academicJournals

Vol. 5(3), pp. 29-34, March, 2013
DOI: 10.5897/JBR2012.035
ISSN 2006 9871 © 2013 Academic Journals
http://www.academicjournals.org/JBR

Journal of Bacteriology Research

Full Length Research Paper

Antibiotic resistance profile of *Escherichia coli* isolated from five major geopolitical zones of Nigeria

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Accepted 13 March, 2013

Information on the resistance profiles of clinical and non clinical human bacteria isolates in the developing countries can serve as important means of understanding the human pathogens drug resistance interactions in the zone. *Escherichia coli* isolated from five geopolitical zones of Nigeria were screened for anti-microbial resistance profile against 14 antibiotics using the disc diffusion method. Overall, the 89 isolates tested were 94.4% that showed resistance to Ampicillin; 85.5% to Cotrimoxazole, 92.1% to Cephalothin; 78.7% to Streptomycin, 70.8% to Nitrofurantoin; 79.8 to Tetracycline; 67.4% to Chloramphenicol; 74.2% to Amoxicillin clavulanic acid; 61.8 to Cefpirome; 52.8% to Cefpodoxime; 46.1% to Cefotaxine; 46.1% to Ceftriaxone; 31.5% to Cefoxitin; 38.2% to Nalidixic acid and 24.7% to Gentamycin. These isolates showed a total of 42 different antibiotics resistance profiles, with all the isolates showing resistance to at least four or more of the drugs tested. Statistical analysis showed there was no statistical difference in the proportion of isolates that were resistant to ≥1 antibiotic for human clinical (84.6%) and non-clinical (91.7%) isolates. This result indicates that antimicrobial use in humans has driven the emergence of multi-drug resistant clones in developing countries such as Nigeria that has resulted in an increasingly high prevalence of multiple resistance.

Key words: *Escherichia coli*, resistance profiles, antibiotics, isolates.

INTRODUCTION

The increasing occurrence of drug-resistant *Escherichia coli* isolates of human origin is a global public health problem. Drug resistant *E. coli* infections are presently associated with higher morbidity, mortality and invariably higher expenditure in treatment when compared with infections by strains susceptible to the drugs. Drug resistant commensal *E. coli*, which may not directly cause disease, remains significant in public health as a reservoir of drug resistance genes. These genes can be transferred to human pathogens, such as *E. coli*, *Salmonella*, or to other Gram-negative bacteria in the gut.

Emergence of drug resistance in *E. coli* may arise either because an erstwhile susceptible strain acquires resistance *de novo* or already drug-resistant clones are selected by drug administration and elimination of the susceptible strains. Examples of the latter phenomenon include the outbreak in South London, United Kingdom, involving trimethoprim-sulfamethoxazole-resistant (TS-R) strains of *E. coli* O15:K52:H1 (1986 to 1987); disseminated urinary tract infections (UTIs) in the United States due to TS-R strains of *E. coli* clonal group A (CGA) (1990s); and widespread infections in Canada, Europe, and Asia due to strains of *E. coli* sequence type 131 (ST131; O25:H4) that exhibit extended-spectrum cephalosporin (ESC) resistance through the production of CTX-M-15, an extended-spectrum beta-lactamase (2000s)

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(Mshana et al., 2009).

There is emerging evidence that anti-microbial use in humans may be the major selective force for multi-drug resistant clones in enterobacteriaceae in many developing countries that has resulted in an increasingly high prevalence of multidrug resistance in these countries (Kariuki et al., 1996; Okoli et al., 2002; Ling et al., 1987). This is contrary to the information from the developed countries where overwhelming evidence seems to suggest that antimicrobial use in agriculture is the major driving force in the selection and dissemination of bacterial resistance (Khachatourians, 1998; Apley et al., 1998; Witte, 1998).

For *E. coli* and other classes of enterobacteriaceae in which asymptomatic colonization of the intestine usually precedes infection, acquisition of one or several new genes, rather than point mutation in existing genes has been shown to be the common anti-microbial resistance mediation route (Lipsitch and Samore, 2002). For example, segments of new genes could be replaced with alleles having entirely different sequences from the new type at multiple positions. These new mechanisms thus arise and spread in the bacterial population under conditions of anti-microbial selective pressure (Lipsitch and Samore, 2002).

Again, it has been shown that exposure of most *E. coli* strains to anti-microbial agents usually occurs during treatment directed at infections caused by other unrelated organisms. Anti-microbial treatment that alters the population of *E. coli* in a given host or environment, will usually affect bacterial contacts of the host or environment (Halloran and Struchiner, 1991). Thus, use of a particular antibiotic in for example human hosts, in an environment may increase the risk of colonization by or infection with resistant organisms in other humans or even animals that have not received that set of antibiotics but are sharing common environment with the humans (Lipsitch and Samore, 2002). Anti-microbial use may also increase the density of resistant organisms within a host that already harbor such organisms at a lower density resulting in enhanced shedding of these organisms and increased risk to other hosts (Donskey et al., 2000).

Problems associated with inappropriate use of antibiotics continues to pose very serious hindrance to adequate and qualitative therapeutic management particularly in a developing country such as Nigeria. It is particularly worrisome that despite numerous studies and identification of specific problems, the problem of antibiotics resistance still persists like a canker worm at all levels of our healthcare delivery system. The consequences of aberrant drug utilization (overuse, under use, etc.) cannot be overemphasized, so also the utility of prescription database as a monitoring tool; it is therefore imperative that we continuously perform these studies. In this study, we evaluated the prevalence of antibiotic resistant *E. coli* collected from the major tertiary hospitals located in the five geopolitical zones of Nigeria. We also studied the susceptibility pattern of commensal isolates, we got from apparently healthy individuals.

MATERIALS AND METHODS

Study population

The study population included humans (who were either ill or presumptively healthy) from five geopolitical zones of Nigeria viz: South-East, South-West, South-South, North-Central and North-North. In the South-South and South-East, clinical specimens were collected at the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State and the Abia State University Teaching Hospital, Aba, Abia State, respectively. The Lagos State University Teaching Hospital, Ikeja, Lagos was the site of specimen collection for the South-West, while the National Hospital, Abuja and Military Reference Hospital, Kaduna State were the sources of specimens from the North-Central and North-North, respectively. All samples from these hospitals were clinical specimens from patients who were having gastroenteritis or similar illness. Apparently, healthy undergraduate students of Madonna University Elele, Rivers State were included in the study for the isolation of human commensal *E. coli.* These individuals reported no exposure to antibiotics for six months prior to sampling and each person received an explanation of the study objectives and consent form for inclusion in the study. All sampling procedures were in accordance with guidelines of the National Health Research Ethics Committee, Nigeria (www.nhrec.net).

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

Human fecal specimens were streaked directly on Eosin Methylene Blue agar (EMB) (Oxoid, England). No antibiotic was included in the EMB agar plates used for the cultivation. The inoculated plates were incubated overnight at 37°C. A single colony on EMB with green metallic sheen taken to be *E. coli* was selected from an individual fecal sample for further characterization. *E. coli* was fully identified using conventional microbiological tests- Indole positive, Methyl red positive and Citrate negative (Cheesbrough, 2000).

Antibiotics susceptibility testing

The antibiotics susceptibility pattern of the isolates was determined using the disk diffusion method (Cheesbrough, 2000), on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2006). Susceptibility was tested against ampicillin (10 µg), amoxycillin/ clavulanic acid (20/10 µg), cefpodoxime (10 µg), cefoxitin (30 µg), cefpirome (30 µg), ceftriaxone (30 µg), cefotaxine (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (30 µg) and streptomycin (10 µg), sulfamethoxazole-trimethoprim (10 µg), tetracycline (30 µg), (Oxoid, England). *E. coli* ATCC 25922 was included as a reference strain.

RESULTS

The antibiotic resistance pattern of the *E. coli* isolates is shown in Table 1. Overall, 42 different antibiotics resistance profiles were observed, with all the isolates showing resistance to at least four or more of the drugs

Table 1. Antibiotics resistance profiles of *E. coli* isolated from human specimens.

Antibiotics resistance profile	Percentage
AMP, SXT, NA, TE, S, C, KF, F, AMC, CPO	18.0
AMP, SXT, GN, NA, S, C, KF, F, AMC, CPO	2.2
AMP, SXT, NA, TE, S, C, KF, F, AMC	3.4
AMP, SXT, GN, NA, TE, S, C, KF, CPO	3.4
AMP, SXT, TE, S, C, KF, F, AMC, CPO	6.7
AMP, SXT, GN, TE, S, C, KF, F, AMC	3.4
AMP, SXT, GN, TE, S, C, KF, F, CPO	9.0
AMP, SXT, NA, TE, C, KF, F, CPO	3.4
AMP, SXT, NA, TE, S, C, KF, CPO	1.1
AMP, GN, TE, C, KF, F, AMC, CPO	1.1
AMP, SXT, TE, C, KF, F, AMC, CPO	2.2
AMP, SXT, TE, S, C, KF, AMC, CPO	1.1
AMP, SXT, TE, S, C, KF, AMC, CPO	10.1
AMP, SXT, TE, S, C, KF, F, AMC	00
SXT, TE, S, C, KF, F, AMC, CPO	1.1
AMP, SXT, NA, S, KF, AMC, CPO	00
AMP, SXT, S, KF, F, AMC, CPO	3.4
AMP, SXT, TE, C, KF, AMC, CPO	1.1
AMP, NA, S, KF, F, AMC, CPO	1.1
AMP, NA, TE, S, C, AMC, CPO	00
AMP, TE, C, KF, F, AMC, CPO	1.1
AMP, SXT, TE, S, KF, AMC, CPO	7.9
AMP, SXT, TE, S, KF, F, CPO	1.1
AMP, GN, NA, TE, S, KF	1.1
AMP, GN, TE, S, C, F, CPO	00
AMP, SXT, KF, F, AMC, CPO	00
AMP, SXT, S, C, KF, AMC	00
AMP, SXT, TE, S, KF, AMC	7.9
AMP, C, KF, F, AMC, CPO	3.4
AMP, NA, TE, S, C, KF	1.1
AMP, KF, F, AMC, CPO	1.1
AMP, SXT, C, KF, CPO	00
AMP, SXT, GN, KF, F	00
AMP, SXT, GN, TE, CPO	00
AMP, SXT, KF, AMC, CPO	1.1
SXT, NA, C, KF, CPO	1.1
SXT, TE, KF, F, CPO	00
SXT, NA, TE, KF	1.1
SXT, S, AMC, CPO	00
AMP, TE, AMC, CPO	00
AMP, C, KF, CPO	00
SXT, TE, S, F	1.1

AMP = Ampicillin, SXT= cotrimoxazole, GN = gentamycin, NA = nalidixic acid, TE = tetracycline, S = streptomycin, C = chloramphenicol, KF = cephalothin, F = nitrofurantoin, AMC = amoxycillin, CPO = cefpirome.

tested. Among the 89 isolates tested, 94.4% showed resistance to ampicillin; 85.5% to cotrimoxazole, 92.1% to cephalothin; 78.7% to streptomycin, 70.8% to nitrofurantoin; 79.8 to tetracycline; 67.4% to chloramphenicol; 74.2% to amoxicillin clavulanic acid; 61.8 to cefpirome; 52.8% to cefpodoxime; 46.1% to cefotaxine; 46.1% to ceftriaxone; 31.5% to cefoxitin; 38.2% to nalidixic acid and 24.7% to gentamycin.

Comparing samples from clinical and non clinical human specimens, the results showed that, the isolates were resistant to ampicillin (93.9% and 100%), cotrimoxazole (87.9 and 50%), cephalothin (92.8 and 83.3%), streptomycin (78.3 and 83.3%), nitrofurantoin (71.1 and 66.7%), tetracycline (81.9 and 50%), chloramphenicol (67.5 and 66.7%), amoxicillin clavulanic acid (74.7 and 66.7%), cefpirome (60.2 and 50%), cefpodoxime (51.8 and 50%), cefotaxine (45.8 and 33.3%), ceftriaxone (44.6 and 66.7%), cefoxitin (28.9 and 66.7%), nalidixic acid (38.6 and 33.3%) and gentamycin (22.9 and 33.3%), respectively (Figure 1).

Taking the susceptibility pattern source after source; the isolates from the south east zone of Nigeria showed a unique pattern of resistance, they were resistant to ampicillin and cotrimoxazole (91.3% each); gentamycin and nalidixic acid (17.4% each); tetracycline and amoxicillin clavulanic acid (87% each); chloramphenicol and ceftriaxone (47.8% each); streptomycin, cefpodoxime and cefotaxine (65.2% each) (Table 2). A similar pattern was observed in the isolates from the south west zone; 93.1% of the isolates were resistance to cotrimoxazole, tetracycline and cephalothin respectively. Also, common resistance pattern of 66.7% to cefpodoxime and nitrofurantoin; 33.3% to ceftriaxone and gentamycin; and 53.3% to cefoxitin and nalidixic acid, respectively, was observed among these isolates (Table 2). The isolates from the south-south zone showed a unique resistance to cephalosporins; 41.2% to cefoxitin, ceftriaxone and cefotaxine, respectively and a very high resistance of 76.5% to cefpirome- a third generation cephalosporin (Table 2). Statistical analysis showed there was no statistical difference in the proportion of isolates that were resistant to ≥1 antibiotic for human clinical (84.6%) and non-clinical (91.7%) isolates (Fisher's exact test, P = 1.0).

DISCUSSION

The introduction of antibiotics is one of the most important medical interventions with regards to reducing human morbidity and mortality. However, the intensive use of antibiotics (which was estimated in 2002 to be 100,000 to 200,000 tonnes per annum (Andersson and Hughes, 2010), worldwide has dramatically increased the frequency of resistance among clinical and commensal isolates and threatens a loss of therapeutic options. Resistance dramatically reduces the possibility of treating infections effectively and increases the risk of complications and of a fatal outcome.

In this study, we examined antimicrobial resistance in

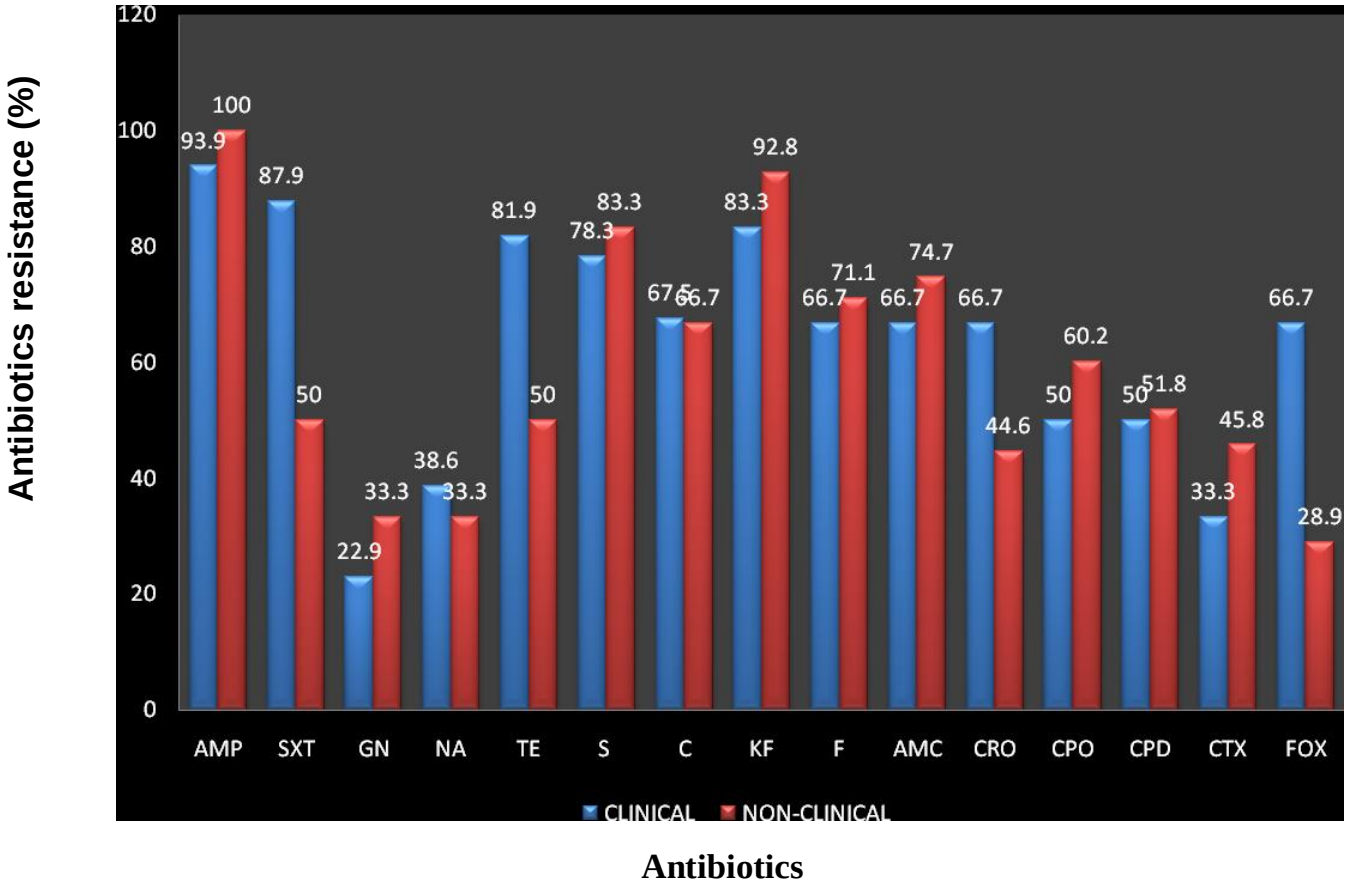

Figure 1. Comparison of percentage antibiotics resistance of *E.coli* Isolates from clinical and non-clinical human specimens. AMP = Ampicillin, SXT = cotrimoxazole, GN = gentamycin, NA = nalidixic acid, TE = tetracycline, S = streptomycin, C = chloramphenicol, KF = cephalothin, F = nitrofurantoin, AMC = amoxycillin clavulanic acid, CRO = ceftriaxone, CPO = cefpirome, CPD = cefpodoxime, CTX = cefotaxine, FOX = cefoxitin.

Table 2. Antibiotics resistance profiles of *E. coli* isolated from human specimens in different regions of Nigeria.

Sample source	Antibiotics														
	AMP	SXT	GN	NA	TE	S	C	KF	F	AMC	CRO	CPO	CPX	CTX	FOX
SE	91.3	91.3	17.4	17.4	87.0	65.2	47.8	95.7	70.0	87.0	47.8	74.0	65.2	65.2	39.0
SW	86.7	93.3	33.3	53.3	93.3	100	73.3	93.3	66.7	60.0	33.3	46.7	66.7	40.0	53.3
SS	100	76.5	29.4	23.5	58.8	88.2	76.5	88.2	58.8	82.4	41.2	76.5	47.1	41.2	41.2
NC	100	100	20.0	100	80.0	80.0	80.0	100	100	80.0	40.0	60.0	60.0	40.0	20.0
NN	96.6	79.3	27.6	44.8	75.8	72.4	70.0	90.1	72.4	65.5	52.2	55.2	41.4	41.4	10.3

AMP = Ampicillin, SXT = cotrimoxazole, GN = gentamycin, NA = nalidixic acid, TE = tetracycline, S = streptomycin, C = chloramphenicol, KF = cephalothin, F = nitrofurantoin, AMC = amoxycillin, CPO = cefpirome, SE = South-East, SW = South-West, SS = South-South, NC = North-Central, NN = North-North.

clinical *E. coli* isolates from human clinical specimens collected from five major tertiary hospitals located in the five geopolitical zone of Nigeria. Because of the geographic sampling of the isolates, this surveillance provides a representative sample of the resistance trends in Nigeria. Generally, when compared with the other antimicrobial agents used in this study, the isolates were susceptible to gentamicin, nalidixic acid and cefoxitin. However, increasing resistance frequencies were noted for ampicillin, cotrimoxazole, cephalothin, streptomycin, tetracycline and nitrofurantoin.

High antimicrobial resistance to tetracycline was noted

among the isolates (79.8%). The presence and frequency of tetracycline resistance agree with findings of other studies on antibiotic resistance in *E. coli* (van den Bogaard et al., 2001; Saya et al., 2005). The patterns of resistance to tetracycline have been attributed in part to widespread and lengthy use of tetracycline in human medicine (Piddock, 2006; Vu and Nikaido, 1985; van den Bogaard et al., 2001). Since tetracycline is a naturally derived compound, bacteria can be exposed to these agents in nature and outside any human use for disease treatment, for prophylaxis, or for livestock growth promotion. Tetracycline is a commonly used first line antibiotic for humans 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 (van den Bogaard et al., 2001). This makes it more possible for a susceptible bacterium to acquire resistance factors by conjugation, or other genetic exchange mechanisms.

The high resistance to cefpodoxime (50% in clinical and 51.8% in non-clinical specimens) and cefpirome (50% in clinical and 60.2% in non-clinical specimens), observed in this study is of public health concern. This is probably the first documented evidence of such high resistance figure against any third or fourth generation cephalosporins in *E. coli* isolates from Nigeria. In fact, Okoli et al. (2005) reported the highest resistance rate of 31.5% to cefuroxime (second generation cephalosporins). It is probable that even though these drugs belong to the third and fourth generation cephalosporins, they may have been introduced into clinical practice in the country. Current information indicates that, third and fourth generation cephalosporins resistance can also be plasmid mediated, involving the *blaCMY-2* plasmids, which is quite distinct from the known cephalosporins resistance genes. The gene has been isolated from *E. coli* and *Salmonella* spp. and has been identified in USA (Daniels, 2009). It is probable that this plasmid-mediated gene may be contributing to the wide distribution of high bacterial resistance to cephalosporins observed in this study. This assumption was later confirmed by DNA microarray, as a remarkable number of this gene was detected in this study (data not shown)

Multi-drug resistance (resistance to at least three antimicrobials) was found in *E. coli* from both clinical and commensal sources, but was higher in frequency and proportion in clinical isolates. However, when these multi-drug resistant strains were compared, it is clear that they do not have common sources of resistant bacteria. Most clinical multi-drug resistant isolates exhibited resistance to a combination of antimicrobials that included streptomycin, tetracycline and cephalothin, while most commensal isolates were resistant to ampicillin and tetracycline or nalidixic acid and gentamicin. This suggests that clinical isolates that were Tet-R are more likely to become resistant to additional antimicrobial agents. Resistance to tetracycline may be conserved in bacterial populations over time, regardless of selection pressure, which might result in an overall increase in resistance over time. In addition, it is likely that clinical sources could act as reservoirs for Tet-R for environmental contamination and human colonization. Furthermore, the difference in resistance between the two groups of isolates can be explained in terms of the interactions of the organisms (associated with the host) and potential horizontal gene transfer in their respective environments.

The very low resistance recorded against gentamicin here is in agreement with results of similar studies in Nigeria (Chah et al., 2003, Okoli et al., 2002). They are however at variance with the results reported by Uwaezuoke et al. (2000). It is possible that these organisms originated as direct human contaminants of the feed ingredients through handling. Gentamicin is marketed mostly as a 2 ml injectable solution in a glass vial. This probably makes the drug unpopular and therefore safe from common abuse in Nigeria.

In conclusion, 42 resistance profiles were observed in the *E. coli* isolates, with predominant patterns being distributed widely across both sample sources, indicating a striking diversity of resistance patterns in Nigeria. Multi-drug resistance was a common feature in these isolates, highlighting the fact that the resistance genes for these drugs are linked to plasmids (Schroeder et al., 2002). Moreover, the wide spread resistance to cotrimoxazole may be implying the presence of class 1 integrons, which are also important in conferring resistance to multiple antimicrobials (Jonhson et al., 2007). This is strong evidence that antimicrobial use in humans has driven the emergence of multi-drug resistant clones in developing countries such as Nigeria which has resulted in an increasingly high prevalence of multiple resistance.

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