

## Original Research Article

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

### ABSTRACT

**Aim:** To investigate the antimicrobial resistance profile of faecal *Escherichia coli* to common antimicrobial agents in healthy adults of 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.

**Methodology:** The stool samples collected were inoculated and screened for *E. coli* using standard microbiological protocols. The antimicrobial susceptibility test of the *E. coli* isolates was done using disc diffusion technique.

**Results:** A total of 110 (84.6%) *E. coli* isolates were obtained from all the samples comprising of 38 (34.5%) from the villagers and 72 (65.5%) from the university students. The overall resistance profiles of 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 *E. coli* isolates from the villagers were observed to exhibit significantly higher resistances to nalidixic acid, chloramphenicol, cefuroxime and co-trimoxazole than those from the students ( $P < 0.05$ ). A total of 76 (69.1%) isolates were multi-resistant with those from the villagers significantly higher ( $P = 0.013$ ).

**Conclusion:** These observations call for measures to reduce the level of antimicrobial resistance among the flora of healthy individuals.

**Keywords:** Multi-resistance, Faecal, *Escherichia coli*, Healthy, Adults.

### 1. INTRODUCTION

Antibiotic resistance is a problem of global proportions affecting virtually all bacteria which commonly cause human illnesses [1-3]. Antibiotic resistance is a phenomenon that develops when a progeny of bacteria emerges in the presence of inhibitory concentration of an antimicrobial agent, and according to Darwinian theory that is based on the spontaneous mutations that confer resistance by natural selection to the bacteria. This type of resistance is alarmingly not limited to a single class of antimicrobial agents or bacteria [4, 5].

Antibiotic resistance is highly prevalent worldwide. In many industrialized countries, it 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* forms an important part of the normal commensal flora of the intestinal tract of humans and animals, and it can also be found in water, soil and vegetation. Most strains of this organism 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 resistance in a population [15-16].

Comment [u1]: Capitalize the S,S

Comment [u2]: How?

Comment [u3]: Rephrase, add reference

Comment [u4]: Bacteria resistance

Comment [u5]: Delete

Comment [u6]: Delete

Comment [u7]: reference

Comment [u8]: form

Comment [u9]: *E. coli* is part of the most important normal flora of the GIT.....

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The acquisition of resistance by commensal bacteria is a serious concern, because intestinal flora acts as potential reservoir pools of resistance genes which may be transferred to pathogenic bacteria within the host and thereby contributing to the general increase and dissemination of bacterial resistance worldwide [17]. Commensal *E. coli* strains can efficiently exchange genetic materials with pathogens such as *Salmonella*, *Shigella*, *Vibrio cholera* and other pathogenic *E. coli* [18, 19]. Hence, the study of the resistance profile of *E. coli* species being the most common commensal of the gut and a predominant antimicrobial resistance carrier of the enterobacteria in faecal flora is very important [20].

Comment [u11]: How?

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 multidrug resistance profile in this region. Thus, we report the carriage of multiple antimicrobial resistant *E. coli* by apparently healthy adults in Amassoma community in 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. Of all the volunteers, 45 (34.6%) were villagers while a total of 59 (45.4%) were female. The study was conducted from February to June 2010.

Comment [u12]: good

### 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 37 °C for 24 hours. The colonies that gave characteristic greenish metallic sheen on EMB agar plates were confirmed to be *E. coli* through conventional biochemical tests.<sup>13</sup> These isolates were then stored on fresh slants of nutrient agar (Oxoid, U.K) for antimicrobial susceptibility testing.

Comment [u13]: good

### 2.3 Antimicrobial susceptibility testing

The antimicrobial susceptibility test was carried out with twelve (12) antibiotic discs from Oxoid, UK using the modified Kirby-Bauer disc diffusion technique [13]. The standardized suspension of each isolate that matched 0.5 McFarland standard was used to swab the surface of an over dried 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 37 °C 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 of the eight classes of antimicrobial drugs tested were defined as having multiple antimicrobial resistance in this study.

Comment [u14]: Why the choice of antibiotics

Comment [u15]: Rephrase; that match; are you assuming

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## 2.4 Statistical Analysis

Frequencies were obtained and percentages were calculated for study variables. Chi square (two tailed) were used for comparing the variables and A p-value of less than or equal to 0.05 is considered to be statistically significant ( $p \leq 0.05$ ).

Comment [u17]: a

## 3. RESULTS

A total of 110 (84.6%) samples from all the screened 130 healthy subjects yielded *E. coli* isolates. There appeared to be equal proportion of yield in both groups with the university students having 72 (84.7%) while the villagers had 38 (84.4%) *E. coli* isolates.

Generally, the isolates from the healthy volunteers exhibited high resistance to ampicillin 95.5%, tetracycline 72.7%, augmentin 70.9%, co-trimoxazole 54.5% and cefuroxime 44.5%; but however, showed low resistance to ceftazidime 15.5%, ciprofloxacin 14.5%, gentamicin 10% and ofloxacin 4.5%. The *E. coli* isolates from the villagers were significantly more resistant to cefuroxime, chloramphenicol, co-trimoxazole and nalidixic acid than those from the students ( $p < 0.05$ ) (Table 1).

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

| Antimicrobial Agents | Number of Resistant Isolates (%) |                 |                  | Chi square test   |                 |         |
|----------------------|----------------------------------|-----------------|------------------|-------------------|-----------------|---------|
|                      | Overall Resistance               | Students N = 72 | Villagers N = 38 | Degree of freedom | $\chi^2$ -value | p-value |
| Ampicillin           | 105 (95.5)                       | 67 (93.1)       | 38 100.0)        | 1                 | 2.765           | 0.096   |
| Augmentin            | 78 (70.9)                        | 48 (66.7)       | 30 (78.9)        | 1                 | 1.818           | 0.177   |
| Cefuroxime           | 49 (44.5)                        | 26 (36.1)       | 23 (60.5)        | 1                 | 6.002           | 0.014*  |
| Ceftazidime          | 17 (15.5)                        | 10 (13.9)       | 7 (18.4)         | 1                 | 0.391           | 0.532   |
| Gentamicin           | 11 (10.0)                        | 8 (11.1)        | 3 (7.9)          | 1                 | 0.286           | 0.593   |
| Ciprofloxacin        | 16 (14.5)                        | 10 (13.9)       | 6 (15.8)         | 1                 | 0.072           | 0.788   |
| Ofloxacin            | 5 (4.5)                          | 3 (4.2)         | 2 (5.3)          | 1                 | 0.069           | 0.793   |
| Nalidixic acid       | 33 (30.0)                        | 17 (23.6)       | 16 (42.1)        | 1                 | 4.051           | 0.044*  |
| Nitrofurantoin       | 31 (28.2)                        | 17 (23.6)       | 14 (36.8)        | 1                 | 2.151           | 0.142   |
| Chloramphenicol      | 43 (39.1)                        | 20 (27.8)       | 23 (60.5)        | 1                 | 11.203          | 0.001*  |
| Co-trimoxazole       | 60 (54.5)                        | 33 (45.8)       | 27 (71.1)        | 1                 | 6.380           | 0.012*  |
| Tetracycline         | 80 (72.7)                        | 49 (68.1)       | 31 (81.6)        | 1                 | 2.293           | 0.130   |

\* = Statistically significant ( $p < 0.05$ ).

Multiple antimicrobial resistance in this study was defined as resistance of an isolate to at least one antimicrobial agent in at least three (3) classes of antimicrobial agents used [22]. An overall of 76 (69.1%) isolates were multi-drug resistant, 44 (61.1%) of them were from students while 32 (84.2%) were from the villagers. The only 4 (5.6%) fully susceptible isolates in this study were from the students (Table 2). The difference in levels of multi-resistance between the studied groups was significant ( $p = 0.013$ ). Ampicillin was in all the antimicrobial resistance combinations and the most dominating combination pattern was found among the isolates that were resistant to 3 to 5 antimicrobial agents (Table 3).

Comment [u19]: why

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**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 (%) |                    | <i>p</i> –value* |
|--------------------------------------|----------------------------------|--------------------|------------------|
|                                      | Students (N = 72)                | Villagers (N = 38) |                  |
| 0                                    | 4 (5.6)                          | 0                  | 0.013            |
| 1                                    | 10 (13.9)                        | 1 (2.6)            |                  |
| 2                                    | 14 (19.4)                        | 5 (13.2)           |                  |
| 3                                    | 17 (23.6)                        | 8 (21.1)           |                  |
| 4                                    | 13 (18.1)                        | 9 (23.7)           |                  |
| 5                                    | 8 (11.1)                         | 3 (7.9)            |                  |
| 6                                    | 3 (4.2)                          | 12 (31.6)          |                  |
| 7                                    | 3 (4.2)                          | 0                  |                  |
| 8                                    | 0                                | 0                  |                  |
| ≥ 3                                  | 44 (61.1)                        | 32 (84.2)          |                  |

\*{Degree of freedom = 1; Chi square value = 6.215; *p*-value = 0.013 (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                                          |

Legend: 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 is a potential reservoir of antibiotic resistance genes which can be transferred from non-pathogenic commensals to virulent microorganisms [23-25]. *E. coli*, an important part of the faecal flora is associated with varying humans' infections ranging from urinary tract infection to bacteremia and it is the main carrier of antimicrobial resistance genes among the faecal flora [26-28].

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.* [29] in Bolivia, van de Mortel *et al.* [30] in Venezuela, Okeke *et al.* [31] in Ile-ife, Nigeria and Nys *et al.* [32] in Ghana. These high resistances depicted by the organism might be as a result of the bacterial innate resistance characteristics being a Gram negative organism with an outer membrane of lipopolysaccharide and lipoprotein layers that serves as barrier to most antimicrobial agents including tetracycline. This organism also produces  $\beta$ -lactamases while some of its strains produce extended spectrum  $\beta$ -lactamases (ESBL) which inactivate the  $\beta$ -lactam antibiotics and other antimicrobial agents [24,25,32,33]. These isolates, most likely had developed resistance due to selective pressure from excessive and inappropriate use of ampicillin and tetracycline in this environment, which by cross-resistance affected augmentin, a  $\beta$ -lactam antibiotic. The findings of Onanuga and Temedie in Amassoma, Bayelsa state (same study area) who reported that multidrug resistant intestinal *S. aureus* were prevalent among self-mediated healthy individuals confirm this observation [34].

The isolates were moderately resistant to co-trimoxazole, cefuroxime and chloramphenicol. Previous reports by Bartoloni *et al.* [29] in Bolivia and Olowe *et al.* [35] 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 the agents are available in an uncontrollable manner at cheaper prices in the nation which necessitated the development of bacterial resistance in form of inactivation of the agents and their reduced permeability to the bacterial target sites. Resistance to cefuroxime, which is usually available in oral and injectable forms at relatively higher prices, might be due to excessive production of  $\beta$ -lactamases which is a common occurrence among some strains of this organism [32, 33]. Thus, these agents are not suitable for empiric treatment of this organism's infections and should not be used as sole agent but in combination with a more effective agent with synergistic effect.

However, low levels of resistance were showed by the isolates to nalidixic acid, nitrofurantoin, ceftazidime, ciprofloxacin, gentamicin and ofloxacin. The observed frequency of resistance to nalidixic acid and nitrofurantoin might be due to the fact that they are relatively inexpensive agents that have been in Nigeria markets for a long time, but their usage have been greatly reduced due to their very low prescription patterns. This and the uncomfortable side effects of the agents might be reasons for the observed reduced level of resistance as compared to other agents that are frequently prescribed and used. The proven activities of ceftazidime and gentamicin which is similar to the findings of Bartoloni *et al.* [29] might be as a result of their availability as only injectables, which need the assistance of experts for their administrations and this reduce their frequent usage. The fluoroquinolones (ciprofloxacin and ofloxacin) still have proven activity on this organism as previously reported by Bartoloni *et al.* [29] 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 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 encouraged the proliferation of various substandard brands of the ciprofloxacin at varying affordable prices in the 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 the resistance to co-trimoxazole, cefuroxime, chloramphenicol and nalidixic acid ( $p < 0.05$ ). This result is similar to the studies of van de Mortel *et al.*<sup>30</sup> and Nys *et al.* [12]. The differences observed might be due to the

**Comment [u21]:** How? This comment is too general please

**Comment [u22]:** Are you saying that ESBL gene is innate? Those that mean that all G negative bacteria are generally resistant

**Comment [u23]:** I think this could be the basic difference in the resistant pattern expressed between the student and the villagers. The students are more exposed and sensitized to the misused of antibiotic awareness than the villagers. Villagers also been more exposed to environmental pollutants from the farm lands, crops etc might have influenced the disparity in resistance pattern

**Comment [u24]:** Compare with your abstract

**Comment [u25]:** Did your work include mode of action. Or bacteria/antibiotic interaction. For you to have made this conclusion? Please use might be

**Comment [u26]:** Your abstract is contradicting to your discussion

**Comment [u27]:** Sensitivity of the isolates to

220 availability of more standard antibiotics in the urban areas than in the villages and more proper education on  
221 the use of antibiotics among urban dwellers than the villagers. Also, crowding together, with poor hygiene and  
222 poor sanitary facilities for sewage disposal among the villagers might encourage the exchange of antibiotic-  
223 resistant bacteria in the population.

Comment [u28]: Very good

225 Multiple antimicrobial resistance among isolates of *E. coli* has been widely reported [14, 35, 36]. In this study,  
226 69% of the total isolates from both students and villagers were multi-resistant. This high rate of multiple drug  
227 resistance has been attributed to excessive and indiscriminate use of antimicrobial agents in humans and  
228 animals thus provide selective pressure that can lead to increase in the development of resistance [19, 26, 31].

Comment [u29]: What are your  
recommendations

## 230 5. CONCLUSION

231  
232 The high level of multi-drug resistance among the *E. coli* isolates in this study signifies the possibility of  
233 increased treatment failure with most of these agents in the empiric therapies of its infections. Thus, there is  
234 need for routine monitoring of antimicrobial susceptibility testing of pathogens to guide effective treatment of  
235 infections and the continuous education of patients on the proper use of antibiotics as means of controlling  
236 resistance among enteric organisms cannot be overemphasized.

241 **ETHICAL APPROVAL:** All authors hereby declare that the research was approved by the appropriate  
242 ethics committee and have therefore been performed in accordance with the ethical standards laid down in the  
243 1964 Declaration of Helsinki.

Comment [u30]: Where is your ethical approval  
number

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