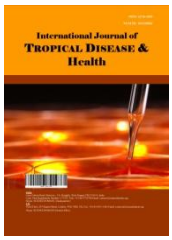

International Journal of TROPICAL DISEASE & Health

42(23): 41-48, 2021; Article no.IJTDH.81474
ISSN: 2278–1005, NLM ID: 101632866

Prevalence of Diarrheagenic Bacteria in Stool Samples of Adult Patients Attending Dutsin-Ma General Hospital, Katsina State, Northwestern Nigeria

Khalifa Jamil Saleh [a*], Anas Umar [a] and Hannatu Idris Abdullahi [a]

[a] Department of Microbiology, Faculty of Life Science, Federal University Dutsin-Ma Katsina State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2021/v42i2330563

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/81474

Original Research Article

Received 21 October 2021
Accepted 25 December 2021
Published 27 December 2021

ABSTRACT

Enteric infections and diarrheal diseases (EIDD) pose serious threats to human health globally, closely associated with poor environmental sanitation, poor water supply and unhygienic nature of individuals, which are common in developing countries. Viruses and Parasites are the major microbial agents known to cause Diarrhea and Bacteria especially *Escherichia coli, Salmonella Spp.* and *Shigella Spp.* This study was carried out to investigate the prevalence of *Escherichia coli* and *Salmonella* species among adults attending Dutsin-Ma General Hospital, Katsina State, and to determine the antimicrobial susceptibility profile of the bacterial isolates to commonly prescribed antibiotics against them. A total of 30 fresh diarrheic samples were collected from the study participants, 17 samples from male and 13 from female participants in sterile sample containers between May and July, 2021. The samples were put in ice packed container and then transported to the laboratory of the Microbiology Department, Federal University, Dutsin-Ma for processing and analyses. Out of the 30 samples examined, 29 (96.67%) were found to be positive, 16(53.33%) *E. coli* and 13(43.33%) *Salmonella* species, respectively. The result of antibiotic susceptibility test showed that both *E. coli* and *Salmonella* species had highest resistance to ampicillin but were found to be sensitive to chloramphenicol, hence, it could be used as a best drug of choice for the treatment of diarrhoea caused by the pathogens.

**Corresponding author: Email: khalifajamilsaleh@gmail.com;*

Keywords: Escherichia coli; Salmonella; Diarrhea; stool samples.

1. INTRODUCTION

Enteric infections and diarrheal diseases (EIDD) pose serious threats to human health globally, closely associated with poor environmental sanitation, poor water supply and unhygienic nature of individuals, which are common in developing countries [1]. EIDD affect a large number of people globally and constitute a leading cause of morbidity and mortality in developing countries, with higher rates among children and aged individuals [2]. Diarrhoea is characterized by stools of decreased consistency and increased volume due to imbalance of secretion and absorption of water and salts in the intestine [3]. Diarrheal diseases are so common worldwide and account among for high mortality and morbidity among young children and elderly people particularly in developing countries like Nigeria. Risk factors include ingestion of contaminated water; poor environmental sanitation, poor personal hygiene, and malnutrition, which remain excessively high. Consumption of tainted food and water infected with *E. coli* and *Salmonella* spp. which can lead to infective bacterial diarrhoea due to the organisms. Antibiotic resistance is a worldwide public health problem, particularly in settings where there is availability of a few treatment options for treatment of infective bacterial diarrhoea.

Diarrhoea can be caused by a number of microbial agents including viruses, bacteria and parasites [4]. Of all the bacterial agents, *Escherichia coli, Salmonella,* and *Shigella* species are one of the most common etiologic agents of diarrhea. *E coli* is Gram-negative, oxidase-negative, rod-shaped bacterium from the family Enterobacteriaceae [5] that lives as a normal flora of human and other mammalian intestine [6]. A total of six *E. coli* pathotypes have been found to have been implicated in diarrhoea, and hence, they area called diarrhoeagenic *E. coli*, including enteropathogenic *Escherichia coli*, Shiga like toxin producing *E. coli* (STEC), enteroinvasive E*. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli* and adherent invasive *E. coli* [1]. These are characterised by expression of different group specific virulence factors. The most commonly identified pathotype is STEC which was previously called as enterohemorrhagic and verocytotoxin producing *E. coli*.

Salmonella is a genus of Gram-negative rod-shaped bacteria belonging to the *Enterobacteriaceae* family, and causes a wide range of diseases to humans, that include enteric fever, gastroenteritis, endocarditis, and bacteraemia. It is among the most widely distributed and most common food borne diseases. In many countries, it is a major public health burden and causes significant health cost. Non-Typhoidal Salmonella serovars such as *Salmonella* serovar *Typhimurium*, *Salmonella* serovar *Paratyphi,* and *Salmonella* serovar *Choleraesuis* among others are kown to be implicated in the cause of diarrhoea [7].

Salmonella enterica and *Salmonella bongori*, are the major divisions of the genus *Salmonella* and differ in their 16S rRNA. Based on their Biochemical properties and their Genomic make up, *Salmonella Spp* are categorized into six (6) sub-groups that include; Enterica; Salamae; Arizonae and Diarizonae; Houtenae; and Indica.

Salmonella enterica subspecies enterica is responsible for approximately 99% of Salmonella infections in humans and warm-blooded animals. In contrast, the other five Salmonella subspecies and *Salmonella bongori* are rare in humans and are found mainly in the environment and in cold-blooded animals [8].

Based on the clinical disease in humans, *Salmonella* strains can be grouped into typhoidal Salmonella and non-typhoidal Salmonella (NTS). The NTS strains are found in animals and cause gastroenteritis in humans. The disease occurs worldwide and is characterized by vomiting, abdominal pain and cramps, myalgias and non-bloody diarrhea. The disease is limited to the lamina propria of the small intestine and usually antimicrobial therapy is not given. Extraintestinal features include bacteremia, septic arthritis, urinary tract infections, and osteomyelitis which may be seen in 5% of cases [9]. Some individuals may become asymptomatic carriers and shedding may last for several weeks to a few months.

Moreover, Antimicrobial drug resistance is a global health problem that continues to expand as microorganisms continue to adapt to antibiotics used to treat them [2] especially in settings where there is availability of a few treatment options for treatment of infective bacterial diarrhoea [10]. The emergence of

antimicrobial resistance, including resistance to the new and potent antimicrobial agents, is a major public health concern especially developing countries, like Nigeria , where bacterial infections are still among the major causes of death especially among young children, is of particular concern. Antibiotic resistant diarrhoeagenic *E. coli* and *Salmonella* species cause severe diarrhoeal disease, and could be associated with treatment failures among diarrhoeal patients [11]. There is however a shortage of information reported by different studies on resistance antibiotics by *E. coli* and *Salmonella* spp. isolated from diarrheic stools of patients in Dutsin-Ma. These highlighted factors are what make it imperative to carry out this study. This research was, therefore, aimed at determining the prevalence of diarrhoeagenic bacteria (*E. coli* and *Salmonella)* in stool samples of adult patients attending Dutsin-Ma General Hospital, Katsina State, Northwestern Nigeria.

2. MATERIALS AND METHODS

2.1Study Area

The study was conducted in Dutsin-Ma town, Dutsin-Ma Local Government Area of Katsina State. Dutsin-Ma Local Government lies on the latitude 12°26N and longitude 07°29E.

2.2 Study Design

The study was a cross-sectional; hospital-based conducted over a period of 3 months, between May and July, 2021. A total of thirty (30) stool samples were collected from diarrheic children attending the selected hospital.

2.3 Study Population

A total of thirty (30) diarrheic patients, 17 males and 13 females attending Dutsin-Ma General Hospital participated in the study.

2.4 Sample Collection

A total of thirty (30) diarrheic stool samples were collected from the patients. The samples were collected into sterile, transparent, wide mouthed bottles. The name, age, and gender of the participants were properly labelled on the universal bottles after which they were placed into an ice parked container and transported to the laboratory of the Department of Microbiology, Federal University Dutsin-Ma for processing and microbiological analyses.

2.5 Sample Processing and Analysis

2.5.1 Isolation and identification of *Escherichia coli:* Isolation and identification of *Escherichia coli*

Sterile swab sticks were used in streaking diarrheic stool samples collected from diarrheal patients on MacConkey agar. Subsequently the plates were incubated at 37°C for 24 hours in an incubator. Afterwards, the plates were observed for colony formation after 24 hours of incubation. After 24hrs of incubation at 37^{o}C, colonies with characteristic pink colour colonies on MacConkey were aseptically picked and streaked on EMB Agar and plates were incubated for 24 hours. Colonies revealing Green Metallic Sheen were confirmed as Escherichia coli and were streaked on nutrient agar slant and stored for further biochemical characterization of the isolates.

2.5.2 Isolation of *Salmonella* species

One gram (1g) of diarrheic stool sample from diarrheal patients was enriched in 5ml of Rappaport Vassiliadis R10 broth at 37°C for 24 hours followed by sub-culturing on both MacConkey Agar and *Salmonella-Shigella*(SS) agar and the plates were incubated at 37°C for 24 hours. Afterwards, the plates were observed for colony formation after 24-48 hours of incubation. Pure cultures were prepared from *Salmonella* like colonies, i.e. colorless colonies on MacConkey agar, and colorless colonies with black spot at the center on SS agar, were aseptically picked and stored on nutrient agar slant for further biochemical characterization of the isolates [12]

2.6 Gram Staining

The organisms were Gram stained according to and appeared pink to red Gram negative rods.

2.7 Biochemical Test and Characterization of the Isolates

Escherichia coli and *Salmonella* species isolated were subjected to a battery biochemical tests which included;, urease test, indole test, methyl-red, vogues proskauer test, citrate test (IMViC), motility test, oxidative fermentation test, triple sugar iron agar test for biochemical characterization, and identified using standard bacteriological procedures [12].

2.8 Antibiotic Susceptibility Testing

Following biochemical characterization of the isolates, antimicrobial susceptibility testing using disk diffusion method was then carried out to test the susceptibility profile of the organisms to most commonly prescribed antibiotics against them. A bacterial lawn was prepared by transferring overnight grown bacterial colonies to a glass tube containing about 5 ml sterile normal saline water with a sterile inoculating loop. The bacterial suspension was vortexed and visually matched with 0.5 Mac Farland standard for turbidity. Sterile swab stick was then immersed in the suspension and spread onto Mueller Hinton agar (Oxoid, UK) to obtain a semi-confluent growth. Discs impregnated with predetermined amounts of antibiotics were placed onto the bacterial lawn and the plates were inverted and incubated for 18-24 hours at 37°C. After the incubation, the diameter of the inhibition zones was measured in millimeters using ruler and interpreted as sensitive, intermediate, and resistant using the criteria described by the Clinical and Laboratory Standards Institute. The antibiotics discs (Oxoid, UK) used included: amoxicillin (10µg), ampicillin (10µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (10µg), nalidixic acid (30µg) and imipenem (10µg), respectively.

3. RESULTS

Analysis of 30 fresh faecal samples revealed positive growth of diarrhoea causing bacteria (*E. coli* and *Salmonella* species) in 29(96.67%) samples out of the total samples analyzed. *Escherichia coli* (53.33%) had higher prevalence than *Salmonella* species (43.33%) as presented in table 1. Patients of age group 25– 36 years had the highest prevalence of 37.50% of diarrhoea causing *E. coli*, while the lowest prevalence (18.75%) of *E. coli* was in the age groups of 18-24 and 49-69 years, respectively, it was more prevalent in males than in female patients (Table 2). Table 3 shows age and gender factors of *salmonella* isolates among the stool samples of the patients. Table 4 shows the antibiotic profile of both *E. coli I* and *Salmonella* species. Highest resistance was observed in ampicillin antibiotic while chloramphenicol was found to be most effective drug of all the tested drugs, hence, considered the best drug for treatment of diarrhoea caused by the bacterial agents.

Table 1. Percentage Distribution of *Escherichia* coli and *Salmonella spp.* for the Study Participants

Bacterial Isolates	Positive samples (%)	Negative samples (%)	Total samples (%)
Escherichia coli	16(53.3)	14(46.7)	30(100)
Salmonella	13(43.3)	17(56.7)	30(100)
Total	29(96.6)	31(103.4)	30(100)

Table 2. Distribution of *Escherichia coli* among the study participants with respect to age and gender

Age group (years)	Male	Female	Total (%)
18-24	2	1	3(18.75)
25-36	4	2	6(37.5)
37-48	1	3	4(25)
49-60	2	1	3(18.75)
Total	9	7	16

Table 3. Distribution of *Salmonella* species among the study participants with respect to their age and gender

Age group (years)	Male	Female	Total (%)
18-24	2	1	3(23.07)
25-36	2	1	3(23.07)
37-48	2	2	4(25)
49-60	2	1	3(23.07)
Total	8	6	13

Table 4. Antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* spp. in diarrheic stools from the patients

Antibiotic	Resistance	Intermediate	Sensitive
Amoxicillin	10(55.6)	5(27.8)	3(16.6)
	7(100)	0(0)	0(0)
Ampicillin	17(94.4)	1(5.6)	0(0)
	5(71.4)	2(28.6)	0(0)
Ceftriaxone	8(44.4)	5(27.8)	5(27.8)
	2(28.6)	2(28.8)	3(42.9)
Chloramphenicol	0(0)	0(0)	18(100)
	0(0)	0(0)	17(100)
Ciprofloxacin	3(16.6)	5(27.9)	14(45.5)
	0(0)	0(0)	7(100)
Imipenem	10(55.6)	5(27.8)	3(16.7)
	3(21.4)	4(28.6)	7(50)
Nalidixic acid	2(11.1)	2(11.1)	14(77.8)
	2(28.6)	0(0)	5(71.41)

4. STUDY LIMITATION, DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Study Limitation

This study was only conducted on adult patients and molecular analysis was not done due to financial constraint.

4.2 Discussion

The result of chi-square analysis at 95% confidence interval showed that there is no significant difference between the presence and absence of diarrhoeagenic bacteria (*E. coli* and *Salmonella* spp.) among the study participants (P= 0.159; χ^2cal.= 2.003, χ^2tab. = 3.842.In this research, 53.3% prevalence of *Escherichia coli* was observed from the patients' stool samples. (Table 1), which is lower compared to 72.22% prevalence of *Escherichia coli* in a study conducted by the Saleh et al*.,* [13] higher compared to 41.4% prevalence of *E. coli* reported by Korie et al. [14] and 59% prevalence of *E. coli* from diarrheic stools reported by Dormanesh et al. [15] in a study conducted in Dutsin-Ma, Enugu, and Iran respectively. Nan-Ok et al. [16] also reported 22.0% of *E. coli* from diarrheic stool specimen in a study conducted in Korea. However, 43.3% prevalence of *Salmonella* spp. was observed in this present study, which is higher than the 22.22% prevalence of *Salmonella* found by Saleh et al*.,* [13] and higher than 5% prevalence of *Salmonella* spp. reported by Mzungu et al. [17] a study conducted in Dutsin-Ma. Tesfahun et al. [18] reported 10.8% occurrence of *Salmonella* spp. from diarrheic stools in a study conducted in Ethiopia while Kabir et al.[19] reported 17% prevalence of *Salmonella* spp. from stool specimen of patients with gastroenteritis in a study conducted in Lagos, Nigeria. The disparity in the presence of *E. coli* over *Salmonella* may be attributable to differences in the study designs, patients´ immunity, different environmental conditions and their behavioral pattern.

Patients of age group 25– 36 years had the highest prevalence of 37.50% of diarrhoea causing *E. coli*, which is lower to the findings of Saleh et al., [13] for age group 21-26 (11.5%) and age group 26-30 (3.9%) in a study conducted by the same author, while the lowest prevalence (18.75%) of *E. coli* was in the age groups of 18-24 and 49-69 years, respectively (Table 2), it was more prevalent in males then in female patients (Table 2). Statistical distribution of *E. coli* showed that there is no significant difference in terms of the distribution of the organism with regards to the patients' age group (P= 0.625; Fcal.= 0.667, Fcrit.= 9.277). Likewise, there is no significant difference between the distribution of *E. coli* in the diarrhoea samples of the male and female subjects (P= 0.604; Fcal.= 0.333, Fcrit.= 10.128).

Similarly, Patients of age group 37-48 years had the highest prevalence of 25.0% of diarrhoea causing *Salmonella* spp., whereas the lowest prevalence of 23.07% was observed in the age groups of 18-24, 25-36 and 49-69 years, respectively (Table 3), it was more prevalent in males then in female patients (Table 2). Statistical distribution of *Salmonella* spp. showed that there is no significant difference in terms of the distribution of the organism with regards to the patients' age group (P= 0.50; Fcal.= 1.0, Fcrit.= 9.277). Also, there is no significant difference between the distribution of the organism in the diarrhoea samples of the male and female patients (P= 0.058; Fcal.= 9.0, Fcrit.= 10.128).

The study reported highest resistance (82.90%) of the organisms to ampicillin (Table 4). Statistical analysis of the antimicrobial susceptibility test results showed that there is no significant difference between the average percentage resistance, intermediary and sensitivity with regards to *E. coli* (P= 0.440; Fcal.= 0.880, Fcrit.= 3.885); and also with regards to *Salmonella* spp. (P= 0.23; Fcal.= 1.665, Fcrit.= 3.885). Similarly, with regards to antibiotics tested, it was found out there was no significant difference observed in the case of both *E. coli (*P= 1.0; Fcal.= 0.004, Fcrit.= 2.996) and *Salmonella* spp. (P= 1.0; Fcal.= 0.000003, Fcrit.= 2.996).

This is similar to the study conducted by Ochoa et al. [20] who reported 85% resistance to ampicillin by diarrhoeagenic *E. coli* among children in Peru. It also agrees with other study of Estrada-Garcia et al.,[21] who recorded 85% resistance to ampicillin antibiotic in Tanzania among young children. The highest resistance to ampicillin showed by the bacteria in the study could be attributed to continuous use of such drugs which might most likely be associated with treatment failure and serious antimicrobial resistance. It might also be because of the fact

that these drugs are relatively cheap and available. This could have rendered them readily accessible to the patients, thereby increasing their misuse and overuse, thus enabling the bacteria to acquire resistance genes, for examples, possession of penicillinases producing genes. The enzymes hydrolyze the beta lactam ring in the antibiotics, and as such render them ineffective, hence, the resistance.

4.3 Conclusion

Conclusively, from the present study, *Escherichia coli* and *Salmonella* spp. were frequently isolated among age groups (18-60years) with prevalence rate of 53.3% and 43.3% respectively. Diarrhoeal diseases caused by enteric infections remain a leading global health problem. About two to four billion episodes of infectious diarrhoea have been estimated to occur annually in developing countries, resulting to death of large number of individuals across age groups, which could be due to resistance of the bacterial agents to antibiotics used for their treatment.

4.4 Recommendation

1. Potential source of infection as well as food and meat inspection must be followed up.
2. A further study should be conducted to ascertain why there is high resistance to Ampicillin in this study population.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval (MOH/ADM/508/1152/1/466) was obtained from Katsina State ministry of health for permission to obtain diarrhoea samples from patients at the selected Hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ustin AP, Wolf J, Bartram J, Clasen T, Cumming O, Freeman MC, Gordon B, Hunter P, Medlicott K, Johnson R. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. International Journal of Hygiene and Environmental Health 2019;222(5):765-777.
2. Nataro JP, Kaper JB Diarrheagenic Escherichia coli. Clin. Microbiol Rev. 1998;11:14-201.
3. World Health Organization. Diarrhoeal diseases; 2019.
4. Bonkoungo IJ, Haukka K, Osterblad M, Hakaken A, Traore BN, Sitonen A. Bacterial and Viral etiology of Childhood in Ougadougou, Burkina-Faso. Biomedical Paediatrics 2013;13(36):1471-2431.
5. Croxen MA, Law RJ, Scholz R, Keeney KM, Woldarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews. 2013;26(4):822-880.
6. Bekal S, Brousseau R, Masson L, Prefontaine G, Fairbrother J, Harel J. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. J Clin Microbiol. 2003;41:2113–25. DOI:10.1128/JCM.41.5.2113-2125.2003.
7. Gal-mor O., Boyle EC., and Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. Frontiers in Microbiology 5(391) 2014.
8. Eng S, Puspurajah P, Ab Mutalib N, Ser H, Chan K, Lee L. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance Frontiers in Life Science. 2015;8(3):284-293
9. Rohilla R, Bhatia M, Gupta P, Singh A, Shankar R, Omar BJ. *Salmonella* osteomyelitis: A rare extraintestinal manifestation of an endemic pathogen Journal of Laboratory Physicians. 2019;11(2):164–170.

10. Adesoji AT, Ogunjobi AA, Olatoye IO, Call DR. Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in southwestern Nigeria. Annals of Clinical Microbiology and Antimicrobials. 2015;14:35. PubMed| Google Scholar.
11. *ASM science | Eosin-Methylene Blue Agar Plates Protocol*. Retrieved June 12; 2020. Available:https://www.asmscience.org/content/education/protocol/protocol.2869
12. Monica Cheesbrough. District Laboratory Practice in Tropical Countries, Part 2 Second Edition, Cambridge University Press, England 2010. Microscopical Techniques used in Microbiology. 2010:35-45.
13. Saleh KJ, Doyinsola AM, Adamu AS. International Journal of Biology Sciences. Search for *E. coli* 0157: h7 in stool sample from diarrheagenic patient attending Dutsinma General Hospital. 2021;3(1):30-36.
14. Korie FC, Ikefuna A, Ibe BC. Bacterial agents associated with acute diarrhoea in Under-5 children in Enugu, Nigeria. IOSR Journal of Dental and Medical Sciences. 2011;2(6):40
15. Dormanesh B, Siroosbakhat S, Karimi GP, Afsharkhas L. Shiga toxigenic *Escherichia coli* in Iranian pediatric patients with and without Diarrhea: O-Serogroups, virulence factors and antimicrobial resistance properties. Iran Red Crescent Medical Journal. 2015;17(10):297–306.
16. Nan-Ok K, Su-Mi J, Hae-Young N, Gyung TC, Cheon-Kwon Y, Won KS, Sahyun H. Enteric bacteria isolated from diarrheal Patients in Korea in 201 Osong Public Health and Research Perspectives. 2015;6(4):233–240.
17. Mzungu I, Inabo HI, Olonitola SO, Aminu M. Antibiotic susceptibilities of Salmonella species prevalentamong children of 0-5 years with diarrhea in Katsina state, Nigeria. Arch Med Biomed Res. 2016;3(1):39-51.
DOI: 10.4314/ambr.v3i1.
18. Tesfahun L, Tsige K, Ketema B. Prevalence and Antimicrobial Resistance in Salmonella and Shigella Species Isolated from Outpatients, Jimma University Specialized Hospital, Southwest Ethiopia. Canadian Journal of Infectious Diseases and Medical Microbiology. 2016:8.
Article ID 4210760,
Available:http://dx.doi.org/10.1155/2016/4210760
19. Kabir OA, Stella IS, Akeeb OO, Kehinde A, Akitoye OC. Trends of multiple drug resistance in Salmonella Enterica Serovar Typhi in Lagos, Nigeria. East and Central African Journal of Surgery. 2007;12:1
20. Ochoa TJ, Molina M, Del Valle LJ. High frequency of antimicrobial resistance of diarrheagenic E; 2009.
21. Estrada-García T, Cerna JF, Pacheco-Gil L, Velázquez RF, Ochoa TJ, Torres J, DuPont HL. Drugresistant Diarrheogenic Escherichia coli, Mexico. Emerg Infect Dis. 2005;11:1306–1308.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/81474