

Full Length Research Paper

Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, south western Nigeria

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We screened 211 clinical samples of which total of 135 *Escherichia coli* isolates from different human clinical specimens comprising urine, stool, wound swabs, high vaginal swabs, ear swabs and blood obtained from patients at Ladoke Akintola University Teaching Hospital, Osogbo, Osun State, Nigeria. The isolated *E. coli* were screened for their antibiograms and plasmid profiles. Seven antimicrobial drugs were used during the study. The prevalence of strains resistance to antimicrobials were; Tetracycline (91.6%), Ampicillin (86.7%), Sulphamide (77.8%) and Gentamicin and Nalidixic acid which were (39.3%) and (4.1%) respectively. A total of seven antibiotic resistance profiles were obtained with over 64% of the isolates showing multi-drug resistance. Plasmids of three size ranges were detected in all of the isolates. Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range < 6 – 25 kb. Very large resistance levels > 85% were detected against Tetracycline, Sulphamide, and Cotrimoxazole while Nalidixic acid showed least resistance of 4.1% among the isolates. Majority of the isolates were positive for betalactamase production when subjected to starch paper method.

Key word: *Escherichia coli*, betalactamase, antibacteria resistance.

INTRODUCTION

Escherichia coli is one of the main causes of nosocomial infections in humans. *E. coli* is also a common inhabitant of the human and animal gut and is considered an indicator of fecal contamination in food. The organism is of clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections. It is one of the organisms most frequently isolated from different clinical cases of diarrhea and others (Okeke et al., 2000; Olowe et al., 2003; Tobih et al., 2004). Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control

(s), prescription programs, making policy decisions and assessing the effectiveness of both (Omigie et al., 2006). Despite the fact that antibiotics were initially developed for the treatment of infectious disease in people, multi-drug resistance to these antibiotics are becoming a global concern. In Nigeria, few reports have been documented on the multidrug resistance microorganism especially the gram-negative bacilli, and where documented, it is basically for such an environment. The study therefore is the first of such detailed studies on multidrug resistance profile in this location. Few data have evaluated antimicrobial resistance in this region. Most available data are specific to pathogenic organisms and trends over time in this region are rarely followed. This study monitored trends in antibiotic resistance prevalence *E. coli* isolates from different clinical isolates from humans by measuring resistance to seven antimicrobial drugs in *E. coli* from

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

Specimen	Number screened	Number of positive samples (%)
Urine	84	53 (63.1)
Wound swab	36	31(86.1)
Ear swab	42	18(42.9)
Stool	41	28(68.3)
Blood	5	3(60.0)
HVS	3	2(66.7)

Table 2. Antimicrobial susceptibility pattern of clinical isolates of *E. coli* from clinical samples in Osogbo. (N = 135)

Antibiotic test	Sensitive	Resistant
Cotrimoxazole	56(41.5%)	79(58.5%)
Sulphonamide	30(22.2%)	105(77.8%)
Cefuroxin	57(42.2%)	78(57.8%)
Gentamicin	82(60.7)	53(39.3%)
Ampicillin	18(13.3)	117(86.7%)
Tetracycline	11(8.4%)	124(91.6%)
Nalidixic acid	116(85.9)	19(14.1%)

Table 3. Summary of antimicrobial resistance profile.

Number of antibiotic	Number of strains showing resistance
One antibiotic	5 (3.7%)
Two antibiotics	8 (5.9%)
Three antibiotics	34 (25%)
Four antibiotics	16 (11.9%)
Five antibiotics	20 (14.8%)
Six antibiotics	36 (26.70%)
Seven antibiotics	16 (11.9%)

203 clinical specimens over a period of two years. This is the first intensive studies of such in this area.

MATERIALS AND METHODS

Sample collection

A total of 211 clinical specimens comprising of urine, stool, wound swabs, high vaginal swabs, ear swabs and blood collected from Ladoke Akintola University of Technology Teaching Hospital were screened for *E. coli*. The specimens were confirmed to be true-to type using standard microbiological methods. All isolates were identified using conventional techniques (Chessbrough, 2000). Betalactamase production was investigated using starch paper method of Odugemi et al. (1977). Plasmid profiles of the isolates were also analysed.

Culture conditions

All the samples were inoculated on blood and MacConkey's agar

except urine samples which were inoculated on CLED agar. Inoculated plates were incubated at 37°C in ambient air for 16 - 18 h (up to 24 h).

Identification of organisms

After overnight incubation, the culture plates were examined for growth. Identification was performed both microscopically and macroscopically by using the standard microbiological and biochemical techniques (Cowan and Steel 1970)

Antibiotic susceptibility

Susceptibility of isolates to antibiotics was tested using the disc diffusion method on Mueller Hinton agar against the following seven antibiotics: Cotrimoxazole(30 µg, Sulphonamide (10 µg), Cefurotoxin(30 µg), Gentamycin (10 µg), Ampicillin (10 µg), Tetracycline (30 µg) and Nalixidic acid (10 µg)

Starch paper test

This was carried out using the starch paper technique (Odugbeni et al., 1977).

Plasmid analysis

Plasmids were extracted from cultured cells using the alkaline SDS method (Johnson, 1998; Dombrovskii, 1990). The DNA was electrophoresed in 0.8% agrose gel stained with ethidium bromide and visualised by UV transillumination.

RESULTS

The various results for the tests done are shown below. Table 1 shows the distribution of *E. coli* from various clinical specimens. Table 2 shows the results of the antimicrobial resistance of *E. coli*. Over 86% of the strains were sensitive to nalidixic acid, 60% to gentamicin, 42.2% to cefuroxin, 41.5% to co-trimoxazole and 22.2% to sulphnamide. High resistance was observed. Ninety-one point six percent (91.6%) were resistant to tetracycline and 86.7% were resistant to Ampicillins. Resistance to sulphnamide was over 77.8%.Only five isolates were resistant to at least one drug, 5(3.7%) of these isolates obtained demonstrated this phenotype. Sixteen isolates were resistant to all drugs tested. Two of these had only low levels of resistance to nalidixic acid and co-trimoxazole and ampicillin in one case. Two isolates recovered in this study had high-level resistance to all the drugs tested.

Table 3 shows a detailed resistance pattern to antimicrobial agents. As strains susceptible to all drugs became less common, the proportion of isolates resistant to multiple antibiotics increased. The results show that about 65.2% of the *E. coli* isolates are multidrug resistant, i.e. are resistant to four or more antibiotics, while 53.3% show resistant to three or more antibiotics. All the isolates were resistant to at least one drug. The proportion of isolates resistant to three or more drugs increased steadily confirming steady rise to multidrug resistance pattern.

Table 4. Beta-lactamase production.

Starch paper	Positive (%)	Negative (%)
135	134(99.3)	1(0.7)

Table 5. Plasmid size range.

Plasmid size (kb)	Number of isolates	Level of resistance
≤ 6.557	10	Medium
6.6557 - 21.773	10	Medium
21.773 - 23.222	62	High

Table 4 shows that almost all the isolates tested in this work produced beta-lactamase. This provide high tendency for resistance among the isolates.

Table 5 shows plasmid profiles of isolates. Out of the 135 *E. coli* isolates, 82 (60.7%) were found to possess plasmids, which ranged in sizes from 6.557 to 23. 222 kb. Some isolates possessed single sized plasmids while others had multiple plasmids with different sizes.

DISCUSSION

E. coli has widely been implicated in various clinical infections as hospital acquired and community infections as reported by Shah et al. (2002). 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. Our data shows that the prevalence of resistance to most drugs tested in *E. coli* isolates from patients also coincides with study of apparently healthy student reported previously (Lamikanra et al., 1997). Multiple drug resistance among UTI isolates in USA was reported to be 7.1% (Tenner et al., 1992; Sahn et al., 2001). Other related cases of drug resistance pattern in blood, high vaginal swab, and diarrhea has been reported previously by other authors (Tobih et al., 2006), Olowe et al., 2003; Aibinu et al., 2004). This work is also similar to what was observed by Aibinu et al. (2004) who reported 100% resistance of their *E. coli* isolates to ampicillin and amoxicillin. The increases in prevalence of resistance to tetracycline were also statistically significant. In most drugs tested, the proportion of resistant isolates has increased rapidly. The prevalence of resistance in these study shows that resistant profile of *E. coli* reached > 50% for all drugs except nalidixic acid. For tetracycline, the proportions of resistant strains show 91.6%. This is in harmony with what was observed by Lamikanra et al., (1997). These data confirms that indiscriminate use of antibiotics in this region and along with poor hygiene and infection control (risk factors for antibiotic resistance in bacteria), are highly prevalent in Nigeria and other deve-

loping countries (Hart et al., 1998; Okeke et al., 1999). The five drugs for which a considerable rise in resistance was seen were ampicillin, sulfonamides, co-trimoxazole, cefuroxime, and tetracycline. They are extensively used in Nigeria and other developing countries (Hart et al., 1998; Okeke et al., 1999). 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, multi-drug 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. The presence of beta-lactamase production by starch paper method is a long way to confirmation of the fact that these isolates may definitely be harboring plasmids in them and a pointer to beta-lactamase production as previously described (Odugbemi et al., 1977). Isolates that showed multiple drug resistance were also found to harbour plasmids with sizes ranging from 6.557 to 23.222 kb. 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 harbour detectable plasmids which ranged in sizes from 0.564 to >23 kb. 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 3.9 and 50 kb 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 9.06 and 7.248 kb. These five inexpensive drugs are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops and other distributors (Hart et al., 1998; Okeke et al., 1999). Ingestion of antibiotics is known to provide selective pressure ultimately leading to a higher prevalence of resistant bacteria (Levin et al., 1997; Levy et al., 199). As recent antibiotic use was a criterion for exclusion from the study, selection of the resistance strains isolated in the study may have occurred before the volunteer hosts were colonized. The source of resistant organisms in our study population is not known, but possible sources are food, water, and person-to-person transfer. Sub-optimal sanitary conditions and overcrowding in student hostels may facilitate the spread of these organisms. We observed rapid increases in the prevalence of resistance in *E. coli* to most of the older, less expensive antimicrobial drugs used in the management of infections in Nigeria. Not only are these strains potential causes of infection, but they are also potential reservoirs of resistance genes that could be transferred to pathogens. For this reason, the trends seen with clinical *E. coli* may also occur with other pathogenic organisms. Studies in other developing countries have shown that the trend in enteric pathogens is toward increasing antibiotic resistance (Hoge et al., 1998). Our study emphasizes the

need to monitor commensal organisms as well as pathogens by susceptibility testing to guide treatment. Control of antibiotic resistance is needed to conserve the usefulness of the remaining drugs. The data suggest that nail-dixic acid and possibly trimethoprim may be useful in treating infections caused by pathogenic *E. coli* and other related bacteria in Nigeria. The future usefulness of these drugs will, however, depend on effective interventions to halt the selection and spread of resistance among enteric organisms. Since antimicrobial resistant patterns are constantly evolving, and it is a 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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