

The Existence of Gram-Negative Bacteria that do not Digest Lactose in the Feces and their Resistance to Specific Drugs

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ABSTRACT

The study's objectives were to identify non-lactose fermenting Gram-negative bacteria from stool samples from many diarrheal patients and to use the disc diffusion method to perform an antibiotic sensitivity test to assess the degree of antibiotic resistance. Between December 2022 and March 2023, 100 stool samples were taken from a few patients at Al-Imam Alhasan Hospital. The MacConky agar medium was used to cultivate the samples. 77% of the bacteria were lactose-fermenting, while 22% were not. Out of 100 isolates, 18 fermented lactose. The percentages of the isolates were as follows: *Pseudomonas* bacteria in 4 out of 18 isolates 23%, *Shigella* bacteria in 2 out of 18 isolates (12.3%), *Salmonella* bacteria in 10 out of 18 isolates 54%, and *Proteus* bacteria in 2 out of 18 isolates (12.5%). The results of a sensitivity test using the diffusion method for ten antibiotics revealed that by measuring the diameter of colony growth inhibition around the antibiotic in millimeters and comparing the results to the international specifications (2020, Clinical and Laboratory Standards Institute), the bacteria were either sensitive (S) or resistant (R). The most inhibiting antibiotic, ciprofloxacin, was 90% inhibiting, followed by ceftriaxone at 80% and ampicillin at 15%.

Keywords: Bacteria resistance, Ceftriaxone, Ciprofloxacin, Gram-negative bacteria

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INTRODUCTION

In animals, including humans, it is colored. Breakdown it is the outcome of the procedure. Stool: Primarily brown, but the color shift could be caused by the food consumed or a medical condition that alters color (Machine) Wayback.^[1] According to Wagner *et al.*,^[2] its consistency is soft, and changes in consistency to liquid (Diarrhea) or solid (constipation) are pathological diseases. Therapy and need stool is occasionally seen as proof of infection with certain illnesses, and this is verified.^[3] Next, a culture in the laboratory to identify the kind by identifying and treating microorganisms using a general stool examination (GSE). Stool components: Water, which is about 75% of the composition of the stool, live and dead bacteria from the lining of the intestines, dead cells from the lining of the uterus, fibers, fats, and a little salt and protein, undigested food residues, mucus from the intestines and pigments secreted by liver cells.^[4,5]

Diarrhea is defined as having watery stools three or more times a day, or more frequently than is typical for that person.^[6] Fever or stomach pain may also accompany diarrhea. Typically, the patient may feel weak and may also have vomiting, stomach or abdominal pain and cramps, and, in extreme chronic or acute bouts of diarrhea, weight loss.^[7,8] Although there are several causes of diarrhea, infection and the spread of infectious organisms including bacteria, viruses, and parasites are the most significant (reference). Inability to digest certain foods such as lactose, surgical procedures to remove part of the intestine, effects of surgical procedures such as gallbladder removal, toxins such as pesticides, mycotoxins, arsenic, and many others, and many medications such as cholesterol-lowering drugs and antacids.^[9,10]

Diarrhea-causing bacteria there are numerous genera of bacteria that cause diarrhea, but we will focus on the study's focus, which is the negative bacteria that do not ferment lactose (Thompson, Gregory e, md, and David w). Kovanda *et al.*, (2019)^[11] study confirmed that *Salmonella* bacteria were the most likely to cause diarrhea cases among these bacteria, with the remaining genera being *Shigella*, *Proteus Morganella*, *Serichia Pseudomonas*,

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Providencia, and others. The study's objectives were to identify non-lactose fermenting Gram-negative bacteria from stool samples from many diarrheal patients and to use the disc diffusion method to perform an Antibiotic Sensitivity test to assess the degree of antibiotic resistance.^[12]

MATERIALS AND METHODS

Sample Collection

Stool samples were collected from patients suffering from diarrhea at Al-imam AL-Hasan General Hospital after ensuring that they were free of parasites following a GSE. The samples were collected from the patients in sterile containers and then cultured immediately afterward to avoid contamination of the samples.

Sample Culture

The culture procedure was performed for samples taken directly from patients using a flame-sterilized loop in a special culture chamber (hood) on MacConkey agar media and then incubated in an incubator at 37°C for 24 h.

Identification of Samples

The growing bacteria samples were identified based on morphological characteristics: By observing the morphological characteristics of the bacteria growing in colonies such as texture, odor, colony shape, color, transparency of colony borders, growth density, and their ability to ferment lactose sugar in McConkey media. Microscopic examination: Microscopic examination was done by making smears of growing bacterial colonies. On a slide, then fixed and stained with Gram stain to identify negative bacteria from positive ones according to their ability to stain with the stain. Biochemical tests it is a group of tests that are performed to help diagnose undiagnosed bacteria using many chemicals. They are primarily related to the chemical nature of the enzymes that these bacteria secrete into the surrounding environment according to their biological activity. These tests include:

Antibiotic Sensitivity Test

The sensitivity test of the bacterial isolates growing on the appropriate growth media was performed by isolating two colonies using a loop and transferring them to a tube containing 3–5 mL of distilled water, noting the change in turbidity. Then a cotton swab was dipped in the solution and spread by the planning method on the solid Mueller–Hinton medium in different directions to ensure the correct spread growth of the bacteria. Then, using sterile forceps, the antibiotic discs were transferred and fixed on the surface of the medium in a good and at a distance of 15 mm from the edge of the dish and 20 mm between the centers of the discs, then the dish is left for half an hour to ensure the spread of the antibiotic, then the dishes are incubated in the incubator at a temperature of 37 m for 18 h, after which the diameter of inhibition for each antibiotic is measured using a ruler and compared.^[13]

RESULTS AND DISCUSSION

The results of this study, which included collecting 100 samples from patients with diarrhea at Al-imam al-Hasan General Hospital for the period from December 2022 to March 2023, and after the diagnosis that was based on biochemical tests as in [Table 1 and Figure 1] and phenotypic and microscopic diagnosis and confirmation with several 20E api, there were only 18 non-lactose fermenting isolates out of 80% isolates 10% *Salmonella* isolates, 4 *Pseudomonas* isolates, 2 *Proteus* isolates, 2 isolates *Shigella*) and as shown in the sensitivity test [Table 2] the highest inhibition rate was for ciprofloxacin antibiotic at 95% and the lowest inhibition rate was for ampicillin antibiotic at 10% inhibition. The highest rates of resistance to antibiotics were due to *Pseudomonas* bacteria due to low sensitivity to antibiotics and the formation of new resistance mechanisms to antibiotics, which are worrisome characteristics in these bacteria; these results consist with other researcher.^[14–16] We noticed through this study that a high percentage of bacterial infections causing diarrhea are due to Gram-negative intestinal bacteria that do not ferment lactose, but they may vary depending on age, season of infection, personal hygiene, health status of the infected person, and dietary habits.

Results of a Study of Phenotypic Traits

The results of the study of the morphological characteristics of the growing colonies showed the appearance of *Salmonella* colonies in the form of large colonies 231–33 mm, circular,

Table 1: Biochemical test for samples (*Salmonella*, *Proteus*, *Pseudomonas*, and *Shigella*)

No	Test name	<i>Salmonella</i>	<i>Proteus</i>	<i>Pseudomonas</i>	<i>Shigella</i>
1	Gram stain	–ve	–ve	–ve	–ve
2	Catalase	–ve	+ve	–ve	–ve
3	Oxidase	–ve	–ve	–ve	–ve
4	PV	–ve	+ve	+ve	+/-
5	Citrate	–\+	–ve	–\+	–ve
6	Indole	+ve	–ve	+ve	+ve
7	MR	–ve	–ve	+ve	+ve
8	Urease	–ve	+ve	–ve	–ve
9	TSI	K/A Gas H ₂ S, CO ₂	A\A, KA Gas H ₂ S, CO ₂	K/K no gas	K/A no gas

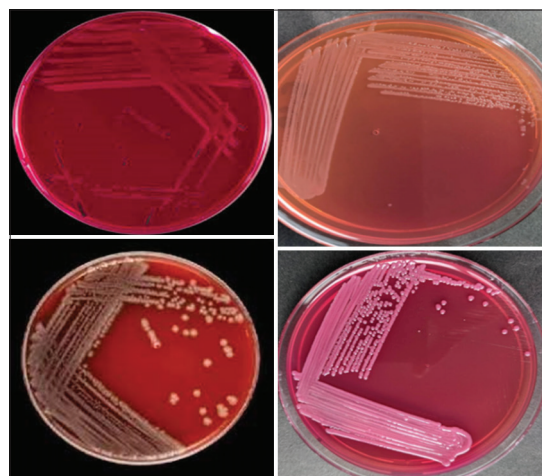


Figure 1: Four types of (*Salmonella*, *Proteus*, *Pseudomonas* and *Shigella*) bacteria that were isolated from stool

slightly convex, and transparent on MacConkey medium, not fermenting lactose sugar. *Proteus* bacteria produce pale, medium-sized, slightly convex, transparent, moist colonies that do not ferment lactose and cover the entire plate due to their characteristic undulation phenomenon.^[17] *Shigella* bacteria were circular, convex, colorless, semi-transparent, smooth-surfaced, with flat edges, and did not ferment lactose and convex and did not ferment lactose. *Pseudomonas* bacteria appeared in the form of large, transparent, convex colonies that did not ferment lactose.

Results of Studying Microscopic Characteristics

The results of microscopic examination of the isolates showed that the *Salmonella* bacteria, *Proteus* bacteria, and *Pseudomonas* bacteria are Gram-negative and motile, with the exception of the *Shigella* bacteria, which are non-motile.

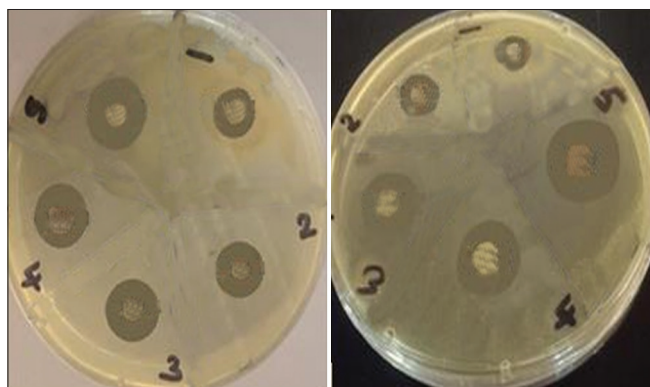
As previously stated, the disk diffusion method was used to confirm the antibacterial qualities. The effectiveness of the ten antibiotics used to stop bacterial growth against four microorganisms was measured using a ruler. The use of ampicillin was examined for bacterial activity. The inhibitory zone of *Shigella*, *Salmonella*, *Proteus*, and *Pseudomonas*, which are Gram-positive and Gram-negative bacteria, is depicted in Figure 2. These results consist with other studies.^[15,16] In comparison, the extract's inhibition zones measured 12.2 and 10.4 mm. Ampicillin's inhibition zones against four different

Table 2: Sensitivity test results for the antibiotic

No.	Name of the antibiotic	<i>Shigella</i> spp. resistance % n=2	<i>Pseudomonas</i> spp. resistance % n=4	<i>Proteus</i> spp. resistance % n=2	<i>Salmonella</i> spp. resistance % n=10
1	AK	(0) 0	(3) 75	(1) 50	(3) 30
2	TM	(1) 50	(2) 50	(0) 0	(1) 10
3	GEN	(1) 50	(2) 50	(1) 50	(1) 10
4	CTR	(0) 0	(1) 25	(0) 0	(2) 20
5	CFP	(1) 50	(2) 50	(1) 50	(3) 30
6	CIP	(0) 0	(1) 25	(0) 0	(0) 0
7	NIT	(1) 50	(3) 75	(1) 50	(2) 20
8	AMP	(2) 100	(4) 100	(1) 50	(9) 90
9	C	(1) 50	(2) 50	(0) 0	(4) 40
10	Pi	(2) 100	(3) 75	(2) 100	(7) 70

Table 3: Displays the antibiotic's zone of inhibition

Antibiotic	<i>Shigella</i>	<i>Pseudomonas</i>	<i>Proteus</i>	<i>Salmonella</i>
AK	14.6 mm	11.1 mm	16.1 mm	13.6 mm
TM	17.9 mm	16.5 mm	15.5 mm	17.6 mm
GEN	15.8 mm	14.8 mm	14.3 mm	13.6 mm
CTR	16.1 mm	15.8 mm	13.9 mm	15.2 mm
CFP	14.6 mm	13.9 mm	12.8 mm	16.7 mm
CIP	26.8 mm	20.5 mm	19.7 mm	19.3 mm
NIT	17.1 mm	16.3 mm	12.7 mm	15.7 mm
AMP	11.4 mm	10.5 mm	9.7 mm	8.6 mm

**Figure 2:** Displays the antibiotic's zone of inhibition for the ten antibiotic that used

bacterial species ranged between 11.2 and 8.6 as shows in Table 3.

CONCLUSION

This study showed that a high percentage of diarrhea cases caused by bacterial infections are due to non-lactose-fermenting Gram-negative intestinal bacteria. The study also showed that some of these species show resistance to some of the antibiotics used to treat them, such as *Pseudomonas* bacteria, due to the difference in their resistance mechanisms and their renewal. Follow healthy eating habits because most infections with this bacterium occur through contaminated water and food. Avoid the random use of treatments and antibiotics without consulting a specialist doctor, as this may result in drug interactions or bacteria acquiring new resistance mechanisms.

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