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RESEARCH ARTICLE

DIARRHEA CAUSING *ENTEROBACTERIACEAE* AND THEIR ANTIBIOTIC RESISTANCE PATTERNS AMONG DIARRHEA PATIENTS FROM SANA'A CITY, YEMEN

Gamil Taher Ahmed AbdulMughni[1], Khaled Abdulkarim Al-Moyed[1,2], Eshrak Adulmalik Al-gunaid[3], Huda Zaid Al-Shami[2], Naif Mohammed Al-Haidary[2], Anowar Gasem Al-Madhaji[2], Hassan Abdulwahab Al-Shamahy[1,2]

[1]*University of 21 September for Medical and Applied Sciences, Sana'a, Yemen.*
[2]*Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University.*
[3]*The National Center of Public Health Laboratories (NCPHL), Sana'a, Yemen.*
[4]*Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.*

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***Address for Correspondence:**
Dr. Hassan Abdulwahab Al-Shamahy, University of 21 September for Medical and Applied Sciences, Sana'a, Yemen. Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University, Yemen. Tel: +967-1-239551; E-mail: *shmahe@yemen.net.ye*

Abstract

Background and aims: The rise of antibiotic-resistant strains of the bacteria causing diarrhea remains a major worldwide health concern, especially in children under five. The purpose of this surveillance study was to ascertain the prevalence and antibiotic susceptibility profile of *Enterobacteriaceae*, which cause diarrhea.

Materials and Methods: The National Center for Public Health Laboratories (NCPHL) in Sana'a city was the site of this cross-sectional investigation. Patient stool samples exhibiting diarrhea were gathered and cultivated using standard differential/selective medium. Standard biochemical assays and serological investigations were employed to identify the isolates. With the aid of Kirby-Bauer disk diffusion, the antibiogram was identified.

Results: *Enterobacteriaceae* isolates were obtained from 972 stool samples collected; 15.3% (149/972) were diarrheagenic *Enterobacteriaceae* bacteria, including *E. coli*, *Salmonella*, and *Shigella*. There were 8.3% (81/972) *Salmonella* spp., 5.7% (56/972) *Shigella* spp., and 1.2% (12/972) *E. coli*. *E. coli* strains were grouped, with the most prominent being serogroups O153 (3, 2%) and O25 (2, 1.4%). We detected high resistance of *Salmonella* isolates to tetracycline (29/81, 35.8%) but relatively low resistance to ciprofloxacin (7/81, 8.6%) and ceftriaxone (5/81, 6.3%).

Conclusion: The many *Enterobacteriaceae* linked to instances of diarrhea in Sana'a, Yemen, were demonstrated in this study, along with the necessity of differential diagnosis for improved treatment results. *Salmonella, Shigella, and Escherichia coli* have all been linked to episodes of diarrhea in Sana'a city. To stop Yemen's antimicrobial resistance from spreading, regular antimicrobial susceptibility testing, efficient monitoring, and national surveillance of AMR bacteria should be put into place.

Keywords: Antibiotics, bacteria, diarrhea, *Enterobacteriaceae*, Yemen.

INTRODUCTION

Though largely treatable and preventive, diarrheal disease ranks second globally in terms of causes of death for children under five, taking the lives of approximately 525,000 children each year[1]. The World Health Organization defines diarrhea as the excessive and frequent evacuation of watery stools, which typically indicates a gastrointestinal illness or condition lasting three to seven days[1]. Numerous viral, bacterial, and parasite pathogens are the primary cause of diarrheal disorders, and their etiology varies based on a number of factors, including host characteristics, socioeconomic circumstances, and geographic and climatic settings[2]. Children under the age of two are known to suffer from diarrhea primarily from viral diseases such rotavirus and norovirus, with the latter resulting in the greatest death rate among children[3]. It has also been documented that parasites such *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium parvum* can induce episodes of severe diarrhea[4-6]. *Escherichia coli* is the most prevalent bacterium responsible for diarrhea in children in developing nations and is an emerging enteropathogen that is resistant to antibiotics in industrialized nations, according to research[7]. However, *Vibrio cholerae* has been identified as the primary bacteria pathogen responsible for diarrhea-associated disorders; certain strains, such Ogawa, have the potential to spread epidemics if left unchecked[8-11]. This is mostly due to unhygienic conditions like feces entering rivers and streams that serve as drinking water for people and cattle, a shortage of potable water, and incorrect handling of household food, which can result in an increase in diarrhea cases[12-14].

There have been documented cholera epidemics and outbreaks in Yemen in the years 2016, 2017, 2018, 2019, and 2024. All parts of the nation were impacted by the 2016–2019 pandemic, which resulted in roughly 1,28,9220 cases overall and 3243 deaths[8-10].

Nonetheless, it has been demonstrated that bacteria other than *V. cholerae* can also cause diarrhea, such as *Campylobacter* species, *Salmonella* species, *Shigella* species, and pathogenic *E. coli* O15:H7 (though this issue is still open), all of which can be just as deadly if left untreated[15,16].

As advised by the WHO, zinc and oral rehydration solutions (ORS) are commonly effective treatments for infections caused by these bacteria. Nevertheless, in cases of severe, bloody, or chronic diarrhea, certain individuals may also benefit from antimicrobial medication[17]. Treatment of bacterial diarrhea would be difficult due to rising reports of increased antibiotic resistance among Yemeni enterobacteria[18-20]. There is serious worry over reports of over 40% of children receiving antibiotic treatment developing non-bacterial diarrhea, of easy access to medications in Yemen through unapproved ways, and of incorrect drug prescriptions[21-23]. Studies have linked bacteria, which have a 3% prevalence rate and a strong resistance to often overused medications like tetracycline, to instances of diarrhea in Yemen[21-25]. In patients presenting to the National Center for Public Health Laboratories (NCPHL) in Sana'a City, the objective of this study was to identify *Enterobacteriaceae* organisms linked to diarrhea and ascertain the isolates' profile of antibiotic susceptibility.

MATERIALS AND METHODS

Study design and identification of microorganisms: This study used clinical samples of diarrheal stool that were obtained between January 1, 2021, and March 1, 2024 (data were complete and available for this period) at the National Center for Public Health Laboratories (NCPHL) in Sana'a, Yemen. The study was cross-sectional in nature. The National Center for Pathology Laboratory Health (NCPHL) serves as the nation's reference laboratory. Its microbiology section comprises six benches and employs twelve bacteriologists, each of whom processes six samples per day. Al-Gumhouri University Hospital and Al-Kuwait University Hospital, two significant Sana'a hospitals, contributed samples.

Bacteria isolation and characterization

Patient stool samples exhibiting diarrhea were gathered and cultivated using standard differential/selective medium. Standard biochemical assays and serological investigations were employed to identify the isolates. Every stool sample was inoculated on three different media: MacConkey agar, which was used to isolate *E. coli* and non-lactose fermenters, and Selenite Faecal Broth, SFB, an enrichment medium used to isolate *Salmonella* and *Shigella* spp. The enteric bacteria were then isolated by aerobic incubation at 37°C for 18–24 hours. On *Salmonella Shigella* Agar (SS), a loopful of SFB and APW broth cultures were sub-cultured and incubated as before. Suspected colonies of *E. coli*, *Shigella*, and *Salmonella* spp. were identified and purified using standard biochemical techniques, the Gram staining response, and colonial features. Using commercially available serological assays, antigenically identified isolates of *Salmonella*, *E. Coli*, and *Shigella* were obtained.

Antimicrobial Susceptibility Testing

Only isolates that were believed to be diarrheagenic bacteria were included in our study for antibiotic susceptibility testing. Following the guidelines set forth by the Clinical and Laboratory Standard Institute (CLSI)[26], the susceptibility to antimicrobial medicines was ascertained using the Kirby Bauer disk diffusion method. The following were tested on isolates: 30 µg of ceftriaxone, 5 µg of norfloxacin, 30 µg of cefotaxime, 30 µg of amikacin, 30 µg of gentamycin, 50 µg of clotrimazole, 30 µg of nalidixic acid, 30 µg of tetracycline, 30 µg of ceftazidime, 30 µg of ciprofloxacin, 300 µg of nitrofurantoin, and 30 µg of cefazolin. On Mueller Hinton Agar plates, zones of inhibition were measured using the Kirby Bauer technique. Susceptibility, resistance, and intermediate susceptibility were ascertained using the CLSI breakpoints[26].

Ethical Consideration: This cross-sectional study, which took place between January 2021 and March 2024, was approved by the Medical Ethics and Research Committee of Sana'a University's Faculty of Medicine and Health Sciences (approval number: 317; dated January 1, 2021). The ethical rules that were established by the review committee were consistently followed.

Statistical Analysis: Epi-Info Statistics version 7 was used to screen information. A statistical study was conducted to calculate the frequency of different bacteria causing diarrhea, and the degree of drug resistance of the isolated bacteria.

RESULTS

Table 1 show the sex and age distribution of diarrheal patients attending the National Laboratory Center for Stool Culture in Sana'a City, tested for anemia. The male patients counted 51.7%, and the female patients counted 48.3%. The mean age of our patients was 30.05 years, with an SD of 14.2 years, and their ages ranged from 3 to 68 years. Most of our patients were in the age group of 16–25 years (36.9%), followed by the group of 26–35 years (18.8%), while other age groups were less frequent. *Enterobacteriaceae* isolates were obtained from 972 stool samples collected; 15.3% (149/972) were diarrheagenic *Enterobacteriaceae* bacteria, including *E. coli* (from bloody stool as well as stool from children under five), and 2 other *Enterobacteriaceae* genera, namely: *Salmonella* and *Shigella*.

Table 1: General characteristics of participate in the study, n=149.

Characters	N (%)
Sex	
Male	77 (51.7)
Female	72 (48.3)
Ages (years)	
≤15 years	17 (11.4)
16-25	55 (36.9)
26-35	28 (18.8)
36-45	24 (16.1)
≥46	25 (16.8)
Mean age	30.05 Years
SD	14.2 Years
Min-Max	3- 68 Years

There were 8.3% (81/972) *Salmonella* spp., 5.7% (56/972) *Shigella* spp., and 1.2% (12/972) *E. coli*, from bloody or infant stool (infant <5 years) (Table 2).Upon serological analysis, *E. coli* strains were grouped, with the most prominent being serogroups O153 (3, 2%) and O25 (2, 1.4%), known to be enterotoxigenic *E. coli* (Table 2). Fifty-six (37.6%) *Shigella* species were identified as *Shigella boydii type 2* and *Shigella bodii type 17* were the most common *Shigella* isolated *(*11, 8.1% and 16, 10.7%, respectively), *Shigella flexneri* type II and *Shigella flexneri* type I (8, 5.3% and 4, 2.7%, respectively), *Shigella dysenteriae* type II (8, 5.3%), *Shigella dysenteriae* type I (6, 4%), and *Shigella sonnei* (21.4%). Among *Salmonella* isolates, serogroups O3.10 (16.1%), O4 (9.4%), and O7 (7.4%) were the most dominant. Twelve *E. coli* isolates were subjected to antimicrobial susceptibility testing; when these isolates were examined against all of the antimicrobials we had chosen, at least one antimicrobial resistance was found (Table 3). The isolates of *E. coli* exhibited the highest levels of resistance to cefazolin (7/12, 58.3%) and tetracycline (10/12, 83.3%). The isolates showed a high susceptibility to aminoglycosides that are given to treat *E. coli* infections, including gentamicin (11/12, 91.7%), amikacin (8/12, 66.7%), and nitrofurantoin (10/12, 83.3%). For one fluoroquinolone, ciprofloxacin (4/12, 33.3%), we found high resistance. When tested against our chosen antimicrobials (Table 4), all 56 *Shigella* isolates were shown to be 100% tetracycline resistant. We found three isolates of *Shigella* that were resistant to multiple drugs. Six (10.7%) isolates were resistant to both norfloxacin and ciprofloxacin, while three (5.4%) isolates were resistant to just one of the three medications (norfloxacin, ciprofloxacin, and tetracycline). It was found that the *Salmonella* isolates had a significant tetracycline resistance (29/81, 35.8%), but a comparatively low ciprofloxacin (7/81, 8.6%) and ceftriaxone (5/81, 6.3%). When tested against gentamycin and norfloxacin, the isolates of *Salmonella* revealed complete susceptibility; nevertheless, no strains of the bacteria that were resistant to multiple drugs were discovered.

Table 2: *Enterobacteriaceae* isolates and serotypes (n = 149).

Species	n (%)
E. coli serogroups	N = 12 (8.1)
O8	1 (0.7)
O159	1 (0.7)
O153	3 (2)
O25	2 (1.4)
O169	1 (0.7)
O18	2 (1.4)
O44	1 (0.7)
O164	1 (0.7)
Shigella serogroups	N = 56 (37.6)
Shigella flexneri type I	4 (2.7)
Shigella flexneri type II	8 (5.3)
Shigella dysenteriae type I	6 (10.7)
Shigella dysenteriae type II	8 (5.3)
Shigella boydii type 2	1 2 (8.1)
Shigella boydii type 17	16 (10.7)
Shigella sonnei Phase I	2 (1.4)
Salmonella serogroups	N = 81 (54.4)
O3, 10	24 (16.1)
O35	4 (2.7)
O4	14 (9.4)
O7	11 (7.4)
O8	4 (2.7)
O9	5 (3.4)
O13	6 (4)
Non-reactive	13 (8.7)
Total	149 (100)

DISCUSSION

Several significant discoveries have been made from our regular surveillance study of diarrhea from Sana'a city from January 2021 to March 2024. This study's isolates of *Enterobacteriaceae* were diverse and frequent, which is consistent with findings from China, Kenya, and India[15,16,27]. *Shigella*, *Salmonella*, and diarrheagenic *E. coli* frequencies are in line with earlier research conducted in African nations[28,29]. Lompo *et al*.'s results[30] did not, however, identify *Salmonella* from stool samples that had diarrhea. *Enterobacteriaceae* isolates were obtained from 972 stool samples collected; 15.3% (149/972) were diarrheagenic *Enterobacteriaceae* bacteria, including *E. coli* (from bloody stool as well as stool from children under five), and 2 other *Enterobacteriaceae* genera, namely: *Salmonella* and *Shigella*. There were 8.3% (81/972) *Salmonella* spp., 5.7% (56/972) *Shigella* spp., and 1.2% (12/972) *E. coli*, from bloody or infant stool (infant <5 years) (Table). When compared to other research (4%) the amount of diarrheagenic bacteria we identified in our study was noticeably higher[15,28]. The high incidence could potentially be attributed to the unhygienic living conditions and lack of potable drinking water at our sample site. As was previously mentioned, street food samples have been found to have diarrheagenic bacteria. This could lead to an increase in occurrences of bacterial diarrhea in places where living circumstances are unhygienic. This finding is crucial in a nation where the majority of diarrheal illnesses are believed to be exclusively caused by *V. cholerae*[8-10].

Table 3: Resistance patterns of enteropathogenic *E. coli* strains to 12 antibiotics, (n=12 trains).

Antibiotis	Resistant N (%)	Modreate N (%)	Sensitive N (%)
CTR: Ceftriaxone (30 µg)	2 (16.7)	1 (8.3)	9 (75)
NOR: Norfloxacin (5 µg)	2 (16.7)	1 (8.3)	9 (75)
AMK: Amikacin (30 µg)	3 (25)	1 (8.3)	8 (66.7)
CTX: Clotrimazole (50 µg)	1 (8.3)	2 (16.7)	9 (75)
GEN: Gentamycin (30 µg)	1 (8.3)	0 (0.0)	11 (91.7)
CTL: Cefotaxime (30 µg)	6 (50)	2 (16.7)	4 (33.3)
NAL: Nalidixic acid (30 µg)	5 (41.7)	1 (8.3)	6 (50)
TET: Tetracycline (30 µg)	10 (83.3)	1 (8.3)	1 (8.3)
CAZ: Ceftazidime (30 µg)	2 (16.7)	1 (8.3)	9 (75)
CPR: Ciprofloxacin (5 µg)	4 (33.3)	2 (16.7)	6 (50)
NIT: Nitrofurantoin (300 µg)	1 (8.3)	1 (8.3)	10 (83.3)
CZL: Cefazolin (30 µg)	7 (58.3)	2 (16.7)	3 (25)

Shigella, *Salmonella*, and enterotoxigenic *E. coli* are common, which highlights the necessity of increasing laboratory-based surveillance and accurately determining the cause of sickness in order to treat diarrheal illnesses. It is quite concerning that strong medications used to treat *Shigella* and *Vibrio*, like ciprofloxacin, norfloxacin, and nalidixic acid, are showing signs of developing resistance. We discovered that all (100%) of the *Shigella* isolates were tetracycline resistant.

Table 4: Resistance patterns of *Shigella* species strains to 12 antibiotics, (n=56 trains).

Antibiotis	Resistant N (%)	Modreate N (%)	Sensitive N (%)
CTR: Ceftriaxone (30 µg)	7 (12.5)	0 (0.0)	49 (87.5)
NOR: Norfloxacin (5 µg)	3 (5.4)	3 (5.4)	50 (89.3)
AMK: Amikacin (30 µg)	6 (10.7)	3 (5.4)	47 (83.9)
CTX: Clotrimazole (50 µg)	13 (23.2)	12 (21.$)	31 (55.4)
GEN: Gentamycin (30 µg)	5 (8.9)	4 (7.1)	47 (83.9)
CTL: Cefotaxime (30 µg)	13 (23.2)	27 (48.2)	16 (28.6)
NAL: Nalidixic acid (30 µg)	10 (17.9)	3 (5.4)	43 (76.8)
TET: Tetracycline (30 µg)	56 (100)	0 (0.0)	0 (0.0)
CAZ: Ceftazidime (30 µg)	0 (0.0)	4 (7.1)	52 (92.9)
CPR: Ciprofloxacin (5 µg)	15 (28.8)	3 (5.4)	38 (67.9)
NIT: Nitrofurantoin (300 µg)	6 (10.7)	4 (7.1)	46 (82.1)
CZL: Cefazolin (30 µg)	14 (25)	23 (41.1)	19 (33.9)

Tetracycline has been widely used in Yemen throughout the years, and there have been numerous reports of antimicrobial resistance to these medications[18,19,31,32]. This could be the cause of this, due to easy access to the medication through pharmacies; there may also be some contribution from the careless use of the drug in the animal husbandry industry. In addition, the strains of *Shigella* were resistant to amikacin (10.7%), ciprofloxacin (28.8%), nalidixic acid (17.9%), and norfloxacin (5.4%), the latter two of which were once highly effective medications for treating adult cases of *Shigella* dysentery[33]. The findings that *Shigella* sp. exhibits strong resistance to tetracycline, amikacin, and nalidixic acid and high susceptibility to ceftriaxone are in line with the findings of Pourakbari *et al.*,[34] and Basnet *et al.*,[33]. The emergence of high resistance to parenteral ceftriaxone is concerning because it is highly effective and advised for the treatment of hospitalized children with severe shigellosis[35]. *E. coli* strains were grouped based on serological analysis; the most prevalent serogroups were O153 (3, 2%) and O25 (2, 1.4%), which are recognized as enterotoxigenic *E. coli* strains.

Table 5: Resistance patterns of *Salmonella* strains to 12 antibiotics, (n=81 trains).

Antibiotis	Resistant N (%)	Modreate N (%)	Sensitive N (%)
CTR: Ceftriaxone (30 µg)	5 (6.2)	11 (13.6)	65 (80.2)
NOR: Norfloxacin (5 µg)	0 (0.0)	0 (0.0)	81 (100)
AMK: Amikacin (30 µg)	5 (6.2)	2 (2.5)	74 (91.4)
CTX: Clotrimazole (50 µg)	0 (0.0)	2 (2.5)	79 (97.5)
GEN: Gentamycin (30 µg)	0 (0.0)	0 (0.0)	81 (100)
CTL: Cefotaxime (30 µg)	14 (17.3)	7 (8.6)	60 (74.1)
NAL: Nalidixic acid (30 µg)	7 (8.6)	0 (0.0)	74 (91.4)
TET: Tetracycline (30 µg)	29 (35.8)	21 (25.9)	31 (38.3)
CAZ: Ceftazidime (30 µg)	0 (0.0)	7 (8.6)	74 (91.4)
CPR: Ciprofloxacin (5 µg)	7 (8.6)	5 (6.2)	69 (85.2)
NIT: Nitrofurantoin (300 µg)	5 (6.2)	19 (23.5)	57 (70.4)
CZL: Cefazolin (30 µg)	10 (12.3)	21 (25.9)	50 (61.7)

Numerous virulent genes that can cause mild to severe infections have been found to be present in the enterotoxigenic *Escherichia coli* (ETEC) serotype O153[36]. This *E. coli* strain has been reported to have been isolated from patients during an outbreak, which may be a sign of its propensity to spread epidemics[37]. Public health may be seriously jeopardized by the spread of these aggressive strains of *E. coli* inside nearby populations. Antimicrobial resistance is becoming more common in populations, as evidenced by the study's detection of multi-drug resistant organisms.

The careless use of antibiotics as growth supplements in the farming and animal husbandry industries in Yemen is a significant issue since it reduces yield by preventing infection. This procedure accelerates the development of antibiotic resistance by pre-exposing medications to these microorganisms[38,39].

In this surveillance project, a village with restricted access to pipe-borne water was selected as the sentinel site. Another issue in the neighborhood is the improper disposal of human waste. The increased incidence of diarrhea could be explained by open defecation into bushes and bodies of water as well as a shortage of drinkable water[13]. It is commonly established that some behaviors can stop the spread of bacterial germs that cause diarrhea, including washing your hands, preparing your food properly, and disposing of your trash and human waste properly.

Limitations of the study

We noted a few restrictions in the research. Compared to serological analysis alone, molecular techniques like PCR would have been more sensitive and provided a better knowledge of the diarrheagenic *E. coli* and ESBLs obtained. Since samples were only taken from patients who consented to be included in the study, we are unable to provide information on the overall number of people who present to medical institutions with diarrhea.

CONCLUSIONS

This study has demonstrated the variety of bacteria (*Salmonella*, *Shigella*, and certain *E. coli*) linked to diarrheal illness and the importance of accurate differential diagnosis for improved therapeutic results. All isolates exhibited resistance, which raises concerns for public health because the medications used to treat Shigellosis, Salmonellosis, and other diarrheal illnesses demonstrated significant resistance. The high rates of resistance found in *Shigella* isolates serve as a warning and highlight the necessity of putting preventive measures in place to reduce transmission. To stop AMR microorganisms, regular antibiotic susceptibility testing, efficient monitoring, and national surveillance should be put into place.

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AUTHOR'S CONTRIBUTION

Al-Shamahy HA: writing original draft, methodology, investigation. **AbdulMughni GTA:** formal analysis, data curation, conceptualization. **Al-gunaid EA:** writing, review and editing, methodology. **Al-Shami HZ:** editing, review. **Al-Haidary NM:** literature survey. **Al-Madhaji AG:** formal analysis, data curation. **Al-Moyed KA:** conceptualization. All authors have revised and approved the final version of the manuscript.

DATA AVILIABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. World Health Organization. WHO Diarrhoeal Disease Key Facts 2017. Available at: *https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease* (Accessed January 30, 2024).
2. Hodges K, Gill R. Infectious diarrhea: Cellular and molecular mechanisms. Gut Microbes 2020; 1, 4–21. *https://doi.org/10.4161/gmic.1.1.11036*
3. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD. World Health Organization–Coordinated Global Rotavirus Surveillance Network. Global, regional, and national estimates of rotavirus mortality in children< 5 years of age, 2000–2013. Clin Infect Dis 2016; 62, S96–S105. *https://doi.org/10.1093/cid/civ1013*
4. AL-Mekhlafi AM, Al-Moyed KA, Al-Shamahy HA, Al-Haddad AM, Al-Ankoshy AAM, Al-Shamahi EH. Prevalence of intestinal protozoa, helminthes, and coccidian infections among primary school children in Thala'a district at Amran governorate, Yemen. Universal J Pharm Res 2023; 8(3):1-6. *https://doi.org/10.22270/ujpr.v8i3.943*
5. Othman AM, Al-Mekhalfi AM. Prevalence of intestinal helminthiasis and their association with eosinophilia among schoolchildren in wadidhahar district at Sana'a governorate, Yemen. Universal J Pharm Res 2020; 5(4):1-8. *https://doi.org/10.22270/ujpr.v5i4.433*
6. Shamsan ENA, De-ping C, Al-Shamahy HA, Al- Hajj MMA, Bo-fan J, Yaogang Z. Coccidian intestinal parasites among children in Al-torbah city in Yemen: In country with high incidence of malnutrition. Universal J Pharm Res 2019; 4(4):1-6. *https://doi.org/10.22270/ujpr.v4i4.301*
7. Zhou Y, Zhu X, Hou H, Lu Y, Yu J, Mao L, *et al*.. Characteristics of diarrheagenic *Escherichia coli* among children under 5 years of age with acute diarrhea: A hospital based study. BMC Infect Dis 2018; 18, 1–10. *https://doi.org/10.1186/s12879-017-2936-1*
8. Al-Mohanadi EMA, Moharem ASS, Al-Moyed KAK, Al-Shamahy HA, Al-Haidari SA, Al-Hadad AM. Cholera in Sana'a, Yemen: Clinical features, risk factors and antibiotic sensitivity of *Vibrio cholerae*. Universal J Pharm Res 2022; 7 (3):1-6. *https://doi.org/10.22270/ujpr.v7i3.772*
9. Sheiban AA, Al-Shamahy HA, Alattab NM, Abbas AKM. Epidemicity of *Vibrio cholera* in Sana'a city, Yemen: Prevalence and potential determinants. Universal J Pharm Res 2018; 2(6):1-6. *https://doi.org/10.22270/ujpr.v2i6.R1*
10. Ogaili MAO, Al-gunaid EA, Al-Shamahy HA, Jaadan BM. Survey of safety practices in diarrheal treatment centers:

Cholera treatment centers in Yemen. Universal J Pharm Res 2020; 5(4). *https://doi.org/10.22270/ujpr.v5i4.432*

11. Danso EK, Asare P, Otchere ID, *et al*. A molecular and epidemiological study of *Vibrio cholerae* isolates from cholera outbreaks in southern Ghana. PLoS One 2020; 15:e0236016. *https://doi.org/10.1371/journal.pone.0236016*
12. Al-Ghaithi GAA, Al-Moyed KA, Al-Shamahy HA, Al-Haddad AM. Prevalence of *Salmonella* and intestinal parasites among food handlers predispose consumers to significant health risks. Universal J Pharm Res 2023; 8(1):1-6. *https://doi.org/10.22270/ujpr.v8i1.890*
13. Larbi RT, Atiglo DY, Peterson MB, *et al*. Household food sources and diarrhoea incidence in poor urban communities, Accra Ghana. PLoS One 2021; 16:e0245466. *https://doi.org/10.1371/journal.pone.0245466*
14. Takyi SA, Amponsah O, Yeboah AS, Mantey E. Locational analysis of slums and the effects of slum dweller's activities on the social, economic and ecological facets of the city: Insights from Kumasi in Ghana. Geo J 2021; 86: 2467–2481. *https://doi.org/10.1007/s10708-020-10196-2*
15. William A, Satija S, Kaur R. Bacterial etiology of diarrhoea in a tertiary care hospital. Int J Recent Sci Res 2020; 11: 39022–39024. *https://doi.org/10.24327/ijrsr.2020.1106.5426*
16. Wang LP, Zhou SX, Wang X, *et al*. Etiological, epidemiological, and clinical features of acute diarrhea in China. Nat Commun 2021; 12, 1–12. *https://doi.org/10.1038/s41467-021-22551-z*
17. World Health Organization. The treatment of diarrhoea: A manual for physicians and other senior health workers. World Health Organization 2005; Report No: 9241593180.
18. Al-Shami HZ, Al-Haimi MA, Al-Shamahy HA, *et al*. Patterns of antimicrobial resistance among major bacterial pathogens isolated from clinical samples in two tertiary's hospitals, in Sana'a, Yemen. Universal J Pharm Res 2021; 6(5):60-67. *https://doi.org/10.22270/ujpr.v6i5.674*
19. Alyahawi A, Alkaf A, Alnamer R, Alnosary T. Study of resistance for recently marketed carbapenem drug among hospitalised patients in Sana'a, Yemen. Universal J Pharm Res 2018; 3(5):1-6. *https://doi.org/10.22270/ujpr.v3i5.203*
20. Saleh AAM, Al-Shamahy HA, Al-Hrazi RMA, *et al*. Biofilm formation and antibiotic susceptibility of uropathogens in patients with catheter associated urinary tract infections in Ibb city -Yemen. Universal J Pharm Res 2020; 4(6). *https://doi.org/10.22270/ujpr.v4i6.329*
21. AL-Magrami RTF, Al-Shamahy HA. *Pseudomonas aeruginosa* skin-nasopharyngeal colonization in the in-patients: prevalence, risk factors and antibiotic resistance in tertiary hospitals in Sana'a city-Yemen. Universal J Pharm Res 2019; 3(6). *https://doi.org/10.22270/ujpr.v3i6.219*
22. Alshamahi EYA, Al-Shamahy HA, Musawa YA, Al-Shami HZ. Bacterial causes and antimicrobial sensitivity pattern of external ocular infections in selected ophthalmology clinics in Sana'a city. Universal J Pharm Res 2020; 5(3), July 2020. *https://doi.org/10.22270/ujpr.v5i3.409*
23. Al-Safani AA, Al-Shamahy H, Al-Moyed K. Prevalence, antimicrobial susceptibility pattern and risk factors of MRSA isolated from clinical specimens among military patients at 48 medical compound in Sana'a city-Yemen. Universal J Pharm Res 2018; 3(3):40-44. *https://doi.org/10.22270/ujpr.v3i3.165*
24. Al-Tahish GAA, Al-Yosaffi EA, Al-Shamahy HA, *et al*. Prevalence of blatem, blashv, and blactx-m genes among esbl-producing *Escherichia coli* isolated from the blood samples of icus patients of university hospitals in Sana'a city, Yemen. Universal J Pharm Res 2024; 8(6):1-6. *https://doi.org/10.22270/ujpr.v8i6.1032*
25. Al-Yosaffi EA, Al-Shamahy HA, Othman AM, Al-Haddad AM, Al-Moyed KA. Antibiotic sensitivity of bacterial bloodstream infections in the intensive care unit patients of university hospitals in Sana'a city, Yemen. Universal J Pharm Res 2023; 8(5):1-8. 2023. *https://doi.org/10.22270/ujpr.v8i5.1004*
26. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2020. *https://clsi.org/media/3481/m100ed30_sample.pdf*
27. Mbuthia OW, Mathenge SG, Oyaro MO, Ng'ayo MO. Etiology and pathogenicity of bacterial isolates: A cross sectional study among diarrheal children below five years in central regions of Kenya. Pan Afr Med J 2018; 31:88. *https://doi.org/10.11604/pamj.2018.31.88.15644*
28. Akuffo R, Armah G, Clemens M, *et al*. Prevalence of enteric infections among hospitalized patients in two referral hospitals in Ghana. BMC Res Notes 2017; 10, 1–7. *https://doi.org/10.1186/s13104-017-2621-x*
29. Kalule JB, Smith AM, Vulindhlu M, *et al*. Prevalence and antibiotic susceptibility patterns of enteric bacterial pathogens in human and non-human sources in an urban informal settlement in Cape Town, South Africa. BMC Microbiol 2019; 19: 1–11. *https://doi.org/10.1186/s12866-019-1620-6*
30. Lompo P, Tahita MC, Sorgho H, *et al*. Pathogens associated with acute diarrhea, and comorbidity with malaria among children under five years old in rural Burkina Faso. Pan Afr Med J 2021; 38:259. *https://doi.org/10.11604/pamj.2021.38.259.15864*
31. Al-Eryani SA, Alshamahi EYA, Al-Shamahy HA, Alfalahi GHA, Al-Rafiq AA. Bacterial conjunctivitis of adults: Causes and ophthalmic antibiotic resistance patterns for the common bacterial isolates. Universal J Pharm Res 2021; 6, (1). *https://doi.org/10.22270/ujpr.v6i1.535*
32. Al-Khamesy KSA, Al-Shamahy HA. Assessment of the present bacteriological profile and antibiotic sensitivity pattern in chronic suppurative otitis media in Sana'a, Yemen. Universal J Pharm Res 2023; 8 (5):1-6. *https://doi.org/10.22270/ujpr.v8i5.1010*
33. Basnet B, Niroula D, Acharya J, Basnyat S. Antibiotic susceptibility pattern of *Shigella* spp. isolated from patients suspected of acute gastroenteritis. J Instr Sci Technol 2021; 26: 1–7. *https://doi.org/10.3126/jist.v26i2.41261*
34. Pourakbari B, Mamishi S, Mashoori N, *et al*. Frequency and antimicrobial susceptibility of *Shigella* species isolated in children medical center hospital, Tehran, Iran, 2001-2006. Braz J Infect Dis 2010; 14: 153–157. *https://doi.org/10.1016/S1413-8670(10)70029*
35. Ashkenazi S, Levy I, Kazaronovski V, Samra Z. Growing antimicrobial resistance of *Shigella* isolates. J Antimicrob Chemother 2003; 51: 427–429. *https://doi.org/10.1093/jac/dkg080*
36. Díaz-Jiménez D, García-Meniño I, Herrera A, *et al*. Genomic characterization of *Escherichia coli* isolates belonging to a new hybrid aEPEC/ExPEC pathotype O153: H10- A-ST10 eae-beta1 occurred in meat, poultry, wildlife and human diarrheagenic samples. Antibiotics 2020; 9:192. *https://doi.org/10.3390/antibiotics9040192*
37. Kennedy C, Walsh C, Karczmarczyk M, *et al*. Multi-drug resistant *Escherichia coli* in diarrhoeagenic foals: Pulsotyping, phylotyping, serotyping, antibiotic resistance and virulence profiling. Vet Microbiol 2018; 223: 144–152. *https://doi.org/10.1016/j.vetmic.2018.08.009*
38. Wilcox MH. The tide of antimicrobial resistance and selection. Int J Antimicrob Agents 2009; 34, S6–S10. *https://doi.org/10.1016/S0924-8579(09)70550-3*
39. Andoh L, Dalsgaard A, Obiri-Danso K, *et al*. Prevalence and antimicrobial resistance of *Salmonella* serovars isolated from poultry in Ghana. Epidemiol Infect 2016; 144: 3288–3299. *https://doi.org/10.1017/S0950268816001126*