)	**9(50)**	8(47.1)	**9(50)**
Septrin (SXT)	3(17.6)	**9(50)**	14(82.4)	**9(50)**
Chloramphenicol(CH)	3(17.6)	**16(88.9)**	14(82.4)	**2(11.1)**
Sparfloxacin (SP)	8(47.1)	**9(50)**	9(52.9)	**9(50)**
Ciprofloxacin (CPX)	3(17.6)	**15(83.3)**	14(82.4)	**3(16.7)**
Amoxacillin (AM)	6(35.3)	**5(27.8)**	11(64.7)	**13(72.2)**
Augmentin (AU)	3(17.6)	**6(33.3)**	14(82.4)	**12(66.7)**
Gentamicin(CN)	6(35.3)	15(83.3)	11(64.7)	**3(16.7)**
Perfloxacin (PEF)	7(41.2)	9(50)	10(58.8)	**9(50)**
Ofloxacin (OFX)	6(35.3)	7(38.9)	11(64.7)	**11(61.1)**

Table 3: Multiple Antibiotic Resistance (MRA) indices of *E. coli* Isolated from Stool Samples from both Male and Female Students

MAR index	Male n(17)	**Female n(18)**
0.0	0(0)	**0(0)**
0.1	0(0)	**0(0)**
0.2	0(0)	**0(0)**
0.3	0(0)	**0(0)**
0.4	1(5.9)	**3(16.7)**
0.5	2(11.8)	**5(27.8)**
0.6	3(17.6)	**4(22.2)**
0.7	3(17.6)	**1(5.6)**
0.8	3(17.6)	**0(0)**
0.9	2(11.8)	**0(0)**
1.0	2(11.8)	**0(0)**

4. Discussion

The widespread use of antibiotics is increasingly perceived as a threat to public health. While rich countries are concerned with hospital-acquired pathogens, poor countries are additionally challenged with multi-resistant pathogens in the community (Amabile- Cuevas, 2010).

Several factors, such as urban migration, overcrowding, and improper sewage and garbage disposal increase the level of faecal pollution in a population. These encourage the exchange of antibiotic resistant organisms between people and also the exchange of resistance genes among bacteria, thereby increasing the prevalence of resistant strains (Okeke *et al.,* 1999). This occurs as bacteria that normally reside in the human colon can transfer resistance genes among themselves (Ochman *et al., 2000*).

Most hostels in educational institutions in Nigeria are overcrowded, especially in Lagos State which is cosmopolitan in nature, hence attracting more people across the country. Consequently, population density may be a factor influencing prevalence of antibiotic resistance in the fecal flora of healthy individuals as observed in this study.

Bruinsma *et al*., (2003) observed in their study that living in an environment with resistant bacteria is a real risk for acquiring such bacteria and that the closer the contact, the greater the risk. It can thus be deduced that in communities where people live in close proximity with one another, and there are persons harboring resistant bacteria or resistant genes, there is a risk factor of acquisition of antibiotic resistant bacteria**.**

Increasing use of disinfectants in everyday household products has also been found to put increasing selective pressure on the bacterial population, resulting in more resistant organisms in the homes that use these products. This change in the normal flora can have adverse consequences if and when the members of the household become ill with an infectious disease. The use of these products may reduce the number of harmless bacteria and increase the selection for antimicrobial resistant organisms. In the population considered for this study most students use medicated soaps and other antiseptics regularly as a preventive measure against infections.

Triclosan, for example is a chemical used in hospital products, but now introduced into homes via hundreds of household items. Now it has been discovered that, a genetic locus in *E.coli* provides resistance to triclosan and to multiple antibiotics via a multidrug efflux pump Thus, confirming that there is a link between resistance to antibacterial products and resistance to antibiotics (McMurry *et al*., 1998a; McMurry *et al*., 1998b).

The isolation of antibiotic resistant *E. coli* from healthy male and female students is supported by Calva *et al*., (1996) and Lamikanra *et al*., (1989) who found out that the residents of developing countries often carry antibiotic-resistant fecal commensal organisms.

The Multiple Antibiotic Resistance (MAR) index is a tool that reveals the spread of bacteria resistance in a given population. It shows that the isolates have been previously exposed to antibiotics (Ngwai *et al*., 2010). MAR above 0.2 implies that the strain of such bacteria originates from an environment where several antibiotics are used (Ehinmidu, 2003).

Thus from Table 3 it can be deduced that all the isolates have been exposed to more than three antibiotics, this is corroborated by the study conducted by Levy *et al*., (1988) where they found out that commensal fecal flora of human showed resistance to 2, 3, and 4 different antibiotics among volunteers that have not ingested any antibiotic, whereas in those subjects consuming an antibiotic, multiple drug resistance in 50% of the coliform flora was significantly higher.

Furthermore, the result of the MAR obtained in this study showed that most of the isolates were resistant to multiples of antibiotics, this agrees with the observation made by Lamikanra and Okeke, (1997) where they discovered that strains isolated from Lagos were more likely to show resistance to 4 to 6 of 7 antibiotics tested, whereas strains from rural areas were in most cases resistant to only 0 to 3 antibiotics.

Gentamicin is an antibiotic sold in ampule hence believed not to be as abused as those sold in capsules and tablets. This is supported by the study as result shows that only 16.7% of the female subjects were resistant to Gentamicin, while 64.7% of the male population was found to be resistant to it. This could probably be as a result of exposure of the male students to the antibiotic which is often used in the treatment of gonorrhea. The disease is symptomatic in males but asymptomatic in females, so males are more likely to abuse it than females. The parenteral administration of the antibiotic could control the misuse of the drug. Restriction of /and sale of antibiotic over the counter could significantly reduce the spread and emergence of resistant bacteria.

High incidence of resistance to Ciprofloxacillin could also be due to overuse of chloroquine to prevent and treat malaria. Mark (2008) reported that *E coli* strain resistant to Ciprofloxacillin was detected in the digestive tracts of villagers from rain forest community in Guayana despite the fact that they have never been given the drug. Most have however been given chloroquine. This could be a risk factor in malaria endemic areas.

Our findings reveal that resistance to Streptomycin, Septrin, Chloramphenicol, Ciprofloxacin, Amoxacillin and Augmentin were significantly high, this agrees with the result of a study in Nigeria, where resistant *E. coli* isolates from persons in an urban metropolis (Lagos) were significantly more likely to be resistant to Ampicillin and Streptomycin than isolates from residents of nearby smaller towns and villages (Lamikanra and Okeke, 1997).

5. Conclusion

The bacteria of the Enterobacteriaceae family have emerged as major pathogens of interest because of their ability to resist multiple antibiotics which confers on its a survival advantage with the propensity to acquire such traits as resistance determinant to various antimicrobials and other virulence factors, these organisms will continue to create new therapeutic problem and dilemmas.(Akortha and Filgona, 2009). Hence, the study suggests that the use of antibiotics should be monitored to prevent the development of multiple antibiotic resistant organisms especially in individuals living in crowded facilities.

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