Antimicrobial Susceptibility and Plasmid Profiles of Escherichia coli Isolates Obtained from Different Human Clinical Specimens in Lagos – Nigeria

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ABSTRACT: A total of 86 *Escherichia coli* isolates from different human clinical specimens comprising urine, stool, wound swabs, high vaginal swabs, urethral swabs, endocervical swabs, ear swabs, semen, used catheter tips, cerebrospinal fluid and blood obtained from patients at two large referral hospitals in Lagos, Nigeria were screened for their antibiograms and plasmid profiles. A total of seven antibiotic resistance profiles were obtained with over 66% of the isolates showing multi-drug resistance. Plasmids of three size ranges were detected in 54 (62.7%) of the isolates. Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 6.557 – 23.130kb. Very high resistance levels (>75%) were detected against tetracycline, augmentin and amoxicillin while nitrofurantoin and ofloxacin recorded the least resistance levels of 6% and 19% respectively among the isolates. [The Journal of American Science. 2006;2(4):70-75].

Keywords: Antibiogram; plasmid profile; clinical specimen.

INTRODUCTION

Escherichia coli is a bacterial organism that belongs to the family Enterobacteriaeceae. *E. coli* is one of the main causes of both nosocomial and community acquired infections in humans. The organism is therefore of clinical importance and can be isolated from various clinical specimens. It is one of the organisms most frequently isolated from blood (Karlowsky, *et al*., 2004).

It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Hassan, 1985). This therefore demands the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities. According to Aibinu *et al*. (2004), *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim - sulfamethoxazole. The widespread occurrence of drug resistant *E. coli* and other pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both (Omigie *et al*., 2006).

In recent years, the application of molecular techniques for isolation and differentiation of bacterial isolates in hospitals have provided a set of powerful new tools that can augment both epidemiological investigations and patient treatment (Villari *et al*., 1998; Gakuya *et al*., 2001).

MATERIALS AND METHODS

Sample collection

A total of 156 clinical specimens comprising urine, stool, wound swabs, high vaginal swabs, urethral swabs, used catheter tips, endocervical swabs, ear swabs, semen, cerebrospinal fluid and blood of patients attending Lagos State University Teaching Hospital

[LASUTH] and General Hospital, Ikeja were screened for *E. coli*. The specimens were processed at the General Hospital, Ikeja, Lagos, using standard microbiological methods. All isolates were identified using conventional techniques (Chessbrough, 2000). Plasmid profile was carried out at the Nigerian Institute for Medical Research, Yaba, Lagos.

Antibiotic susceptibility testing

Susceptibility of isolates to antibiotics were tested using the disk diffusion method on Mueller Hinton agar against the following eight antibiotics, namely amoxicillin (25 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), gentamicin (10 µg), nitrofuratoin (30 µg), ofloxacin (30 µg), augmentin (30 µg) and tetracycline (30µg). The sensitivity tests were standardised using *Staphylococcus aureus* (NCTC no. 6571) and *E. coli* NCTC no. 10418). Inhibition zones sizes were interpreted using standard recommendations of NCCLS (2000).

Plasmid analysis

Plasmids DNA were extracted from cultured cells using the alkaline SDS method (Johnson, 1998). The DNA were electrophoresed on 0.8% agarose gel stained with ethidium bromide and visualized by UV-transillumination. Plasmid sizes were estimated by comparing with previously characterized plasmids.

RESULTS

The various results for the tests done are shown below. Table 1 shows the distribution of *E. coli* from various clinical specimens.

Table 1. Distribution of *E. coli* from the various clinical specimens

Specimens	Number Screened	Number of Positive samples
Urine	66	39
High vaginal swab	16	4
Wound swab	20	14
Ear swab	4	2
Used catheter tips	14	9
Endocervical swab	5	1
Urethral swab	3	1
Semen	9	3
Stool	7	4
Blood	9	5
Cerebrospinal fluid	3	1
Total:	**156**	**86**

Table 2 shows the results of the antimicrobial resistance of *E. coli*. Over ninety percent of the strains were sensitive to nitrofuratoin, 57% to nalidixic acid, 51.2% percent to gentamicin, 77.9% to ofloxacin, 48.8% percent to cotrimoxazole. High resistance to penicillin was observed. Ninety three percent were resistant to Agumentin and 88.4% were resistant to Amoxicillins. Resistance to Tetracycline was over 90%.

Table 2. Antibiotic sensitivity/resistance of *E. coli* strains isolated from the various human clinical specimens (n=86).

Antibiotics Tested	**Sensitive (%)**	**Resistant (%)**
Amoxicillin	10 (11.6)	76 (88.4)
Cotrimoxazole	42 (48.8)	44 (51.2)
Nitrofuratoin	80 (93.0)	6 (7.0)
Gentamicin	44 (51.2)	42 (48.8)
Nalidixic acid	49 (57.0)	37 (43.0)
Ofloxacin	67 (77.9)	19 (22.1)
Augmentin	6 (7.0)	80 (93.0)
Tetracycline	5 (5.8)	81 (94.2)

Table 3. Antibiotic resistance of *Escherichia coli* isolates from various various human clinical specimens

Antibiotic tested	Ear swab n = 2	Wound swab n = 14	Urine n = 39	HVS n = 4	Stool n = 4	Semen n = 6	Blood n = 5	Cathete n = 9	CSF n = 1	Urethra n = 1	ECS n = 1	Total n=86
Tet	2(100%)	14(100%)	37(94.9%)	3(75.0%)	4(100%)	6(100%)	4(80%)	8(88.9%)	1(100%)	1(100%)	1(100%)	81
Amp	2(100%)	14(100%)	31(79.5%)	3(75.0%)	4(100%)	6(100%)	4(80%)	9(100%)	1(100%)	1(100%)	1(100%)	76
Nal	0(0%)	7(50%)	14(35.9%)	2(50%)	1(25%)	4(66.7%)	2(40%)	5(55.6%)	1(100%)	0(0%)	1(100%)	37
Cot	1(50%)	10(71.4%)	16(41.0%)	2(50%)	2(50%)	2(33.3%)	5(100%)	4(44.4%)	1(100%)	0(0%)	1(100%)	44
Ofl	1(50%)	1(7.1%)	10(25.6%)	1(25%)	1(25%)	2(33.3%)	0(0%)	0(0%)	1(100%)	1(100%)	1(100%)	19
NIH	0(0%)	0(0%)	3(7.7%)	0(0%)	1(25%)	0(0%)	1(20%)	1(11.1%)	0(0%)	0(0%)	0(0%)	6
Aug	2(100%)	14(100%)	36(92.3%)	2(75.0%)	4(100%)	6(100%)	4(80%)	8(88.9%)	1(100%)	1(100%)	1(100%)	80
Gert	1(50%)	12(85.71%)	14(35.9%)	1(25%)	1(25%)	2(33.3%)	3(60%)	5(55.6%)	1(100%)	1(100%)	1(100%)	42

Key: Amx = Amoxicillin, Aug = Augmentin, Cot = Cotrimoxazole, Gen = Gentamicin,
Nal = Nalidixic acid, Nit = Nitrofurantoin, Tet = Tetracycline.
CSF = Cerebrospinal fluid, ECS = Endocervical swab, HVS = High vaginal swab.

Detailed results of the antibiotic resistance screening tests and the summary of the antibiogram profiles obtained are presented in tables 4 and 5 respectively. The results show that about 66.26% of the *E. coli* isolates are multidrug resistant, i.e. are resistant to four or more antibiotics

Table 4. Antimicrobial resistance profiles (Antibiograms) of *E. coli* isolated from various human clinical specimens.

Antimicrobial resistance profiles	**Number of strains showing profile**
Tet Aug Amx Cot Gen Nal Ofl	11
Tet Aug Amx Cot Gen Nal	9
Tet Aug Amx Cot Nal Ofl	3
Tet Aug Amx Gen Nal Ofl	2
Tet Aug Amx Cot Gen Ofl	1
Tet Aug Amx Cot Gen Nit	3
Tet Aug Amx Cot Gen	7
Tet Aug Amx Gen Nal	2
Tet Aug Amx Gen Ofl	1
Tet Aug Amx Cot Nal	2
Tet Aug Cot Gen Nal	1
Tet Amx Gen Nal Nit	1
Tet Aug Amx Nal	3
Amx Cot Gen Nal	1
Tet Aug Gen Nit	1
Tet Aug Amx Cot	5
Tet Aug Cot Ofl	1
Tet Aug Amx Gen	2
Tet Aug Amx Nit	1
Aug Gen Nal	1
Tet Aug Nal	1
Tet Aug Amx	19
Tet Aug	4
Tet Amx	1
Amx	2
Cot	1

Table 5. Summary of Antimicrobial resistance profiles (Antibiograms) of *E. coli* isolated from various human clinical specimens.

Number of antibiotics resistant to	**Number of strains showing pattern**
One antibiotic	3 (3.49%)
Two antibiotics	5 (5.81%)
Three antibiotics	21 (24.41%)
Four antibiotics	14 (16.27%)
Five antibiotics	14 (16.27%)
Six antibiotics	18 (20.93%)
Seven antibiotics	11 (12.79%)

Out of the 86 *E. coli* isolates, 54 (62.7%) were found to possess plasmids, which ranged in sizes from 2.322 kb to 23. 130 kb. Some isolates possessed single sized plasmids while others had multiple plasmids with different sizes as shown in table 6.

Table 6. Sizes and frequency of plasmas detected in *E. coli* isolated from different human clinical isolates and correlation with resistance profiles.

Plasmid sizes (kb)	**No. (%) of isolates**	**Level of resistance profile**
≤2.322 kb	5 (9.3%)	Low (1 – 2 antibiotics)
>2.322 – 6.557 kb	22 (40.7%)	Medium (3 – 4 antibiotics)
>6.557 – 23.130 kb	27 (50%)	High (5 - 7 antibiotics)

DISCUSSION

Epidemiological surveillance of antimicrobial resistance is indispensable for empirical treatment infections, implementing control measures, and preventing the spread of antimicrobial resistant microorganisms (Goosens and Sprenger, 1998).

Pathogenic isolates of *E. coli* have relatively high potentials for developing resistance (Karlowsky *et al*., 2004). High resistance of *E. coli* to antimicrobial agents tested was observed in this study. This is similar to what was observed by Aibinu *et al.,* (2004) who reported 100% resistance of their *E. coli* isolates to ampicillin and amoxicillin. Resistance to amoxicillin observed in this study was similar to what was observed in South Africa, Israel, (62% - 84%) and Hong Kong, Philippines (64 - 82%) (Stelling *et al*., 2005). Densenclos *et al*. (1988)) reported 53% of their *E. coli* isolates were resistant cotrimoxazole and 67% to tetracycline. Their finding is in harmony with the report of this study, showing 69% and 88% resistance to cotrimoxazole and tetracycline respectively. The reason for this high resistance to commonly used antibiotics may be due to widespread and indiscriminate use in our environment.

Isolates in this study were highly sensitive to nitrofurantion (80%). Extreme sensitivity of *E. coli* isolates to nitrofurantion has earlier been reported by Bonten *et al*. (1990).

In recent years, use of fluoroquinolones has increased in many countries and emergence of resistance of bacterial isolates to fluoroquinolones has been observed. Consistent stepwise increase in *E. coli* resistance to ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by Bolon *et al*., (2004). Ciprofloxacin resistance in Portugal was 25.8% and Italy 24.3% while in Germany and Netherlands it was 15.2% and 6.8% respectively (Oteo *et al*., 2005). In pervious years, *E. coli* was 100% susceptible to the fluoroquinolones. In 1996, Egri-Okwaji reported 100% susceptibility of *E. coli* isolates to ofloxacin. In another study carried out by Kesah *et al*. (1999), resistance of *E. coli* to fluroquinolone was 2%. The percentage of ofloxacin resistance observed in this study was 22.1%, which is on the high side. Similar high resistance of *E.*

coli to ofloxacin has also been documented by Alex *et al*. (2001); they observed that 24% of 189 *E. coli* isolates were resistant to ofloxacin.

The reason for the high resistance to ofloxacin observed in this study may be due to increasing an irrational consumption rate, transmission of resistant isolates between people and consumption of food from animals that have received antibiotics. Self-medication and non-compliance with medication and sales of substandard drug may account for the rise in antibiotic resistance observed in this community.

Multiple drug resistance among UTI isolates in USA was reported to be 7.1% in 2000 (Sahm *et al*., 2001). Such multi drug resistance has serious implications for the empiric therapy of infections caused by *E. coli* and for the possible co-selection of antimicrobial resistance mediated by multi drug resistance plasmids (Sherley *et al*., 2004). From table 3, multidrug resistant *E. coli*, i.e. isolates resistant to four or more antibiotics, were observed to be very common in the study area as 67% of isolates showed multidrug resistance. Isolates that showed multiple drug resistance were also found to harbour plasmids with sizes ranging from 2.322kb to 23.130kb. This is similar to what was observed by Smith *et al*., (2003) who reported that 47 of the *E. coli* isolated from animals in Lagos harboure detectable plasmids which ranged in sizes from 0.564kb to >23kb. This indicates that animals could be a source of dissemination of this plasmid resistant *E. coli* in the environment. Danbara *et al,* (1987) also reported plasmids of sizes between 3.9kb and 50kb in *E. coli* strains isolated from Traveller's diarrhoea. Similarly, Todorova *et al*., (1990) showed that 92% of *E. coli* serotype 0164 strain possessed two small plasmids of molecular sizes 9.06kb and 7.248kb.

Since antimicrobial resistant patterns are constantly evolving, and present global public health problem, there is the necessity for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empiric therapies.

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