International Journal of TROPICAL DISEASE & Health
4(12): 1245-1253, 2014
ISSN: 2278–1005

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Pattern of Antimicrobial Resistance of *Escherichia coli* from Healthy Adults in Amassoma, South-South Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author AO designed the study, performed the statistical analysis, wrote the protocol and the final draft of the manuscript. Author EB managed the literature searches, carried out the laboratory work under the supervision of author AO and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2014/12715
Editor(s):
(1) William Ebomoyi, Department of Health Studies College of Health Sciences, Chicago State University, USA.
Reviewers:
(1) Anonymous, University of Milan, Italy.
(2) Igwe James Chibueze, Medical Biotechnology Department, National Biotechnology Development Agency, Abuja, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=673&id=19&aid=6370

Original Research Article

Received 15th July 2014
Accepted 23rd September 2014
Published 6th October 2014

ABSTRACT

Aim: To investigate the antimicrobial resistance profile of faecal *Escherichia coli* to common antimicrobial agents in healthy adults in Amassoma, South Southern Nigeria.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Pharmaceutical Microbiology and Biotechnology, Niger Delta University, Bayelsa State, between February and June 2010.

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Methodology: The stool samples collected were inoculated and screened for *E. coli* using standard microbiological protocols. The antimicrobial susceptibility test of the isolates was done using disc diffusion technique.
Results: A total of 110 (84.6%) *E. coli* isolates were obtained from all the samples comprising 38 (34.5%) from the villagers and 72 (65.5%) from the University students. The overall resistance profiles of all the isolates were: ampicillin-95.5%, tetracycline-72.7%, augmentin-70.9%, co-trimoxazole-54.5%, cefuroxime-44.5%, chloramphenicol-39.1%, nalidixic acid-30.0%, nitrofurantoin-28.2%, ceftazidime-15.5%, ciprofloxacin-14.5%, gentamicin-10.0% and ofloxacin-4.5%. The isolates from the villagers exhibited significantly higher resistances to some of the antibiotics than those from the students ($P<0.05$). The prevalence of multiple drug resistance among all the isolates was 76 (69.1%).
Conclusion: The observed high level of multiple drug resistance among the flora of healthy individuals call for measures to control the sales of antimicrobial agents in this country as a strategy toward the containment of antibiotic resistance.

Keywords: Multi-resistance; faecal; Escherichia coli; healthy; adults.

1. INTRODUCTION

Antibiotic resistance is a problem of global health affecting virtually all bacteria which commonly cause human illnesses [1-3]. Antibiotic resistance is a phenomenon that occurs when bacterial progeny emerges by natural selection in the presence of sub-inhibitory concentration of an antimicrobial agent and the emerging bacteria in this environment tend to be resistant to the agent [4]. This type of bacterial resistance is not limited to a single class of antimicrobial agents [5].

Antibiotic resistance in many industrialized countries is usually due to heavy use (over-prescription) of antibiotics, excessive use of disinfectants for routine hygiene and use of antibiotics for veterinary medication or growth promoters [6-10]; while in developing countries, it is particularly due to inappropriate use of antimicrobials, misdiagnosis and use of antimicrobials of substandard quality [11,12].

Escherichia coli, an important part of the normal flora of the gastrointestinal tract of humans and animals, can also be found in faecally contaminated water, soil and vegetation. Most strains of *E. coli* are non-pathogenic but a few of them are associated with infections such as urinary tract infection, wound infection, bacteremia, neonatal meningitis, diarrhoeal diseases and sepsis [13-14]. The carriage of resistance by commensals has been proposed as an indication of the burden of antibiotic misuse and resistance in a population [15-16].

The acquisition of resistance by commensal bacteria is a serious concern, because intestinal flora that had been exposed to sub-therapeutic doses of antibiotics can act as potential reservoir of resistance genes which may be transferred to susceptible pathogenic bacteria within the host, leading to the general increase of bacterial resistance worldwide [17]. Commensal *E. coli* strains can efficiently exchange genetic materials with pathogens such as *Salmonella, Shigella, Vibrio cholerae* and other pathogenic *E. coli* [18,19]. Hence, the study of the resistance profile of faecal *E. coli* species is very important since it is the commonest commensal of the gut and a predominant antimicrobial resistance carrier of the intestinal enterobacteria [20].

There are few studies that have been documented on the resistance patterns of this organism in various regions of Nigeria but this study is the first of such detailed studies on multiple drug resistance profile in this region. Thus, we report the carriage of multiple antimicrobial resistant *E. coli* by apparently healthy adults in Amassoma community in the South-South region of Nigeria.

2. MATERIALS AND METHODS

2.1 The Study Population

Stool samples were collected with the aid of sterile swab sticks from 130 apparently healthy adult volunteers comprising of students of the Niger Delta University, Amassoma and the villagers in the community. The volunteers, who were 16-40 years of age, gave informed consent in accordance with the institutional ethical standards on human experimentation and had not taken any antimicrobial agents within the previous one month before the survey. Amassoma is a village in the Wilberforce Island of Bayelsa state in the South-South part of Nigeria. It houses a fast growing state University with students from mostly Southern part of Nigeria. The villagers who were basically fishermen, crop farmers and traders, participated poorly in the study by refusing to provide their stool samples. The study was conducted from February to June 2010.

2.2 Isolation and Characterization of *E. coli*

The collected 130 stool samples were transported immediately in iced-packs to the laboratory where each of the samples was inoculated separately on MacConkey (Oxoid, UK) and Eosin Methylene Blue (EMB) (Oxoid, UK) agar plates, then streaked and incubated at 37ºC for 24 hours. The colonies that gave characteristic greenish metallic sheen on EMB agar plates were further confirmed as *E. coli* using conventional biochemical IMViC (Indole, Methyl red, Voges Proskauer, Citrate, Urease) tests [13]. These isolates were then stored on fresh slants of Nutrient agar (Oxoid, U.K) for antimicrobial susceptibility testing.

2.3 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was carried out with twelve (12) discs (Oxoid, UK) of commonly used antimicrobial agents using modified Kirby-Bauer disc diffusion technique [13]. The standardized suspension of each isolate that was matched with 0.5 McFarland standard was used to swab the surface of Mueller Hinton (Oxoid, UK) agar plate and the following discs were placed on the plates (in duplicates) after 20 minutes of inoculation: ampicillin (10 µg), augmentin (30 µg), chloramphenicol (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), nitrofurantoin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg) and tetracycline (30 µg). The plates containing the discs were allowed to stand for at least 30 minutes before incubated at 37ºC for 24 hours. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards [21]. The isolates of *E. coli* that were resistant to at least one antimicrobial agent in at least three classes of antimicrobial agents tested were defined as having multiple antimicrobial resistance in this study [22].

2.4 Statistical Analysis

Frequencies were obtained and percentages were calculated for study variables. Fisher Exact Probability test (two tailed) was used for comparing the variables and a p-value of less than or equal to 0.05 was considered significant ($p \leq 0.05$).

3. RESULTS

The volunteers in this study comprised 45 (34.6%) villagers and 85 (65.4%) University students. The total number of female and male were 59 (45.4%) and 71 (54.6%) respectively. One hundred and ten (84.6%) samples from all the screened 130 healthy subjects yielded *E. coli* isolates which were 72 (84.7%) from the students and 38 (84.4%) from the villagers. The patterns of antimicrobial resistance of the isolates in the two groups of subjects are shown in Table 1.

Table 1. The distribution of antimicrobial resistant *Escherichia coli* isolates among the volunteers

Antimicrobial agents	Number of resistant isolates (%)			*p-value*
	Overall resistance	Students N = 72	Villagers N = 38	
Ampicillin	105 (95.5)	67 (93.1)	38 100.0)	0.162
Augmentin	78 (70.9)	48 (66.7)	30 (78.9)	0.194
Cefuroxime	49 (44.5)	26 (36.1)	23 (60.5)	0.017*
Ceftazidime	17 (15.5)	10 (13.9)	7 (18.4)	0.584
Gentamicin	11 (10.0)	8 (11.1)	3 (7.9)	0.745
Ciprofloxacin	16 (14.5)	10 (13.9)	6 (15.8)	1.0
Ofloxacin	5 (4.5)	3 (4.2)	2 (5.3)	1.0
Nalidixic acid	33 (30.0)	17 (23.6)	16 (42.1)	0.052
Nitrofurantoin	31 (28.2)	17 (23.6)	14 (36.8)	0.182
Chloramphenicol	43 (39.1)	20 (27.8)	23 (60.5)	0.001*
Co-trimoxazole	60 (54.5)	33 (45.8)	27 (71.1)	0.016*
Tetracycline	80 (72.7)	49 (68.1)	31 (81.6)	0.177

** = Statistically significant (p<0.05)*

The multiple antimicrobial resistance which is defined as the resistance of an isolate to at least one antimicrobial agent in at least three (3) classes of antimicrobial agents tested [22] was used in this study to measure multiple drug resistance of each isolate. Tables 2 and 3 show the multiple drug resistant profiles and the common antibiogram combination patterns of the *E. coli* isolates respectively.

Table 2. The Multiple antimicrobial resistant profiles of *E. coli* isolates from the healthy subjects

Number of classes of agents resisted	Number of resistant isolates (%)			*p* –value*
	Overall (N = 110)	Students (N = 72)	Villagers (N = 38)	
0	4 (3.6)	4 (5.6)	0 (0.0)	
1	11 (10.0)	10 (13.9)	1 (2.6)	
2	19 (17.3)	14 (19.4)	5 (13.2)	

Table 2 continued……

3	25 (22.7)	17 (23.6)	8 (21.1)	
4	22 (20.0)	13 (18.1)	9 (23.7)	
5	11 (10.0)	8 (11.1)	3 (7.9)	
6	15 (13.6)	3 (4.2)	12 (31.6)	
7	3 (2.7)	3 (4.2)	0 (0.0)	
8	0 (0.0)	0 (0.0)	0 (0.0)	
≥ 3	76 (69.1)	44 (61.1)	32 (84.2)	0.017

**statistically significant*

Table 3. The Common antibiotic resistance combination patterns of the *E. coli* isolates

Number of agents resisted	Number of resistant isolates	Common antibiogram	Number of isolates with common antibiogram
1	7	Amp	7
2	9	Amp, Aug	4
		Amp, Tet	3
3	17	Amp, Aug, Tet	5
		Amp, Tet, Cot	5
		Amp, Nal, Nit	2
4	15	Amp, Aug, Tet, Cot	5
		Amp, Cef, Aug, Tet	4
5	18	Amp, Chl, Aug, Tet, Cot	5
		Amp, Aug, Nal, Tet, Cot	2
		Amp, Cef, Aug, Cez, Tet	2
6	13	Amp, Chl, Cef, Aug, Tet, Cot	2
		Amp, Cef, Aug, Cez, Tet, Cot	2
7	9	Amp, Chl, Cef, Aug, Nit, Tet, Cot	2
8	11	Amp, Chl, Cef, Aug, Nal, Nit, Tet, Cot	4
		Amp, Chl, Cef, Aug, Cez, Nit, Tet, Cot	2
9	7	Amp, Chl, Cef, Aug, Cez, Nal, Nit, Tet, Cot	2
		Amp, Chl, Cef, Aug, Nal, Cip, Nit, Tet, Cot	2

Amp- ampicillin, Aug- augmentin, Chl- chloramphenicol, Cef- cefuroxime, Cez- ceftazidime, Nal- nalidixic acid, Nit- nitrofurantoin, Cip- ciprofloxacin, Tet- tetracycline Cot- cotrimoxazole

4. DISCUSSION

The normal faecal flora has been shown to be a potential reservoir of antibiotic resistance genes which can be transferred from non-pathogenic commensals to virulent microorganisms [23,24]. *E. coli*, an important part of the faecal flora is associated with varying humans' infections ranging from urinary tract infection to bacteremia and it has been shown that it is the main carrier of antimicrobial resistance genes among the faecal flora [25,26].

This study revealed that the non-pathogenic faecal *E. coli* isolates in the healthy adults were highly resistant to ampicillin, augmentin and tetracycline. These patterns of resistance have been previously reported in healthy subjects by Bartoloni et al. [27] in Bolivia, van de Mortel et al. [28] in Venezuela and Okeke et al. [29] in Ile-ife, Nigeria. Ampicillin and tetracycline are among the older generations of antibiotics among humans and the observed high resistance

to them might be due to selective pressure from their excessive and inappropriate uses in this environment which by cross-resistance affected augmentin, a β-lactam antibiotic. The findings of Onanuga and Temedie in Amassoma, Bayelsa state on the behavioural uses of antibiotics among healthy individuals support this observation [30].

The isolates were moderately resistant to co-trimoxazole, cefuroxime and chloramphenicol. Previous reports by Bartoloni et al. [27] in Bolivia and Olowe et al. [31] in Osogbo, Nigeria support these findings. The observed rate of resistance to these agents (co-trimoxazole and chloramphenicol) may be as a result of the fact that they are readily available in the country at cheaper rates which favour their excessive and inappropriate uses, thus leading to the development of bacterial resistance. Resistance to cefuroxime, which is usually available in oral and injectables dosage forms at relatively higher prices, might be due to excessive production of β-lactamases which is a common occurrence among some strains of this organism [32]. Thus, these agents may not be suitable for empiric treatment of this organism's infections. The observed lower level of resistance to nalidixic acid and nitrofurantoin when compared to other cheaper older agents might be due to their low prescription patterns in the country and the accompanying uncomfortable side effects.

However, the isolates were relatively more susceptible to ceftazidime, ciprofloxacin, gentamicin and ofloxacin. The proven activities of ceftazidime and gentamicin which is similar to the findings of Bartoloni et al. [27] might be as a result of their availability as injectables only, which need the assistance of experts for their administrations and this can reduce their frequent uses. The fluoroquinolones (ciprofloxacin and ofloxacin) still have proven activity on this organism as previously reported by Bartoloni et al. [27] and Khan et al. [14] but the rate at which this organism in apparently healthy individuals is developing resistance to these agents is alarming. Resistance to ciprofloxacin in this study was observed to be higher than that of ofloxacin which might be due to the over-prescription pattern of ciprofloxacin over ofloxacin in most hospitals in the country and this practice can encourage the influx of substandard brands of ciprofloxacin at varying cheaper prices into the nation's markets thereby leading to the increasing rate of bacterial resistance [11].

The isolates from the villagers were generally more resistant to all the agents than those from the students who have their abode in the urban areas of the region. The observed differences were significant in their resistances to co-trimoxazole, cefuroxime and chloramphenicol ($p<0.05$). This result is similar to the studies of van de Mortel et al. [28] and Nys et al. [12]. The differences observed might be due to the availability of more standard antibiotics in the urban areas than in the villages and more proper education on the use of antibiotics among urban dwellers than the villagers. Also, crowding together, with poor hygiene and poor sanitary facilities for sewage disposal among the villagers might encourage the exchange of antibiotic-resistant bacteria in the population.

Multiple antimicrobial resistance among isolates of *E. coli* has been widely reported [14,31,33]. In this study, 69% of the total isolates from both students and villagers were multi-resistant. This high rate of multiple drug resistance has been attributed to excessive and indiscriminate use of antimicrobial agents in humans and animals which provide selective pressure that can lead to increase in the development of resistance [19,26,31]. The continuous education of people on the proper use of antibiotics in all human endeavours and the control of their sales by the government are pivotal to the strategies of containment of the resistance.

5. CONCLUSION

The results of this study suggest that high levels of multi-drug resistant *E. coli* isolates are prevalent among healthy individuals in the study area. This signifies that most of the tested commonly used agents may not be suitable for empiric therapies of this organism's infections. Thus, there is need for routine monitoring of antimicrobial susceptibility testing of pathogens so as to guide effective treatment of infections.

ETHICAL APPROVAL

All authors hereby declare that all experiments in this study have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=673&id=19&aid=6370