Antibacterial susceptibility profile of *Escherichia coli* O157:H7 from shallow wells in some parts of Katsina State, Nigeria

Mukhtar Fatima1*, Gambo L. Mukhtar1, Helene I. Inabo2, Edward D. Jatau2 and Adamu M. Shitu2

1Department of Microbiology, Umar Musa Yar’adua University, Katsina, Nigeria
2Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

Ground water pollution and indiscriminate use of antibiotics in food animals and in treatment predisposed consumers to risks of antibiotic resistance. The aim of this research work is to determine the antibacterial susceptibility profile of *Escherichia coli* O157:H7 from shallow wells in some parts of Katsina state, Nigeria. The presence or absence of well covers and distance from pit latrines were observed at collection points. Most of the wells were uncovered or partially covered with old rusted roofing sheets, distance of wells from pit latrines range from 3-9 m which were all below the limit of 30 m set by WHO, Nigerian Environmental Protection Agency and 15.24 m or 50 ft set by United State Environmental Protection Agency (USEPA). The organism was isolated by cultural method using selective media, gram stained and subjected to series of biochemical tests, the isolates were further confirmed serologically using Latex agglutination kit (Oxoid, UK). Antibiotic susceptibility testing was performed using commercial gram negative disks and the multidrug resistance pattern and multiple antibiotic resistance indices of the isolates were also determined. Out of the 300 well water samples analysed 246 wells were positive for presumptive *E. coli* and 7 were serologically confirmed to be *E. coli* O157:H7. All the isolates were resistant to multiple antibiotics; the highest resistance was to tetracycline and Augmentin. However, 100% of the isolates were sensitive to fluoroquinolones and Nitrofurantoin. Absent of pipe borne water, poor sanitary habits and indiscriminate use of drugs pre-disposed the inhabitants of the study area to dangers of multidrug resistant organisms. Provision of adequate potable water, improved sanitation and restricting the illegal use of drugs in food animals and in treatment of infections were recommended to overcome the problems of multidrug-resistant organisms.


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* E-mail: fateemam2@gmail.com

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1. INTRODUCTION

Waterborne diseases due to fecal pollution of human and animal origin, are responsible for approximately 2.2 million deaths annually in children under the age of five years in developing countries [1,2]. Most of these deaths are due to inadequate potable water supplies, poor hygiene practices and insufficient sanitation infrastructures [1,2,3]. The World Health Organization (WHO) estimated that 1.2 billion of the world’s population lack access to safe drinking water and these people use any source of water, usually the most convenient source, regardless of its quality [1]. The United Nations (UN) set a goal in their Millennium Declaration to reduce the amount of people without safe drinking water by half in the year 2015 [4]. Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odor, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes [5].

However, inadequate sanitation and persistent fecal contamination of water sources is responsible for a large percentage of people in Nigeria with inadequate sanitation and persistent fecal contamination of water sources is responsible for a large percentage of people in Nigeria.
both developed and developing countries not having access to microbiologically safe drinking water and suffering from diarrheal diseases [1,2]. Diarrheal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially those in areas devoid of access to potable water supply and sanitation [6,7].

Many infectious diseases are associated with fecal-contaminated water and are a major cause of morbidity and mortality worldwide [5,8]. Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites that are transmitted by the fecal oral route [5,8].

Water of good drinking quality is of basic importance to human physiology and man’s continued existence depends very much on its availability. Safety and quality of drinking water is always an important public health concern [9]. The provision of potable water to the rural and urban population is necessary to prevent health hazards [10]. Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is palatable and safe for drinking [10]. Potable water is defined as water that is free from disease producing microorganisms and chemical substances deleterious to health. Water borne diseases continue to be one of the major health problems especially in developing nations particularly in rural and sub-urban areas in Katsina State.

Nigeria has been ranked as one of the countries without access to improved water sources [11]. Isolation of E. coli O157:H7 and Salmonella species from water source connote a serious health risk to consumers due to the threat of disease and antibiotic resistance which in turn reduces treatment options [12].

The first reported outbreak of E. coli O157 infection in the developing world occurred in 1992 in Southern Africa [13]. Outbreaks have also occurred in Central African Republic in 1996 and Cameroon, in 1997 (14). Such outbreaks have been linked to contaminated bovine food products, contaminated drinking water and flood irrigation with water contaminated by animal faeces or surface runoff and cattle faeces have been implicated as the major source of contamination [13]. The emergence of antimicrobial resistance is usually preceded by antimicrobial misuse; however, surveillance of the spread of antimicrobial-resistant pathogens is expected to play a very important role in reducing the rate of emergence and spread of antimicrobial-resistant pathogens since such early warning signals make timely intervention possible.

EHEC is recognized as the primary cause of haemorrhagic colitis (HC) or bloody diarrhoea, which can progress to the potentially fatal hemolytic uremic syndrome (HUS). EHEC is typified by the production of verotoxin or Shiga toxins (Stx). Although Stx1 and Stx2 are most often implicated in human illness, several variants of Stx2 exist. There are many serotypes of Stx-producing E. coli (STEC), but only those that have been clinically associated with HC are designated as EHEC. Of these, O157:H7 is the prototypic EHEC and most often implicated in illness worldwide (CDC, 1993). The infectious dose for O157:H7 is estimated to be 10 - 100 cells, EHEC infections are mostly food or water borne and have implicated undercooked ground beef [15], raw milk, cold sandwiches and water [16]. Unpasteurized apple juice, sprouts and vegetables [17]. EHEC O157:H7 is phenotypically distinct from E. coli in that they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity.

Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem [18]. Antimicrobial resistance in Enterobacteriaceae poses a critical public health threat, especially in the developing countries [11,18]. Much of the problem has been shown to be due to the presence of transferable plasmids encoding multidrug resistance and their dissemination among different enterobacterial species [19].

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Katsina state, one of the 36 states of Nigeria. Katsina state is situated at 12.99° North latitude, 7.6° East longitude and 464 m elevation above the sea level with two distinct season’s wet and dry seasons. Three (3) Local Governments were selected, namely: Mani, Dan-musa and Danja.

2.2 Sample Collection

Three hundred well water samples were collected in sterile 60ml containers, the collected sample were labeled and transported to the laboratory of the Department of Microbiology Ahmadu Bello University, Zaria in ice packs and analysed within 6 hours [20].

Wells were observed for the presence or absence of well casing, well covers and distance from the laterine or soak away [21].

All the media used in this study were prepared and sterilized according to manufacturer’s instructions. The media used included; Sorbitol MacConkey agar, Nutrient agar, Mueller Hinton agar and EMB agar (Oxoid UK).

One (1ml) of the sample was pre-enriched into 9mls double strength trypton soy broth and was
incubated at 37 °C for 24 h, it was then subcultured onto prepared EMB plates and further incubated at 37 °C for 24 hours. Green metallic sheen colonies with dark centers were gram stained and stored on nutrient agar slants for further characterization by employing Biochemical tests. The biochemical tests employed were; Indole, Methyl-red, Vogesproskauer and Citrate utilization test.

Confirmed E. coli were streaked on sorbitol MacConkey agar. Those that are colourless were tested with latex agglutination kit (Oxoid) for E.coli O157: H7. The positive were taken as E.coli O157:H7 while those that do not show agglutination were taken as non E.coli O157:H7.

2.3 Antibacterial susceptibility Testing

Each of the isolates were subjected to antibacterial susceptibility testing using the Bauer-Kirby method that has been standardized and evaluated by the methods of Clinical and Laboratory Standards Institute [22]. The susceptibility testing was carried out using Mueller Hinton agar. The organisms were tested in vitro for susceptibility to the following commonly used antibacterial drugs: Ceftaxidime (30µg), Cefuroxime (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacine (5µg), Chloramphenicol (30µg), Augmentin (30µg), Tetracyclin (30µg), Nitrofurantoin (300ng) and Sulphametazole-trimethoprim (25µg).

3. RESULTS AND DISCUSSION

Table 1 shows result for mean aerobic counts obtained from the three (3) study locations respectively. Dan-musa local government area had the highest mean aerobic counts of 3.47±0.02, followed by Mani with 2.88±0.02 and Danja local government area had the least mean aerobic counts of 1.99±0.02. The result showed that none of the water samples met the [23,24,11] guideline for potable drinking water quality, which states that the coliform count in drinking water both piped and unpiped should be ≤6cfu/ml and ≤10cfu/ml [25]. Coliform bacteria have been widely used as indicator of the microbiological quality of surface and ground waters [26]. Thus the presence of coliforms is an index of bacteriological quality of water, the isolation of coliforms especially Escherichia coli, from the water sources is attributable to contamination by material of human and animal origin and this is of health significance as this organism have generally been reported as causative agent of gastroenteritis in humans. All the water sampled had very high counts and this indicates that they are unfit for human consumption.

Table 1: Mean viable Count of well water obtained from the three Local Government Areas.

<table>
<thead>
<tr>
<th>Sample location</th>
<th>No. of Samples</th>
<th>Mean±SEM (log_{10}cfu/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dan-musa</td>
<td>100</td>
<td>3.47±0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Mani</td>
<td>100</td>
<td>2.88±0.02</td>
<td></td>
</tr>
<tr>
<td>Danja</td>
<td>100</td>
<td>1.99±0.02</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>2.78±0.02</td>
<td></td>
</tr>
</tbody>
</table>

F= 7.162, Df=2, P-value 0.01
Key: SEM=Standard error mean, df= Degree of freedom, cfu=Colony forming unit

Table 2 shows the distribution of presumptive E. coli frequency of occurrence and percentage of E.coli O157:H7 from the three local government areas. Mani local government had the highest frequency of occurrence of E.coli isolates with 89.0 % and 28.6% E. coli O157:H7 followed by Dan-musa with E.coli 85% and 57.1% E. coli O157:H7while Danja local government area had the least frequency of occurrence of presumptive E.coli 72.0% and 14.3% E. coli O157:H7. The differences in the distribution as observed showed that there is significant statistical association with ($\chi^2= 10.705$, df = 2, $P = 0.005$).

Table 2. Distribution of the presumptive isolates, the number and percentage of E.coli O157:H7 isolates.

<table>
<thead>
<tr>
<th>Location</th>
<th>no. of samples</th>
<th>no. of E.coli isolated (%)</th>
<th>no. of E.coli O157:H7 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mani</td>
<td>100</td>
<td>89</td>
<td>2(28.6)</td>
</tr>
<tr>
<td>Dan-musa</td>
<td>100</td>
<td>85</td>
<td>4(57.1)</td>
</tr>
<tr>
<td>Danja</td>
<td>100</td>
<td>72</td>
<td>1(14.3)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>246</td>
<td>7(100)</td>
</tr>
</tbody>
</table>

($\chi^2=10.705$, df=2, $P=0.005$)
Table 3 shows the result of the measured distances of wells positive for *E. coli* O157:H7 from pit latrines, the distances were between 3-9m, which were all below the limit set by [24] and that of Nigerian Environmental Protection Agency of 30m each and 15.24m of the United State Environmental Protection Agency [27]. Most of the wells were uncovered while some were partially covered with woods or old rusted roofing sheets.

### Table 3: Distance between pit latrines and wells positive for *E. coli* O157:H7

<table>
<thead>
<tr>
<th>Location</th>
<th>Well Identity</th>
<th>Distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dan-musa</td>
<td>Dm3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Dm21</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Dm38</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Dm47</td>
<td>3</td>
</tr>
<tr>
<td>Mani</td>
<td>Mn79</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Mn84</td>
<td>9</td>
</tr>
<tr>
<td>Danja</td>
<td>Dj9</td>
<td>4</td>
</tr>
</tbody>
</table>

*Mn = Mani; Dj = Danja; Dm = Dan-musa*

Table 4 shows the association between distance of wells from pit latrines and occurrence of *E. coli* O157:H7. Significant association was found (*p*<0.05). The strength of the association (Odds ratio) was further determined (2.741). This showed that *E. coli* O157:H7 is two times more likely to be found in wells with close proximity to pit latrines. It was generally observed that wells located close to pit latrines are the wells positive for the presence of this pathogenic bacterium (*E. coli* O157:H7). This finding corroborates that of [28] who stated that the greater the distance between the latrine and the water point, the lower the risk of contamination. Also, if the time taken for a pathogen to be transferred to the water point is large, the pathogen would have died off and that the water would no longer be a threat to public health.

### Table 4. Association between occurrence of *E. coli* O157:H7 and distance of wells from pit latrines.

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th><em>E. coli</em> O157:H7 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>6-10</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>11-15</td>
<td>-</td>
</tr>
<tr>
<td>&gt;15</td>
<td>-</td>
</tr>
</tbody>
</table>

(*χ²=9.166, df= 3, *p* = 0.027; OR= 2.741, 95% CI= 0.809-7.6854)

Fig. 1 show percentage antibiotic resistance; virtually all the isolates were resistant to multiple antibiotics at various percentages. However, 7 (100%) of the *E. coli* O157:H7 isolates were resistant to Augmentin and Tetracycline, 86% resistant to Cefuroxime, Sulphametazole-trimethoprim and Gentamicin; 72% resistant to Cefixime. However, Cefazidime, Chloramphenicol, Nitrofurantoin and Ofloxacil were 100% effective against the isolates, Cefotaxime and Ciproflloxacin were 72% and 71% effective to the tested isolates. Tetracycline which has the highest resistance in this study is one of the most commonly available for use as growth promoter and routine chemoprophylaxis among livestock in Nigeria. They are readily available in different dosage forms and in combination with other antibiotics and vitamins.

Table 5 shows the effectiveness of fluoroquinolones, and is in agreement with the findings of [29], suggesting the use of this class of antibiotics. Resistance of *E. coli* O157:H7 isolates to Augmentin, Tetracycline and Cotrimoxazole is similar to a result of [30], in Ogun state, Nigeria. Resistance to such antibacterials may be due to their wide application in dairy operations. The findings of this study is in agreement with the previous work by [31], [32] (in Nasarawa State, Nigeria), [33] and [34].

In Table 5 Eight resistant phenotypes were obtained with varying combinations of 5,6,7 and 8. The predominant MAR phenotypes were CTX, AUG, GEN, TET, CXM having 41.0% and CRX, AUG, GEN, TET, CXM, SXT having 50.0%.

### Table 5. Multidrug Resistance Pattern of *E. coli* O157:H7 showing the resistant Phenotypes

<table>
<thead>
<tr>
<th>S/N</th>
<th>No. of Resistant Isolates</th>
<th>Resistant Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>CTX,AUG,GEN,TET,CXM</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>CRX,AUG,GEN,TET,CXM,STX</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>CRX,AUG,CIP,GEN,TET,CXM,STX</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>CRX,CTX,AUG,CIP,GEN,TET,CXM,STX</td>
</tr>
</tbody>
</table>

*AUG=Augmentin; GEN=Gentamicin; CIP=Ciproflloxacin; TET=Tetracycline; SXT= Sulphamethoxazole-trimethoprim; CXM=Cefixime; CRX=Cefuroxime; CTX=Cefotaxime*
Fig. 1. Percentage antibacterial resistance of *E. coli* O157:H7

From Fig. 2, multiple antibiotic resistance indices (MARI) were determined for the *E. coli* O157:H7 isolates. All the isolates showed a MARI of ≥ 0.4, this depicts the source as high risk source and that the antibacterials are in constant abuse in the study location.

Fig. 2. Multiple antibiotic resistance indices and the percentage of *E. coli* O157:H7 isolates.

**CONCLUSION**

The absence of municipal piped water has forced residents of the study area to rely on untreated ground water sources such as wells commonly dug by inhabitants, and the population of people obtaining drinking water from such wells are at risk of water borne
diseases. Results of this study showing that the water samples failed to meet the WHO standards for microbial contamination is not surprising since the commonest causes of ground water pollution can be easily observed in these locations. These activities include exposure to contamination due to human activities. The wells are often not covered and residents could be found washing clothes and dirty kitchen wares and sometimes bathing around the wells. Also animals/livestock are reared within compounds and householders of owners and are allowed to roam freely in search for food and water, hence they consequently serve as sources of faecal contamination of water sources.

The high prevalence of antibiotic resistant bacteria in Nigeria and other developing countries has been associated with several factors including indiscriminate use due to unregulated access of non-professional to different classes of antimicrobial over-the-counter. There is increasing concern about the rapid spread of antimicrobial resistant pathogenic bacteria in the environment and their ecotoxic effects. The results obtained from this study is a warning signal to all stakeholders in community health in the study area to direct action at protecting drinking water from fecal contamination, it also suggest that selection pressure imposed by the use of these antibiotics whether therapeutically in human and veterinary medicine or as prophylaxis in animal production is a key driving force in the selection of antibacterial resistance in organisms.

RECOMMENDATIONS

(i) Prompt well water quality assessment should be undertaken
(ii) The public health workers should ensure that the distance of pit latrine to shallow wells meet the recommended distance of 30 m by WHO
(iii) Government should ensure adequate and efficient public water supply through the provision of pipe bore water.
(iv) There is need for Government to legislate and enforce laws to limit the prescription and dispensing of antibacterials and other drugs to only qualified professionals.

REFERENCES


