Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 6
Open Access

Determination of the Lactic Acid Bacteria (LAB) as an alternative for probiotics and their inhibitory role against the pathogenic bacterial profile in the stool samples of newborns

Karkaz M.Thalij [1] Noor S.Ahmed [1] Muthanna A. Sultan[2]
[1*]Department of Food Science, College of Agriculture, University of Tikrit, Tikrit - Iraq
[2*]Department of Microbiology, College of Veterinary Medicine, University of Tikrit, Tikrit - Iraq

Cite this paper as: Karkaz M.Thalij , Noor S.Ahmed, Muthanna A. Sultan (2024) Determination of the Lactic Acid Bacteria (LAB) as an alternative for probiotics and their inhibitory role against the pathogenic bacterial profile in the stool samples of newborns. Frontiers in Health *Informatics*, *13(6)* 1201-1212

Abstract
This study aimed to isolate, identify, and diagnose the pathogenic bacterial profile from the 150 stool samples of the newborns, represented by 75 of these samples (50 %) of healthy newborns and 75 samples from another group of newborns affected with diarrhea. The samples were then cultivated on Mannitol salt agar (MSA), MacConkey agar (MA), and MRS agar within 37 º C for 24 hrs., which was followed by an evaluation of lactic acid bacteria (LAB) to detect the probiotics properties as well as the inhibitory role of the metabolites activity against the pathogenic isolates. The results of this study have shown that the bacterial isolates from the healthy newborn at 75 samples were at 82 isolates; they were distributed for LAB at 53, *E.coli* at 21, two of *Staphylococcus aureus*, one of *Shigella sonnei* and five isolates of *Enterobacter cloacae*. At the same time, the samples from diarrhea newborns were isolated from 93 bacterial isolates, distributed at 10 of LAB, 42 of *E.coli*, and 12 of *Staph. aureus*, 17 of *S. sonnei* and 12 isolates from *E.chloacae*. The LAB species appeared as *Lactobacillus gasseri*, *Bifidobacterium bifidus*, *Lactobacillus acidophilus*, and *Lactobacillus casei* at 21 (39.6%), 10 (18.8%), 10 (18.8%) and 12 (22.6%) respectively. The species of *Lb. gasseri* has been shown to act as probiotics and appeared to be the best at resisting bile salts and pH levels. The pathogenic isolates appeared to be resistant to most antibiotics, while the E.coli isolates were the most resistant, and the LAB appeared susceptible to all antibiotics used. In addition, the inhibitory activity of the LAB isolates or its metabolites as probiotics against *E.coli* isolates have been shown at a high range of zone inhibition diameters of 35mm. In conclusion, the data obtained from this study suggests that the microbial profile of the stool from the healthy newborn contains significantly a higher number of LAB compared with newborns affected with diarrhea, which is the last (LAB isolates) probiotic properties which could be leading to maintain the immune homeostasis of the body and improving the health status.
Keywords: Probiotics, Diarrhea, Newborn babies, Pathogenic bacteria, Inhibition.

Introduction

Diarrhea can be categorized into acute and chronic forms based on duration and underlying causes. Often, acute diarrhea is caused by viral and bacterial infections and parasitic infections such as rotavirus, E.coli, and Shigella, respectively. On the other hand, chronic diarrhea may last more than two weeks, as it is caused by persistent infections, inflammatory bowel diseases, or other chronic conditions (UNICEF/WHO, 2023).

Globally, diarrheal illnesses continuously pose a severe threat to public health, especially for newborns and early children. It is a common gastrointestinal tract (GIT) illness that causes different forms of diarrhea based on the causative agent. GIT still affects newborns and early children and is a primary worldwide health concern (Khalil et al., 2020). To

reduce morbidity and mortality linked to diarrheal diseases, public health initiatives have investigated treatment strategies by considering the microbiological profile of diarrheal infections during childhood (Kotloff and Blackwelder, 2024).

In addition to the infectious causes, diarrhea can have many other causes, including non-infectious ones such as drug side effects and dietary indiscretions (Gupta et al., 2023). The beneficial effect of both probiotic and healthy commensal bacteria and their products toward the microenvironment of the digestive tract resulted in improving the systemic immunity of the host, which, based on that the researchers have had intestine is considered one of the GIT homeostasis as one of the critical factors for the healthy status of the body preventing the sequela of the other diseases (Alaobady and Thalij, 2024).

This study aimed to examine the distribution of bacterial isolates found in stool samples from infants who have had diarrhea. These samples' ‹bacterial diversity sheds light on this susceptible group's epidemiology and etiology of diarrheal illnesses.

Materials and Methods

One hundred fifty samples were collected; seventy-five samples were from healthy newborns, and others were from newborns with diarrhea, the age of four days to 60 months. Samples were immediately transferred to the laboratory unit for Blood mannitol salt, MacConkey, and MRS agar culturing.

The collection of samples was conducted at Tikrit Teaching Hospital from 1st February until the last of April 2024, considering the database for each patient, such as age, gender, and other databases.

Isolation and Identification of Bacteria

Samples were inoculated into McConkey, Mannitol salt, and MRS agar, then incubated at 37 ºC for 24 hours. Colonies were then purified and used for identification tests. The suspected colonies were stained using the gram stain method, and their shapes, colors, and arrangements were observed under the light microscope.

All bacteriological isolates were examined and confirmed by applying the biochemical tests according to Baron and Bergey's manual of determinative bacteriology and other references (Winn et al., 2006; Leboffe and Pierce, 2011).

Anti‹bacterial Susceptibility Test: The antibacterial susceptibility test was done using the Kirby-Bauer method according to (Bauer et al., 1966), which was modified by the World Health Organization (Vandepitte et al., 2003) as follows: The adjusted suspension was used within 15 minutes to inoculate the plates by dipping a sterile cotton-wool swab into the suspension and removing the excess liquid by turning the swab against the side of the container above the level of the liquid. Streak the swab evenly over the entire plate surface by swabbing in three directions, rotating the plate through an angle of 60º after each application. Finally, pass the swab around the edge of the agar surface. Allow the plate to dry with the lid closed before applying the discs. The antibacterial discs were placed on the inoculated plates using a pair of sterile forceps and then Incubated under 35-37 ºC overnight for 18-20 hrs. After incubation, the diameter of each zone (including the diameter of the disc) was measured and recorded in mm (CLSI, 2023).

Antibiotics Sensitively Assay

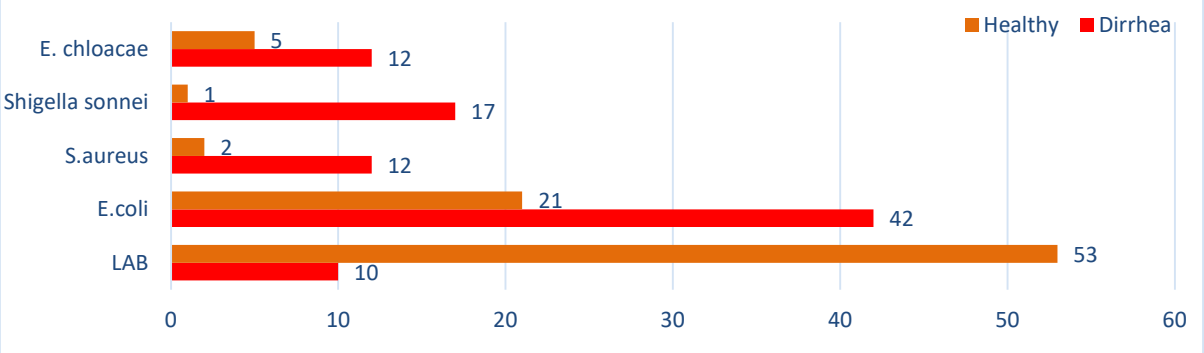
Bacterial isolates from *E. coli, Staphylococcus aureus, Shigella sonnei, Enterobacter chloacae. Lactobacillus gasseri, Bifidobacterium bifidus, Lactobacillus acidophilus,* and *Lactobacillus casei* were tested for susceptibility to the different antibiotics using the Kirby-Bauer technique (Jorgensen and Turnidge 2007). The antibiotics used contain Ampicillin, Azithromycin, Clindamycin, Amikacin, Trimethoprim, Ciprofloxacin, and Nalidixic acid. A single colony from each bacterial isolate was transferred to a new test tube containing 5ml nutrient broth and incubated at 37 ℃ for 24 hrs to make a bacterial inoculum; then, the balances were adjusted by comparing it to McFarland tube concentration 0.5. A sterile cotton swab was dipped in the inoculum and uniformly separated across the surface of a Muller-Hinton

agar plate. The antimicrobial-containing disks are then put into the agar using forceps squeezed firmly to establish contact with the agar, and the plates are inverted and incubated at 37 ℃ for 18 hours. Following incubation, the inhibition zone diameter (IZD) surrounding each disk is measured in (mm), and the isolates are classified as sensitive, intermediate, or resistant to a specific medication based on comparisons with conventional inhibition zone diameters (CLSI, 2022).

Results and Discussion

The Bacterial Contamination Isolates

The results showed in Figure 1. that the bacterial isolates of the healthy newborn were at 82 isolates; they were distributed for *Lactoacid bacillus* LAB at 53, *E.coli* at 21, two of *Staphylococcus aureus*, one of *Shigella sonnei* and five isolates of *Enterobacter cloacae*. While the stool samples from d newborns affected with diarrhea were isolated from 93 isolates, they were distributed at 10 of LAB, 42 of E.coli, and 12 of Staph. aureus, 17 of *S. sonnei* and 12 isolates from *E.chloacae*.



	LAB	E.coli	S.aureus	Shigella sonnei	E. chloacae
Healthy	53	21	2	1	5
Dirrhea	10	42	12	17	12

Fiq 1. Distribution of contaminated microbial isolates from stool samples of children patients with diarrhea

The results have showed that the LAB isolate was the dominant one between the isolates in stool samples of children without diarrhea (53) compared to the stool samples of children infected with diarrhea, which at (10) isolates (Fig. 2). The results of our study has also been shown that the number of pathogenic bacteria of all species has increased in infected newborn babes at 83 isolates at the expense of the number of LAB which at ten isolates, which explains why diarrhea in children occurs through the influence of one of the factors causing its occurrence, such as the transmission of the pathogenic bacteria from the infected people whom in contact with children or through drinking contaminated water and other beverages or could be the contamination of the instruments that was used during preparation of the meals for the children,All the reasons that just mentioned have played such an important factors mediating the in inhibition of the LAB isolates and thus reducing their numbers in the intestine.

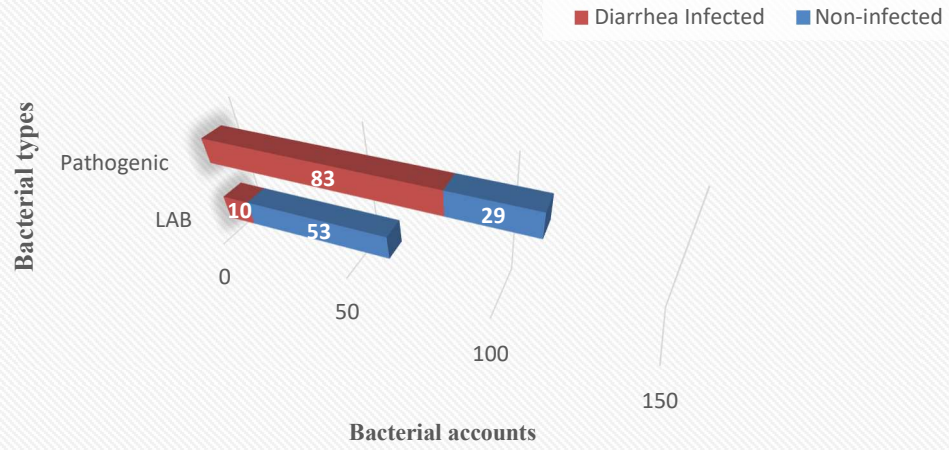


Fig.2. Disturbiution of LAB and Pathogenic Isolates from Haelthy and Diarrhea Infection Newborns Stools Samples

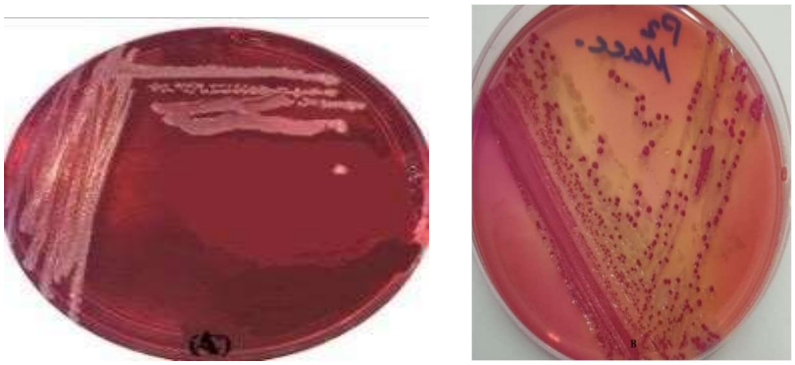
The results are consistent with those of Gupta et al. (2023), as he found in his study that the causes of acute diarrhea in children were viruses and bacterial pathogens.

Pathogenic Bacterial Isolates

The first identification of bacterial isolates was done after incubating aerobically on Mannitol Salt agar and MacConkey agar plates at 37 ° C for 24 hours. Some colonies were shown mannitol fermenting in mannitol salt agar medium (Fiq.3) and appeared as gram-positive colonies at 1–2 mm in diameter. The shape was that of grape-like clusters, and when it was viewed through a microscope, it also showed large, round, golden-yellow colonies. It produces yellow colonies with yellow zones on mannitol salt agar plates, which are transparent due to the fermentation of mannitol, which leads to a drop in the medium's pH. (Ayeni et al., 2017). These characters were indicated for the isolate as Staphylococcus aureus.

Fiq 3. The colony of Staphylococcus aureus cultivation on mannitol salt agar at 37 ºC for 24 hrs.

The bacteria that were isolated on MacConkey agar after incubation at 37 ° C for 24 hrs the isolate was demonstrated lactose fermentation and gave a bright pink halo (Fig 4 A). In contrast, pink colony growth due to the conversion of neutral red indicator dye when it is below pH 6.8 and the ability to ferment some carbohydrates (Table 1) were these characteristics of gram-negative indicated for these isolates as *E. coli* bacteria. These results were agreed upon by Mahmood et al**. (2016).**



Fiq 4. Colony of bacterial species that's growth on MacConkey agar at 37 ° C for 24 hrs. A: *E.coli* B: *Shigella sonnia.*

The other isolates that grew on MacConkey agar appeared as gram-negative (Fig 4B). The species comprises non-motile bacteria, does not form gas from glucose, inositol, raffinose, and xylose, and can ferment lactose, mannitol, cellobiose, and arabinose. This species' character was referred to as *Shigella sonnia.*

The last species isolated from the specimens was gram-negative, which appeared catalase-negative and oxidase-positive. Also, for the IMVC test as (- - + +), H2S is negative, urease lysis is variable, and gelatinase production is negative and capable of motility. The sugar fermentation is positive for all carbohydrates (Table 1). These characteristics were called Enterobacter cloacae species (Holt et al., 1994).

Table 1. Biochemical parameters for Enterobacteriaceae species

Parameters types		***E.coli***	***Shigella sonnia***	***Enterobacter cloacae***
Catalase		+	-	+
Oxidase		-	-	-
Indole		+	-	-
Methyl red		+	+	-
Voges-Proskauer		-	-	+
Citrate utilize		-	-	+
H2S production		-	-	-
Urease lysis		-	-	+/-
Gelatinase production		-	-	-
Motility		+	-	+
Sugar Fermentation	**Glucose**	+	-	+
	Lactose	+	-	+
	Mannitol	+	+	+
	Cellobiose	-	+	+
	Inositol	-	-	+
	Raffinose	-	-	+

	Xylose	+	-	+
	Arabinose	+	+	+

(+) means positive, and (–) means negative result.

Lactic Acid Bacterial Isolates

In this study, four lactic acid bacteria (LAB) species were isolated from the intestines of children which are *Lactobacillus gasseri, Bifidobacterium bifidus, Lactobacillus acidophilus,* and *Lactobacillus casei*. The phenotypic and biochemical tests are summarized in the (Table 2).

Table 2. Phenotypic and biochemical tests of lactic acid bacteria isolated from the intestines of children.

+ mean positive results, - mean negative results, +/- variable.

Tests types	***Lb.casei***	***Lb.acidophilus***	***Bifi.bifidus***	***Lb. gasseri***
Colony Shape	**White to cream, large in size, round, flat, smooth.**	**white to creamy white, small to medium size, oval, flat, Soft and shiny.**	**White to creamy, small in size, round shape, regular edges, smooth and shiny.**	**White to grey color, small to medium size, round, smooth and shiny**
Microscopic Shape	**G+, Bacillus or in short chains.**	**G+, Bacillus or in short chains.**	**G+, Bacillus of Y or V chains. Rounded tips**	**G+, Bacillus or in short chains.**
Catalase	-	-	-	-
Oxidase	-	-	-	-
Gelatine liquefication	-	-	-	-
Glucose /CO_2 produce	+/-	+/-	+/-	+/-
Lactose	+	+	+	+
Sorbitol	+	-	-	-
Xylose	+	-	+	-
Raffinose	+	-	-	-

The LAB isolates exhibited distinct colony morphologies and microscopic shapes. *Lb. gasseri* and *Lb. acidophilus* appeared as Gram-positive bacilli in short chains, while *Bifido. bifidus* displayed Y or V-shaped bacilli with rounded tips. These variations in colony morphology and microscopic shape were consistent with previous studies on LAB characterization. These isolates have shown the inability to produce the catalase, oxidase, and gelatine liquefaction enzymes, confirming their strict anaerobic nature and failure to break down hydrogen peroxide. They also showed that the glucose fermentation was positive, and all LAB isolates showed a negative ability to produce CO2, indicating a variety of metabolic capacities under various conditions.

The capacity of *Lactobacillus casei* to fermentate sorbitol, xylose, and raffinose was distinctive and demonstrated the versatility of its metabolism of carbohydrates. Metabolic diversity is essential for LAB to colonize and persist in different intestinal environments.

Recent studies have highlighted the benefits of LABs and their practical uses in the industry. The studies have demonstrated that the LAB strains have been maintaining the immune homeostasis of the body and improving the

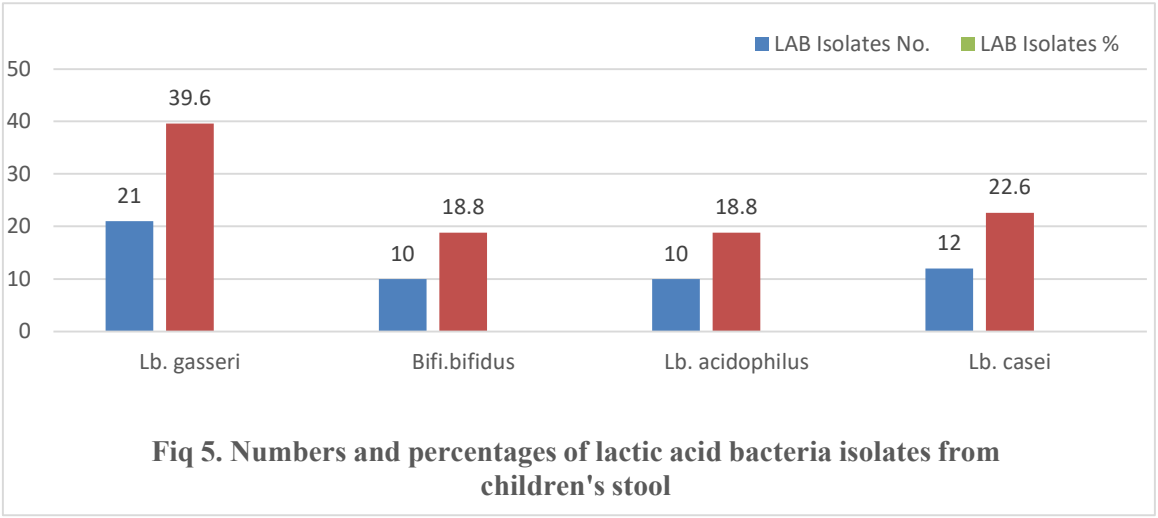
healthy status of the gut by inducing the production of short-chain fatty acids (SCFAs)

Another characteristic of the LAB isolate was its antioxidant and antibacterial role, which demonstrated the potential role of LAB in functional food design and healthcare, preventing infections and promoting overall health. Additionally, LABs are explored for their wound-healing properties, contributing to faster recovery through keratinocyte activation and collagen deposition mechanisms.

LAB characterization from children's intestines reveals their diverse phenotypic and biochemical profiles. The insights gained from this study, combined with recent research, underscore the potential of LAB in therapeutic and industrial applications. Understanding these properties is essential for developing targeted probiotic treatments and functional foods to improve human health.

Namouras of Lactic Acid Bacteria Isolates

The account of each LAB species isolated from the newborn baby's stool samples is shown in Figure 5. They showed that the most common type of lactic acid bacteria is *Lb. gasseri* at 21 (39.6%) isolates, followed by *Lb. casei*, with 12 (22.6%) isolates, then both *Bifido. bifidus* and *Lb. acidophilus*. Which were at 10 (18.8%) for each one.



Fiq 5. Numbers and percentages of lactic acid bacteria isolates from children's stool

The results indicated that the highest numbers of LAB were of the *Lb. gasseri*, which means the health of the digestive system of the children from whom it was isolated. This was confirmed because this species was isolated from healthy children. The state of the positive balance of the intestinal microbiota, which increased in the number of LAB isolates, indicated the conditions before treatments that most negatively affect the health status of the intestine in both children and adults.

Recent studies have indicated the ability of these types of bacteria to inhibit pathogenic bacteria that cause diarrhea (Thalij et al., 2022), and the results also agreed with what was reported by (Lee et al., 2024), who found that the ability of LAB to inhibit the pathogenic bacteria.

The ability of LAB to tolerate the bile salts:

The results of our study have shown that the isolated species of LAB of *Lb. casei*, *Bifido. bifidum, Lb.acidophilus,* and *Lb. gassaria* were resistant to bile acids at concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 mg/ml, as shown in (Table 3).

Table.3 The ability of lactic acid bacteria to tolerate different concentrations of bile salts.

Isolates types	Bile salts conc. (mg/ml)					
	0.1	0.25	0.5	1.0	2.5	5.0
Lb. casei	*****	***	**	**	*	-
B. bifidum	*****	****	***	**	*	-
Lb.acidophilus	*****	*****	*****	***	*	*
Lb. gassaria	*****	*****	****	****	**	*

-Nontolerance *Week tolerance ** Meddle tolerance, * Good tolerance, **** Very good tolerance, ***** Excellent tolerance.**

The results showed that low concentrations of bile salts were resistant to the LAB and that increasing the concentration at 2.5 and 5.0 caused a decrease in resistance and its inhibition at the 5 mg/ml for each of the *Lb.casei and Bifido.bifidum and its resistance to both Lb.acidophilus* and *Lb. gassaria*.

The ability of lactic acid bacteria species to resist bile salts comes from their ability to block their effects by multiple mechanisms and their possibility of possessing genes that express resistance to those salts.

The ability of LAB to tolerance the pH levels:

The effects of different pH levels on the growth rates of lactic acid bacteria species have been shown in Table (4). Our results have shown that the types of lactic acid bacteria of *Lb. casei, Bifido. bifidum, Lb.acidophilus,* and *Lb. gassaria* have been showing resistance to a pH level of 2, while all of them did not resist a pH level of 1 except the *Lb. gassaria*. The ability of species of lactic acid bacteria to withstand low levels of pH comes from the fact of effect of those levels, their enzymatic activity, and metabolic processes, which depend entirely on the level of pH, which causes an impact on the enzymes of lactic acid bacteria, as their ability to stimulate chemical reactions and facilitate growth is affected. Changing it over a wide range could cause denaturation of the protein that makes up the enzyme and, thus, loss of its effectiveness.

Table 4. The ability of lactic acid bacteria to tolerate different pH levels

Isolates Type	pH levels						
	1	2	3	4	5	6	7
Lb. casei	-	**	***	*****	*****	*****	*****
B. bifidum	-	**	***	*****	*****	*****	*****
Lb.acidophilus	-	**	****	*****	*****	*****	*****
Lb. gassaria	*	**	****	*****	*****	*****	*****

-non tolerance *Week tolerance ** Meddle tolerance, * Good tolerance, **** Very good tolerance, ***** Excellent tolerance.**

However, the results showed that the species of lactic acid bacteria resisted inhibition from low pH levels, which could be due to their genetic makeup, which determines the type of amino acids used. These species use it in their metabolic processes for their vital reactions.

Antibiotic susceptibility to bacterial isolates

The antibiotic susceptibility test toward the bacterial isolates contamination of stool newborn specimens is illustrated in Table (5). The results showed that the *E.coli* isolate could resist all antibiotics such as Ampicillin (AM), Amikacin (Ak), Ceftriaxone (CRO), Nalidixic acid (NA NA), Azithromycin (AZM), Trimethoprim (TMP), and intermediate for Clindamycin (DA).

All species of pathogenic bacteria are from *Sh.sonnei, Entero. cloacae,* and *Staph. aureus* appeared to range from

intermediate to resistant to antibiotics. The results also showed that all LAB species were susceptible to all antibiotics used in the study.

Pathogenic isolates appeared resistant to most antibiotics widely used in the treatment of infections; this status of bacterial isolates' ability to be resistant to antibiotics indicated their importance because they were capable of causing infections and diseases after transmission by various processes; these results agreed with Thalij et al. (2019).

Table 5. Antibiotics susceptibility toward bacterial isolates.

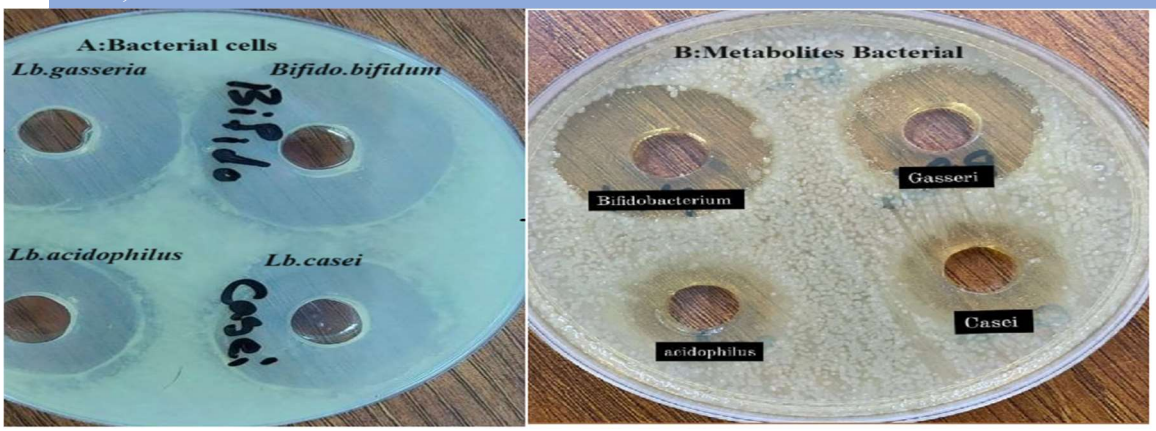
Isolates species	**Microbial species sensitivity to antibiotics types (%)**						
	AM	**AK**	**CRO**	**NA**	**DA**	**AZM**	**TMP**
E.coli	R(100)	R(95)	R(80)	R(90)	I(60)	R(90)	R(90)
Sh. sonnei	R(85)	R(85)	I(60)	R(95)	R(97)	I(60)	R(82)
Entero. cloacae	R(80)	I(58)	I(70)	I(70)	I(65)	I(70)	R(95)
Staph. aureus	R(85)	I(60)	I(65)	I(70)	R(75)	I(67)	R(83)
Lb. casei	S (5)	S(15)	S (2)	S (5)	S(15)	S(10)	S(5)
B. bifidum	S (10)	S(5)	S (5)	S (10)	S(5)	S(5)	S(10)
Lb.acidophilus	S (5)	S(8)	S (5)	S (5)	S(10)	S(5)	S(8)
Lb. gassaria	S (8)	S(10)	S (10)	S (8)	S(15)	S(5)	S(5)

R=resistance I=intermediate, S=sensitive, Am = Ampicillin (10µg), Ak= Amikacin (10µg), CRO = Ceftriaxone (10µg), NA= Nalidixic acid (30µg), DA= Clindamycin (10µg), AZM = Azithromycin (15µg), TMP= Trimethoprim (10µg).

The LAB also appeared that the subspeciality to the antibiotics these were referred to did not contain the genes that encode resistance to antibiotics, as well as the inability of these genes to be transmitted to it from pathogenic microbes, which made it classified as safe microbes for use in food. (Obioha et al., 2023).

Assessment of probiotics cells or their metabolites on inhibition of *E.coli* isolates

Table 6. It shows the effectiveness of the probiotic species *Lb. casei*, *Bifido. bifidum*, *Lb.acidophilus*, and *Lb. gassaria* in the ability of cells or their metabolites to inhibit the selected isolates of E.coli bacterial isolates, which were numeric 1, 2, and 3. The results showed the most significant effectiveness of both cells and metabolites of *Bifido. bifidum* and *Lb. gassaria* in the inhibition of isolates of E.coli-1 and two bacteria (Fiq 6 A and B), which had a high inhibition at 26 to 35 mm for the cells and between 16 to 25 mm for their metabolites and less than that for isolate No. 3. The diameters of inhibition also ranged for each of the cells of the probiotics of the *Lb.casei* and *Lb.acidophilus* at 16 to 25 mm, and their metabolites at 10 to 15 mm.



Fiq 6. Effect of probiotics cells and their metabolites on inhibition of *E.coli* isolates

The results agreed with what was stated by Aleman and Yadav (2024), who found that bacterial species of probiotics had an inhibitory effect on pathogenic bacteria. Probiotics play a crucial role in maintaining gut health and preventing bacterial infections. These beneficial microorganisms, often comprising strains of beneficial bacteria, naturally reside in the gut and are consumed as dietary supplements. Probiotics exhibit significant inhibitory effects on pathogenic bacteria through several mechanisms, such as the production of antibacterial compounds such as organic acids, lactic acid, and acetic acid. These acids lower the surrounding environment's pH, creating unfavorable conditions for the growth of pathogenic bacteria.

Table 6. Effect of probiotics cells and their metabolites on inhibition of *E.coli* isolates.

Pathogenic bacteria Isolates	**Probiotics species**							
	Lb. casei		***Lb. acidophilus***		***Lb. gassaria***		***Bifido. Bifidum***	
	metabolites	**Cells**	**metabolites**	**Cells**	**metabolites**	**Cells**	**metabolites**	**Cells**
E.coli-1	+	++	+	++	++	+++	++	+++
E.coli-2	+	++	+	++	+	++	++	+++
E.coli-3	+	++	+	++	+	++	+	++

+ mean inhibition zone at 10-15 mm, ++ mean inhibition zone at 16-25mm, +++ mean inhibition zone at 26 -35mm.

In addition, they were producing bacteriocins, which target and kill pathogenic bacteria directly. Some probiotic strains inhibit pathogenic bacteria's communication system (quorum sensing) by producing compounds that interfere with signaling molecules, disrupting vital processes such as biofilm formation and toxin production.

The increased effect of live cells from probiotics compared to their metabolites could be due to the ability of bacterial cells to produce the aforementioned metabolic compounds during their presence in a competitive environment with bacterial cells and other microbial cells, unlike the case of metabolites whose concentrations are limited in their effect and which can interfere. Some components of the medium cause a decrease in its inhibitory effect (**Alaobady and Thalij 2024)**.

CONCLUSIONS

In conclusion, the study successfully isolated and identified bacterial species from the gut of healthy and diarrheal newborns. The research highlighted the presence of lactic acid bacteria (LAB) such as *Lactobacillus gasseri, Bifidobacterium bifidus, Lactobacillus acidophilus, and Lactobacillus casei*, with *Lactobacillus gasseri* showing the most substantial probiotic potential. The findings also indicated significant antibiotic resistance among pathogenic

bacteria, particularly *E. coli*, while LAB demonstrated antibiotic susceptibility. Importantly, in cell form or through their metabolites, LAB exhibited a robust inhibitory effect on pathogenic *E. coli* isolates, suggesting their potential as effective probiotics.

Ethics approval and consent to participate: The authors declare their responsibility for any of the ethical issues that may arise after the publication of this manuscript.

Consent for publication: Researchers have no objection to publishing the research.

Availability of data and material: The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Competing interests: There is no conflict of interest in this research.

Funding: The research was completed without any financial support.

Authors' contributions: Karkaz M. Thalij designed the experiments, supervised all the research work and statistical analyses, and prepared the manuscript. Noor S. Ahmed participated in running all the experiments works. Muthana A. Sultan performed the experiments and helped to assist in certain aspects of the experiments. All authors read and approved the final manuscript.

Acknowledgment: Our thanks and appreciation to the laboratory staff in the Food Science Department, Tikrit University, IRAQ., for helping to provide most of the requirements for a successful search.

Ethics Statement: There is no conflict of interest. The feces samples of newborns were collected for screening the diarrhea infections conducted after the Scientific Research Ethics Commate, Tikrit University approval at 17/10l2024.

according to order 3l7l63 on 17-Jan-2023.

REFERENCES

1. **Alaobady F.H. and Thalij K.M. (2024). Evaluation of the Antioxidant and Inhibitory Activity of Fermented Aloe vera Extract Against the Pathogenic Bacteria Isolated from Diarrhoea Infections.Tikrit Journal for Agricultural Sciences Vol. 24 ((1).246-262.**
2. Alaobady F.H. and Thalij K.M. (2024). Evaluation of the Antioxidant and Inhibitory Activity of Fermented Aloe vera Extract Against the Pathogenic Bacteria Isolated from Diarrhoea Infections.Tikrit Journal for Agricultural Sciences Vol. 24 (1).246-262.
3. Aleman R.S. and Yadav A. (2024). Systematic Review of Probiotics and Their Potential for Developing Functional Nondairy Foods. *Appl. Microbiol.* 2024, *4*(1), 47-69; https://doi.org/10.3390/applmicrobiol4010004.
4. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility Testing; Thirty informational supplements. Approved standard M100-S25. CLSI, Wayne, PA. 2020.
5. Gupta, A., Polyak, C. S., Bishop, R. D., Sobel, J., Mintz, E. D., and Kulkarni, M. A. (2023). Burden of viral and bacterial pathogens causing acute diarrhea in children aged less than five years: A systematic review and meta-analysis. Clinical Infectious Diseases, 77 (1), 52-61. doi: [10.1093/cid/ciab064](https://doi.org/10.1093/cid/ciab064).
6. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods, p: 1152–1172. In Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC 2007.
7. **Khalil, I. A., Troeger, C., Blacker, B. F., Rao, P. C., Brown, A., Atherly, D. E., and Hay, S. I.** (2020). Morbidity, mortality, and long-term consequences associated with diarrhea from Cryptosporidium infection in children younger than 5 years: a meta-analyses study. **The Lancet Global Health, 8**(5), e598-e608. doi: 10.1016/S2214-109X(20)30030-9.

8. Kotloff, K. L., and Blackwelder, W. C. (2024). Burden and etiology of diarrhoeal disease in infants and young children in developing countries: A prospective, case-control study. The Lancet Global Health, 12 (3), e354-e365. doi: [10.1016/S2214-109X(24)00025-9](https://doi.org/10.1016/S2214-109X(24)00025-9).
9. Lee, B.H.; Hu, Y.F.; Chu, Y.T.; Wu, Y.S.; Hsu, W.H.; Nan, FH (2024). Lactic Acid Bacteria-Fermented Diet Containing Bacterial Extracellular Vesicles Inhibited Pathogenic Bacteria in Striped Beakfish (Oplegnathus fasciatus). Fermentation, 10, 49. https://doi.org/10.3390/ fermentation10010049.
10. Mahmood K. Salih, Nizar I. Alrabadi, Karkaz M. Thalij, Ali S. Hussien (2016). Isolation of Pathogenic Gram-Negative Bacteria from Urinary Tract Infected Patients. Open Journal of Medical Microbiology. Vol. 6: 59-65.
11. Obioha P.I., Anyogu B, Awamaria B., Ghoddusi H.B. and Ouoba L.I.I. (2023). Antimicrobial Resistance of Lactic Acid Bacteria from Nono, a Naturally Fermented Milk Product. Antibiotics 2023, 12(5), 843; https://doi.org/10.3390/antibiotics12050843.
12. Thalij, K. M., Sheet, B. S., & Samir, Z. T. (2022). The role of Lactobacillus casei on some physiological and biochemical parameters in male laboratory rats infection with salmonellosis. International Journal of Health Sciences, 6(S2), 5188–5199. https://doi.org/10.53730/ijhs.v6nS2.6296.
13. Thalij, K.M.; Mahmood, N.T.; Awth, H.A. (2019). Evaluation of Antibiotics Sensitivity for Bacteria Isolated from Urinary Tract Infections of Patients in Tikrit City, Iraq. EC Microbiology. 15 (10):974-979.
14. UNICEF/WHO. (2023). Diarrhea: Why children are still dying and what can be done. Available at: [UNICEF Diarrhoea Report](https://www.unicef.org/reports/diarrhoea-why-children-are-still-dying-and-what-can-be-done).