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# Sorbitol MacConkey agar and wastewaters` coliphages

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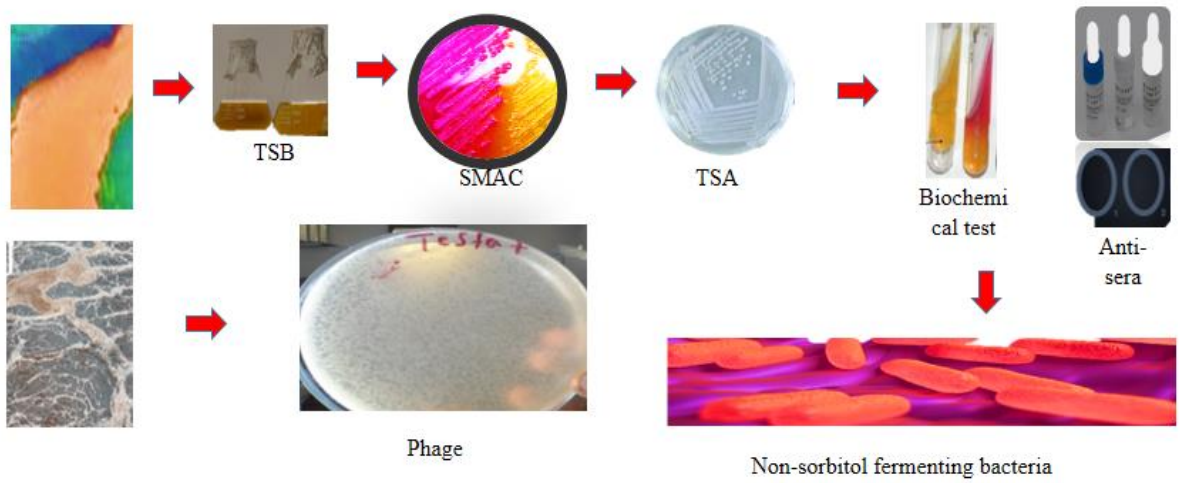
## GRAPHICAL ABSTRACT

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## ABSTRACT

A total of 114 samples collected from hospital wastewaters and rivers in Addis Ababa, Ethiopia were tested for non-sorbitol fermenting bacteria and coliphages. Sorbitol MacConkey agar is mainly used in the detection of *E. coli* O157:H7. However, other emerging diarrhoeagenic enteropathogens such as *Plesiomonas shigelloides*, *Edwardsiella tarda*, *Providencia alcalifaciens*, *Escherichia albertii, Escherichia vulneris* and *Escherichia fergusonii* were detected in the samples using this medium. Information for most of the emerging enteropathogens is scarce in most countries including Ethiopia. A total of 20 different genera, 38 species of non-sorbitol fermenting bacteria were isolated. *Escherichia coli* O157 could not be detected from any of the samples. All these backgrounds may mask the detection of *Escherichia coli* O157. Even if sorbitol MacConkey agar has several background limitations, different emerging diarrhoeagenic non-sorbitol fermenting bacteria were detected in the majority of the rivers and hospitals` wastewaters samples. The correlation between coliphages and non-sorbitol fermenting bacteria were not significant. As several bacteria have been isolated on sorbitol MacConkey agar medium, it is essential that the most selective laboratory techniques will be desired for outbreak investigation of *E. coli* O157, but other non-sorbitol fermenting enteropathogens should also be detected using sorbitol MacConkey agar in low resources countries.

## 1. Introduction

Due to the growth of human population, fecal pollution of the waterways has increased in the urban cities (Mullan, Dueker and Juhl, 2017). Different wastewaters and rivers contain various harmful pathogens (Uddin *et al.*, 2019). Contamination of water by pathogenic bacteria is a worldwide public health problem. Regardless of the advances in water and food sanitation and management, water and foodborne diseases

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continue to happen even in several industrialized countries (Guzman-Herrador, *et al*., 2012; Canizalez-Roman, *et al.*, 2013; Boisen, *et al*., 2015). The diseases can be acquired *via* a direct contact with water used for irrigation or *via* consumption of foods such as vegetables and fruits contaminated with irrigation waters. Wastewater and contaminated surface water used for irrigation of vegetables and other food products of plant origin used for human consumption, are sources of foodborne outbreaks (Canizalez-Roman *et al.*, 2019).

In most resource limited countries, there is no effective infrastructure for water management. Majority of the population in these countries relay on highly contaminated and untreated surface water (Limaheluw *et al*., 2019). There are no facilities to treat wastewater that is directly discharged into rivers (Islam, Sokolova and Hofstra, 2018). Such polluted water bodies are dangerous to human beings, animals, birds and fish, and water from these sources is not suitable for agriculture, drinking, industry and recreation purposes (Uddin *et al.*, 2019).

The water bodies are extremely contaminated with pathogenic and indicator organisms including *Escherichia* coli (*E. coli*) (Luther *et al.*, 2016). *E. coli* is a member of Enterobacteriaceae family which is oxidase negative, Gram negative, non-sporulating facultative anaerobic and rod shaped bacterium (Croxen *et al.*, 2013). Most of the *E. coli* strains are parts of the normal flora of the gut of humans and warm-blooded animals (Canizalez-Roman *et al.*, 2019). There are some pathogenic variants of *E. coli* associated with diarrhoeal illness. Six of the major diarrhoeagenic variants are shiga toxin producing *E. coli,* enterotoxigenic *E. coli,* enteropathogenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli* and adherent invasive *E. coli* (Croxen, Law, Scholz, Keeney, *et al.*, 2013). Of these pathotypes, only shiga toxin producing *E. coli* has been routinely tested by most public health and clinical laboratories (Gould *et al.*, 2012).

There are other Enterobacteriaceae that cause gastrointestinal infection and various extraintestinal diseases especially in immunosuppressed individuals. Some of these pathogens are *Escherichia fergusonii* (*E. fergusonii)* (Mahapatra and Mahapatra, 2005), *Escherichia vulneris* (*E. vulneris)* (Jain *et al.*, 2016), *Edwardsiella tarda* (*E. tarda)* (Schlenker, Fellow and Surawicz, 2009), *Providencia alcalifaciens* (*P. alcalifaciens)* (Shah, Odoyo and Ichinose, 2019), *Plesiomonas shigelloides* (*P. shigelloides)* (Janda, Abbott and Mciver, 2016), *Raoultella ornithinolytica* (*R. ornithinolytica*) (Seng *et al.*, 2016), and *Escherichia albertii (E. albertii*) (Namdari, 1985). These emerging enteropathogenic bacteria possess sufficient clinical importance and high sufficient frequency (Janda and Abbott, 2011).

*E. coli* is used routinely in monitoring fecal pollution in various environmental waters (Wanjugi *et al.*, 2016). Recently, somatic and male coliphages testing has been included in international guidelines as the major water safety parameters. The enumeration of somatic and male coliphages by the *E. coli* host strain CB390 is cost effective (Campos *et al.*, 2019). These bacteriophages are non-pathogenic viruses that infect *E. coli*. The phages are proposed as fecal and viral pollution indicators. Bacteriophages are classified into two operational groups, somatic and male or F- specific bacteriophages. Somatic phage infects *E. coli via* the cell wall whereas male coliphage infects *E. coli via* sex pili. The combination of both coliphages is called total coliphages (Mcminn *et al.*, 2017).

Since advanced laboratory equipment and methods are not always possible in resource limited countries because of shortage of well-trained human resources and high cost (Hussein *et al.*, 2019), non-sorbitol fermenting *E. coli* has been isolated by conventional culture techniques from various sources (Lupindu *et al.*, 2016; Iijima *et al.*, 2017). No information is available for the most emerging enteropathogenic bacteria in most countries including Ethiopia. The objective of the present study was to evaluate sorbitol MacConkey selective agar for the growth of non-sorbitol fermenting bacteria and the contamination level of hospitals wastewaters and rivers water samples in Addis Ababa, Ethiopia.

## 2. Materials and methods

### 2.1. Study design and sample collection

A cross-sectional study was conducted between February and April, 2017 on hospital wastewaters and rivers that pass through the sub-cities of Addis Ababa, Ethiopia. A total of 114 water samples, 30 samples from hospitals wastewaters and 84 samples from 32 polluted rivers were collected using sterile glass containers. All the samples were collected manually and transported to Ethiopian Public Health Institute using cold chain. The samples were processed within 24 h of collection. The criteria for choosing rivers and wastewaters are due to the extremely pollutions of these sources by various discharges, poor sanitation, and settlements mainly in sub-Saharan Africa (Odero *et al.* 2023). These sources are used for irrigation that pose health problems (Nezami and Aghlmand, 2023). Research findings related to wastewaters and rivers significantly govern their pollutions (Pan *et al*, 2020). Wastewaters and surface waters used as a reservoir for most outbreak associated pathogens Rozman *et al*., 2020.

In order to isolate non-sorbitol fermenting bacteria, including O157 *E. coli*, hospitals wastewaters and polluted rivers water samples of 40 ml were inoculated and mixed in equal volume of 40 ml tryptic soya broth (TSB, Oxoid), followed by incubation at 44.5 °C for 24 h. Then, sorbitol MacConkey agar (SMAC) medium was inoculated and incubated at 37 ºC for presumptive identification of non-sorbitol fermenting bacteria (LeJeune J *et al.*, 2001).

### 2.1. Enumerations of coliphages

The somatic and male-specific coliphages simultaneous detection from hospitals wastewaters and polluted rivers water samples was performed using host *E. coli* CB390 (obtained from North Carolina *University***,** Chapel Hill) using single agar layer (SAL) plaque assay. The log phase of the host containing ampicillin, 0.15% with magnesium chloride in tryptic soy agar (double strength) [Difco] was applied. Plaques, lysis zone formation in bacterial host lawn, were enumerated after 16-24 h at 37 ºC per plate for positive total coliphages and the absence of clear zone was taken as coliphage negative. Enumeration of the phages was employed per 100 mL of the sample (Sobsey, 2014).

### 2.2. Biochemical tests

For the identification of non-sorbitol fermenting isolates, colorless bacterial colonies were subcultured onto trypticase soya agar and incubated at 37 °C for 24 h. Then, the isolates were subjected to different biochemical tests and incubated at 37 °C for 24-48 h. The biochemical tests used for the isolation of these bacteria were sorbitol, indole, citrate utilization, motility, Voges–Proskauer, methyl red, triple sugar iron, urease, Beta-galactosidase, Ornithine, lysine iron agar and hydrogen sulphide. The identification of the bacteria was interpreted using online Enterobacteriaceae, Pseudomonas and non-fermenters identification software database (https://www.tgw1916.net/bacteria_logare_desktop.html).

### 2.3. Serological test

Biochemically confirmed non-sorbitol fermenting *E. coli* colonies were serotyped by commercial *E. coli* O157 antisera (Oxoid, UK). The colonies were emulsified using normal saline on microscopic slide to form suspension and a drop of *E. coli* O157 antisera was added and mixed. The mixture was shaken for 2 minutes and tested for agglutination. The presence of agglutination showed positive result, whereas its absence showed negative result following the manufacturer instructions. *E. coli* O157:H7 (ATCC 43889) were confirmed positive with O157 and H7 antisera.

### 2.4. Data analysis

SPSS version 20 (SPSS Inc. Version 20, Chicago, Illinois) was used to analyze the data. Kruskall Wallis test was used to see the differences in non-sorbitol fermenting bacteria and total phages values by sub-cities and water sources. The level of significance was established at p value $\leq$ 0.05.

## 3. Results and discussion

### 3.1. Emerging pathogenic non-sorbitol fermenting bacteria

On *E. coli* O157 selective sorbitol MacConkey agar, both non-sorbitol fermenting and sorbitol fermenting bacteria were grown from all the 114 samples of rivers and hospitals` wastewaters. Colorless colonies on sorbitol MacConkey selective agar were examined for 11 conventional biochemical and *E. coli* O157 slide agglutination tests. *E. coli* O157 antisera was tested only for *E. coli* with colorless colonies.

*E. coli* O157 was not detected in any of the sample. However, *Plesiomonas shigelloides*, *Edwardsiella tarda*, *Providencia alcalifaciens*, *Escherichia albertii*, *Escherichia fergusonii*, and *Escherichia vulneris* were detected in rivers and hospitals` wastewaters (Table 1). The evidence about the absence of *E. coli* O157 in rivers and hospital wastewaters samples in the present study may not reflect the absence of clinical infections in human beings (Bonetta *et al.*, 2016). This may be due to *E. coli* O157 selective cultivation shortcomings in these samples with heavy growth background (Müller and Ehlers, 2005). The capability of *E. coli* O157 to be isolated from mixed culture is reliant on the number of background microbes (Durso, 2013). This is predominantly true while examining hospital wastewaters or polluted

rivers` samples that have the highest number of these background floras.

### 3.2. Non-sorbitol fermenting bacteria confused with *E. coli* O157

The background growth number of sorbitol fermenting colonies on sorbital MacConkey agar was very high compared to non-sorbital fermenting colonies for each sample. A total of 20 different genera, 38 species of non-sorbitol fermented bacteria were isolated on this medium. Non-sorbitol fermenting *E. coli was detected in* 41 of 84 of rivers and 19 of 30 hospital wastewaters samples. Whereas non-sorbitol fermenting bacteria other than *E. coli* were detected in 43 rivers and 11 of wastewaters samples.

**Table 1**. Occurrence of non-sorbitol fermenting enteropathogenic bacteria and coliphages in rivers and hospitals` wastewaters (n, %).

| S. no. | Pathogenic bacteria (n, %) | Total detection rate (n, %) | In rivers (n, %) | In HWW (n, %) | Phage in bacterial presence |
|---|---|---|---|---|---|
| 1 | *E. fergusonii* | 29 (25.4) | 22 (19.3) | 7 (6.1) | 11 (9.6) |
| 2 | *E. vulneris* | 22 (19.3) | 11 (9.6) | 11 (9.6) | 6 (5.2) |
| 3 | *E. tarda* | 5 (4.4) | 0 | 5 (4.4) | 0 |
| 4 | *P. alcalifaciens* | 4 (3.5) | 3 (2.6) | 1 (0.9) | 3 (2.6) |
| 5 | *P. shigelloides* | 3 (2.6) | 2 (1.8) | 1 (0.9) | 0 |
| 6 | R. ornithineolytica | 3 (2.6) | 3 (2.6) | 0 | 2 (1.8) |
| 7 | *E. albertii* | 1 (0.9) | 0 | 1 (0.9) | 0 |

HWW– Hospital wastewaters, n – frequency, %– Percentage

On SMAC, non-sorbitol fermenting colonies appeared as colorless or amber like, while sorbitol fermenting colonies appeared as pink. In this medium, lactose is replaced by sorbitol. Since most *E. coli* strains can ferment sorbitol, *E. coli* O157 can be differentiated by its sorbitol non-fermenting characteristic (90%). This phenotypic characteristic is well known to isolate the bacteria from environmental samples using this medium (Müller and Ehlers, 2005). However, *E. coli* O157 isolation among non-sorbitol fermenting colonies from samples such as polluted waters is not easy. In SMAC, sorbitol is an important fermentable carbohydrate. Crystal violet and bile salts make the medium selective by inhibiting Gram-positive bacteria and letting Gram negative organisms to grow. Neutral red indicates the pH of the medium (Murray *et al.*, 2007). *Escherichia* spp. and non-*E. coli* bacteria in rivers water and hospitals` wastewaters samples are shown in Table 2.

**Table 2**. Sorbitol non-fermenting bacteria isolated from rivers and hospitals` wastewaters samples.

| No. | Sorbitol negative bacteria | Frequency, % |
|---|---|---|
| 1. | *Acinetobacter johnsonii* | 1 (0.9) |
| 2. | *Brevundimonas diminuta* | 1 (0.9) |
| 3. | *Burkholderia multivorans* | 1 (0.9) |
| 4. | *Buttiauxella agrestis* | 1 (0.9) |
| 5. | *Citrobacter farmeri* | 1 (0.9) |
| 6. | *Citrobacter koseri* | 2 (1.8) |
| 7. | *Citrobacter murliniae* | 1 (0.9) |
| 8. | *E. adecarboxylata* | 4 (3.5) |
| 9. | *E. hermanni* | 3 (2.6) |
| 10. | *E. coli 2* | 1 (0.9) |
| 11. | *Edwardsiella hoshinae* | 1 (0.9) |
| 12. | *Edwardsiella ictaluri* | 2 (1.8) |
| 13. | *Edwardsiella tarda* | 5 (4.4) |
| 14. | *Enterobacter gergoviae* | 1 (0.9) |
| 15. | *Raoultella ornithinolytica* | 3 (2.6) |
| 16. | *Klebsiella pneumoniae subsp. ozanae* | 1 (0.9) |
| 17. | *Kluyvera ascorbata* | 2 (1.8) |
| 18. | *Kluyvera georgiana* | 1 (0.9) |
| 19. | *Morganella morganii biogroup 1* | 1 (0.9) |
| 20. | *Morganella morganii subsp. sibonii* | 1 (0.9) |
| 21. | *Photorhabdus asymbiotica subsp. asymbiotica* | 3 (2.6) |
| 22. | *Plesiomonas shigelloides* | 3 (2.6) |
| 23. | *Proteus myxofaciens* | 2 (1.8) |
| 24. | *Providencia alcalifaciens* | 4 (3.5) |
| 25. | *Providencia rettgeri* | 4 (3.5) |
| 26. | *Providencia stuartii* | 2 (1.8) |
| 27. | *Pseudomonas xiamenensis* | 2 (1.8) |
| 28. | *Serratia bongori* | 1 (0.9) |
| 29. | *Serratia entomophila* | 1 (0.9) |
| 30. | *Stenotrophomonas maltophilia* | 1 (0.9) |
| 31. | *Xenorhabdus bovienii* | 1 (0.9) |
| 32. | *Xenorhabdus japonica* | 1 (0.9) |
| 33. | *Yersinia ruckeri* | 2 (1.8) |
| 34. | *Shimwellia blattae* | 1 (0.9) |
| | *Total* | 54 (47.4) |

### 3.3. Non-sorbitol fermenting isolates, non-*E. coli* O157

In the majority of cases, SMAC has been recommended for *E. coli* O157 isolation due to its simplicity and low cost (Ammon, Petersen and Karch, 1999). However, several bacteria other than *E. coli* O157, mainly other *E. coli* strains and *Proteus* species, do not ferment sorbitol that can be initially confused with *E. coli* O157 (Chapman *et al.*, 1991). The reports of SMAC selectivity for *E. coli* O157 vary extensively. In study done by (Osuolale and Okoh, 2017), the pathogen was isolated from 41.7% to 45.8% of samples tested, whereas other researchers, (Osuolale and Okoh, 2017), isolated *E. coli* O157 from SMAC in none of the samples. The bacterium might have been missed and can consume substantial cost and time of the testing laboratories (Chapman *et al.*, 1991).

Also, *E. coli* O157 is able to enter into a state that is viable but non-culturable. This state is a phase in which the bacterium remains alive, but unable to form colonies on routine culture media which usually support their growth. Entry into a state of viable but non-culturable is a survival mechanism for non-sporulating bacteria under stressful environment conditions. This state can lead to false negative result and such pathogens mainly remain food safety and public health hazard due to the incapability of standard detection techniques to isolate the bacteria correctly (Liu *et al.*, 2016; Cao *et al.*, 2019).

Furthermore, *E. coli* O157 strains do not ferment sorbitol, a means commonly used to distinguish it from other *E. coli*. However, scientific studies conducted by various researchers revealed that *E. coli* O157 strains detected were unusually fermenting sorbitol after overnight

incubation. Marejková *et al*., 2013 found that 5 of 39 (12.8%) *E. coli* O157 detected were fermented sorbitol after 24 h incubation.

This leading waterborne *E. coli* O157 has been frequently detected from patients with gastroenteritis and from various environmental samples including polluted waters (Uddin *et al.*, 2019). Its prevalence in wastewater has been assessed in various investigation, presenting several positive samples for this bacterium (Garci and Blanch, 2006). The presence of *E. coli* O157 was also observed in rivers or other water samples (Kristiani *et al.*, 2019). In a study conducted in Mexico, *E. coli* O157 was detected in 14% of various water samples including river water and irrigation canal (Canizalez-Roman *et al.*, 2019). Its occurrence in these waters can reveal clinical infections that have been prevalent in human beings (Bonetta *et al.*, 2016).

*E. coli* O157 is a common pathogen that is predominantly carried by cattle and other ruminants. An important mode of infection by *E. coli* O157 in human or animal is contaminated water primarily used for irrigation (Myataza *et al.*, 2017). Rivers are highly contaminated with *E. coli* O157 as a result of ongoing sewage water discharge into them (Uddin *et al.*, 2019). *E. coli* O157 is one of the major water and foodborne pathogenic bacteria that may lead to numerous health disorders. The major prevalent subgroup producing toxin is Enterohemorrhagic *E. coli,* serotype O157. Shiga toxin producing *E. coli* is known as enterohemorrhagic *E. coli* or verocytotoxin producing *E. coli* and it includes O157 serotypes*.* The pathotypes related to intestinal illness are known as diarrhoeagenic *E. coli*. *E. coli* O157 occurs due to fecal runoff into streams and rivers (Tsiraki *et al.*, 2018).

Food and waterborne illness outbreaks due to this zoonotic pathogen have been frequently reported globally (Kristiani *et al.*, 2019). In 14 years survey in Nordic countries, pathogenic *E. coli* has been the third most common waterborne outbreak involved (Kuusi, 2012). About 25% of African countries have reported *E. coli* O157 isolation from food, humans, animals or the environment (Yakubu *et al.*, 2018). Shiga toxin producing *E. coli* incidence considerably higher in developing regions, but there are no sufficient surveillance data existing for these regions. This pathogen is known to cause intestinal and extraintestinal diseases in human being that can affect gastrointestinal, bloodstream, urinary tract, and central nervous systems (Kristie *et al.*, 2013).

Of 38 species of non-sorbitol fermenting bacteria isolated on SMAC medium in the current study, the major isolated bacteria on this medium were non-*E. coli* O157 *Escherichia* spp. (53.5%) followed by *Providencia* spp. (8.8%) and *Edwardsiella* spp. (7%). *E. fergusonii* was the major bacteria isolated from Escherichia genera (25.4%).

In a study assessed sorbitol non-fermenters on selective agar of *E. coli* O157 (Müller and Ehlers, 2005), majority of the sorbitol fermenting bacteria isolated were Burkholderia glumae (19.5% of isolates). Other non-sorbitol fermenting bacteria including *Proteus* spp., *Providencia* spp. and *Morganella* spp. found in environmental samples can be confused with the potential pathogen *E. coli* O157 (Chapman, 2000).

The presence of other enteropathogenic bacteria such as *E. fergusonii, E. vulneris, E. tarda*, *P. alcalifacien*, *P. shigelloides*, *R. ornithinolytica*, and *E. albertii* in 58.8% of hospitals wastewaters and rivers` water samples in the present study suggests that these water bodies may be the main reservoirs of the bacteria, and designates a high risk for the pathogens.

The predominant bacterial pathogen detected in the rivers and hospitals` wastewaters samples was *E. fergusonii (*25.4%). *E. fergusonii* is a rod shaped, Gram negative non-lactose fermenting Enterobacteriaceae which is an emerging human pathogen. It has been isolated from stool, extraintestinal samples and food of animal origin. It has also been associated with hemolytic uremic syndrome and acute kidney injury after bacteremia (Chun and Hong, 2019).

*E. fergusonii* was found in rivers samples in large number than hospitals wastewaters. In the presence of *E. fergusonii*, total coliphges were found in 9.6% of the samples. The presence of *E. fergusonii* pathotypic strains in water sources and their ingestion by humans and animals can cause public health problems. Maheux *et al* isolated *E. fergusonii* from water, characterized the bacterium and found pathogenic genes in 83% of the strains with *E. albertii* (Maheux *et al.*, 2014).

The second predominant enteropathogen detected in the rivers and hospitals` wastewaters samples in Addis Ababa, Ethiopia was *E. vulneris (*19.3%). *E. vulneris* is an opportunistic pathogen of human that causes unusual complicated diarrhoea with severe complications including sepsis in children. It has also been observed in other immunosuppressed individuals and adults (Jain *et al.*, 2016). In the present study, *E. vulneris* was found in rivers water samples in equal proportion to hospitals wastewaters samples. In the presence of *E. vulneris*, total coliphges were found in 5.2% of the samples. It has been detected in environmental samples (Yu *et al*., 2021), and it is reported from humans with gastroenteritis as an important health issue (Jain *et al.*, 2016).

The other emerging enteropathogen, *E. tarda* was found in hospitals wastewaters in Addis Ababa. *E. tarda* is a family of Enterobacteriaceae, motile, rod shape, facultatively anaerobic and Gram negative bacterium (Hirai, Asahata-tago and Ainoda, 2015). *E. tarda* is the only pathogenic species in the *Edwardsiella* genus for human being (Humphries and Linscott, 2015). *E. tarda* causes Salmonella like gastroenteritis with bloody diarrhoea in humans which can finally lead to systemic infections and extraintestinal illness (Schlenker *et al*., 2009). In the presence of *E. tarda*, there was no detection of total coliphages. About 80% of *E. tarda* illnesses reported in human are attributed to gastrointestinal tract. *E. tarda,* one of the marine bacteria (Zhang *et al.*, 2020) that has worldwide distribution, but it is commonly found in tropical and subtropical zones. The risk factors for acquiring diarrhoea due to *E. tarda* are consumption of contaminated foods such as fish and ingestion of contaminated water. These contaminated water and food ingestions introduce the bacterium into the gastrointestinal tract (Humphries and Linscott, 2015). In immunocompromised individuals including patient with liver cirrhosis, *E. tarda* causes severe water and foodborne infection that results in death (Hirai *et al*., 2015).

Furthermore, *P. alcalifaciens* was detected in the rivers` water and hospitals` wastewaters samples in the present study (3.5%). *P. alcalifaciens* is a member of Enterobacteriaceae family that raises public health concern in developing and developed countries (Shah *et al*., 2019). It is an emerging pathogen and well known to cause gastroenteritis in travellers and children. *P. alcalifaciens* has been associated at least with three outbreaks of gastrointestinal illness, comprising large outbreak involving more than 270 children in Japan that offers clear confirmation that *P. alcalifaciens* is a causative agent of the gastroenteritis. Foodborne outbreak caused by *P. alcalifaciens* was reported in 2013 in Kenya Japan and Turkey. *P. alcalifaciens* is usually considered to be a non-pathogen in the intestine, and most laboratories do not distinguish it as a severe cause of diarrhoea (Shah *et al.*, 2015; Shah *et al*., 2019).

Similarly, *P. shigelloides* was detected in the rivers` water and hospitals` wastewaters samples in 2.6%. *P. shigelloides* is the only bacterium of the genus Plesiomonas and it is the only oxidase positive Enterobacteriaceae, facultative anaerobic gram negative rod organism (Pence, 2016). *P. shigelloides* is an enteric pathogen that causes water and foodborne intestinal and various extraintestinal illnesses in any age group, with immunosuppressed persons and numerous studies have recorded infections in children (Tseng *et al.*, 2020).

*P. shigelloides* has global distribution, occurs mostly when freshwater temperature rises and its proliferation is increased through sewage contamination. The pathogen has found a high rate of isolation with other enteric pathogens with a high rate of outbreaks, at least 11 in different countries (Abbott and Mciver, 2016). Its water and foodborne outbreaks occur mainly due to the consumption of food products that are contaminated with wastewaters (Humphries and Linscott, 2015). *P. shigelloides* appear as secretory gastroenteritis, dysenteric colitis, or persistent diarrhoea forms (Kelly, 2011). *Plesiomonas* was found to be the 3rd and 4th ranking in gastroenteritis cause in Nigeria, respectively (Onyemelukwe, 2014) and China (Chen *et al.*, 2013).

*R. ornithinolytica* was detected only in rivers` water samples in the current study. *R. ornithinolytica* is an encapsulated non-motile Gram negative Enterobacteriaceae, aerobic rod bacteria. *R. ornithinolytica* was formerly known as *K. ornithinolytica*. Based on current molecular approaches, this organism was reclassified as Raoultella. *R. ornithinolytica* is found in various water environments. The pathogenic ability of *R. ornithinolytica* isolates in human illness has become significant (Seng *et al.*, 2016). In the cases of infection study in four university hospital centers, France, 13% of 112 cases of *R. ornithinolytica* identified were responsible for gastrointestinal infections. Among the reported cases, the death rate of infections to human caused by this virulent pathogen was comparatively high (20%) (Seng *et al.*, 2016).

This study reveals that *E. albertii* was found in wastewaters in Addis Ababa, Ethiopia. *E. albertii* is an emerging enteric pathogen of human. The significant phenotypic characteristics differentiating *E. albertii* from *E. coli* comprise a negative reaction for indole and the inability to ferment D-sorbitol and lactose (Nimmervoll *et al.*, 2014). Phylogenetic studies show that *E. albertii* is a member of *Shigella boydii* 13 that known not to belong to true shigellae (Hyma *et al.*, 2005). *E. albertii* is a pathogen isolated from the stool of humans with gastroenteritis which causes diarrhoea. *E. albertii* has been isolated from active surveillance in Brazil (Ori *et al.*, 2019). This pathogen has also been recently reported from an outbreak of gastroenteritis in humans in Japan involving 48 individuals (Ooka, 2013).

### 3.4. Occurrence of coliphages in wastewaters and rivers

Out of the total samples examined in Addis Ababa, Ethiopia, coliphages were detected in 47 (41.2%) samples, 10% hospitals' wastewaters and 52.4% polluted urban rivers sources. The presence of coliphages in hospitals' wastewaters and polluted urban rivers` water samples indicates fecal pollution and hence presence of bacterial pathogens (*World Health Organization*, 2011). The presences of sorbitol non-fermenting *E. coli* and sorbitol non-fermented bacteria other than *E. coli* in the presence of coliphages are shown in Table 3.

The correlation coefficient r of the Spearman's rank results was significant at the 0.01 level (2-tailed). The correlation test showed that there were not statistically significant correlations between coliphages and non-sorbitol fermenting *E. coli* or non-sorbitol fermenting non-*E. coli*. Rho values of non-sorbitol fermenting *E. coli* and non-sorbitol fermenting non-*E. coli* to total coliphages were -0.052 and 0.052, respectively. This indicates, there was no significant relationship between coliphages and sorbitol non-fermenting bacteria in the current study. P-values for non-sorbitol fermenting *E. coli* and non-sorbitol fermenting non-*E. coli* using Kruskal Wallis test for rivers and hospitals` wastewaters samples by sample type and sub-city were >0.05. This revealed that non-sorbitol fermenting *E. coli* and non-sorbitol fermenting non-*E. coli* differed statistically by sample type and sub-city.

**Table 3**. The number of non-sorbitol fermenng *E. coli* and non-sorbitol fermenting bacteria other than *E. coli* in the presence of coliphages in rivers and hospitals` wastewaters (PFU/100mL).

| Detected bacteria | Sample type | Total coliphages ranges | | | | Total |
|---|---|---|---|---|---|---|
| | | <1.0×10[1] | 10-99 | 100-999 | 10[3]-5200 | |
| Sorbitol non- fermenting *E. coli* | Rivers | 21 | 6 | 11 | 3 | 41 |
| | HWW | 16 | 0 | 2 | 1 | 19 |
| Sorbitol non- fermenting other bacteria | Rivers | 19 | 6 | 11 | 7 | 43 |
| | HWW | 11 | 0 | 0 | 0 | 11 |
| Total | | 67 | 12 | 24 | 11 | 114 |

PFU–Plaque forming unit, HWW–Hospital wastewater

## 3. Conclusions

Even if *E. coli serotype* O157 could not be detected from any of the rivers` water and hospitals` wastewaters samples in Addis Ababa, seven different emerging diarrhoeagenic non-sorbitol fermenting bacteria and coliphages were detected in the majority of the samples. As several other non-sorbitol fermenting bacteria have been isolated on sorbitol MacConkey medium, it is essential that the most selective laboratory desired for surveillance and outbreak investigations of *E. coli* O157. Unless the contamination level of the hospital wastewaters and rivers water samples are monitored for various microbes by health sectors, food safety and environmental authorities, polluted waters can pose a high risk for humans and animals health and affects crops production and other water sources including drinking water. Hence efforts should be continued for better detection of *E. coli* O157 and other non-sorbitol fermenting enteropathogens from waste water and rivers for early detection of outbreaks related to *E. coli* O157 pathogens. In addition, further investigations are needed to discover certain risk assessment tools related to simple and cheap methods of pathogen detection in rivers and wastewater.

### Author Contributions

Tesfaye Legesse: Writing the manuscript, the concept, design, analysis and interpretation of data.
Teshome Belachew, Adey Feleke, Fitsum Tigu, Dagim Jirata, Kassu Desta T, Samson Girma, Firehiwot Abera, Kaleab Sebsibe, Tatek Kasim, Waktole Gobena, Elias Seyoum, Dejenie Shiferaw, Getinet Fikresilasie, Geremew Tassew, Tigist Yohannis, and Asnake Desalegn: drafting, revision and approval of the final version.

### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Data Availability Statement

Data are however available from the authors upon reasonable request and with permission of Ethiopian Public Health Institute.

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