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ORIGINAL ARTICLE

Food Microbiology, Safety and Toxicology

Investigating bacterial gastroenteritis prevalence in the population of Tizi-Ouzou city under the context of social distancing and barrier measures

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ABSTRACT

Background: Epidemics of gastroenteritis are responsible for more than one million consultations in general medicine each year. **Aims:** This study aimed to isolate and identify the main bacteria at the origin of gastroenteritis, and to follow the distribution of these pathogenic bacteria within the population to predict hygiene conditions in the city of Tizi-ouzou. **Material and Methods:** Our study was conducted during the summer of 2022 on 106 liquid and semi-liquid stool samples. **Results:** The results of direct examination and fecal culture revealed the absence of pathogenic organisms, which constitutes a promising result. **Conclusions:** The findings correlate with a rise in COVID-19 cases during the survey period. Hence, the probable reason for the absence of pathogenic germs is the efficacy of social distancing and barrier measures, specifically in curbing the transmission of fecal-oral contaminations.

Keywords: Bacterial gastroenteritis, stool culture, diarrhea, COVID pandemic.

ARTICLE INFORMATION

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1 Introduction

An individual's health is contingent upon factors such as their biology, lifestyle, and environment. This is due to the consistent exposure of the human body to pathogenic microorganisms, some of which have the potential to cause infectious diseases like gastroenteritis [1]. Gastroenteritis is an inflammatory bowel disease of common infectious etiology manifested by diarrhea, often accompanied by nausea, vomiting, abdominal pain and fever [2]. Diarrhea, as a main symptom of gastroenteritis, stands as a prevalent reason for medical consultations in industrialized countries. Conversely, in less developed regions such as Africa, Asia, and Latin America, it leads to an annual toll of 5 to 10 million deaths. Notably, in children and the elderly, its elevated morbidity and mortality rates make it a primary factor for hospitalizations [3].

While gastroenteritis typically presents as an individual occurrence, it can also manifest epidemiologically, such as in hospital or residential outbreaks or cases of food poisoning. Its primary cause is typically infectious, stemming from three categories of microorganisms. Yet, bacteria are the most common culprit. However, it can also result from diverse factors like toxins, pharmacological agents, food-related issues, or serve as a manifestation of digestive or systemic diseases [4, 5]. In some cases, one can get allergic gastroenteritis (eosinophilic gastroenteritis). Gastrointestinal infections are more prominent in geographic regions with poor sanitation and health systems. Their severity varies considerably, ranging from simple discomfort to systemic involvement resulting in death from dehydration within hours. In the most severe cases, appropriate and timely treatment significantly reduces mortality [6]. Despite this health concern, it has been the subject of relatively few etiological studies in Algeria. This is the reason we were interested in this subject of bacterial gastroenteritis, and we carried out a follow-up of the population to determine the prevalence of the pathogenic bacteria responsible for gastroenteritis, during COVID-19 outbreak, at the University Hospital Center of Tizi-ouzou.

2 Material and Methods

2.1 Purpose, duration and location of the study

The main objective of our study was to prospect and search for pathogenic bacteria causing gastroenteritis from an extremely diversified flora, for patients admitted to the Tizi-Ouzou University Hospital during the summer period of 2022. The second objective being to follow up on the state of health and monitor the distribution of germs responsible for diarrhea within the population, thus making an observation on the sanitary conditions.

As viral and cell biology analyzes were not available at the central microbiology laboratory of the university hospital, the analysis was limited only to the bacteriological flora. This study was carried out at the microbiology laboratory of university hospital. The samples were collected from different departments of the hospital and from a private laboratory.

2.2 Sampling

According to Berthélémy [7], in order to obtain an adequate sample several steps must be followed with respect to sampling, transportation, diagnostic approach and microscopic examination. The samples were collected during the first days of the disease and before the start of antibiotic therapy. The prescription of a single stool culture is generally sufficient. The stools were collected as soon as they were issued. An aliquot of the stool was removed using a spatula or a spoon-flask. A rectal swab can be useful especially in infants and young children, and in particular in the context of post-diarrhea hemolytic uremic syndrome (HUS) [8].

Transportation

The samples were sent quickly to the laboratory with their clinical information sheets. In case of delayed technical management, the stools were kept at +4°C and inoculated within a maximum of 12h.

Diagnostic approach

Among the many bacterial species in the flora, only some are considered pathogenic agents. Thus, the interest of a stool culture lies essentially in the search for the infectious etiology of a diarrheal syndrome.

Macroscopic examination

Macroscopic examination of the stool is important to orient the pathophysiology of the diarrhea. First, the consistency should be noted; if the stool is solid, it is advisable to look for the presence of blood, pus or mucus. Liquid stools with the presence of bloody mucus suggest a dysenteriform syndrome, whereas a colorless or "rice water" appearance suggests a choleriform syndrome.

Fresh direct examination allows the detection of leukocytes, red blood cells, microbial flora and the mobility of certain bacteria in the stool. It is possible if the stools are diarrheic or afecal, there are leukocytes present in case of diarrhea with invasive germs (*Salmonella spp*, *Shigella spp*, *Campylobacter spp*.). In case of diarrhea with enterotoxigenic germs (*Vibrio cholerae*, *Aeromonas spp*, *Clostridium difficile*), there are no leukocytes.

Examination of the smear after Gram staining makes it possible to appreciate the importance and the balance of the flora between Gram$^+$ and Gram$^-$ bacteria. A balanced flora is composed mainly of Gram$^-$ bacilli, but always with the presence of Gram$^+$ bacilli. Any significant disturbance of this balance must be reported. The pathognomonic form of *Campylobacter spp* makes it possible to make the diagnosis from the direct examination of the stool. For solid stools, they were diluted 1/10 in distilled water; vortex well; smear the suspension on a slide and stain. For liquid stools, place a drop of stool directly on the slide and stain.

2.3 Stool culture

Stool culture was practiced on liquid, soft, mucous or hemorrhagic stools, or on very specific indications for solid stools. This corresponds to the inoculation of generally selective media to isolate and then identify the infectious agent.

2.3.1 Salmonella

The search for *salmonella* and *shigella* was carried out on a selective medium of the Hektoen type and an enrichment medium with selenite SFB. *Salmonella* appears as green colonies with a black center on Hektoen medium (H_2S^+ and lactose$^-$), and oxidase.

2.3.2 Shigella

There is no enrichment medium for *Shigella*. Using an ounce of platinum, the stools were inoculated onto the Hektoen selective medium. The dish was then incubated at 37°C for 24 h. *Shigella* appear as green colonies lactose$^-$, sucrose$^-$, salicin$^-$, H_2S^-, and oxidase.

The identification of suspicious colonies of *Salmonella* or *Shigella* on Hektoen was performed by carrying out an oxidase and urease test on the suspicious colonies; then an API 20E gallery or a gallery composed of the following media (Kligler, Moeller with lysine, urea-tryptophan, ONPG test). It is advisable to carry on the identification of the only colonies verified as "negative urease". Under these conditions, the identification gallery can only be inoculated with a suspension prepared from a "urease negative" suspension and not from a colony. The API 20E strip was inoculated after adding 4 mL of sterile distilled water to a negative urease suspension. The presence of phenol red from the urea-tryptophan medium can give a pink tint to the ONPG test. Perform slide agglutinations using specific immunosera to identify which of the four *Shigella* serotypes has caused the infection.

2.3.3 Yersinia

According to Robins-Browne, Rabson [9], a Rappaport broth should be inoculated from direct stools. It was incubated at 4°C for 3 weeks, and sub-cultured every 5 days on CIN agar. *Yersinia enterocolitica* appears as round pink colonies about 2 mm in diameter, with dark pink centers surrounded by an area of precipitated bile. Confirmatory testing is required. Based on colony morphology on CIN agar, suspect colonies were isolated and incubated in urea broth at 37°C for 24 h. All urease-positive isolates were inoculated using an API 20E gallery according to the standard protocol [10].

2.3.4 *Listeria monocytotogenes*

Fraser broth was inoculated from the direct stool. After 5 days of incubation at -4°C, isolation was performed following the steps described by [11].

Perform a Gram stain for hemolytic colonies. They are Gram-positive bacilli measuring 1 to 2 µm by 0.5 µm, with parallel edges and organized in a palisade [12]. *Listeria monocytogenes* is catalase$^+$ and Gram$^+$ such as all other species of Listeria. This pathogenic species is hemolytic, has a positive reaction to the Camp test against *Staphylococcus aureus* and finally uses rhamnose but not xylose [13,14].

2.3.5 Campylobacter

Suspend the sample in Preston Enrichment Broth, incubate for 48 h at 42°C under anaerobiosis. Then subculture on Butzler's selective medium and incubate for 48 h at 42°C in an anaerobic jar with a CO_2 generator bag. *Campylobacter* is characterized by their "seagull flight" morphology, due to their mobility thanks to a polar ciliature. Then Confirm identification with a Campy API Gallery.

2.3.2 Enteropathogenic *E. coli* (EPEC)

The search for EPEC was conducted on the stools of children under two years of age. Inoculation was performed on the Hektoen from direct stools; incubate for 24 h at 37°C. Identification was led by slide agglutination reaction with sera corresponding to enteropathogenic serotypes.

2.3.6 Search of *Vibrio cholerae*

The search for the cholera vibrio was carried out in accordance with the protocol of [14]. Alkaline Bile Nutrient Agar was used instead of alkaline agar.

3 Results

During this study, 106 stool samples were collected. **Figure 1** illustrates the distribution of fecal samples according to the type of bacteria suspected.

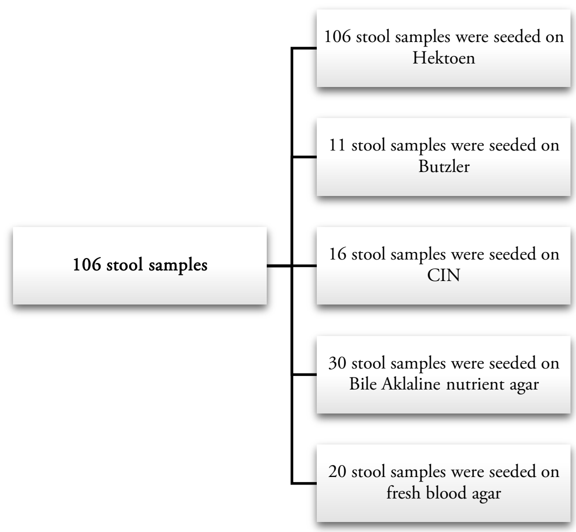


Figure 1. Distribution of stools samples

Among the total number of samples, only negative results were obtained for the investigated bacteria (*Salmonella, Shigella, EPEC, Yersinia, Campylobacter, Listeria, Vibrio cholerae*). Amongst 106 samples, a percentage of 59% was observed in male patients against 41% in female patients, when the highest rate was observed in males. **Table 1** shows the different results obtained on various culture media in comparison with positive controls.

Among 106 samples inoculated on Hektoen agar, 82% of the colonies were orange (Lactose$^+$) which means that they are not *Salmonella* or *Shigella*.

Table 1: Different results obtained on various culture media

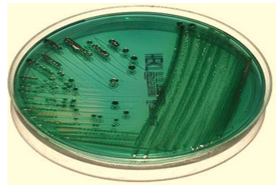
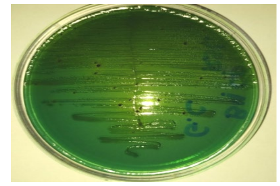
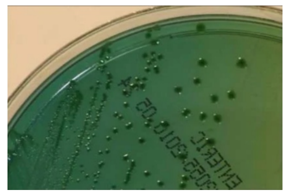
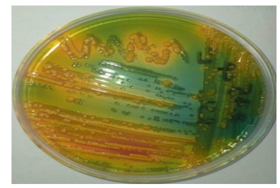
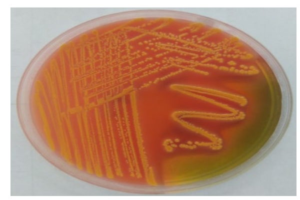
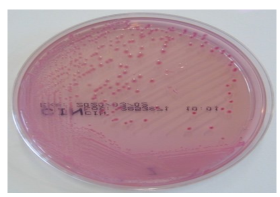
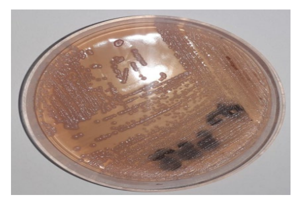
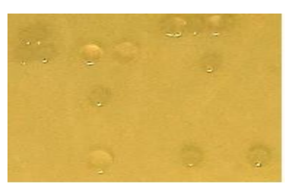
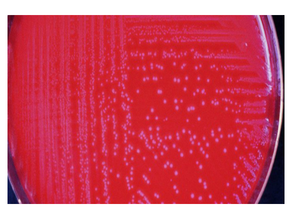
Media	Colony Appearance	Control picture	Culture results
Hektoen agar	*Salmonella* colonies appear blue-green to blue in color, with most strains being black in the center or over their entire surface.		
	Shigella colonies are green in color, moist and convex.		
	Escherichia coli colonies are yellow to salmon pink colored		
CIN agar	*Yersinia* colonies appear small (1 mm in diameter) translucent with a red center or entirely red (mannitol^{+}).		
Butzler agar	*Campylobacter* colonies are flat, shiny with a characteristic spreading along the isolation streak if the agar is moist.		
Alkaline Bile Nutrient Agar	Colonies are transparent		
Listeria agar	Colonies are β-hemolytic		

Table 2: *Salmonella* and *Shigella* research results on Hektoen agar

Suspect	Type of stool	Type of colonies	Oxidase	Urease	Culture on TSI agar	API 20^E
1	Loose stool	Green Colonies	-	-	Lactose $^-$	*Morganella*
2	Mucous diarrhea	Green Colonies	-	-	Lactose $^-$	*Proteus sp*
3	Diarrhea	Green Colonies	-	-	Lactose $^-$	*P. mirabilis*
4	Loose stool	Green Colonies	-	-	Lactose $^-$	*Citrobacter*
5	Loose stool	Green Colonies	+			
6	Diarrhea	Green Colonies	-	-	Lactose $^-$	*Enterobacter*
7	Diarrhea	Green Colonies	-	-	Lactose $^-$	*E. coli*
8	Diarrhea	Green Colonies	-	-	Lactose $^-$	*Hafnia alvei*
9	Loose stool	Green Colonies	-	-	Lactose $^-$	*E. coli*
		Green colonies (black centers)	-	-	Lactose$^-$, H_2S^+	*C. youngae*
10	Loose stool	Green Colonies	+			
11	Diarrhea	Green Colonies	-	-	Lactose $^-$	*K. Orithinolytica*
12	Loose stool	Green Colonies	-	-	Lactose $^-$	*E. coli*
13	Glairous	Green Colonies	-	+	Lactose $^+$	
14	Diarrheal	Green Colonies	-	+	Lactose $^+$	
15	Soft	Green Colonies	-	-	Lactose $^+$	

This type of colony was taken into consideration only if the patient is a child under two years old, because in this case, it can be an EPEC. 14% of the samples present either green colonies (Lactose$^-$) or green with a black center (Lactose$^-$ / H_2S^+). These two types of colonies are characteristic of *Shigella* and *Salmonella* respectively; the results are displayed in **Table 2**. 4% of the samples were completely sterile.

The stool cultures of 80 patients were Lactose$^+$, indicating the absence of pathogenic germs in adults. The stool culture from seven children under two years old, presenting Lactose$^+$ tests, are subjected to further tests to confirm that they are not enteropathogenic *Esherichia coli*, these results are detailed in **Table 3**.

Table 3. EPEC research results

Patients	Urease test	Culture on TSI agar	Serum agglutination results
15	-	Lactose$^+$	No agglutination
16	-	Lactose$^+$	No agglutination
17	-	Lactose$^+$	No agglutination
18	-	Lactose$^+$	No agglutination
19	-	Lactose$^+$	No agglutination
20	-	Lactose$^+$	No agglutination
21	-	Lactose$^+$	No agglutination

The search for *Yersinia* was made on 16 samples seeded on CIN agar, four suspicious colonies were subjected to the urease test and the results are presented in **Table 4**.

Table 4. Yersinia search results on CIN agar

Patients	Urease test	API 20^E results
22	+	*Enterobacter aerogenes*
14	+	*Proteus*
4	+	*Escherichia coli*
11	+	*Escherichia coli*

Search for *Vibrio cholerae* was carried out after incubation of the Alkaline Peptone Water (EPW) enrichments for 24 h, the latter were launched on Alkaline Bile Nutrient Agar (ABNA). Among the 30 samples, 12 with positive oxidase tests were confirmed with an API 20^{NE}. The results are summarized in **Table 5**.

Of the total number of samples, only two yielded β-hemolytic colonies characteristic of Listeria. A Gram stain was performed and the results showed Gram$^+$ cocci, unlike *Listeria* which is a Gram$^+$ bacillus. The results were also negative for campylobacter, no suspicious colonies on the selective culture medium.

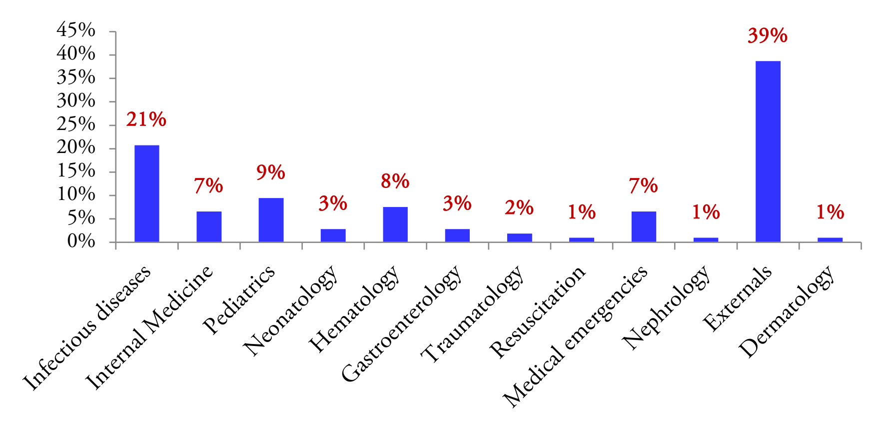
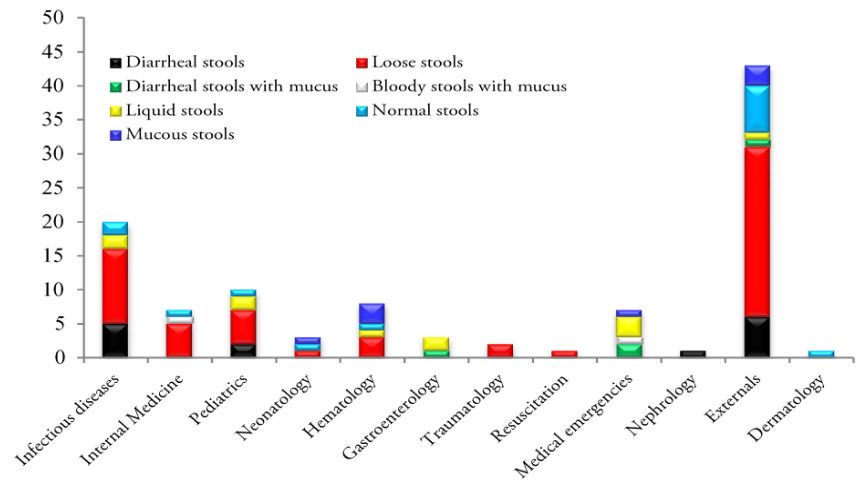


Figure 2. Distribution of patients by medical department



The results presented in **Tables 2 - 5** showed the absence of the pathogenic germs sought (*Salmonella, Shigella, EPEC, Yersinia, Vibrio cholerae, Listeria* and *Campylobacter*). This is not a first, indeed in a large survey of diarrheal diseases among the rural indigent population of Guatemala, 439 of 578 fecal specimens from patients with diarrhea did not produce any bacteria recognized as a pathogen. Yet the disease remained severe and often fatal [16].

The study period coincided with the extension of the confinement period and the obligation of strict health distancing measures under COVID-19. This period saw a lack of demand for stool culture; in fact, only 90 samples presented themselves to the microbiology laboratory during the two months of the study. The highest rate was recorded at the External department level with a percentage of 39%, then the Infectious Diseases department with a percentage of 21% (**Figure 2**).

Table 5. Search for *Vibrio cholerae*

Patients	Oxidase tes	API 20^{NE} results
4	+	*Aeromonas hydrophila*
23	+	*Pseudomonas aeroguinosa*
24	+	*Aeromonas hydrophila*
25	+	*Aeromonas hydrophila*
26	+	*Aeromonas hydrophila*
12	+	*Aeromonas hydrophila*
27	+	*Aeromonas hydrophila*
28	+	*Proteus spp*
1	+	*Aeromonas hydrophila*
29	+	*Aeromonas hydrophila*
30	+	*Aeromonas hydrophila*
31	+	*Aeromonas hydrophila*
32	+	*Aeromonas hydrophila*
33	+	*Burkhalderia cepacia*

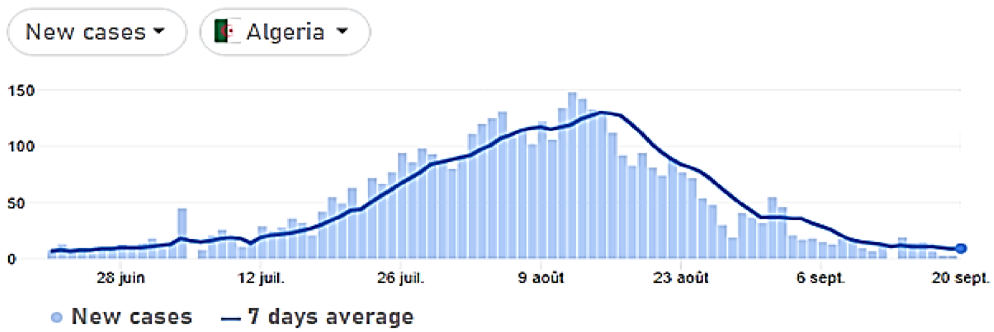


Figure 4. Statistics of COVID-19 daily cases recorded in Algeria during the summer 2022

The Infectious Diseases department and Externals showed the highest proportion of diarrheal stools. On the other hand, the other departments marked a percentage lower than 10%.

This study period was also characterized by a significant peak in COVID cases according to **JHU CSSE COVID-19 Data** (https://coronavirus.jhu.edu/map.html)**,** as shown in **Figure 4**.

4 Discussion

In the light of the obtained results, the viral context of gastrointestinal infections is essential. Diarrhea is one of the most frequent symptoms of patients with COVID-19. Studies showed that diarrhea occurs in about 2-50% of patients with COVID-19 [17]. A retrospective cohort study of 183 patients found a 37.1% incidence of diarrhea [18].

In fact, a study by Xiao et al. [19] analyzed stool samples from 73 patients with COVID-19 with the aim to assess the clinical significance of measuring SARS-CoV-2 RNA in stool. Diarrhea was found in 26 patients and stool tests remained positive for 12 days after illness onset. This indicates that viral gastrointestinal infection and potential fecal-oral transmission may persist even after viral clearance in the respiratory tract.

Patients with COVID-19 were treated with antiviral drugs, antibiotics, and corticosteroids. Antiviral drugs such as RNA polymerase inhibitors favipiravir and remdesivir can cause diarrhea [20]. Diarrhea is also a frequent adverse reaction to antibiotics such as cephalosporins, macrolides, and fluoroquinolones, primarily due to disruption of normal gut microbiota. Additionally, treatment of COVID-19 patients with broad-spectrum antibiotics may potentially increase the risk of C. difficile infection, including in survivors, even long after recovery from COVID-19. The use of proton pump inhibitors during COVID-19 infection often induces diarrhea by altering the gastrointestinal microbiota through different mechanisms, including a direct consequence of elevated gastric pH itself [21]. The composition of the intestinal flora could also be modified by an increase in pro-inflammatory mediators due to viral inflammation [22]. Gastroenteritis associated with contaminated food remains a common cause of illness. According to the WHO initiative to estimate the global prevalence of foodborne diseases, 31 global hazards caused 600 million cases of foodborne illness and 420,000 deaths in 2010; diarrheal pathogens are the main cause of these conditions [23]**.** The abrupt closure of restaurants and fast-food outlets eliminated the potential cause of foodborne infections, including gastroenteritis. Food businesses are reopening with stricter rules. Indeed, safety and health rules were top priorities, which also lead to fewer foodborne disease outbreaks. The COVID-19 epidemic has led to an increase in sanitary and personal hygiene measures, which seems to affect the rate of food contamination. The Food Safety Information Committee released a progress report on Australia's food safety record, showing that *campylobacter* and *salmonella* levels had halved from the two years prior confinement was introduced. Finnish Center for Health and Welfare has seen a substantial drop in the number of foodborne outbreaks in Finland, only four suspected outbreaks were recorded in March and May 2020 – far fewer than in previous seasons [24].

Social distancing constitutes an effective barrier to potential infectious risks that can affect a community. The fear of a coronavirus infection has led the population to include preventive actions in their routine, such as the use of disinfectant gel which has become systematic everywhere. Thus, well before COVID-19, in 2010, a pilot study carried out in two primary schools in France made it possible to highlight a significant reduction in the incidence of gastroenteritis thanks to the systematic and controlled use of hydroalcoholic solutions [25]. According to a study conducted in Spain with the aim of evaluating the effectiveness of a handwashing program with disinfectants, to prevent school infections, revealed a reduction of 32% [26].

The measures introduced in the context of the COVID-19 pandemic (confinement, physical distancing, reinforcement of hand hygiene) have effectively reduced the transmission routes of pathogenic bacteria. According to a surveillance analysis carried out in Germany, a reduction in the number of cases of gastrointestinal diseases was observed in all age groups. All gastrointestinal conditions included showed a significant reduction in the number of cases compared to the expected number of cases for previous years. Shigella (-82.9), Salmonellosis (-45.5%), Campylobacter (-22.2%), Yersiniosis (-7.0%) and Listeriosis also showed significant decreases [27].

In fact, the proportion of emergency department visits for gastroenteritis dropped dramatically in early 2020 to a historic low, and then stabilized at 0.5% in April 2020, according to French Sentinel Network data in winter 2019. The year 2020 was marked by the lowest cumulative incidence of acute diarrhea among general practices in the last ten seasons. This decrease in France was undoubtedly linked to the measures introduced in the context of the COVID-19 pandemic (containment, social distancing, and personal hygiene). Such a level has not been observed for all surveillance indicators in retrospective data over the last ten years. One study showed that a decrease in observance of these COVID-19 protective measures and the end of containment were associated with an increase in the incidence of gastroenteritis, particularly in young children [28].

Thus, the reasons for the absence of pathogenic bacteria are multifactorial, and cannot be fully explained by this study. Although these results suggest an improvement of the sanitary conditions in our city.

5 Conclusion

In the current study, we were interested in bacterial gastroenteritis. Several bacteria cause gastroenteritis, however our research was more focused on the most pathogenic bacteria in case of bacterial gastroenteritis: *Escherichia coli*, *Shigella*, *Salmonella*, *Yersinia*, *Listeria*, *Vibrio cholerae* and *Campylobacter*. The study was conducted on the different types of stools except for solids. 106 stools samples were received from various hospital departments. The most observed sex was male with a prevalence of 53%, followed by female with a prevalence of 41%. Unexpected results were obtained which proved to be conclusive and satisfactory. We found no pathogenic bacteria among those we were investigating. This proves that the distancing measures and the barrier gestures prevented bacterial contaminations, especially since the period of our study coincided with a peak of COVID-19.

Despite the comprehensive nature of our research, several limitations must be acknowledged. First, the study sample size was limited by the number of hospital admissions under confinement conditions, which potentially limited the generalizability of the results. Furthermore, the lack of available or reliable data was a challenge, hampering the depth of our analysis; notably the absence of previous research studies on this topic has further limited our ability to contextualize and expand on existing knowledge. Additionally, time constraints limited the rigor of our investigation. Further notable limitation was the unavailability of viral analysis data, which prevented us from integrating this crucial aspect into our study. These limitations highlight the need for caution in extrapolating our results and highlight the importance of future research efforts to address these constraints.

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Conflicts of Interest: The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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