The effect of enterocin produced from *Enterococcus faecalis* and *Lactobacillus gasseri* filtrates on Enteropathogenic *Escherichia coli* causing diarrhoea in Iraqi children

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Abstract

Enteropathogenic *Escherichia coli* (EPEC) is one of the most prominent causative agents of diarrhea. This study aimed to isolate and identify pathogenic *E. coli* and to show their resistance to antibiotics, in addition to evaluating the inhibitory effect of enterocins produced by *Enterococcus faecalis* and *Lactobacillus gasseri* against these isolates. The results revealed the isolation of 103 EPEC isolates (68.66%), with isolation rates reaching 73% from stool samples and 60% from rectal swabs. Colonies exhibited diverse growth characteristics on selective and enriched media, and microscopic and biochemical examinations confirmed the diagnosis. Antibiotic susceptibility testing revealed that most EPEC isolates were multidrug-resistant (MDR), with the highest resistance to cefixime (39.80%) and Levofloxacin 40 (38.83%). Conversely, they showed the highest sensitivity to Amikacin 86 (83.49 %), and Azithromycin and Gentamicin 77 (74.75%). As for enterocin-producing bacteria, *L. gasseri* accounted for 40% of the isolated cases, while *E. faecalis* accounted for 26.6%. Diffusion tests in solid and liquid media showed that the filtrates from these isolates possessed inhibitory activity against EPEC, with inhibition zone diameters ranging from 8–14 mm for *L. gasseri* and 8–12 mm for *E. faecalis*. These results confirm that enterocins represent promising alternatives or complements to conventional antibiotics against multiresistant *E. coli* isolates, paving the way for their use as probiotics or future therapeutic agents, provided their safety and efficacy are clinically verified.

Keywords: Enteropathogenic *Escherichia coli*, bacterial diarrhoea, *Enterococcus faecalis*, *Lactobacillus gasseri*, enterocin.

Introduction.

Diarrheal diseases continue to pose a major threat to global health, particularly in low- and middle-income countries where sanitation and access to healthcare may be limited. Among the key bacterial agents responsible for infantile diarrhoea is Enteropathogenic *Escherichia coli* (EPEC), a pathotype of *E. coli* that causes significant morbidity due to its unique ability to form attaching and effacing (A/E) lesions on intestinal epithelial cells. This virulence mechanism enables EPEC to adhere tightly to host cells, resulting in the disruption of the intestinal barrier and leading to watery diarrhoea, particularly in infants and young children. Compounding the clinical challenge is the increasing resistance of EPEC strains to commonly used antibiotics,

necessitating the exploration of novel antimicrobial strategies [1-6]. Natural antimicrobials such as enterocins have garnered significant attention. Enterocins are ribosomally synthesized antimicrobial peptides primarily produced by lactic acid bacteria, including species such as Enterococcus faecalis and Lactobacillus gasseri. These peptides exhibit strong bactericidal or bacteriostatic activity, largely by disrupting the bacterial cell membrane or interfering with key metabolic processes. Enterocin A and B, produced by *E. faecalis*, and gassericin A and T, produced by *L. gasseri*, are among the most studied enterocins. Their amphipathic structures enable them to insert into bacterial membranes, resulting in pore formation, membrane depolarisation, and cell death. These properties make enterocins promising alternatives or adjuncts to traditional antibiotics, particularly in the control of multidrug-resistant pathogens [7].

Enterococcus faecalis is a facultative anaerobic Gram-positive coccus commonly found in the human gastrointestinal tract. It is known for producing several types of enterocins, including Enterocin A and L50, which have been shown to inhibit a wide range of bacterial pathogens. Although *E. faecalis* is sometimes associated with nosocomial infections, certain well-characterized strains are considered safe and even beneficial when used as probiotics. Similarly, *Lactobacillus gasseri* is a Gram-positive, anaerobic rod that is a natural inhabitant of the human gut and vaginal microbiota. It produces gassericins, which not only possess antibacterial activity but also contribute to the modulation of host immune responses and inhibition of pathogen adhesion [8].

To evaluate the inhibitory potential of enterocins against EPEC, researchers typically prepare cell-free filtrates (CFFs) from cultured *E. faecalis* and *L. gasseri* by centrifugation and membrane filtration to remove bacterial cells. These CFFs are then subjected to pH neutralization and catalase treatment to eliminate the effects of acidic metabolites and hydrogen peroxide, ensuring that any observed antimicrobial effect can be attributed primarily to the proteinaceous enterocins. In vitro assays such as agar well diffusion and minimum inhibitory concentration (MIC) tests have demonstrated significant inhibitory activity of these filtrates against EPEC strains. Notably, when filtrates from both *E. faecalis* and *L. gasseri* are used in combination, a synergistic effect is often observed, enhancing the inhibition of EPEC growth. The exact mechanism by which enterocins affect Gram-negative bacteria like EPEC is still under investigation. Still, it is hypothesized that their amphipathic nature allows them to interact with and penetrate the outer membrane, especially when combined with acidic or oxidative stressors [9]. The practical applications of these findings are numerous. Enterocin-producing strains, such as *E. faecalis* and *L. gasseri*, may be utilised as probiotics to reduce EPEC colonisation in the gut. Alternatively, purified or semi-purified enterocins (postbiotics) could be incorporated into therapeutic regimens or used in food preservation to prevent contamination. Furthermore, enterocins may act synergistically with antibiotics, enhancing their efficacy and potentially reducing the emergence of resistance [10].

Despite their promise, several challenges remain. Safety concerns regarding the use of *E. faecalis*, particularly given its association with opportunistic infections, must be addressed through the selection of specific strains and rigorous safety assessments. Additionally, issues related to the stability, delivery, and regulation of enterocin-based products must be resolved before widespread clinical or commercial application can be realized. Resistance to enterocins, though less common than antibiotic resistance, is still a possibility and warrants careful monitoring. So, enterocins from *E. faecalis* and *L. gasseri* represent a promising class of natural antimicrobials with potent activity against EPEC and other enteric pathogens. Their unique mechanisms of action, combined with their origin from generally recognized as safe (GRAS) organisms, position them as valuable tools in the ongoing fight against diarrheal diseases and antibiotic resistance [11]. The current study aims to examine the effect of enterocin produced from *Enterococcus faecalis* and *Lactobacillus gasseri* filtrates on Enteropathogenic *Escherichia coli* causing diarrhoea in Iraqi children.

Experimental

- **Stool sample collection:** One hundred stool samples were collected from individuals between the ages of 18 and 65 years, and fifty rectal swab samples were taken from children between the ages of 1 and 3 years who had watery diarrhoea, some of whom also had blood. In Baghdad, both male and female patients of both sexes were visiting the Medical City's facilities. To diagnose and isolate *E. coli*, samples were collected between August 28, 2023, and November 28, 2024, and were sent directly to the lab in a cool box. Fifty diarrhoea samples were also collected from healthy individuals.
- **Microscopic and morphological examination of stool samples and rectal swabs:** Physical examination (appearance, colour, and odour) of stool samples was performed. Samples were also examined microscopically using saline and iodine solutions in wet slide smears. Each sample was analysed for the presence of pus cells using a high-magnification lens (40X).
- **Identification of bacterial isolates:** All samples were cultured directly on MacConkey agar, Eosin Methylene Blue (EMB) agar, Bile Esculin Azide agar (BEAA) and Blood agar. Plates were incubated at 37°C for 18–24 hours [12]. After incubation, Gram stain was used to detect *E. coli* isolated from culture media [13]. Biochemical tests were performed to diagnose *E. coli* isolates using the VITEK 2 system (bioMérieux) [14]. Motility Test medium was used to study the motility of isolated bacteria [13].
- **The test for EPEC isolates' sensitivity to and resistance to antibiotics:** in accordance with the reference [15, 16], the modified Kirby-Bauer method (Disk Diffusion Method) was used to examine the susceptibility and resistance of *E. coli* isolates to different antibiotics.
- ***Enterococcus faecalis* and *Lactobacillus* isolation and diagnosis**: they were isolated from 150 stool and rectal samples by inoculating 100–200 µL of each sample into the appropriate enrichment medium. *E. faecalis* was cultured in bile-esculin-azide broth and incubated at 37 °C for 18–24 hours under aerobic conditions. Before plating, the broth's darkening from esculin hydrolysis was thought to be a common, though not a necessary, indicator. In order to lower the redox potential, 0.05% L-cysteine was optionally added when the sample was inoculated into MRS broth for *L. gasseri*. Incubation in an anaerobic jar (GasPak) or under microaerophilic conditions with 5% CO_2 at 37 °C for 24–48 hours was used to adjust the headspace to low oxygen levels. Bile or sodium azide was also omitted to prevent lactobacilli from being suppressed. After that, they diagnosed using Indole, Methyl Red, Vogues-Proskauer, and Citrate (IMViC tests) and the VITEK2 system [17-20].

- **Methods for detecting bacteriocins**

    1. **Cup Method assay:** To detect the ability of the isolates to produce enterocin, the following method was followed [21]: The previously grown *E. faecalis* cultures were cultured in a prepared heart and brain infusion broth medium at 14 hours of age using the diffusion method on MRS agar medium. The plates were then incubated at 37°C for 14 hours. After incubation, 5 mm diameter discs were made using a cork piercer in this medium. 0.1 ml of cultures of each of the test bacterial isolates was placed on the surface of the prepared nutrient agar, spread with a

diffuser. The number of cultured cells was confirmed at 10^8 cells/ml by comparison with the standard McFarland solution. The plates were then incubated at 37°C for 14 hours, after which the diameter of the inhibition zone around the discs was measured. It was compared with the control treatment containing untreated MRS broth medium. The experiment was repeated using *Lactobacillus gasseri*.

2. **Diffusion Well Method**: According to [21], to detect enterosine production in the liquid medium (MRS) of *E. faecalis*, the plates containing the nutrient agar medium were planted by spreading 0.1 ml of the inoculum of the test bacterial isolates using a sterile glass diffuser (each one separately) after the number of cultured cells was fixed at 10^8 cells/ml, by making holes with a diameter of 1 mm on the surface of the medium, each hole was filled with 100 microliters of the liquid culture of *E. faecalis* isolates. The plates were then incubated at 37°C for 14 hours. The inhibition zones around the holes containing the productive isolate were measured and compared with the control treatment containing MRS broth medium not inoculated with bacteria. The experiment was repeated using *Lactobacillus gasseri*.

Results and Dissections

After performing morphological, microscopic, and biochemical examinations, (103) isolates of Enteropathogenic *Escherichia coli* (EPEC) were obtained, representing (68.66%). Out of 100 faecal samples, EPEC was isolated in 73 cases (73%). Out of 50 rectal swabs, EPEC was isolated in 30 cases (60%), as shown in Table 1. While *Lactobacillus gasseri* and *Enterococcus faecalis* were found in 60 (40%) and 40 (26.66 %), respectively, of the clinical models studied.

Table (1): Percentages of EPEC isolates isolated from patients with diarrhoea using selective and enriched culture media

Number of negative samples (%)		**Number of positive samples containing EPEC isolates (%)**		**Total (%)**	
Number	**(%)**	**Number**	**(%)**	**Number**	**(%)**
47	(31.33)	103	(68.66)	150	(100)

Culture media that were enriched, specific, and selective were used to identify the bacterial isolates. Colonies of *E. coli* showed up as pink on both selective and differential MacConkey agar.

Bacterial isolates produced shiny metallic green colonies on Eosin Methylene Blue (EMB) agar. This characteristic distinguishes *E. coli* from other members of the Enterobacteriaceae family, as the medium contains the dyes Eosin and Methylene Blue, which precipitate in the acidic medium after binding to each other, thereby encouraging their association with bacteria and subsequent uptake by them. They give a metallic green sheen, indicating that they have produced organic acids as a result of the fermentation of lactose and sucrose.

In the present study, *E. coli* colonies appeared on Blood agar as large, circular, grey to white, and moist colonies. 30 isolates (29.12%) showed β-hemolysis, and 73 isolates (70%) did not show γ-hemolysis on the medium. This indicates that the isolates that produced hemolysin are hemolysin producers.

The bacteria grown on MacConkey agar were stained after incubation for 18-24 hours using Gram stain and examined under a conventional light microscope. Bacilli and Coccobacilli cells appeared short, Gram-negative, and arranged in double or single shapes, which were assumed to be *E. coli*. In the current study, several biochemical tests were conducted on all bacterial isolates, which gave a positive result for the catalase test, which proves that these bacteria have the ability to convert hydrogen peroxide into H_2O and O_2. The isolates yielded a positive result for the Indole test. This test is important for detecting and distinguishing *E. coli* from other members of the Enterobacteriaceae family. The isolates also yielded a positive result for the Methyl red test, as *E. coli* has the ability to consume and ferment glucose and peptose, forming acids. On the other hand, the isolates yielded negative results for the Voges-Proskauer test, citrate utilization test, oxidase test, and urease test.

The appearance of *E. faecalis* colonies and the change in the colour of the medium to black after incubation indicate the ability of the cocci isolates to produce the enzyme esculinase, which hydrolyzes esculin into glucose and esculin, which combines with iron ions to form a black complex. The isolates also exhibited growth in high concentrations of bile salts. In comparison, its colonies appeared on blood agar medium as grey, beta-hemolytic. In the current study, *Enterococcus faecalis* is characterized by being Gram-positive cocci, arranged in single cocci, pairs, and short chains, and some in a coccobacillary form [Table 2]. It is characterized by being negative for the oxidase test and not producing cytochrome, so it gives a negative test for the catalase test, although some are weakly positive. Biochemical reactions represent a diagnostic feature of the genus *Enterococcus faecalis*, distinguishing it from other streptococci. *Enterococcus faecalis* is the only species that ferments pyruvate. Bacterial isolates also demonstrated the ability to produce the enzyme hippuricase, which hydrolyzes hippurate, producing glycine and benzoic acid, while the remaining isolates tested negative for enzyme production.

Under the light microscope, *Lactobacillus gasseri* appears Gram-positive, rod-shaped, and appears in pairs or short chains. MRS agar and broth stimulate its growth, resulting in the formation of rough, round, white colonies. Biochemically, nitrate reduction is usually not observed, and catalase and oxidase tests yielded negative results. Citrate reduction is recorded as negative in IMViC (Indole, Methyl Red, Voges-Proskauer, Utilization of Citrate) reactions, while methyl red is recorded as variable or slightly positive depending on the isolates, and indole and Voges-Proskauer tests are recorded as negative. The results of fermentation of sugars (Glucose, Fructose, Lactose, Mannitol, Sucrose, Maltose) were (+,+,+,-,-,-) respectively [Table 2]. Both species (*Enterococcus faecalis* and *Lactobacillus gasseri*) give the same results in IMViC (– + – –), so differentiation between them must be based on other biochemical tests, such as growth in 6.5% NaCl for *Enterococcus faecalis*, while carbohydrate fermentation is used for *L. gasseri*.

Table 2: Biochemical and cultural characteristics of bacteria *Lactobacillus gasseri* and *E. faecalis*

Test name	*Lactobacillus gasseri*	*Enterococcus faecalis*

Gram stain	Gram-positive bacilli	Gram-positive cocci (pairs/ short chains)
Catalase	-ve	-ve
Oxidase	-ve	-ve
IMViC tests	-,+,-,- ve respectively	-,+,-,- ve respectively
Hydrolysis of Bile esculin	-ve	**+ve**
PYR test	-ve	**+ve**
Growth in 6.5% NaCl	-ve	**+ve**
Growth at 10 °C	Poor/-ve	**+ve**
Growth at 45 °C	Poor/-ve	**+ve**
Gas from glucose fermentation	Variable (often no gas; homofermentative)	-ve
Hemolysis on blood agar	α or γ	Usually γ (non-hemolytic)
Optimum growth atmosphere	Microaerophilic/anaerobic	Aerobic or facultative anaerobic

Antibiotic Resistance and Sensitivity in Enteropathogenic *E. coli*

Antibiotic susceptibility testing was performed for EPEC isolates, which included 8 antibiotics (Oxoid): Ceftazidime (5 µg/disc), Ceftriaxone (30 µg/disc), Cefixime (30 µg/disc), Amikacin (10 µg/disc), Gentamycin (5 µg/disc), Levofloxacin (30 µg/disc), Ciprofloxacin (5 µg/disc), and Azithromycin (15 µg/disc). All EPEC isolates were multidrug-resistant (MDR). The results showed that 41 (39.80%) bacterial isolates were highly resistant to Cefixime, and also resistant to the antibiotic Levofloxacin at a rate of 40 (38.83%). While the percentages of resistance to the antibiotics Ceftriaxone and Ciprofloxacin were 30 (29.12%). Regarding the antibiotic Azithromycin, the bacterial isolates showed the least resistance to it, with a percentage of 21 (20.38%). The bacterial isolates showed the highest sensitivity to the antibiotic amikacin at a rate of 86 (83.49 %), and Azithromycin and Gentamicin at a rate of 77 (74.75%) [Table 3 and Figure 1].

Table (3): Number of EPEC bacterial isolates and the percentage of their resistance and sensitivity to the antibiotics under study

Antibiotic	Code	E. coli					
		(S)		(I)		(R)	
		No.	(%)	No.	(%)	No.	(%)
Cefixime	CFM	57	55.33	2	1.94	41	39.80
Ceftriaxone	CRO	67	65.04	3	2.91	30	29.12
Ceftazidime	CAZ	73	70.87	2	1.94	25	24.27
Gentamicin	CN	77	74.75	3	2.91	20	19.41
Levofloxacin	LEV	57	55.33	3	2.91	40	38.83
Amikacin	AK	86	83.49	2	1.94	22	21.35
Ciprofloxacin	CIP	67	65.04	3	2.91	30	29.12
Azithromycin	AZM	77	74.75	2	1.94	21	20.38

R: Resistance, I: Intermediate, S: Sensitive

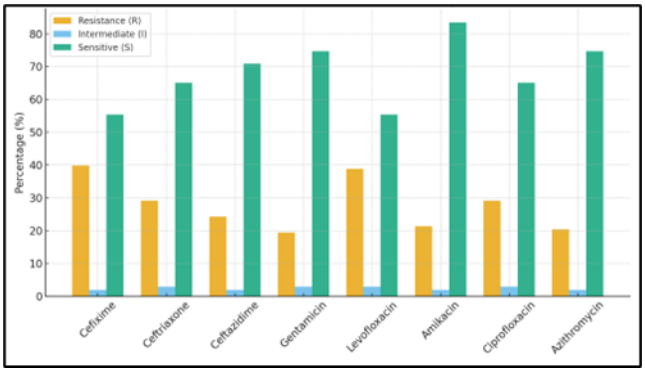

Figure 1: Resistance (R), intermediate (I) and sensitivity (S) rates of EPEC isolates against the different antibiotics under study

In the present study, the susceptibility of local *E. faecalis* isolates to enterocin production was tested by Cup Agar in solid MRS medium and BHI broth against test bacterial isolates, which included Gram-negative bacteria causing diarrhoea in children. The anthrosin-producing *E. faecalis* isolates showed variation in inhibitory effect against the test bacteria, with the inhibition diameter ranging between 8 and 12 mm for the agar disc method. A discrepancy in the inhibitory effect of the same isolates on the two media used was also observed. Four *E. faecalis* isolates demonstrated an inhibitory effect on one or more of the test bacteria on enterocin production on solid media using the agar disc method. These results are consistent with local and international studies. All of these isolates were susceptible to enterocin. When studying the sensitivity of enterocin-producing *Lactobacillus* isolates to diarrhoea-causing bacteria using the well diffusion method, it was found that the inhibition zone was between 8-14 mm (Figures 2 and 3). Therefore, the current study concluded that pathogenic *E. coli* isolates exhibit high resistance to antibiotics, highlighting the need to develop therapeutic alternatives. Entercins produced from *E. faecalis* and *L. gasseri* have proven to be inhibitory against these isolates, making them a promising option for combating bacterial diarrhoea in the future.

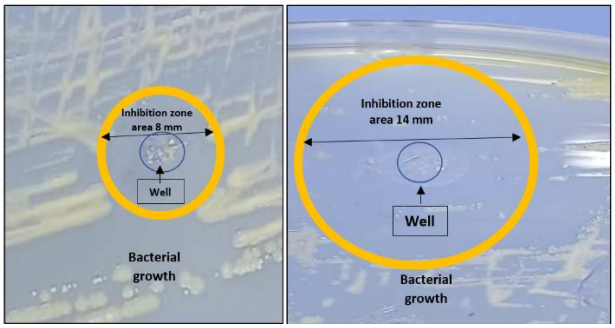

Figure 2: Susceptibility of enterocin-producing *Lactobacillus* isolates to diarrhoea-causing bacteria using the Well Diffusion Method

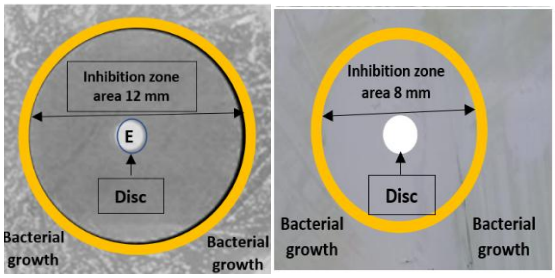

Figure 3: The inhibition zone diameter for the Cup plate method (disk diffusion test) against *E. coli* on MRS agar in the presence of *Enterococcus faecalis* filtrates ranged from 8 to 12 hours.

Awayid and Khamas (2013) [21] found that 300 samples were collected from different hospitals in Baghdad. To identify each isolate up to the species level, VITEK 2 performed cultural, microscopical, and biochemical analyses. According to the findings, 40 isolates of *Enterococcus* faecalis were found, with 25 of these coming from diarrhoea-causing bacteria and 15 from normal flora. Of the thirty-one isolates, eight were of Salmonella spp., five were of *E. coli* O157:H7, and eighteen were of *E. coli*. The API 20E strip was used for testing, and all isolates underwent biochemical, microscopical, and culture analyses, and 31 isolates to antibiotics (11–9) was evaluated. The isolates exhibited multiple antibiotic resistance, according to the results. *Salmonella spp*. and all isolates of *E. coli* and *E. coli* O157:H7 were resistant to a number of antibiotics, including negative bacteria and those in the Enterobacteriaceae group. Assessing the inhibitory activity of broth and agar in two media, MRS, BHI, and two techniques against diarrheal bacteria in order to determine whether local isolates of *E. faecalis* are capable of producing enterocin. With an inhibitor zone ranging from 12 to 20 mm, the results demonstrated a range of local isolates' inhibitory efficacy against bacteria that cause diarrhoea in children and young sheep. The best media from the brain-heart infusion were MRS liquid and solid. In another study, the ability of local *E. faecalis* isolates to produce enetocin on solid media was tested using Cup Agar on solid MRS and brain and heart infusion agar against test bacterial isolates, which included Gram-negative bacteria causing diarrhoea. Anthrosin-producing *E. faecalis* showed varying inhibitory effects against test bacteria, with the inhibition diameter ranging from 13 to 21 mm for the agar disc method. While the effectiveness of 4 isolates of *E. faecalis* against one or more isolates of test bacteria was demonstrated, on the production of enterocin on solid media using the agar disc method, these results are consistent with the results obtained by Facklam and Elliott, 1995 when they used several methods to investigate the production of bacteriocin, which confirmed the efficiency of the agar disc method to detect the inhibitory activity of the produced bacteriocin.

Discussion:

Colonies of *E. coli* showed as pink on both selective and differential MacConkey agar due to the fact that, as other studies have previously documented, *E. coli* ferments lactose [22]. They give a metallic green sheen, indicating that they have produced organic acids as a result of the fermentation of lactose and sucrose [23]. The IMViC tests in the current study are consistent with the results of previous local and international studies [24, 25]. Black colonies of *E. faecalis* appeared on the culture medium, indicating the production of the enzyme esculinase, which hydrolyzes esculin into glucose and esculin and then combines with iron ions to form a black complex.So, the present results are compatible with other studies [26].

The present study was similar in its results to a study conducted in 2017 [27] and [28]. Beta-hemolysis was observed in 25.58% of *E. coli* isolates, and the appearance of a clear zone around the colonies on Blood agar may be due to the expression of the (*hlyA*)-hemolysin α gene possessed by the bacteria [29]. The morphological studies of bacteria in the current studies were consistent with what was previously mentioned [30-32].

Recent studies indicate an increase in the rates of resistance of *Escherichia coli* isolated from diarrheal cases to various antibiotics between 2022 and 2025. A study in Iraq in 2025 showed that *E. coli* strains exhibited high resistance to ceftazidime, ceftriaxone, and cefixime, with the resistance rate exceeding 90%, while resistance to amikacin was low at 4.6%, and resistance to gentamicin reached 41.3% [33]. Another study in 2024 showed 18.97% resistance to levofloxacin and 25.79% to ciprofloxacin among strains isolated from diarrheal patients [34]. As for azithromycin, another study in Iraq in 2025 showed 100% resistance among *E. coli* strains isolated from diarrheal cases [35]. A study published in 2025 also indicated that Extended-Spectrum Beta-Lactamases (ESBL) producing strains were more resistant to antibiotics than non-producing strains, emphasizing the importance of identifying these genes in assessing bacterial resistance [36]. Another study in 2025 showed that resistance patterns vary significantly between different regions, necessitating local studies to determine the prevailing resistance patterns [28]. Based on these findings, it is recommended to conduct periodic susceptibility tests, implement strategies to combat antibiotic resistance, and develop strict policies for the use of antibiotics in human medicine, veterinary medicine, and agriculture [34]. In addition, a study published in 2025 showed that 100% of *E. coli* strains isolated from diarrheal cases in Baqubah were resistant to penicillin, indicating widespread resistance of this strain to basic antibiotics [38]. Diarrheal diseases are a major cause of infection, morbidity, and mortality among children, and diarrheal *Escherichia coli* is a major factor in some developing countries. The prevalence of diarrheal *E. coli* in children under 5 years of age was estimated and the effect of some antibiotics was evaluated. Researchers collected 112 samples from children at two health centers in Wasit Governorate, Iraq, and cultured them on different culture media. Forty-three (38.4%) *E. coli* isolates were identified using conventional testing. The isolates showed resistance to penicillin (100%), high resistance to cephalothin (76.7%) and amoxicillin-clavulanate (62.8%), while they were sensitive to gentamicin (60.4%). The results showed that diarrheal *E. coli* was the most prevalent among the infected children, with varying antibiotic responses [39].

Yang *et al*., (2018) [40] stated that solid media stimulates antagonism to the production of bacteriocins. In a study conducted by Al-Barzangi (2001) [41] on bacteriocins produced by *E. faecalis*, the highest percentage of isolates producing and effective against Gram-positive bacteria was found to be 33%, while the ability of the remaining producing isolates to affect other types of bacteria belonging to Gram-positive bacteria varied. while none of the isolates studied showed effectiveness against *E. coli*. Many studies that have addressed the mechanism of action of bacteriocins indicate that its effect on sensitive isolates may be due to the stimulation of some enzymes (Autolysine) that are, under normal conditions, linked to fatty acids that are part of the wall composition and that work to destroy the membranes of bacterial cells by forming ion channels that lead to the removal of polarity of the cell membranes (Depolorization) [41]. While the resistance of other isolates lies in their possession of the gene that encodes immunity to enterosin, or the occurrence of some simple changes in the composition of the membranes and walls of these cells, or their lack of receptors specific to this enterosin [42-44]. Jabir's study (2017) [45] revealed that among 20 samples taken from acne patients, 15 isolates were identified as *Propionibacterium acnes*, while the remaining 5 isolates were *Staphylococcus* aureus and

Staphylococcus epidermidis. Antibiotic susceptibility testing revealed variations in resistance and sensitivity among the isolates. The filtrate *of Lactobacillus gasseri* showed clear inhibitory activity against *Propionibacterium acnes*. Especially when the concentrated filtrate was used twice with a 48-hour incubation period, the largest inhibition zones were recorded, while the effect was less pronounced at 24 and 72 hours. This activity is attributed to the production of organic acids such as lactic and acetic acids, as well as bacteriocins that affect the cell membrane and metabolic activities of bacteria, suggesting the potential of probiotics as an adjunctive therapeutic option in controlling acne. After 24 hours, the inhibition zones ranged from 10 to 15 mm using the non-concentrated and double-concentrated filtrate. After 48 hours, the best result was achieved, with inhibition zones ranging from 13 to 16 mm for the non-concentrated filtrate. They ranged from 18 to 28 mm for the double-concentrated filtrate, with the largest inhibition zone (26–28 mm) recorded for some isolates. The research demonstrated that the filtrate of *Lactobacillus gasseri* has a protective effect against pathogenic *Aeromonas spp*., both inside and outside the body.

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Conclusion

This study demonstrates a high prevalence of multidrug-resistant enteropathogenic *Escherichia coli* among stool and rectal swab samples, highlighting a growing therapeutic challenge. The elevated resistance to commonly used antibiotics contrasts with retained susceptibility to amikacin, azithromycin, and gentamicin. Enterocins produced by Lactobacillus gasseri and Enterococcus faecalis showed clear inhibitory activity against EPEC isolates. These findings support the potential role of enterocins as effective adjuncts or alternatives to conventional antibiotics. Further in vivo and clinical studies are required to confirm their safety, stability, and therapeutic applicability.

Conflicts of interest

There are no conflicts to declare

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تأثير الإنتيروسين المُنتج من مُرشحات بكتيريا المكورة المعوية البرازية (Enterococcus faecalis) وبكتيريا لعصية اللبنية الجاسيرية (Lactobacillus gasseri) على بكتيريا الإشريكية القولونية المُمرضة للأمعاء لمُسببة للإسهال لدى الأطفال العراقيين

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الخلاصة:

تُعدّ الإشريكية القولونية الممرضة للأمعاء (EPEC) من أبرز مسببات الإسهال. هدفت هذه الدراسة إلى عزل وتحديد سلالات الإشريكية القولونية الممرضة، وبيان مقاومتها للمضادات الحيوية، بالإضافة إلى تقييم التأثير المثبط للإنتيروسينات التي تنتجها بكتيريا المكورة المعوية البرازية (Enterococcus faecalis) وبكتيريا العصية اللبنية الجاسيرية (Lactobacillus gasseri) ضد هذه السلالات. كشفت النتائج عن عزل 103 سلالات من الإشريكية القولونية الممرضة للأمعاء (68.66%)، حيث بلغت نسبة العزل 73% من عينات البراز و 60% من مسحات لمستقيم. أظهرت المستعمرات خصائص نمو متنوعة على أوساط زرع انتقائية ومُخصبة، وأكدت الفحوصات المجهرية والكيميائية الحيوية التشخيص. أظهر اختبار حساسية المضادات الحيوية أن معظم سلالات الإشريكية القولونية الممرضة للأمعاء مقاومة للأدوية المتعددة(MDR) ، مع أعلى مقاومة للسيفيكسيم(39.80 %) والليفوفلوكساسين40 (38.83 %). في المقابل، أظهرت هذه العينات أعلى حساسية 86 (83.49%) ، وللأزيثروميسين والجنتاميسين77 (% 74.75). أما بالنسبة للبكتيريا المنتجة للإنتيروسين، فقد شكلت بكتيريا L. gasseri نسبة 40% من الحالات المعزولة، بينما شكلت بكتيريا E. faecalis نسبة 26.6% واظهرت اختبارات الانتشار في الأوساط الصلبة والسائلة أن الرشاحة من هذه العزلات تمتلك نشاطًا مثبطًا ضد بكتيرياEPEC ، حيث تراوحت أقطار منطقة التثبيط من 8 إلى 14 ملم لبكتيريا L. gasseri ومن 8 إلى 12 ملم لبكتيريا.E. faecalis

تؤكد هذه النتائج أن الإنتيروسينات تمثل بدائل أو مكملات واعدة للمات الحيوية التقيدية د لات الإشريكية القولونية المقاومة للأدوية المتعددة، مما يمهد الطريق لاستخدامها كمُعززات حيوية أو عوامل علاجية مستقبلية، شريطة التحقق سريريًا من سلامتها وفعاليتها.

الكلمات المفتاحية: الإشريكية القولونية المسببة للأمراض المعوية، الإسهال البكتيري، المكورة المعوية البرازية، العصية اللبنية الجاسيرية، الإنتيروسين.