Antimicrobial resistance profile of *Escherichia coli* isolates recovered from diarrheic patients at Selam Health Center, Addis Ababa, Ethiopia

Abebe Aseffa Negeri¹*, Eyasu Tigabu Seyoum¹, Rajiha Abubeker Ibrahim¹ and Hassen Mamo²

¹Clinical Bacteriology and Mycology Research Case Team, Ethiopian Public Health Institute, Addis Ababa, Ethiopia.
²Microbial, Cellular and Molecular Biology Department, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Received 28 November, 2018; Accepted August 2, 2019

Antimicrobials have been playing an important role in preventing illness and death associated with infections due to bacteria. However, the emergence and spread of resistance by pathogens have decreased the effectiveness of the commonly prescribed antimicrobials. Intestinal *Escherichia coli* are among bacterial pathogens that are endowed with such resistance traits because they are important source and reservoir of genes that encode antimicrobial resistance. To determine the antimicrobial resistance profile of fecal isolates of *E. coli* from diarrheic patients. Stool samples were collected consecutively from 100 individuals who visited Selam Health Center during the study period, April to June 2018. Samples were collected and transported under sterile condition to the National Clinical Bacteriology and Mycology reference Laboratory, Ethiopian Public Health Institute. The samples were streaked on MacConkey agar and incubated overnight at 37°C. *E. coli* isolates were further confirmed using conventional biochemical tests. Antimicrobial susceptibility status was determined using the disk diffusion method on Mueller Hinton agar as recommended by the Clinical Laboratory Standard Institute. The raw data was compiled and entered to spreadsheet and analysis was done using SPSS Version 20 with p-value ≤0.05 considered statistically significant. Out of the 100 patients, 43 were female and the rest were male. Confirmed *E. coli* were isolated from 73 individuals. Antimicrobial susceptibility testing showed that *E. coli* isolated in this study were highly resistant to trimethoprim-sulfamethoxazole 49 (67.1%) and amoxicillin-clavulanic acid 47 (64.4%). No isolates showed resistance to gentamicin and tobramycin. Of all the isolates, 11 (15.1%) were multidrug resistant. No association was observed between antimicrobial resistance status and sex of individuals included in this study. However, there was an association between age and resistance patterns. Resistance to commonly prescribed antibiotics among *E. coli* isolated in this study was high and a considerable proportions of the strains were multidrug resistant. This is an indication for an alarming rate of resistance of intestinal *E. coli* to first line antimicrobials. To reduce the problem, regular monitoring and education for the community are very important.

Key words: *Escherichia coli*, antibiotic susceptibility, multidrug resistant, Ethiopia, biochemical tests, disk diffusion.

INTRODUCTION

Antimicrobials have been playing an important role in preventing illness and death associated with bacteria infections. However, the emergence and spread of resistant pathogenic and commensal bacteria is
increasing all over the world (Aarestrup et al., 2008). Increased resistance to antibiotics has made it difficult to treat infections due to bacteria and even impossible in the extreme cases which in turn results in morbidity and mortality. The problem is particularly serious in developing countries where the availability of alternative antimicrobials is very low and too expensive (Ellipoulous et al., 2003). The World Health Organization (WHO) has raised this issue as a global challenge and a major threat of healthcare in the society today (WHO, 2014).

The emergence of antimicrobial resistance is believed to have a positive association with the way antimicrobials have been used. This is possibly the most important factor that increases the emergence of antimicrobial-resistant microorganisms. These are the result of misuse of antimicrobials by physicians, unskilled practitioners, weak integration between private and governmental health facilities and pharmacy outlets (Bailey et al., 2010; Vila and Pal., 2010).

*E. coli* is Gram-negative facultative anaerobe bacteria and they are the component of the human gastrointestinal tract. Most of them are usually commensal bacteria and seldom cause disease in healthy individuals. However, in immuno-suppressed patients and when they breached the gastrointestinal barriers, commensal *E. coli* can cause infection. Other group of *E. coli* are pathogenic when they gain virulence factors which enable them to cause intestinal and extraintestinal infections including, diarrhea, septicemia, urinary tract infections, and meningitis not only in immunocompromised patients but also in healthy individuals. Lipo polysaccharide (O) and flagellar antigens are the features of pathogenic *E. coli* which can define serotypes or serogroup of these bacteria (Kaper et al., 2004; Skjot-Rasmussen et al., 2012).

The gastrointestinal area provides favorable environmental conditions for the transmission of resistance genes within and between bacterial species through horizontal gene transfer and other mechanisms. The most abundant organisms in the fecal flora of warm blooded animals including humans are *E. coli* (WHO 2014). *E. coli* are used for monitoring antimicrobial drug resistance in fecal bacteria because they are found more frequently in a wide range of hosts, acquire resistance easily, and are reliable indicator of resistance in salmonellae (Tadesse et al., 2012). Apart from their pathogenicity, fecal *E. coli* have been used as sensitive indicators in surveillance and spread of antimicrobial resistance (WHO, 2014; Tadesse et al., 2012).

In Ethiopia, a number of hospital based studies have been conducted on the profile of antimicrobial resistant *E. coli* isolated from different clinical specimens (Tuem et al., 2018). However, studies from primary healthcare settings are limited. Therefore, the aim of this study was to determine the antimicrobial profile of fecal *E. coli* isolated from patients presenting with gastrointestinal problem at Selam Health Center (SHC) in Addis Ababa from April to June 2018. The study provided important information regarding the pattern of antimicrobial resistance in a primary healthcare setting where over 75% of all healthcare antibiotics are prescribed as reported data from other countries (Hopkins, 2016).

**MATERIALS AND METHODS**

**Study subjects and sample collection**

Consecutive non-duplicate 100 diarrheic patients who visited SCH for stool examinations, from April to June 2018 were included in this study. The study participants were informed about the purpose of the study and written consent was obtained from each participant.

All information related to personal identity was kept with strict confidentiality and samples were identifiable only via a generic code. Information collected include age and sex of the patients. Stool samples were collected in sterile cup and then using sterile cotton swabs, the samples were immediately transferred to Cary-Blair transport media and taken to the National Clinical Bacteriology and Mycology Reference Laboratory of the Ethiopian Public Health Institute.

**Plating and identification of *E. coli***

At National Clinical Bacteriology and Mycology Reference laboratory, stool specimens were inoculated on MacConkey agar (Oxoid) to select lactose fermenting *E. coli* using the cotton swabs on the first quadrant of the plate and then streaked using sterile plastic inoculation loop. The plates were incubated overnight at 37°C. After overnight incubation, a pink colony was randomly picked and sub-cultured on the same plate media using a sterile inoculation loop to get well isolated colonies. Colonies suspected as *E. coli* were further confirmed using conventional biochemical tests. Briefly, motile, positive indole test, citrate negative, urea test negative and lysine decarboxylase test positive isolates were characterized as *E. coli* after overnight incubation at the same temperature. Isolates confirmed as *E. coli* were selected for antimicrobial susceptibility testing.

**Antibiotic susceptibility testing**

Susceptibility testing was determined using the disk diffusion method on Muller Hinton agar (MHA) as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2017). Susceptibility test was performed against amoxicillin/clavunate (20/10 µg), gentamicin (10 µg), tobramycin (10 µg), trimethoprim-sulfamethoxazole (Co-trimoxazole) SxT (1.25/23.75 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), cefepime (30 µg) and nalidixic acid (30 µg). Following 16 to 18 h incubation,
the plates were examined, and the inhibitory zone diameters for individual antimicrobial agents were measured and recorded as susceptible and resistant based on the breakpoints for respective antimicrobial susceptibility of CLSI 2017. A standard culture of E. coli (ATCC 25922) was used as a control with each batch of antimicrobial susceptibility test. The isolates showed resistance to three or more different groups of antibiotics designated as multi drug resistant (MDR) E. coli.

**Phenotypic characterization of extended spectrum beta-lactamases (ESBLs)**

E. coli resistance to third generation cephalosporins (ceftaxim) and fourth generation cephalosporin (cefepim) were classified as ESBL producers and were further confirmed for the production of the enzymes using combination disk tests. Briefly, each isolate was sub-cultured on blood agar plate and incubated overnight at 37°C. After overnight incubation, a 0.5 McFarland standard was prepared by the direct colony suspension method in normal saline. Using a sterile cotton swab, the suspension was inoculated on the surface of MHA plate by streaking the entire surface in three different directions as well as the outer rim of the plate. Once the plate was dried, antimicrobial disks were applied. Cefotaxime (30 µg) alone and ceftaxime-clavulanic acid (30/10 µg) and cefazidime (30 µg) alone and ceftazidime-clavulanic acid (30/10 µg) were used. Plates were then incubated at 35°C to 16 to 18 h and the zones of inhibition surrounding each disk were measured.

The interpretation of positive results was based on an increase in zone diameter by ≥ 5 mm for the agents tested (ceftaxime and cefazidime) in combination with clavulanic acid as compared to that agent alone. Parallel to all tests, E. coli ATCC 25922 (ESBL negative and K pneumonia ATCC 700603 (ESBL-positive) were run for quality control.

**Statistical analysis**

The data was captured and computed using Microsoft Excel. Percentage of MDR strains were analyzed using SPSS Version 20. Tables and graphs were used to summarize the results.

**Ethical considerations**

Ethical clearance for this study was obtained from the Ethiopian Public Health institute scientific and ethical review committee.

**RESULTS**

**E. coli isolates and study participant’s characteristics**

A total of 100 stool samples were collected and processed for this study. Of all the study participants, 62% (n= 62) were females (Table 1). Since the selection of colonies from the primary media for further analysis was random, 73 E. coli isolates from the collected stool samples could be obtained, of which 43 isolates were derived from female participants and 30 E. coli isolates were from male patients. The age distribution of the patients with regards to E. coli obtained is shown in Table 2. It was found that the 21-30 year age group was the highest in prevalence (30.2%) among female patients. While 31-40 year age group was the patients with highest frequency (33.3%) among male participants. Less study participants were from patients with age > 50 both in female and male (Tables 1 and 2). Median age of the study participants was 25.64 year and standard deviation=16.40 year.

**Antimicrobial resistance profile of E. coli**

All biochemically confirmed E. coli isolates were tested by agar disk diffusion to determine their susceptibility profile to a panel of eight antimicrobial agents. The antimicrobials tested were from five classes of antibiotics used commonly in clinical practices. These include: Aminoglycosides (Gentamycin and Tobramycin), Cephalosporin (third generation Cefotaxime and fourth generation Cefepime), Penicilllin combinations (Amoxicillin/clavulanate), Quinolones/Fluoroquinolones (Ciprofloxacin) and nalidixic acid and Sulfonamides (Trimethoprim-Sulfamethaxazole) (Co-trimoxazole). The result showed that a greater percentage of E. coli isolates were resistance to Co-trimoxazole (67.1 %) and Amoxicillin/clavulanate (64.4%).

Among the Quinolones/Fluoroquinolone, resistance to nalidixic acid and Ciprofloxacin was 27.4 and 2.7%, respectively. Resistance to cephalosporins, cefotaxime and cefepime was 6.9% and all of them were phenotypically ESBL producers. Apart from ESBL productions which hydrolyze extended spectrum cephalosporins, the isolates were co-resistance with other antimicrobial agents. However, no isolates showed resistance to Aminoglycosides, Gentamicin and tobramycin (Figure 1).

The pattern of E. coli resistance to antibiotics in terms of sex was further analyzed and the finding indicated that antimicrobial resistance in E. coli was not significantly associated with the sex of the participant patients (P-value=0.93) (Table 3).

Another question was whether there were any associations between the age of individuals and antibiotic resistance. Interestingly, highest resistant strains (82.4%) to Co-trimoxazole were obtained from 0-10 age groups. As age increases, the resistance pattern of E. coli to co-trimoxazole was relatively decreasing. However, in the case of amoxicillin/clavulanate, E. coli strains isolated from patients who are younger (0-10 year) and the elderly above 40 year age group, exhibited increased incidence of resistance (Table 4).

**Multi-drug resistance patterns (MDR)**

MDR bacteria are defined as bacterial strains that are non-susceptible to at least one antimicrobial agent in three or more antimicrobial classes (Sweeney et al., 2018). Accordingly, 17.8% (n=13), of 73 studied E. coli strains were susceptible to all antimicrobial classes tested. With regards to resistant E. coli strains, 27.4%


Table 1. Age and sex distribution of the study participants.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male (n=30)</th>
<th>Female (n=43)</th>
<th>Total (N=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>10</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>11-20</td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>21-30</td>
<td>8</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>41-50</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>51-60</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>&gt;60</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>62</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of E. coli in diarrheic patients based on age and sex distribution.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male (n=30)</th>
<th>Female (n=43)</th>
<th>Total (N=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>7(23.3)</td>
<td>10(23.3)</td>
<td>17(23.3)</td>
</tr>
<tr>
<td>11-20</td>
<td>3(10)</td>
<td>6(14)</td>
<td>9(12.3)</td>
</tr>
<tr>
<td>21-30</td>
<td>6(20)</td>
<td>13(30.2)</td>
<td>19(26)</td>
</tr>
<tr>
<td>31-40</td>
<td>10(33.3)</td>
<td>9(20.9)</td>
<td>19(26)</td>
</tr>
<tr>
<td>41-50</td>
<td>2(6.7)</td>
<td>3(7)</td>
<td>5(6.8%)</td>
</tr>
<tr>
<td>51-60</td>
<td>1(3.3)</td>
<td>1(2.3)</td>
<td>2(2.7)</td>
</tr>
<tr>
<td>≥61</td>
<td>1(3.3)</td>
<td>1(2.3)</td>
<td>2(2.7)</td>
</tr>
</tbody>
</table>

Figure 1. Over all antibiotic resistance profile of E. coli: NAL = nalidixic acid, GEM = gentamicin, TOB = tobramycin, AMC = amoxicillin–clavulanic acid, CPR = ciprofloxacin, CTX = cefotaxime, CFP = cefepime, SXT = trimethoprim–sulfamethoxazole.

(n=20) were resistant to a single antimicrobials, 39.7% (n=29) strains were resistant to two antimicrobials and 15.1% (n=11) E. coli strains were resistant to three and more than three antimicrobials of different classes which
Table 3. Comparison of sex-based antibiotic resistance patterns of the *E. coli* strains isolated from patients with diarrhea.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Male (n=30)</th>
<th>Female (n=43)</th>
<th>T-test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>S</td>
<td>%R</td>
</tr>
<tr>
<td>AMC</td>
<td>19</td>
<td>11</td>
<td>63.3</td>
</tr>
<tr>
<td>GEN</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>TOB</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>CTX</td>
<td>3</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>CFP</td>
<td>3</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>CPR</td>
<td>1</td>
<td>29</td>
<td>3.3</td>
</tr>
<tr>
<td>SXT</td>
<td>20</td>
<td>10</td>
<td>67</td>
</tr>
<tr>
<td>NAL</td>
<td>8</td>
<td>22</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 4. Association between age group and antimicrobial resistance of *E. coli* isolates recovered from diarrheic patients.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Resistance percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMC (R%)</td>
</tr>
<tr>
<td>0-10</td>
<td>70.6</td>
</tr>
<tr>
<td>11-20</td>
<td>66.7</td>
</tr>
<tr>
<td>21-30</td>
<td>57.9</td>
</tr>
<tr>
<td>31-40</td>
<td>31.6</td>
</tr>
<tr>
<td>&gt;40</td>
<td>77.8</td>
</tr>
</tbody>
</table>

Figure 2. MDR *E. coli* isolated in this study (≥R3). R0: No resistance, R1: Resistance to one antibiotic, R2: Resistance two antibiotic, ≥R3: Resistance to three or more antibiotics (MDR).

are considered as MDR strains (Figure 2).

DISCUSSION

This study was conducted on stool specimen collected from SHC. Even though, there is no compiled Ethiopian study data on antibiotic prescription dosage, studies in other part of the world indicate that the greatest proportion of antibiotics for human use is prescribed at primary healthcare sector (Fernando et al., 2017), where use is strongly correlated to antibiotic resistance rates highlighting this sector as an important area for research and intervention (Bell et al., 2014). Therefore, antimicrobial resistance of *E. coli* isolates from patients visited the center due to diarrhea were examined. To do so, after overnight incubation as indicated in materials and methods section, a single isolate representing each sample
was randomly picked and sub-cultured for biochemical analysis and susceptibility test. This is because it was reported that most of the *E. coli* strains isolated from one stool samples are identical (Bok et al., 2018).

The pattern of antibiotic resistance of *E. coli* strains was tested against trimethoprim-sulfamethoxazole, β-lactams (cephalosporins and β-lactamases inhibitor combinations, AMC), fluoroquinolones and aminoglycosides. These antibiotics are used to treat community and hospital infections due to *E. coli* (Pitout, 2012).

Resistance to Trimethoprim-sulfamethoxazole was one of the most common antibiotics resistance patterns identified among *E. coli* isolates. Trimethoprim-sulfamethoxazole resistance results from alterations of different substrate enzymes or their overproduction, loss of bacterial drug-binding capacity, and decreased cell permeability and this is often associated with acquisition of the resistance genes sul1 and sul2 (Kozak et al., 2009). Sulfonamide resistance genes are commonly associated with mobile genetic elements, and these elements play a major role in dissemination of multiple antimicrobial drug resistance genes in *E. coli* isolates (Bean et al., 2009). 67.4% of *E. coli* strains examined for this study were resistant to trimethoprim–sulfamethoxazole. This result is almost similar with 66% of compiled study finding from community settings in South Asia and Sub-Saharan Africa (Ingle et al., 2018) and higher than 57.47% of resistance rate reported from Ethiopia (Tuem et al., 2018).

Amoxicillin (AMX), broad-spectrum β-lactam penicillin in combination with the β-lactamase inhibitor clavulanic acid is used for treating lower respiratory tract infections and abdominal infections caused by Enterobacteriaceae and other group of bacteria. The resistant Enterobacteriaceae including *E. coli* isolated from patients with abdominal infections have been typically associated with administration of AMC (Lund et al., 2001). Frequent use of these antibiotics increases the concern for emerging development and spread of antibiotic resistance genes (Duystschaever et al., 2013). The highest incidence of resistant *E. coli* strains to SXT and AMC in this study may be the indication of frequently prescription of these antibiotics by physicians and misuse of the antibiotics in the community.

Cephalosporins are β-lactam antibiotics and are the major drug classes used to treat community-onset or hospital-acquired infections caused by *E. coli* (Pitout, 2010). The production of β-lactamase by *E. coli* is the most important contributing factor to β-lactam resistance. The enzymes β-lactamases inactivate β-lactam antibiotics by hydrolysis, which results in ineffective the compounds (Pitout, 2010). In this study, Cefotaxime and Cefepim third generation and fourth generation cephalosporins respectively, were tested. These antibiotics are called expanded-spectrum cephalosporins because they are developed to treat infection due to Enterobacteriaceae including *E. coli* producing narrow-spectrum β-lactamases such as TEM-1, TEM-2 and SHV-1 enzyme (Bush and Jacoby, 2010). However, the bacteria developed resistance to the expanded-spectrum cephalosporins by producing plasmid-mediated extended-spectrum β-lactamases such as TEM derivatives, SHV derivatives and CTX-M types (Pitout, 2012). Among *E. coli* isolated in this study, 6.9% were resistant to expanded-spectrum cephalosporins. These groups of *E. coli* are assumed to produce extended-spectrum-beta-lactamases and were phenotypically confirmed as all of them were ESBL producer in this study. Extended spectrum beta lactamase producing enterobacteriaceae including *E. coli* are the major public health concern because few antibiotics remain active against these bacteria and can be disseminated easily into the community as the genes encoding these enzymes are found on plasmids (Ruppé et al., 2013).

Reducing the susceptibilities of *E. coli* to fluoroquinolone is due to the up regulation of efflux pumps and plasmid-mediated resistance mechanisms such qnr determinants. In addition, 1-2 point mutations within the quinolone resistance determining regions of gyrA and parC, are required for high level resistance to the fluoroquinolones in *E. coli* (Johnson et al., 2013). The results indicate that 27.4% fecal isolated *E. coli* were resistant to nalidixic acid and these isolates may produce these genes and might have mutation as a result of selective antibiotic pressure.

The patterns of antibiotic resistance in *E. coli* strains were further analyzed in terms of sex of the participants. The results of this study indicate that no direct relation exists between the sex of the patients and the resistance patterns of the isolates. This was explained by studies done elsewhere in that, the susceptibility patterns of bacteria depending on exposure of the individuals to antimicrobial agents which may result in acquiring mutation that confers resistance to these drugs regardless of the sex of the patients (Cho et al., 2011; Sahuquillo-Arce et al., 2011).

The associations between the age of individuals and antibiotic resistance among the most frequent *E. coli* strains resistant to Trimethoprim-Sulfamethoxazole and Amoxicillin/clavulanate documented in this study, can be resulted from intensively prescribed and over abused antimicrobials for mild infection particularly in young individuals. These have been well explained by many studies that, the selective pressure produced by antibiotic prescribing in community contribute to such problem (Cho et al., 2011). The higher resistance of AMC in the greater than 40-year-old group of patients may be explained by the longer exposure of these individuals to these antibiotics which has been reported as the age of the patients are one of the factors for antimicrobial resistance (Garcia et al., 2017).

Multidrug resistance among *E. coli* isolates observed in the present study sends alarming message as these
group of organisms have significant clinical implication.

Conclusion

Resistance to commonly used primary care antibiotics particularly Trimethoprim-Sulfamethoxazole (co-trimoxazole) and Amoxicillin/clavulanate in faecal E. coli isolates from this study was very high. Over prescribing of these antibiotics at primary healthcare for mild or self-limiting infections, may be responsible for the major problem (Costelloe et al., 2010). To reduce the problem, education for prescribers and patients at facility and community level is essential. Moreover, extensive research that can show on the relation between antibiotic prescriptions and resistance burden is needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


