

Original Research Article

EVALUATION OF CHEESE WHEY FOR ANTIBACTERIAL ACTIVITY AGAINST DIARRHOEAGENIC *ESCHERICHIA COLI*

Komolafe, T. O^{1*}, Adebolu T. T², Daniels, A. O³

¹ Science Laboratory Technology Department, Faculty of Applied Science, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

² Microbiology Department, Federal University of Technology Akure, Ondo State, Nigeria.

³ Microbiology Department, Achievers University, Owo, Nigeria.

Correspondence should be addressed to Komolafe Tolulope Omolola: komolafetolulopelola@gmail.com

Article No: 024 | **Accepted:** 28 September 2026 | **Published:** 10 October 2026

Abstract: Diarrhoeal diseases remain a major public health problem and are among the leading causes of morbidity and mortality in children, particularly in developing countries. Among the bacterial pathogens associated with childhood diarrhoea, *Escherichia coli* is frequently implicated, with diarrhoeagenic strains contributing significantly to gastrointestinal infections. Although the infection can be treated with antibiotics however the emergence of multi antibiotic-resistant *E. coli* has complicated treatment therefore the need for alternative antibacterial therapies. This study therefore evaluates cheese whey for antibacterial activity against diarrhoeagenic *E. coli* isolated from stool samples of diarrhoeic children in the bid of searching for alternative therapies for treating bacterial diarrhoea caused by *E. coli*. The influence of types of coagulants in whey production on its antibacterial properties was also studied. A total of 55 stool samples was collected from diarrhoeal children at selected primary health centers and government hospitals in Owo, Ondo State, Nigeria. The collected stools were analyzed using standard microbiological, biochemical and molecular techniques to identify the types of bacteria present. The antibiogram profile of the isolated *E. coli* was determined using standard microbiological assays. The antibacterial activity of commercially prepared whey (CPW) was compared with that of the laboratory prepared whey (LPW) against the isolated *E. coli* using agar well diffusion assay. So also the effect of types of coagulants used in the production of whey on its antibacterial activity using standard methods. *E. coli* was isolated in 47.3% of the stool samples examined. Other bacterial species isolated are *Staphylococcus aureus* (21.8%), *Pseudomonas aeruginosa* (16.4%), and *Salmonella enterica* (14.6%). The antibiogram profile of the isolated *E. coli* revealed high levels of multi-antibiotic resistance (MAR) index (0.4-1.0) among the isolates to pefloxacin, amoxicillin, augmentin and streptomycin but were susceptible to ciprofloxacin. CPW demonstrated antibacterial activity against

some of the isolated *E. coli* whereas LPW exhibited broader and stronger antibacterial activity on all the test *E. coli* isolates except one. The inhibition mediated by LPW was superior to that mediated by ciprofloxacin on 7 (26.9%) of the *E. coli* isolates and was at par with the inhibition mediated by ciprofloxacin on most of the *E. coli* isolates. Whey prepared using *Calotropis procera* as coagulant showed the highest growth inhibitory activity compared with whey prepared using lime and alum. The findings demonstrate that cheese whey possesses antibacterial properties against the isolated diarrhoeagenic *E. coli*, with efficacy influenced by coagulant type. Therefore, cheese whey can be explored in the development of a more effective antibacterial drug that can be exploited in the management of diarrhoea caused by *E. coli* especially in the absence of ciprofloxacin which is the most effective antibiotic against the isolated diarrhoeagenic *E. coli*.

Keywords: Diarrhoeagenic *Escherichia coli*, Antibigram profile, Cheese whey, Antibacterial activity.

INTRODUCTION

Diarrhoeal diseases still constitute one of the most important public health concerns around the globe, especially among infants and toddlers in less developed nations (Behera & Mishra, 2022). Even though there have been substantial developments made regarding the provision of health services, sanitation, and prevention measures, diarrhoea still contributes to the ill health and deaths of many children around the world. According to the World Health Organization (WHO) (2024), diarrhoeal diseases are one of the top contributors to deaths in children below five years old. The impact of this illness is particularly high in low-and-middle income nations due to factors such as poor sanitation, contaminated water, lack of hygiene, malnutrition, and lack of access to health care services which can facilitate the spread of enteric pathogens (Taviani & Pedro, 2022).

Some microorganisms like bacteria, viruses, and protozoans can cause diarrhoea. Out of these microbes, the bacteria known as *Escherichia coli* (*E. coli*) is highly regarded due to its common presence and the potential to cause different gastrointestinal infections. While some strains of the *E. coli* bacteria occur naturally as nonpathogenic symbionts in the intestines of both human beings and animals, other strains of the same bacteria have the potential to cause disease due to their virulence. Such bacteria are classified under diarrhoeagenic *Escherichia coli* (DEC) and are recognized as the important etiological agents of diarrhoea worldwide (Makarova, 2023).

Diarrhoeagenic *E. coli* includes various types like enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (Makarova, 2023). Various virulence genes are found in each type of *E. coli*, which help them adhere, colonize, produce toxins, invade tissues, and develop the disease. Common virulence genes that include *eaeA*, *hlyA*, *bfpA*, *aggR*, and *aaiC* are usually considered

molecular markers in identifying pathogenic *E. coli* (Kumawat & Sharma, 2025). Their existence is normally associated with the virulence capabilities of the organism and their ability to cause diarrhoea.

Antibiotic therapy is common in the management of bacterial diarrhoea, which is usually accompanied by supportive treatment where necessary (Dereje *et al.*, 2023). Unfortunately, the efficiency of antibiotic therapy has been hampered by the growing issue of antimicrobial resistance. The improper prescription and use of antibiotics by patients, the extensive use of antibiotics on animals for meat production purposes, and poor patient compliance with the prescribed treatment plans have all led to resistant pathogens becoming more common. Enteric bacteria, such as diarrhoeagenic *E. coli*, have thus become resistant to various antibiotics, making treatment more difficult and multiplying the risk of complications and mortality (Biswas *et al.*, 2024). This issue which has evolved into a global public health problem therefore necessitates new solutions for the control of bacterial infections (Zulfkar *et al.*, 2025). In searching for new therapies naturally occurring antimicrobials isolated from plants, microorganisms, and food sources have attracted a lot of research interest because of their low toxicity, wide availability, and low resistance induction capability. Among these alternatives, one particular source of naturally occurring antimicrobials is dairy products which has emerged as a promising source of bioactive compounds with antimicrobial properties (El Alami *et al.*, 2025).

Cheese Whey is the byproduct of cheese and casein production that remains after the selective coagulation of casein (Eugter *et al.*, 2012). Whey is yellowish green in color and contains water, lactose, whey proteins, minerals, vitamins, and different types of bioactive compounds. It contains key health-promoting proteins and peptides—such as immunoglobulins, bovine serum albumin, α -lactalbumin, and β -lactoglobulin—that act as antimicrobial, antioxidant, and immune-modulating agents in the body (Vildan *et al.*, 2023). The amount of whey is about 85%-90% of milk and comprises almost 50% of the total nutritional value of milk. For a long time, whey has been regarded as a waste product in the dairy industry, leading to environmental contamination owing to its high organic matter content. However a variety of researches show that whey has a variety of functional characteristics, such as antioxidant, immuno-modulating, anti-inflammatory, probiotic, and antimicrobial effects (Adebolu & Olorunfemi, 2016; Ashraf *et al.*, 2025). The antimicrobial properties of whey are mainly due to the presence of various bioactive substances, such as lactoferrin, lactoperoxidase, immunoglobulins, bacteriocins, hydrogen peroxide, organic acids, and antimicrobial peptides generated by lactic acid bacteria (Luparelli *et al.*, 2025). These substances may inhibit various pathogens by means of different mechanisms, such as membrane destruction, nutritional depletion, inhibition of metabolism, and formation of inhibitory metabolites (Malos *et al.*, 2025). Although there are studies indicating that whey can be used for antimicrobial purposes (Adebolu & Olorunfemi, 2016), yet there is scant literature regarding the antibacterial activity of whey prepared using different coagulants on diarrhoea-producing *E. coli*. Therefore, the aim of this study was to investigate the antibiotic susceptibility pattern of the isolated diarrhoeagenic strains of *Escherichia coli* in this study, assess the antibacterial properties of cheese whey prepared using traditional

technology and control laboratory procedures using different coagulants against the pathogen. In addition, the roles played by the different metabolites produced by lactobacilli isolated from the whey samples on the antibacterial activity of whey on the test bacteria was evaluated. The findings of this research are expected to help develop better alternatives for the treatment of bacterial diarrhoea exploring cheese whey which is normally discarded as a waste product.

METHODOLOGY

Collection of stool samples, isolation and identification of bacteria present

Prior to the commencement of this study, Ethical Clearance Certificate (OSREC/16/07/2025/1047) was collected from the appropriate health authority at the Ministry of Health, Akure, Ondo State, Nigeria. Stool samples (n = 55) was then collected from the patients that reported to the selected hospitals and primary health centres in Owo, Ondo State with the complaints of diarrhoea and fever and who gave their consent to participate in the study. Each stool sample was serially diluted and 1ml was taken from the 10^{-4} dilution and cultured on Nutrient Agar plate under sterile conditions. It was then incubated at 37°C for 18–24 hours. After incubation, the plate was observed for bacterial growth. Colonies were picked with sterile inoculating loop and subcultured on Eosin Methylene Blue (EMB) Agar and MacConkey Agar (MAC). Colonies with a green metallic sheen was picked on EMB and pink, round, medium-sized colonies was picked on MCA, for Gram's staining and biochemical tests to confirm *E.coli* (Cheesbrough, 2006; Tille, 2017). All the other colonies were also subjected to both Gram's staining and biochemical tests for their identification. Molecular classification of the isolated *E. coli* was carried out using PCR by amplifying specific genes associated with virulence and pathogenicity. The following genes; *eaeA*, *bfpA* and *hlyA* were amplified in three of the isolates using standard protocols.

Antibiotic Susceptibility Test

Mueller Hinton Agar (MHA) was prepared and poured into sterile Petri dishes. It was allowed to solidify and dry. The standardized *E. coli* inoculum using McFarland method was swabbed uniformly on the MHA plate. Standard antibiotic discs was placed on the swabbed plates and the plates were incubated at 37°C for 18 hours. The diameter of inhibition zones was measured. The results were interpreted using to CLSI guidelines (2024).

Source of Commercially produced cheese whey

The commercially produced whey (CPW) used in this study was sourced from Fulani women who produce cheese in Owo town, Ondo State, Nigeria. The whey was collected into sterile containers and transported to the laboratory for growth inhibitory activity on the isolated *E. coli* and other tests such as pH determination and titratable acidity assay.

Production of Cheese whey in the Laboratory using different coagulants

Fresh raw milk from a healthy cow was bought from cattle sellers in Owo, Ondo State, Nigeria and was divided into three portions (A, B, C). Each portion of the milk was poured into three different clean pots and heated until warm (45°C). Into the first portion of the milk (A), undiluted juice of *Calotropis procera* leaves was gradually added and stirred gently. Lime juice was gradually added to the second portion (B) of the warm milk while Alum water was gradually added to the third portion (C) of the warm milk. As the milk was gently stirred, curds (cheese) begin to form leaving the liquid part which is the whey. The curds were carefully separated from the liquid using clean cheese cloth. The liquid part was used for the study.

Evaluation of the Antibacterial activity of commercially prepared whey (CPW) and laboratory prepared whey (LPW) on the isolated *E. coli*

Mueller Hinton Agar (MHA) was prepared, poured into Petridish plates and standardized *E. coli* inoculum using McFarland method was swabbed on the surface of the agar. For the evaluation of the antibacterial activity of freshly prepared CPW on the isolated *E. coli*, three 6 mm diameter wells were punched on different locations on the agar. The first well (A) was filled with 0.5mL of the whey sample, the second well (B) was filled with 0.5mL amoxicillin and the last well (C) was filled with 0.5mL ciprofloxacin. Both amoxicillin and ciprofloxacin served as control. For the comparative antibacterial activity of freshly prepared CPW and LPW, three 6mm diameter wells were bored on another MHA agar and 0.5mL CPW was introduced into well 1, 0.5mL LPW was introduced into well 2 and Ciprofloxacin into the third well. All the plates were incubated at 37°C for 24h and the zones of inhibition were measured using a transparent ruler. For the effect of fermentation duration and temperature of holding on the antibacterial activity of CPW on the isolated *E. coli*, two 6mm diameter wells were bored into another MHA plate and the different whey samples were added into the wells as follows: Well 1, 24h fermented whey at 4°C and well 2, 24h fermented whey at 36°C. The plates were incubated as earlier described and zones of inhibition measured. This was repeated daily for 7 days using the fermented whey held at the two temperatures. All assays were carried out in triplicates.

Evaluation of the Bio-metabolites in Whey for Antibacterial activity

For this assay, the types of bacteria in whey was first isolated and identified from 72h fermented whey using standard microbiological tests including culturing whey on Man, Rogosa and Sharpe agar (MRS) for growth of Lactic Acid Bacteria (LAB). The isolated LAB was maintained in MRS broth and incubated for 24 hours at 37°C and then centrifuged at 5000rpm for 10mins. The cell free supernatant (CFS) was collected and filtered using 0.22um membrane to sterilize it. To assess the antibacterial activity of the CFS, the procedure of El Ahmadi *et al.* (2025) was used. In the procedure, the CFS was divided into four tubes and treated as follows:

- Tube 1: To get bacteriocin – catalase and NaOH were added
- Tube 2: To get Hydrogen peroxide – Trypsin and NaOH were added
- Tube 3: To get Organic acid – Trypsin and catalase were added

- Tube 4: To block the Hydrogen peroxide, Organic acid and Bacteriocin – catalase, NaOH and Trypsin were added. After this, *E. coli* inoculum was swabbed on MHA plates. Four 6 mm diameter wells were punched in the agar, each well was filled with 0.5ml of the content of each tube and the plate was incubated at 37°C for 18 hours. Zones of inhibition was looked out for and measured accordingly. All assays were carried out in triplicates. Data are presented as mean $\pm$ standard deviation, analysed by one-way ANOVA with Duncan's Multiple Range Test ($p \leq 0.05$) in IBM SPSS Statistics v23.

RESULTS

Types and Frequency of Occurrence of Bacteria Isolated from Stool Samples of Diarrhoeic Children

Four different types of bacterial species were isolated from the stool samples worked on. These are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas aeruginosa*. The percentage frequency of occurrence is shown in Table 1. *Escherichia coli* was the most frequently encountered bacterial species. It was isolated in 26 (47.3%) of the total stool samples examined. *Staphylococcus aureus* was isolated in 12 of the stool samples representing 21.8% occurrence, *Pseudomonas aeruginosa* in 9 of the stool samples with 16.4% occurrence while the lowest occurrence was observed with *Salmonella enterica* in only 8 of the isolates representing 14.6% occurrence of the total isolates.

Molecular Identity of the *E. coli* Isolated from the Stool Samples

The following virulence genes; *eaeA*, *bfpA* and *hlyA* were successfully amplified in the test organisms. Virulence genes *eaeA* and *bfpA* were observed in all the isolates tested and *hlyA* gene in 2 of the isolates. The molecular identity of the *E. coli* isolated evaluated is shown in Plates 1a – c. Plate 1 (a) shows the amplification of the virulence gene, *eaeA*, which had an estimated band size of 917 base pairs. The gel indicated the successful amplification of the *eaeA* gene for all the selected isolates. Plate 1 (b) displays the amplification of the virulence gene, *hlyA*, which had an estimated band size of 199 base pairs in which successful amplification was achieved only for samples 1 and 3.

Antibiogram Profile of the isolated *E. coli* from the Stool Samples

Table 2 shows the antibiogram profile of the isolated *E. coli* from stool samples of diarrhoeic children against ten commonly used antibiotics. The result showed that the isolates had different degrees of susceptibility, intermediate and resistance to the tested antibiotics. The isolates were highly susceptible to Ciprofloxacin (CPX) with several isolates showing diameters of zones of inhibition higher than the susceptibility cut-off of ≥ 21.0 mm (CSLI, 2025). This shows that ciprofloxacin was the most effective antibiotic against the *E. coli* isolates tested. Isolates PHG1, SLH2, PHS2, GH02, PHI2, PHI4, PHY2i, GH03, PHJ2, SLH2a and PHK9 were highly sensitive. Some of the isolates displayed intermediate susceptibility or resistance to antibiotics such as Gentamicin (CN), Tarivid (OFX), Sparfloxacin (SP), Septrin (SXT) and Pefloxacin (PEF).

Table 1: Frequency of Occurrence of the Isolated Bacteria from the Stool of Diarrhoeic Children

Bacterial Species	Total No. of Samples	No. Positive	% Positive
<i>Escherichia coli</i>	55	26	47.3
<i>Staphylococcus aureus</i>	55	12	21.8
<i>Salmonella enterica</i>	55	8	14.6
<i>Pseudomonas aeruginosa</i>	55	9	16.4

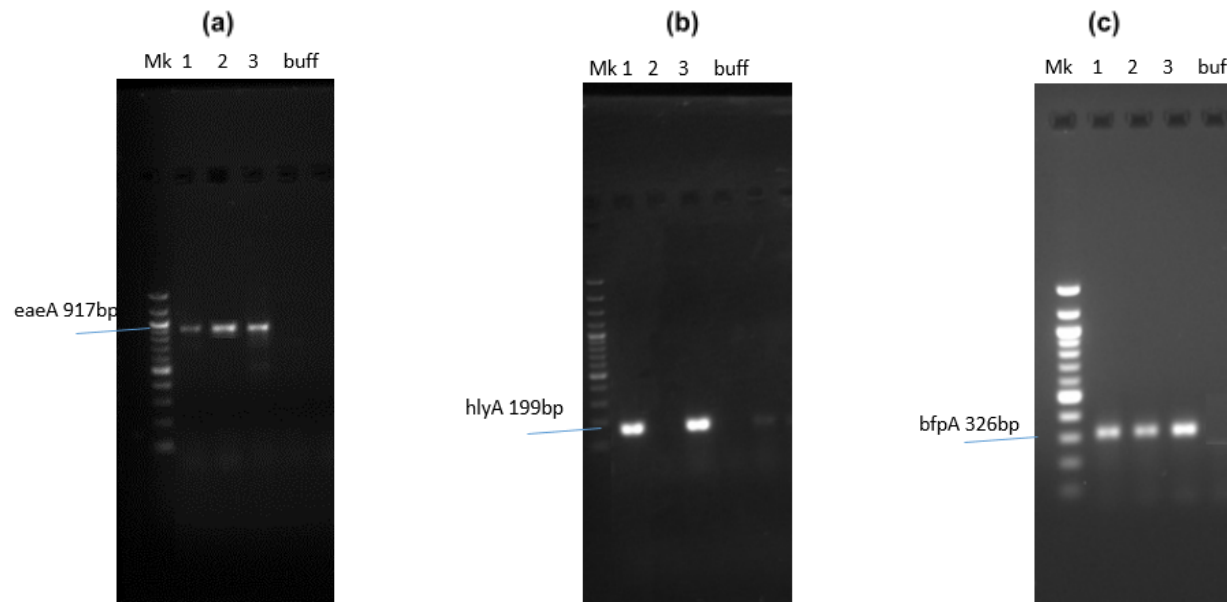


Plate 1: Agarose Gel Electrophoresis of the PCR products of virulence genes (eae, hlyA, bfpA) Amplified from Selected *E. coli* isolates (Band sizes approximately 917bp, 199bp,326bp respectively).

Table 2: Antibigram Profile of the Isolated *E. coli* from the Stool of Diarrhoeic Children

Bacterial Code	Diameter of Zone of Inhibition (mm)									
	A	B	C	D	E	F	G	H	I	J
	S≥15	S≥18	S≥18	S≥24	S≥18	S≥19	S≥15	S≥16	S≥21	S≥16
	I-13-14	I-14-17	I-13-17	I-0	I-14-17	I-16-18	I-12-14	I-13-15	I-17-20	I-11-15
	R≤12	R≤13	R≤12	R≤23	R≤13	R≤15	R≤11	R≤12	R≤16	R≤10
PHG1	15.8±0.4 ^a	13.0±1.0 ^a	15.7±0.6 ^a	16.7±1.2 ^a	14.0±0.0 ^a	17.7±0.6 ^b	14.3±0.6 ^b	15.7±0.6 ^c	22.3±0.1 ^a	18.3±0.6 ^a
SLH2	15.2±0.9 ^a	10.7±1.2 ^a	12.7±2.5 ^a	17.0±1.0 ^b	12.0±0.0 ^b	16.7±2.1 ^a	14.0±0.0 ^a	16.3±0.6 ^a	21.7±1.5 ^c	19.0±1.0 ^b
PHS2	13.0±2.7 ^a	7.3±6.4 ^b	12.7±3.1 ^a	13.3±3.1 ^a	12.0±1.7 ^a	14.0±2.0 ^b	3.3±5.8 ^b	15.7±2.5 ^c	23.0±3.5 ^a	8.7±8.1 ^a
GHO2	16.3±0.6 ^a	14.0±0.0 ^a	14.0±0.0 ^a	17.3±3.2 ^b	13.0±0.0 ^b	13.0±3.0 ^b	7.0±6.1 ^b	16.3±1.5 ^b	22.7±2.5 ^a	17.0±0.0 ^a
PHJ1	13.2±1.0 ^a	16.7±1.5 ^a	13.3±1.2 ^a	15.0±1.0 ^a	15.7±1.2 ^a	16.0±2.0 ^c	8.7±7.6 ^c	14.0±2.0 ^c	21.0±2.0 ^c	15.3±1.2 ^a
SDH7	13.4±0.8 ^a	10.3±9.5 ^a	15.3±1.5 ^a	16.3±0.6 ^b	13.3±1.5 ^a	17.0±1.0 ^a	0.0±0.0 ^a	14.0±1.7 ^c	12.0±0.0 ^a	16.3±0.6 ^a
PHI2	14.0±0.0 ^a	4.3±7.5 ^b	14.7±1.2 ^a	16.7±3.1 ^b	11.0±2.7 ^b	16.0±0.0 ^b	10.3±9.2 ^b	14.7±2.9 ^a	23.0±1.0 ^a	10.7±1.2 ^b
PHI4	9.0±1.7 ^a	7.3±6.9 ^a	11.0±1.0 ^a	7.7±5.8 ^b	7.7±6.4 ^a	10.7±1.2 ^a	10.7±1.2 ^a	7.7±6.4 ^b	22.0±0.1 ^c	3.3±5.8 ^c
PHK1	4.9±4.1 ^a	3.3±5.8 ^a	12.0±2.7 ^a	6.7±5.8 ^a	3.3±5.8 ^b	3.3±5.8 ^c	3.3±5.8 ^a	11.0±0.0 ^b	15.3±0.4 ^a	7.3±6.4 ^a
PHK2	4.3±7.5 ^a	0.0±0.0 ^a	11.3±1.2 ^a	6.7±5.8 ^a	10.7±1.2 ^a	11.3±2.3 ^c	3.3±5.8 ^c	11.3±1.2 ^b	23.3±0.5 ^b	0.0±0.0 ^b
PHK3	10.9±1.0 ^a	4.0±6.9 ^a	10.0±0.0 ^b	8.0±6.9 ^a	4.7±8.1 ^a	11.3±2.3 ^b	10.0±0.0 ^b	13.3±1.5 ^a	11.0±1.7 ^a	3.3±5.8 ^a
PHK4	11.2±1.0 ^a	3.3±5.8 ^a	4.7±6.8 ^b	11.3±2.1 ^a	6.7±5.8 ^a	7.3±6.1 ^a	11.3±1.2 ^a	12.7±1.2 ^b	11.3±1.2 ^b	6.7±5.8 ^a
PHS1	12.9±2.8 ^a	13.3±3.1 ^a	11.3±1.5 ^a	3.3±5.8 ^a	8.7±7.6 ^b	4.7±8.1 ^b	0.0±0.0 ^a	14.0±3.6 ^a	16.0±4.6 ^a	9.7±8.1 ^a

PHY2i	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^c	0.0±0.0 ^b	0.0±0.0 ^b	0.0±0.0 ^b	22.0±0.0 ^a	0.0±0.0 ^b
GHO3	16.3±0.6 ^a	14.0±0.0 ^a	14.0±0.0 ^a	17.3±3.2 ^a	13.0±0.0 ^a	13.0±3.0 ^a	7.0±6.1 ^a	16.3±1.5 ^b	22.7±0.0 ^b	17.0±0.0 ^a
PHJ2	13.2±1.0 ^b	16.7±1.5 ^a	13.3±1.2 ^a	15.0±1.0 ^a	15.7±1.2 ^a	16.0±2.0 ^b	8.7±7.6 ^a	14.0±2.0 ^a	23.0±1.0 ^b	15.3±1.2 ^b
PHK5	4.3±7.5 ^a	0.0±0.0 ^a	11.3±1.2 ^a	6.7±5.8 ^a	10.7±1.2 ^a	11.3±2.3 ^b	3.3±5.8 ^b	11.3±1.2 ^a	23.3±0.0 ^a	0.0±0.0 ^a
PHK6	10.9±1.0 ^a	4.0±6.9 ^a	10.0±0.0 ^a	8.0±6.9 ^a	4.7±8.1 ^a	11.3±2.3 ^c	10.0±0.0 ^b	13.3±1.5 ^a	11.0±1.7 ^a	3.3±5.8 ^b
PHG2	15.8±0.4 ^b	13.0±1.0 ^a	15.7±0.6 ^a	16.7±1.2 ^a	14.0±0.0 ^a	17.7±0.6 ^b	14.3±0.6 ^b	15.7±0.6 ^a	22.3±0.6 ^a	18.3±0.6 ^a
SDH8	13.4±0.8 ^a	10.3±9.5 ^a	15.3±1.5 ^a	16.3±0.6 ^a	13.3±1.5 ^c	17.0±1.0 ^b	0.0±0.0 ^a	14.0±1.7 ^a	12.0±0.0 ^c	16.3±0.6 ^b
PHI6	14.0±0.0 ^a	4.3±7.5 ^b	14.7±1.2 ^a	16.7±3.1 ^a	11.0±2.7 ^a	16.0±0.0 ^c	10.3±9.2 ^a	14.7±2.9 ^a	23.0±1.0 ^a	10.7±1.2 ^b
SLH2a	15.2±0.9 ^a	10.7±1.2 ^a	12.7±2.5 ^a	17.0±1.0 ^a	12.0±0.0 ^b	16.7±2.1 ^a	14.0±0.0 ^a	16.3±0.6 ^a	22.7±1.5 ^c	19.0±1.0 ^a
PHK7	4.9±4.1 ^b	3.3±5.8 ^a	12.0±2.7 ^a	6.7±5.8 ^a	3.3±5.8 ^b	3.3±5.8 ^a	3.3±5.8 ^c	11.0±0.0 ^a	17.3±0.4 ^a	7.3±6.4 ^a
PHS3	13.0±2.7 ^a	7.3±6.4 ^a	12.7±3.1 ^a	13.3±3.1 ^a	12.0±1.7 ^a	14.0±2.0 ^a	3.3±5.8 ^b	15.7±2.5 ^b	14.0±3.5 ^a	8.7±8.1 ^a
PHK8	11.2±1.0 ^b	3.3±5.8 ^a	4.7±6.8 ^a	11.3±2.1 ^a	6.7±5.8 ^b	7.3±6.1 ^a	11.3±1.2 ^b	12.7±1.2 ^b	11.3±1.2 ^b	6.7±5.8 ^b
PHK9	4.9±4.1 ^b	3.3±5.8 ^b	12.0±2.7 ^a	6.7±5.8 ^b	3.3±5.8 ^b	3.3±5.8 ^b	3.3±5.8 ^a	11.0±0.0 ^a	23.3±0.0 ^a	7.3±6.4 ^c

Values are expressed as Mean ± Standard deviation with the mean values having different superscript across the rows are statistically significant at $p < 0.05$.

KEY: A - Gentamicin 30µg, B - Amoxicillin 30µg, C - Chloramphenicol 30µg, D - Pefloxacin 30µg, E - Augmentin 10µg, F - Sparfloxacin 10µg, G - Streptomycin 30µg, H - Tarivid 10µg, I - Ciprofloxacin 30µg, J - Septrin 30µg, PHG – Primary Health Centre, GRA Ijebu-Owo, SLH – St Louis Hospital, Owo, PHE – Primary Health Center, Ehinogbe, PHI – Primary Health, Igboroko nla, PHY – Primary Health Center, Iyere, PHO – Primary Health, Oghaluwa, PHS – Primary Health Center, Isaipen, GHO – General Hospital Owo, PHJ – Primary Health Center, Ijebu Owo, SDH – St Davids Hospital, Owo, PHK – Primary Health Center, Oke Mapo, Owo

The rates of antibiotic resistance and susceptibility of the isolated *E. coli* to the tested antibiotics are shown in Table 3. The results showed that the isolates displayed varying resistance patterns. The highest resistance rate was observed against Pefloxacin (PEF) in which all the isolates (100.0%) are resistant. Resistance was also high against Amoxicillin (AM), Augmentin (AU) and Streptomycin (S) with 84.6% resistance each. High resistance rate of 61.5% was observed against Chloramphenicol (CH), Sparfloxacin (SP) and Septrin (SXT) while the resistance rate of 46.2% was observed against Gentamicin (CN). The resistance rate for Tarivid (OFX) and Ciprofloxacin (CPX) was relatively low at 34.6% each. The multiple antibiotic resistance index (MARI) of the test *E. coli* isolates ranged from 0.4 to 1.0 (Table 4).

Growth Inhibitory Activity of the Commercially Purchased Whey Against *E. coli*

Table 5 shows the growth inhibitory activity of CPW against the isolated *E. coli* using Amoxicillin and Ciprofloxacin as positive controls. The growth of most of the isolates was not inhibited by CPW except isolates PHJ1 and PHY2i which were highly susceptible to CPW than the growth inhibition mediated by Amoxicillin but not as high as the inhibition mediated by Ciprofloxacin.

Table 3: Antibiotic Resistance and Susceptibility Rate of the isolated *E. coli*

Antibiotics	No. Resistant	Resistant (%)	No. Intermediate	Intermediate (%)	No. Susceptible	Susceptible (%)
A	12	46.2	8	30.8	6	23.1
B	23	84.6	1	15.4	2	7.7
C	16	61.5	10	38.5	0	0
D	26	100.0	0	0.0	0	0
E	22	84.6	4	15.4	0	0
F	15	61.5	10	38.5	0	0
G	22	84.6	4	15.4	0	0
H	9	34.6	13	50.0	4	15.4
I	9	34.6	1	3.8	0	61.5
J	16	61.5	2	7.7	8	30.8

KEY: A: Gentamicin 30µg, B: Amoxicillin 30µg, C: Chloramphenicol 30µg, D: Pefloxacin 30µg, E: Augmentin 10µg, F: Sparfloxacin 10µg, G: Streptomycin 30µg, H: Tarivid 10µg, I: Ciprofloxacin 30µg, J: Septrin 30µg

Table 4. MARI Profile of the isolated *E. coli* from the stool of Diarrhoeic Children

Isolate codes	Number of Antibiotics Resistant to	Names of the Antibiotics	MAR Index
PHG1	4	AM, PEF, SP, CPX	0.40
SLH2	5	AM, PEF, AU, SP, CPX	0.50
PHS2	7	AM, PEF, AU, SP, S, CPX, SXT	0.70
GHO2	5	PEF, AU, SP, S, CPX	0.50
PHJ1	4	PEF, SP, S, CPX	0.40
SDH7	5	AM, PEF, SP, S, CPX	0.50
PHI2	6	AM, PEF, AU, SP, S, CPX	0.60
PHI4	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT	1.00
PHK1	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT	1.00
PHK2	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT	1.00
PHK3	9	CN, AM, CH, PEF, AU, SP, S, CPX, SXT	0.90
PHK4	8	CN, AM, CH, PEF, AU, SP, CPX, SXT	0.80
PHS1	7	CH, PEF, AU, SP, S, CPX, SXT	0.70
PHY2i	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT	1.00
GHO3	5	PEF, AU, SP, S, CPX	0.50

Isolate codes	Number of Antibiotics Resistant to	Names of the Antibiotics	MAR Index
PHJ2	4	PEF, SP, S, CPX	0.40
PHK5	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT	1.00
PHK6	9	CN, AM, CH, PEF, AU, SP, S, CPX, SXT	0.90
PHG2	4	AM, PEF, SP, CPX	0.40
SDH8	5	AM, PEF, SP, S, CPX	0.50
PHI6	6	AM, PEF, AU, SP, S, CPX	0.60
SLH2a	5	AM, PEF, AU, SP, CPX	0.50
PHK7	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT	1.00
PHS3	7	AM, PEF, AU, SP, S, CPX, SXT	0.70
PHK8	8	CN, AM, CH, PEF, AU, SP, CPX, STX	0.80
PHK9	10	CN, AM, CH, PEF, AU, SP, S, OFX, CPX, SXT, SXT	1.00

KEY: A: Gentamicin 30µg, B: Amoxicillin 30µg, C: Chloramphenicol 30µg, D: Pefloxacin 30µg, E: Augmentin 10µg, F: Sparfloxacin 10µg, G: Streptomycin 30µg, H: Tarivid 10µg, I: Ciprofloxacin 30µg, J: Septrin 30µg; MAR: Multiple Antibiotic Resistance

Table 5: Growth Inhibitory Activity of Freshly Prepared CPW on the Isolated *E. coli*

Bacterial Codes	Diameter zone of inhibition (mm)		
	WHEY	AM (Amoxicillin)	CIP (Ciprofloxacin)
PHS1	20.0 ± 0.0 ^a	19.5 ± 0.0 ^a	26.0 ± 0.0 ^b
PHG1	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	18.0 ± 0.0 ^a
PHK3	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	21.0 ± 0.0 ^b
PHJ1	15.0 ± 0.0 ^a	0.0 ± 0.0 ^a	18.0 ± 0.0 ^a
PHK1	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	22.0 ± 0.0 ^a
PHI4	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	21.0 ± 0.0 ^a
PHK4	0.0 ± 0.0 ^a	19.5 ± 0.5 ^b	34.0 ± 0.0 ^c
SDH7	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	15.0 ± 0.7 ^a
GHO2	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	18.0 ± 0.0 ^a
PHI2	0.0 ± 0.0 ^a	26.0 ± 0.7 ^b	26.0 ± 0.0 ^b
SLH2	0.0 ± 0.0 ^a	30.2 ± 0.3 ^b	28.8 ± 1.1 ^b
PHK2	0.0 ± 0.0 ^a	17.3 ± 2.5 ^b	37.0 ± 0.0 ^c
PHS2	15.5 ± 0.7 ^a	35.5 ± 0.5 ^c	34.0 ± 0.0 ^b
PHY2i	20.0 ± 0.0 ^b	0.0 ± 0.0 ^a	20.0 ± 0.0 ^a
GHO3	0.0 ± 0.0 ^a	19.0 ± 0.0 ^b	34.5 ± 0.0 ^c

Bacterial Codes	Diameter zone of inhibition (mm)		
	WHEY	AM (Amoxicillin)	CIP (Ciprofloxacin)
PHJ2	15.0 ± 0.0 ^a	0.0 ± 0.0 ^b	26.0 ± 0.0 ^a
PHK5	0.0 ± 0.0 ^a	17.3 ± 2.5 ^a	37.0 ± 0.0 ^b
PHK6	0.0 ± 0.0 ^a	0.0 ± 0.0 ^b	21.0 ± 0.0 ^c
PHG2	0.0 ± 0.0 ^a	0.0 ± 0.0 ^b	22.0 ± 0.0 ^a
SDH8	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	20.0 ± 0.0 ^a
PHI6	0.0 ± 0.0 ^a	26.0 ± 0.7 ^b	26.0 ± 0.0 ^b
SLH2a	0.0 ± 0.0 ^a	30.2 ± 0.3 ^b	28.8 ± 1.1 ^a
PHK7	0.0 ± 0.0 ^a	0.0 ± 0.0 ^b	24.0 ± 0.0 ^c
PHS3	15.5 ± 0.7 ^a	35.5 ± 0.5 ^a	34.0 ± 0.0 ^a
PHK8	0.0 ± 0.0 ^a	19.5 ± 0.5 ^c	34.0 ± 0.0 ^b
PHK9	0.0 ± 0.0 ^a	17.3 ± 2.5 ^a	37.0 ± 0.0 ^b

Values are expressed as Mean ± Standard deviation with the mean values having different superscript across the rows are statistically significant at $p < 0.05$.

KEY: PHG – Primary Health Centre, GRA Ijebu-Owo, SLH – St Louis Hospital, Owo, PHE – Primary Health Center, Ehinogbe, PHI – Primary Health, Igboroko nla, PHY – Primary Health Center, Iyere, PHO – Primary Health, Oghaluwa, PHS – Primary Health Center, Isaipen, GH0 – General Hospital Owo, PHJ – Primary Health Center, Ijebu Owo, SDH – St Davids Hospital, Owo, PHK – Primary Health Center, Oke Mapo, Owo.

Comparative Growth Inhibitory Activity of Freshly Prepared CPW and LPW on the isolated *E. coli*

Comparative growth inhibitory activity of freshly prepared CPW and LPW using *C. procera* juice as coagulant against the isolated *E. coli* is presented in Table 6. The result showed significant differences in antibacterial activity between the two whey preparations. CPW showed lower antibacterial activity against the *E. coli* isolates and the inhibition was observed only in a few isolates such as PHS1, PHJ1, PHS2, PHY2i, PHJ2 and PHS3. The inhibition zones ranged from 15.00 ± 0.00 mm to 20.00 ± 0.00 mm. On the other hand, LPW exhibited appreciable higher antibacterial activity against all the test isolates with inhibitory zones which ranged from 12.00 ± 0.00 mm to 26.00 ± 0.00 mm. The highest inhibition activity was obtained against the isolates PHK6, PHK8 and PHK9 with inhibition zones of 26.0 ± 0.0 mm each, while the lowest inhibition zone was against isolate PHS1 (12.0 ± 0.0 mm). Several number of isolates which were not inhibited by CPW, recorded marked susceptibility to LPW. The inhibition mediated by LPW was superior to that mediated by ciprofloxacin on 7 (26.9%) of the *E. coli* isolates and was at par with the inhibition mediated by ciprofloxacin on most of the *E. coli* isolates.

Growth Inhibitory Activity of Laboratory Prepared Whey (LPW) Using Different Coagulants against Isolated *E. coli*

The antibacterial activity of the different whey samples on the test bacteria was observed to be different depending on the type of the coagulant used. Whey prepared with *C. procera* as coagulant (A) showed significant antibacterial activity against all the test isolates with inhibition zones ranging from 12.00 ± 0.00 mm to 26.00 ± 0.00 mm. Similarly, whey prepared with lime as coagulant (B) exhibited good antibacterial activity against the test isolates with inhibition zones ranging from 15.00 ± 0.00 mm to 25.00 ± 0.00 mm. However, whey prepared with alum as coagulant (C) showed relatively lower antibacterial activity against the isolates with inhibition zones of 8.00 ± 0.00 mm to 18.00 ± 0.00 mm. Table 7 shows the growth inhibitory activity of LPW prepared with different coagulants against the isolated *E. coli*.

Table 6: Comparative Inhibitory Activity of Freshly Prepared CPW and LPW on the Isolated *E. coli*

Bacterial Codes	Diameter zone of inhibition (mm)		
	CPW	LPW	Ciprofloxacin
PHS1	20.0 ± 0.0 ^a	12.0 ± 0.0 ^a	26.0 ± 0.0 ^b
PHG1	0.0 ± 0.0 ^a	18.0 ± 0.0 ^b	18.0 ± 0.0 ^a
PHK3	0.0 ± 0.0 ^a	20.0 ± 0.0 ^a	21.0 ± 0.0 ^b
PHJ1	15.0 ± 0.0 ^b	22.0 ± 0.0 ^a	18.0 ± 0.0 ^a
PHK1	0.0 ± 0.0 ^a	20.0 ± 0.0 ^a	22.0 ± 0.0 ^a
PHI4	0.0 ± 0.0 ^a	22.0 ± 0.0 ^b	22.0 ± 0.0 ^a
PHK4	0.0 ± 0.0 ^a	23.0 ± 0.0 ^a	34.0 ± 0.0 ^c
SDH7	0.0 ± 0.0 ^a	22.0 ± 0.0 ^b	15.0 ± 0.7 ^a
GHO2	0.0 ± 0.0 ^a	24.0 ± 0.0 ^a	18.0 ± 0.0 ^a
PHI2	0.0 ± 0.0 ^a	21.0 ± 0.0 ^c	26.0 ± 0.0 ^b
SLH2	0.0 ± 0.0 ^a	23.0 ± 0.0 ^b	28.8 ± 1.1 ^b
PHK2	0.0 ± 0.0 ^a	22.0 ± 0.0 ^b	37.0 ± 0.0 ^c
PHS2	15.5 ± 0.7 ^a	20.0 ± 0.0 ^a	34.0 ± 0.0 ^b
PHY2i	20.0 ± 0.0 ^a	20.0 ± 0.0 ^a	20.0 ± 0.0 ^a

Bacterial Codes	Diameter zone of inhibition (mm)		
	CPW	LPW	Ciprofloxacin
GHO3	0.0 ± 0.0 ^a	22.0 ± 0.0 ^c	34.5 ± 0.0 ^c
PHJ2	15.0 ± 0.0 ^a	24.0 ± 0.0 ^a	26.0 ± 0.0 ^a
PHK5	0.0 ± 0.0 ^a	23.0 ± 0.0 ^b	37.0 ± 0.0 ^b
PHK6	0.0 ± 0.0 ^a	26.0 ± 0.0 ^a	21.0 ± 0.0 ^c
PHG2	0.0 ± 0.0 ^b	25.0 ± 0.0 ^a	22.0 ± 0.0 ^a
SDH8	0.0 ± 0.0 ^a	22.0 ± 0.0 ^b	20.0 ± 0.0 ^a
PHI6	0.0 ± 0.0 ^a	24.0 ± 0.0 ^a	26.0 ± 0.0 ^b
SLH2a	0.0 ± 0.0 ^b	25.0 ± 0.0 ^a	28.8 ± 1.1 ^a
PHK7	0.0 ± 0.0 ^a	24.0 ± 0.0 ^b	24.0 ± 0.0 ^c
PHS3	15.5 ± 0.7 ^a	22.0 ± 0.0 ^b	34.0 ± 0.0 ^a
PHK8	0.0 ± 0.0 ^a	26.0 ± 0.0 ^a	34.0 ± 0.0 ^b
PHK9	0.0 ± 0.0 ^a	26.0 ± 0.0 ^b	37.0 ± 0.0 ^b

Values are expressed as Mean ± Standard deviation (n = 3). Values with different superscript across the rows are significantly different at p ≤ 0.05

KEY: PHG – Primary Health Centre, GRA Ijebu-Owo, SLH – St Louis Hospital, Owo, PHE – Primary Health Center, Ehinogbe, PHI – Primary Health, Igboroko nla, PHY – Primary Health Center, Iyere, PHO – Primary Health, Oghaluwa, PHS – Primary Health Center, Isaipen, GHO – General Hospital Owo, PHJ – Primary Health Center, Ijebu Owo, SDH – St Davids Hospital, Owo, PHK – Primary Health Center, Oke Mapo, Owo.

Table 7. Growth Inhibitory Activity of Freshly Prepared LPW Using Different Coagulants on the Isolated *E. coli*

Bacterial Codes	Diameter zone of inhibition (mm)		
	A	B	C
PHS1	12.0±0.0 ^a	22.0±0.0 ^a	14.0±0.0 ^b
PHG1	18.0±0.0 ^a	20.0±0.0 ^a	12.0±0.0 ^a
PHK3	20.0±0.0 ^a	20.0±0.0 ^a	13.0±0.0 ^c
PHJ1	22.0±0.0 ^a	22.0±0.0 ^a	12.0±0.0 ^b
PHK1	20.0±0.0 ^a	23.0±0.0 ^b	11.0±0.0 ^b
PHI4	22.0±0.0 ^b	24.0±0.0 ^a	12.0±0.0 ^b
PHK4	23.0±0.0 ^a	20.0±0.0 ^a	16.5±0.7 ^c
SDH7	22.0±0.0 ^a	21.0±0.0 ^a	10.0±0.0 ^a
GHO2	24.0±0.0 ^a	23.3±0.4 ^b	18.0±0.0 ^b
PHI2	21.0±0.0 ^a	24.0±0.0 ^a	12.0±0.0 ^a
SLH2	23.0±0.0 ^a	24.0±0.0 ^a	13.0±0.0 ^a
PHK2	22.0±0.0 ^b	20.0±0.0 ^b	14.0±0.0 ^b
PHS2	20.0±0.0 ^a	15.0±0.0 ^a	14.0±0.0 ^a
GHO3	22.0±0.0 ^a	18.0±0.0 ^a	10.0±0.0 ^a
PHJ2	24.0±0.0 ^a	22.0±0.0 ^b	12.0±0.0 ^c

Bacterial Codes	Diameter zone of inhibition (mm)		
	A	B	C
PHK5	23.0±0.0 ^a	20.0±0.0 ^a	10.0±0.0 ^a
PHK6	26.0±0.0 ^a	22.0±0.0 ^a	14.0±0.0 ^b
PHG2	25.0±0.0 ^a	20.0±0.0 ^a	14.0±0.0 ^a
SDH8	22.0±0.0 ^b	21.0±0.0 ^a	13.0±0.0 ^a
PHK7	24.0±0.0 ^a	22.0±0.0 ^b	12.0±0.0 ^b
PHS3	22.0±0.0 ^a	20.0±0.0 ^a	12.0±0.0 ^a
PHK8	26.0±0.0 ^a	25.0±0.0 ^a	12.0±0.0 ^a
PHK9	26.0±0.0 ^a	22.0±0.0 ^c	8.1±0.1 ^a
PHY2i	20.0±0.0 ^a	20.0±0.0 ^a	10.0±0.0 ^a
PHI6	24.0±0.0 ^b	22.0±0.0 ^a	12.0±0.0 ^a
SLH2a	25.0±0.0 ^a	23.0±0.0 ^a	8.0±0.0 ^a

Mean ± standard deviation (n=3). Values with different superscript across the rows are significantly different at $p \leq 0.05$

Key: A= Whey of cheese using *Calotropis procera* as Coagulant

B= Whey of cheese using Lime as Coagulant

C= Whey of cheese using Alum as Coagulant

Effect of fermentation duration and temperature of holding on the antibacterial activity of CPW and LPW on the isolated *E. coli*

It was observed that the fermentation duration of the prepared whey samples and temperature of holding affected the antibacterial activity of the samples. The growth inhibition of the test bacteria by the whey samples increased as fermentation duration increases to peak by 48h of fermentation on most (12; 46.2%) of the isolates. The inhibition then begins to wane by day 72h until the 144h of fermentation (Table 8). The growth inhibition mediated by the whey held at 36°C was slightly higher than the inhibition mediated by whey held at 4°C on some of the isolates.

Comparative Antibacterial Activity of Bio-metabolites in Whey against the isolated *E. coli*

The relative role of the bio-metabolites present in whey for antibacterial activity against the isolated *E. coli* is presented in Table 9. The result showed that the bacteriocin cell free supernatant (A) exerted the highest antibacterial activity on 7 (26.9%) of the isolates with inhibition zones ranging from 10.00 ± 0.00 mm to 28.00 ± 0.00 mm. The maximum inhibition zone was observed on isolate PHK9 (28.00 ± 0.00 mm) followed by PHI6 (26.00 ± 0.00 mm) and PHI4 (25.00 ± 0.00 mm). Similarly, the cell-free supernatant containing hydrogen peroxide (B) exerted the highest antibacterial activity on 9 (34.6%) of isolates with zones of inhibition ranging from 10.00 ± 0.00 mm to 27.50 ± 0.00 mm. The highest inhibition zone was observed on isolate PHS3 (27.50 ± 0.00 mm) followed by PHK9 (27.00 ± 0.00 mm), PHI4 (25.00 ± 0.10 mm) and PHI6 (24.00 ± 0.00 mm). The cell-free supernatant containing organic acid (C) exhibited less antibacterial activity against the isolates, with inhibition zones ranging from 8.00 ± 0.00 mm to 20.00 ± 0.00 mm while the cell free supernatant in which the organic acid, bacteriocin and hydrogen peroxide were blocked exerted the least effect on a few of the isolates. Overall, the metabolite that exerted its highest effect on most of the *E. coli* isolates was hydrogen peroxide followed by the bacteriocin produced by the isolated *Lactobacillus* sp. from the whey sample. However, organic acid also play significant role because its neutralisation resulted in least inhibition of the isolates in spite of the presence of Bacteriocin and hydrogen peroxide in the cell free supernatant.

Table 8: Effects of fermentation duration and temperature of holding on the growth inhibitory activity of CPW on the isolated *E. coli*

Bacterial code	Temp. (°C)	Fermentation duration (d)/ Diameter zone of inhibition (mm)						
		0	24	48	72	96	120	144
PHS1	4	ND	20.0 ±	20.0 ±	0.0 ±	0.0 ±	0.0 ±	12.0 ±
			0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
	36	20.0 ±	20.0 ±	20.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
PHG1	4	ND	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±
			0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^a
PHK3	4	ND	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±	11.0 ±
			0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±	0.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
PHJ1	4	ND	10.0 ±	12.5 ±	0.0 ±	0.0 ±	0.0 ±	11.0 ±
			0.0 ^a	3.5 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
	36	15.0 ±	15.0 ±	15.0 ±	0.0 ±	0.0 ±	0.0 ±	10.0 ±
		0.0 ^a	0.0 ^b	0.0 ^b	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
PHK1	4	ND	20.0 ±	20.0 ±	12.0 ±	13.0 ±	14.0 ±	0.0 ±
			0.0 ^b	0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^b	0.0 ^a
	36	0.0 ±	10.0 ±	20.0 ±	12.0 ±	10.0 ±	16.0 ±	0.0 ±
		0.0 ^a	14.1 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
PHI4	4	ND	0.0 ±	0.0 ±	16.0 ±	14.0 ±	11.5 ±	10.0 ±
			0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^a	0.3 ^a	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	14.0 ±	15.0 ±	13.0 ±	0.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	1.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
PHK4	4	ND	0.0 ±	0.0 ±	24.7 ±	23.3 ±	24.0 ±	12.0 ±
			0.0 ^a	0.0 ^a	1.1 ^a	0.4 ^a	0.0 ^a	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	26.0 ±	24.5 ±	24.0 ±	10.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^b	0.7 ^b	0.0 ^a	0.0 ^b
SDH7	4	ND	0.0 ±	0.0 ±	14.0 ±	13.0 ±	15.0 ±	0.0 ±
			0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^a	0.0 ^a	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	10.0 ±	14.0 ±	13.0 ±	9.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^a
GHO2	4	ND	0.0 ±	0.0 ±	13.0 ±	14.0 ±	14.0 ±	12.0 ±
			0.0 ^a	0.0 ^a	0.0 ^b	0.0 ^a	0.0 ^b	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	11.0 ±	14.0 ±	14.0 ±	0.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.7 ^a	0.0 ^a	0.0 ^a	0.0 ^a
PHI2	4	ND	0.0 ±	0.0 ±	14.0 ±	12.0 ±	18.0 ±	16.0 ±
			0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
	36	0.0 ±	0.0 ±	0.0 ±	15.0 ±	12.0 ±	16.0 ±	15.0 ±
		0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a

SLH2	4	ND	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
PHK2	4	ND	20.0 ± 0.0 ^a	20.0 ± 0.0 ^a	14.0 ± 0.0 ^b	15.0 ± 0.0 ^a	15.0 ± 0.0 ^b	13.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	20.0 ± 0.0 ^a	20.0 ± 0.0 ^a	12.0 ± 0.0 ^a	14.0 ± 0.0 ^a	14.0 ± 0.0 ^a	12.0 ± 0.0 ^a
PHS2	4	ND	15.0 ± 0.0 ^a	17.5 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
	36	15.5 ± 0.7 ^a	17.5 ± 3.5 ^b	17.5 ± 3.5 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
PHY2i	4	ND	25.0 ± 0.0 ^b	25.0 ± 0.0 ^b	14.0 ± 0.0 ^b	14.0 ± 0.0 ^a	14.0 ± 0.0 ^a	15.0 ± 0.0 ^a
	36	20.0 ± 0.0 ^b	20.0 ± 0.0 ^a	20.0 ± 0.0 ^a	12.0 ± 0.0 ^a	15.0 ± 0.0 ^a	15.0 ± 0.0 ^a	12.0 ± 0.0 ^a
GHO3	4	ND	14.0 ± 0.0 ^a	18.0 ± 0.0 ^a	16.0 ± 0.0 ^a	15.0 ± 0.0 ^a	12.0 ± 0.0 ^b	12.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	16.0 ± 0.0 ^a	15.0 ± 0.0 ^a	14.0 ± 0.0 ^a	15.0 ± 0.0 ^a	13.0 ± 0.0 ^b	9.0 ± 0.0 ^b
PHJ2	4	ND	20.0 ± 0.0 ^a	16.0 ± 0.0 ^a	12.0 ± 0.0 ^b	13.0 ± 0.0 ^a	15.0 ± 0.0 ^a	14.0 ± 0.0 ^a
	36	15.0 ± 0.0 ^a	22.0 ± 0.0 ^b	20.0 ± 0.0 ^a	14.0 ± 0.0 ^a	12.0 ± 0.0 ^a	14.0 ± 0.0 ^a	12.0 ± 0.0 ^a
PHK5	4	ND	26.0 ± 0.0 ^c	22.0 ± 0.0 ^a	14.0 ± 0.0 ^a	17.0 ± 0.0 ^b	14.0 ± 0.0 ^a	13.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	22.0 ± 0.0 ^a	24.0 ± 0.0 ^a	14.0 ± 0.0 ^a	15.0 ± 0.0 ^a	16.0 ± 0.0 ^a	14.0 ± 0.0 ^a
PHK6	4	ND	23.0 ± 0.0 ^b	24.0 ± 0.0 ^a	17.0 ± 0.0 ^a	11.0 ± 0.0 ^a	12.0 ± 0.0 ^a	12.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	28.0 ± 0.0 ^a	26.0 ± 0.0 ^a	16.0 ± 0.0 ^a	9.0 ± 0.0 ^a	10.0 ± 0.0 ^a	10.0 ± 0.0 ^a
PHG2	4	ND	16.0 ± 0.0 ^a	20.0 ± 0.0 ^b	14.0 ± 0.0 ^b	10.0 ± 0.0 ^a	9.0 ± 0.0 ^a	9.0 ± 0.0 ^b
	36	0.0 ± 0.0 ^a	15.0 ± 0.0 ^a	18.0 ± 0.0 ^a	15.0 ± 0.0 ^a	10.0 ± 0.0 ^b	10.0 ± 0.0 ^b	0.0 ± 0.0 ^a
SDH8	4	ND	18.0 ± 0.0 ^b	19.0 ± 0.0 ^b	16.0 ± 0.0 ^a	15.0 ± 0.0 ^a	13.0 ± 0.0 ^a	9.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	10.0 ± 0.0 ^a	17.0 ± 0.0 ^a	18.0 ± 0.0 ^a	17.0 ± 0.0 ^b	15.0 ± 0.0 ^a	12.0 ± 0.0 ^a
PHI6	4	ND	24.0 ± 0.0 ^a	18.0 ± 0.0 ^a	18.0 ± 0.0 ^b	16.0 ± 0.0 ^b	17.0 ± 0.0 ^a	18.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	22.0 ± 0.0 ^a	20.0 ± 0.0 ^a	17.0 ± 0.0 ^a	17.0 ± 0.0 ^a	15.0 ± 0.0 ^a	17.0 ± 0.0 ^a
SLH2a	4	ND	22.0 ± 0.0 ^c	14.0 ± 0.0 ^b	9.0 ± 0.0 ^a	0.0 ± 0.0 ^a	10.0 ± 0.0 ^a	0.0 ± 0.0 ^a

PHK7	36	0.0 ± 0.0 ^a	19.0 ± 0.0 ^b	15.0 ± 0.0 ^a	10.0 ± 0.0 ^a	0.0 ± 0.0 ^a	9.0 ± 0.0 ^b	9.0 ± 0.0 ^a
	4	ND	23.0 ± 0.0 ^b	20.0 ± 0.0 ^a	20.0 ± 0.0 ^a	22.0 ± 0.0 ^a	12.0 ± 0.0 ^a	14.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	25.0 ± 0.0 ^a	24.0 ± 0.0 ^a	22.0 ± 0.0 ^a	22.0 ± 0.0 ^a	10.0 ± 0.0 ^a	12.0 ± 0.0 ^a
	4	ND	26.0 ± 0.0 ^c	25.0 ± 0.0 ^a	10.0 ± 0.0 ^a	9.0 ± 0.0 ^a	9.0 ± 0.0 ^a	9.0 ± 0.0 ^a
PHK8	36	15.5 ± 0.7 ^a	27.0 ± 0.0 ^a	28.0 ± 0.0 ^a	0.0 ± 0.0 ^a	10.0 ± 0.0 ^a	10.0 ± 0.0 ^a	10.0 ± 0.0 ^a
	4	ND	22.0 ± 0.0 ^b	24.0 ± 0.0 ^b	15.0 ± 0.0 ^a	14.0 ± 0.0 ^a	13.0 ± 0.0 ^a	14.0 ± 0.0 ^a
	36	0.0 ± 0.0 ^a	27.0 ± 0.0 ^a	26.0 ± 0.0 ^a	13.0 ± 0.0 ^a	14.0 ± 0.0 ^a	14.0 ± 0.0 ^a	11.0 ± 0.0 ^a
	4	ND	24.0 ± 0.0 ^a	25.0 ± 0.0 ^b	18.0 ± 0.0 ^b	15.0 ± 0.0 ^a	12.0 ± 0.0 ^a	12.0 ± 0.0 ^a
PHK9	36	0.0 ± 0.0 ^a	28.0 ± 0.0 ^b	25.0 ± 0.0 ^a	16.0 ± 0.0 ^a	14.0 ± 0.0 ^a	14.0 ± 0.0 ^a	11.0 ± 0.0 ^b

Values are expressed as Mean ± Standard deviation with the mean values having different superscript across the row are statistically significant at $p < 0.05$.

KEY: PHG – Primary Health Centre, GRA Ijebu-Owo, SLH – St Louis Hospital, Owo, PHE – Primary Health Center, Ehinogbe, PHI – Primary Health, Igboroko nla, PHY – Primary Health Center, Iyere, PHO – Primary Health, Oghaluwa, PHS – Primary Health Center, Isaipen, GHG – General Hospital Owo, PHJ – Primary Health Center, Ijebu Owo, SDH – St Davids Hospital, Owo, PHK – Primary Health Center, Oke Mapo, Owo, ND – Not done.

Table 9: Effects of Bio-metabolites in Whey Responsible for Antibacterial Activity on the Isolated Diarrhoeagenic *E. coli*

S/No	Bacterial Codes	A	B	C	D
1.	PHI2	15.5±0.0 ^a	13.5±0.0 ^a	8.0±0.0 ^a	8.0±0.0 ^a
2.	PHK1	14.0±0.0 ^a	14.0±0.0 ^c	12.0±0.0 ^a	12.0±0.0 ^b
3.	PHK7	12.0±0.0 ^a	10.0±0.0 ^a	8.0±0.0 ^a	8.0±0.0 ^a
4.	PHK5	21.5±0.0 ^a	22.5±0.0 ^a	8.0±0.0 ^b	8.0±0.0 ^a
5.	PHI6	26.0±0.0 ^a	24.0±0.0 ^b	20.0±0.0 ^a	20.0±0.0 ^c
6.	PHK6	16.0±0.0 ^a	17.5±0.0 ^a	12.0±0.0 ^a	12.0±0.0 ^c
7.	PHS3	12.5±0.0 ^a	27.5±0.0 ^a	14.0±0.0 ^a	10.0±0.0 ^b
8.	PHG1	15.0±0.0 ^a	15.0±0.0 ^a	10.0±0.0 ^b	10.0±0.0 ^a
9.	PHS1	15.0±0.0 ^a	16.0±0.0 ^a	10.0±0.0 ^a	10.0±0.0 ^a
10.	PHI4	25.0±0.0 ^a	25.0±0.1 ^a	20.0±0.0 ^c	20.0±0.0 ^a
11.	PHK8	22.0±0.1 ^a	24.0±0.0 ^b	10.0±0.0 ^a	10.0±0.0 ^a
12.	PHK9	28.0±0.0 ^a	27.0±0.0 ^b	12.0±0.0 ^a	10.0±0.0 ^b
13.	SDH8	14.0±0.1 ^a	12.0±0.0 ^a	10.0±0.0 ^b	10.0±0.0 ^b
14.	SDH7	14.0±0.0 ^a	14.0±0.0 ^a	14.0±0.0 ^a	14.0±0.0 ^b
15.	GHO2	10.0±0.0 ^a	14.0±0.0 ^a	15.0±0.0 ^a	10.0±0.0 ^c
16.	PHJ1	10.0±0.0 ^a	15.0±0.0 ^c	10.0±0.0 ^a	10.0±0.0 ^c

S/No	Bacterial Codes	A	B	C	D
17.	PHK3	12.0±0.0 ^a	12.0±0.0 ^a	12.0±0.0 ^c	12.0±0.0 ^a
18.	SLH2	14.0±0.0 ^a	15.0±0.0 ^a	12.0±0.1 ^b	12.0±0.0 ^a
19.	PHY2i	12.0±0.0 ^a	12.0±0.0 ^a	12.0±0.0 ^b	12.0±0.0 ^a
20.	PHS2	15.0±0.1 ^b	14.0±0.0 ^a	12.0±0.0 ^a	10.0±0.0 ^c
21.	PHK4	10.0±0.0 ^a	10.0±0.0 ^a	10.0±0.0 ^a	10.0±0.0 ^b
22.	GHO3	12.0±0.0 ^a	12.0±0.0 ^a	10.0±0.0 ^b	10.0±0.0 ^a
23.	SLH2a	18.0±0.1 ^a	20.0±0.1 ^a	10.0±0.0 ^c	10.0±0.0 ^a
24.	PHJ2	10.0±0.0 ^a	10.0±0.0 ^c	10.0±0.0 ^a	10.0±0.0 ^a
25.	PHG2	16.0±0.1 ^a	18.0±0.1 ^a	10.0±0.0 ^a	10.0±0.0 ^b
26.	PHK2	20.0±0.0 ^a	18.0±0.0 ^a	10.0±0.0 ^a	10.0±0.0 ^b

Mean ± standard deviation (n=3). Values with different superscript across the rows are significantly different at $p \leq 0.05$

Key: A = Bacteriocin , B = Hydrogen Peroxide, C = Organic acid, D = All metabolites (Bacteriocin + Hydrogen peroxide + organic acid) blocked

Comparative Changes in pH of Commercially Prepared Whey and Laboratory Prepared Whey

Table 10 shows the pH changes of CPW and LPW stored at room temperature (36°C) and refrigeration temperature (4°C) for 7 days. The result showed a gradual decrease of pH values during the storage period under both storage conditions. Room temperature storage of whey resulted in slow decrease of the pH from day 1 (6.49) to day 6 (5.73) and slight increase on day 7 (5.84). The pH drop was more pronounced at room temperature than at refrigeration temperature. The pH values were relatively higher at refrigeration temperature during storage and decreased gradually from 6.38 to 6.02 on day 2 to day 6 and slightly increased to 6.07 on day 7. at day 1 at refrigeration temperatures no value was recorded.

Comparative Changes in Total Titratable acidity of Commercially Prepared Whey and Laboratory Prepared Whey

The comparative changes in the total titratable acidity (TTA) of CPW and LPW stored at room temperature (36°C) and refrigeration temperature (4°C) during a period of 7 days is shown in Table 11. The highest TTA value at room temperature was at day 1 (25.20%) and rapidly decreased to 2.43% by day 2. After that, the TTA increased gradually from 2.88% on day 3 to 3.42% on day 5 and then decreased to 2.62% on day 6 and 1.04% on day 7. The TTA values were generally lower during the early storage period under refrigeration temperature from 1.26% to 1.62% between days 2 and 5. The TTA increased to 2.62% on day 6 and further increased to 3.42% on day 7. For refrigeration temperature, no value was detected for day 1.

Table 10: Comparative Changes in pH of CPW and LPW Stored at Two Different Temperatures for 7 Days

Duration (Day)	Room Temperature (36°C)		Refrigeration Temperature (4°C)	
	CPW	LPW	CPW	LPW
1	6.49	6.42	ND	ND
2	6.23	6.17	6.38	6.36
3	6.00	6.20	6.28	6.20
4	5.80	5.96	6.15	6.15
5	5.75	5.80	6.04	6.13
6	5.73	5.77	6.02	6.00
7	5.84	5.75	6.07	6.03

Table 11: Comparative Changes in Total Titratable Acidity (TTA) (%) of CPW and LPW Stored at Two Different Temperature for 7 Days

Duration (Day)	CPW		LPW	
	Room Temperature (36°C)	Refrigeration Temperature (4°C)	Room Temperature (36°C)	Refrigeration Temperature (4°C)
1	25.20	ND	0.06	ND
2	2.43	1.49	0.05	0.04
3	2.88	1.26	0.03	0.03
4	3.20	1.40	0.02	0.03
5	3.42	1.62	0.03	0.05
6	2.62	2.62	0.06	0.02
7	1.04	3.42	0.04	0.02

DISCUSSION

Diarrhoeal diseases are still a major cause of morbidity and mortality in children, particularly in developing countries, where poor sanitation and lack of clean water favour the faecal-oral transmission of enteric pathogens. Diarrhoeagenic *E. coli* is among the most important bacterial causes of diarrhoea due to its virulence potential and increasing resistance to commonly used antibiotics. The development of antibiotic resistance in enteric bacteria has led to the search for alternative antibacterials from natural sources. Cheese whey is a by-product of cheese production and contains a variety of bioactive compounds with known antimicrobial properties (Adebolu and Olorunfemi, 2016). The present study was conducted to determine the antibiotic profile of bacterial isolates from diarrhoeic children and the antibacterial activities of commercial and laboratory prepared cheese whey on diarrhoeagenic *E. coli*.

The predominance of *E. coli* isolated in this study agrees with earlier studies that *E. coli* is the most frequently encountered bacteria in cases of bacterial diarrhoea (Adebolu and Babafemi, 2008; Okebugwu *et al.* 2017; Olayinka *et al.*, 2024). The high prevalence of *E. coli* could be attributed to poor environmental sanitation and poor personal hygiene practices that boost the transmission of enteric pathogens other than contaminated water and food. Isolation of the bacterial agents, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella enterica* also indicates the possible involvement of several bacterial agents in childhood diarrhoeal infections.

The isolation of *S. aureus* in the present study corroborates the findings of Viana *et al.* (2025) who isolated *S. aureus* from the stool samples of diarrhoeic patients and

attributed the presence of the organism to poor hygienic condition and food contamination. The presence of *Pseudomonas aeruginosa* was in agreement with the report of Fakhkhari *et al.* (2022) who reported *P. aeruginosa* as opportunistic enteric pathogen in diarrhoeic stool samples.

The low prevalence of *Salmonella enterica* in the present study is in agreement with the results of Tilahun *et al.* (2025) who reported lower prevalence of *Salmonella* species than *E. coli* in diarrhoeic children.

In this study, the successful amplification of *eaeA* gene in some of the *E. coli* showed the presence of intimin adhesion factor which is important in attachment and colonisation of intestinal epithelial cells, suggesting that the isolates have virulence properties of classical enteropathogenic *E. coli*. This result is consistent with the report of Zelelie *et al.* (2023) who reported high prevalence of *eaeA* gene in diarrhoeagenic *E. coli* isolates from children with diarrhoea. Similar observations were also reported by Manhique-Coutinho *et al.* (2022) who detected *eaeA* gene in pathogenic isolates of *E. coli* from children with diarrhoea.

The amplification of *hlyA* gene in only one of the evaluated *E. coli* isolates suggests the presence of the haemolysin associated virulence determinant. The pathogenicity of Enterohaemorrhagic *E. coli* is associated with the *hlyA* gene which produces toxins and kills host cells. This finding is in agreement with the report of Dela *et al.* (2022) who reported that the distribution of *hlyA* gene was not uniform in the isolates of diarrhoeagenic *E. coli*. Hasan *et al.* (2022) also showed the presence of *hlyA* gene in some strains of pathogenic *E. coli*.

The amplification of *bfpA* gene in all the evaluated *E. coli* isolates indicates the presence of virulence factors of bundle forming pilus which are associated with bacterial adherence and colonisation. This finding is in accordance with the findings of Jaaz & Kadham (2026) who reported *bfpA* gene as an important virulence marker in isolates of enteropathogenic and diarrhoeagenic *E. coli*.

The absence of the *aggR* and *aaiC* genes which are normally found in enteroaggregative *E. coli* according to Del Carpio *et al.* (2025) in any of the evaluated *E. coli* isolates in this study suggests that the isolates were not related to the enteraggregative *E. coli* strain. Similar results was reported by Das *et al.* (2022) in which not all the diarrhoeagenic *E. coli* isolated in their study harboured the virulence genes *aggR* and *aaiC*.

The antibiogram profile of the bacterial isolates obtained in this study revealed variable susceptibility and resistance to the antibiotics tested, indicating the increasing challenge of antimicrobial resistance among enteric pathogens associated with diarrhoeal infections. The present study showed that the *E. coli* isolates were highly resistant to Amoxicillin, Augmentin, Streptomycin, Chloramphenicol and Pefloxacin. This may be due to indiscriminate use, self-medication and misuse of antibiotics in the community. Moreover, the complete resistance of the isolate PHY2i to nearly all the antibiotics except

Ciprofloxacin shows the emergence of multidrug resistant strains among the diarrhoeagenic bacteria. This finding is in accordance with Hetsa *et al.* (2026), which documented high levels of resistance among strains of *E. coli* to common antibiotics like Amoxicillin and Augmentin. Earlier, Medugu *et al.* (2022) reported that there has been an increased incidence of multidrug resistance among enteric bacterial isolates from Nigeria.

In this study, CPW was observed to exert growth inhibitory activity on only some of the isolated *E.coli* strains. Although the growth inhibition was inferior to that mediated by Ciprofloxacin but it was superior to that mediated by most of the other conventional antibiotics including amoxicillin. The inhibition observed can be due to the presence of antimicrobial metabolites, such as organic acids, hydrogen peroxide and bacteriocin-like substances, which are naturally present in whey. The antibacterial activity of CPW against *E. coli* was however observed to be influenced by the type of coagulants used in whey production. Whey produced using *C. procera* exerted the highest growth inhibition of the test bacteria. Apart from the naturally occurring whey metabolites, the increased antibacterial activity could be due to the antimicrobial phytochemicals of *C. procera*. Murshed *et al.* (2025) reported the antimicrobial activity of the extracts of *C. procera* against enteric pathogens. Sakr *et al.* (2026) also reported similar results and they found that the plant-based coagulants could improve the antimicrobial activity of the dairy products.

The comparative antibacterial activity of CPW and LPW showed that the inhibitory activity of LPW was significantly higher against the isolated *E. coli*. On the other hand, LPW was observed to have significant inhibitory activity against all the *E. coli* isolates including seven that recorded lower susceptibility to Ciprofloxacin. The low inhibitory activity observed with CPW suggests that processing conditions may have effect on the concentration of active antimicrobial metabolites.

The difference in antibacterial activity of the whey prepared with different coagulants indicates that the type of coagulant used is a major factor affecting the antimicrobial activity of the whey. Whey prepared from *C. procera* exhibited the highest inhibitory activity followed by lime prepared whey while alum prepared whey showed least antibacterial activity. The higher activity of whey obtained from *C. procera* may be due to the presence of antimicrobial compounds in the latex of the plant such as flavonoids, tannins, alkaloids and saponins. The good performance of lime prepared whey may be related to the acidic conditions during cheese production which may favour the production of antimicrobial metabolites. Whey prepared with alum however showed lower activity indicating less production or preservation of antimicrobial compounds. This is in agreement with the observation of Sangchi *et al.* (2026) who reported that plant-based coagulants have higher antimicrobial properties than chemical coagulants in fermented dairy products. Similar results were reported by Onelli *et al.* (2026) where natural coagulants were superior to inorganic coagulants in improving the antimicrobial quality of cheese whey.

The observed decrease in pH of CPW in storage at room than at refrigeration temperatures suggests that biochemical and microbial activities continue in whey during

storage. The greater decrease in pH at room temperature than at refrigeration temperature is indicative of a greater fermentation activity and rapid accumulation of acidic metabolites at room temperature. The slight increase of pH on day 7 may be due to the consumption of metabolic process or degradation of acid materials during the long-term storage. This is in agreement with the observation of El-Garhi *et al.* (2025) who reported progressive acidification of whey on storage due to microbial fermentation and production of organic acids. Similar results were obtained by Ma *et al.* (2025) who also reported that storage temperature is an important factor affecting pH stability and biochemical modifications in whey products.

The variations in total titratable acidity (TTA) also confirm that active biochemical reactions take place during storage. High initial TTA at room temperature and oscillations during the storage period suggest a dynamic microbial metabolism and variations in the acid production. The slow but steady increase of TTA with increase of storage days during refrigeration period may be indication of slow but continuous accumulation of acids at low temperature. This result is similar with the report of Anwar *et al.* (2025) who reported that condition of fermentation and storage have great influence on development of acidity in dairy products. Xu *et al.* (2025) obtained similar results, during long storage, variation in acidity of whey products was caused by microbial and enzymatic activities.

This study findings showed that bio-metabolites in whey played significant role in its antibacterial activity against the isolated *E. coli*. Following neutralisation and treatment with catalase, the cell-free supernatant of the bacteriocin displayed potent antibacterial activity with the largest inhibition zones in isolates PHK9, PHI6 and PHI4. This suggests that bacteriocin-like substances were important in the inhibition of the isolates. This result agrees with the findings of Chen *et al.* (2025) who reported that bacteriocins produced by lactic acid bacteria have strong antibacterial activity against enteric pathogens. The cell-free supernatant of the hydrogen peroxide also effect showed significant antibacterial activity against the isolates indicating the contribution of hydrogen peroxide to the inhibitory effect of whey. The isolates PHS3, PHK9, PHI4 and PHI6 showed the highest inhibition zones. This is in agreement with the report of Amenu & Bacha (2025) who reported that hydrogen peroxide produced during fermentation contributes significantly to the antimicrobial activity of fermented dairy products. The neutralised cell-free supernatant which showed comparatively lower antibacterial activity suggests that acidic metabolites also played a role in bacterial inhibition. The lower inhibitory activity after neutralisation suggests that organic acid which had been neutralised play a great role because despite the fact the both the bacteriocins and hydrogen peroxide were still present in the cell free supernatant, the growth inhibition was very low against the isolates. Therefore the growth inhibition mediated by whey in this study is attributed to the synergistic action of organic acids, bacteriocins and peroxide.

CONCLUSION

This study investigated the antibiotic profile of *E. coli* isolated from diarrhoeic children and assess the antibacterial activities of commercial and laboratory prepared

cheese whey against the isolated *E. coli*. The isolated *E. coli* isolates were confirmed to be pathogenic by molecular analysis. The successful amplification of virulence genes such as *eaeA*, *hlyA* and *bfpA* suggests the pathogenic potential of the isolates and their ability to cause intestinal infections.

The isolated *E. coli* varied in its susceptibility and resistance to the antibiotics tested in the antibiotic susceptibility test. Ciprofloxacin was effective against most of the isolated bacterial species while high resistance was observed against Amoxicillin, Augmentin, Streptomycin and Chloramphenicol, indicating the increasing threat of antibiotic resistance among enteric pathogens. Some of the isolates also exhibited multiple drug resistance which makes treatment of childhood diarrhoea difficult.

Commercially produced whey was found to inhibit only a few the *E. coli* isolates. On the other hand, the Laboratory prepared whey (LPW) showed a significant antibacterial activity on all the isolated *E. coli*. Whey that resulted using *C. procera* or lime as coagulant exerted higher antibacterial activity on the test organisms than whey got from using alum as coagulant of fresh milk. This indicates that the coagulant used in cheese making is relevant for the antibacterial potential of the resulting whey. Furthermore, the concerted efforts of bacteriocins, hydrogen peroxide and acidic metabolites are responsible for the inhibitory effects of whey on the isolated *E. coli*.

In conclusion, the present study indicates that the laboratory prepared cheese whey, especially the whey prepared with *C. procera* or lime as coagulants, has potential antibacterial properties against diarrhoeagenic *Escherichia coli*. Thus, the present study highlights the possible use of whey as a natural antimicrobial agent as an alternative or complementary measure in management of diarrhoeal infections caused by common enteric bacteria.

References

1. Adebolu, T.T. and Babafemi, E.O. (2008). Epidemiological survey of the most prevalent bacteria responsible for infantile diarrhoea in South west, Nigeria. *Journal of Pure and Applied Microbiology*, 2(1):69-72.
2. Adebolu, T. T., Olorunfemi, O. B. (2016). Effects of fermented cheese whey on the cells of the immune system of apparently healthy albino rats. *Journal of Immunobiology*1(2), 1-2.
3. Amenu, D., & Bacha, K. (2025). Bio-preservation potential and antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from Ethiopian traditional fermented dairy products. *Probiotics and Antimicrobial Proteins*, 17(5), 3402-3415.
4. Anwar, A., Faiz, M. A., Badar, I. H., Jaspal, M. H., & Hou, J. (2025). Influence of fermentation time and storage conditions on the physicochemical properties of different yogurt varieties using starter cultures and probiotic *Lactobacillus rhamnosus* GG. *Processes*, 13(3), 759.
5. Ashraf, A., Sabu, S., Sasidharan, A., & Sunooj, K. V. (2025). Natural feed supplements from crustacean processing side streams for improved growth of finfishes and crustaceans: a review. *Journal of Animal Physiology and Animal Nutrition*, 109(2), 376-401.
6. Behera, D. K., & Mishra, S. (2022). The burden of diarrhea, etiologies, and risk factors in India from 1990 to 2019: evidence from the global burden of disease study. *BMC Public Health*, 22(1), 92.
7. Biswas, S., Bal, M., Pati, S., Rana, R., Dixit, S., & Ranjit, M. (2024). Antibiotic resistance in toxigenic *E. coli*: a severe threat to global health. *Discover Medicine*, 1(1), 72.

8. Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries* (2nd ed.). Cambridge University Press.
9. Chen, X., Bai, H., Mo, W., Zheng, X., Chen, H., Yin, Y., & Peng, H. (2025). Lactic acid bacteria bacteriocins: safe and effective antimicrobial agents. *International Journal of Molecular Sciences*, 26(9), 4124.
10. Das, R., Palit, P., Haque, M. A., Ahmed, T., & Faruque, A. S. G. (2022). Association between pathogenic variants of diarrheagenic *Escherichia coli* and growth in children under 5 years of age in the Global Enteric Multicenter Study. *The American Journal of Tropical Medicine and Hygiene*, 107(1), 72.
11. Del Carpio, A. M., Freire, C. A., Gomes, T. A., Abe, C. M., & Elias, W. P. (2025). Importance of aggR sequence variants detection for accurate molecular diagnosis of enteroaggregative *Escherichia coli*. *Microbiology Spectrum*, 13(11), e01441-25.
12. Dela, H., Egyir, B., Majekodunmi, A. O., Behene, E., Yeboah, C., Ackah, D., & Addo, K. K. (2022). Diarrhoeagenic *E. coli* occurrence and antimicrobial resistance of Extended Spectrum Beta-Lactamases isolated from diarrhoea patients attending health facilities in Accra, Ghana. *PLoS One*, 17(5), e0268991.
13. Dereje, B., Yibabie, S., Keno, Z., & Megersa, A. (2023). Antibiotic utilization pattern in treatment of acute diarrheal diseases: the case of Hiwot Fana Specialized University Hospital, Harar, Ethiopia. *Journal of Pharmaceutical Policy and Practice*, 16(1), 62.
14. El Alami El Hassani, N., Baraket, A., & Alem, C. (2025). Recent advances in natural food preservatives: A sustainable solution for food safety and shelf-life extension. *Journal of Food Measurement and Characterization*, 19(1), 293-315.
15. El-Garhi, H. E. M., Abd-Elghany, A. A., Ibrahim, A. M., El-Aidie, S. A., & Castro-Muñoz, R. (2025). Effect of organic acids on pathogenic and lactic acid bacteria in directly raw milk cheddar cheese. *Probiotics and Antimicrobial Proteins*, 1-21.
16. Eugster, E., Jakob, E., & Wechsler, D. (2012). Cheese, processed cheese, and whey. In *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/14356007.a06_163.pub2
17. Fakhkhari, P., Tajeddin, E., Azimirad, M., Salmanzadeh-Ahrabi, S., Abdi-Ali, A., Nikmanesh, B., & Alebouyeh, M. (2022). Involvement of *Pseudomonas aeruginosa* in the occurrence of community and hospital acquired diarrhea, and its virulence diversity among the stool and the environmental samples. *International Journal of Environmental Health Research*, 32(1), 61-71.
18. Hasan, R. N., Jasim, S. A., & Ali, Y. H. (2022). Detection of fimH, kpsMTII, hlyA, and traT genes in *Escherichia coli* isolated from Iraqi patients with cystitis. *Gene Reports*, 26, 101468.
19. Hetsa, B.A., Yeboah, E.E.A., Ismail, A. Essack S.Y., Amoako D.G. (2026). Pathogenomics of multidrug-resistant *Escherichia coli* from bloodstream infections in South Africa. *BMC Med Genomics* 19, 71 (2026). <https://doi.org/10.1186/s12920-026-02349-y>
20. Jaaz, W. S., & Kadham, E. J. (2026). Identification of virulence factors genes (eaeA) and (bpfA) of Enteropathogenic *Escherichia coli* strains isolated from children with diarrhea in Kerbala city. *Perinatal Journal*, 34(1), 96-102.
21. Kumawat, M., & Sharma, M. (2025). Virulence Gene Profiles of Diarrheagenic *Escherichia coli*: A Comparative Analysis of Pathotypes Isolated from Acute Diarrheal Patients. *International Journal of Research in Medical and Basic Sciences (IJRMBS)*, 11(10), 1-14.
22. Luparelli, A., Trisciuzzi, D., Schirinzi, W. M., Caputo, L., Smiriglia, L., Quintieri, L., & Monaci, L. (2025). Whey proteins and bioactive peptides: Advances in production, selection and bioactivity profiling. *Biomedicines*, 13(6), 1311.
23. Ma, Y., Sun, H., Zhang, S., Yang, C., Musazade, E., Fan, H., & Zhang, Y. (2025). Structural modification of whey protein isolate via electrostatic complexation with Tremella polysaccharides and its effect on emulsion stability at pH 4.5. *International Journal of Biological Macromolecules*, 297, 139870.
24. Makarova, M. A. (2023). A modern view of diarrheagenic *Escherichia coli*—a causative agent of acute intestinal infections. *Journal of Microbiology, Epidemiology and Immunobiology*, 100(4), 333-344.
25. Malos, I. G., Ghizdareanu, A. I., Vidu, L., Matei, C. B., & Pasarín, D. (2025). The role of whey in functional microorganism growth and metabolite generation: A biotechnological perspective. *Foods*, 14(9), 1488.

26. Manhique-Coutinho, L., Chiani, P., Michelacci, V., Taviani, E., Bauhofer, A. F. L., Chissaque, A., & De Deus, N. (2022). Molecular characterization of diarrheagenic *Escherichia coli* isolates from children with diarrhea: A cross-sectional study in four provinces of Mozambique: Diarrheagenic *Escherichia coli* in Mozambique. *International Journal of Infectious Diseases*, 121, 190-194.
27. Medugu, N., Aworh, M. K., Iregbu, K., Nwajiobi-Princewill, P., Abdulraheem, K., Hull, D. M., & Thakur, S. (2022). Molecular characterization of multi drug resistant *Escherichia coli* isolates at a tertiary hospital in Abuja, Nigeria. *Scientific Reports*, 12(1), 14822.
28. Murshed, M., AL-Tamimi, J., Mares, M., Aljawdah, H. M., & Al-Qurishy, S. (2025). Effectiveness of *Calotropis procera* Leaf Extract in Treating Eimeriosis-Induced Inflammation Alteration of the Jejunum in Mice. *Journal of the Hellenic Veterinary Medical Society*, 76(2), 9085-9096.
29. Okebuagwu, Q.C., Adebolu, T.T. and Ojo, B.A. (2017). Types of bacteria associated with diarrhoeal cases among children in Akure, Nigeria and their antibiogram profile. *Journal of Advances in Microbiology*, 2(2):1-10
30. Olayinka, A. A., Oginni-Falajiki, I. O., Okeke, I. N., & Aboderin, A. O. (2024). Diarrhoeagenic *Escherichia coli* associated with childhood diarrhoea in Osun state, Nigeria. *BMC Infectious Diseases*, 24(1), 928.
31. Onelli, R. R. V., de Jesus, J. C., Lins, R. P., da Capela, A. P., Soares, L. S., & Ferrão, S. P. B. (2026). Technological innovation in the production of matured buffalo cheese using artichoke flower extract (*Cynara cardunculus* L. var. *scolymus*) as a coagulant: organic acid profile, proteomic and sensory study. *International Dairy Journal*, 173, 106491.
32. Sakr, S. S., Alharbi, T A., & Hassan, M. F. (2026). Research Article Functional Evaluation of Moringa Seed Extracts as Plant-Based Coagulants for Camel Milk. *Int. J. Dairy Sci*, 21, 14-23.
33. Sangchi, Z. G., Peyravi, M., & Jahanshahi, M. (2026). A Comparative Study of Bio-Based Antimicrobial Membranes Induced by Medicinal Plant-Derived Biocide for Dairy Wastewater Treatment. *Journal of Polymers and the Environment*, 34(3), 51.
34. Saxena, S., Khetra, Y., Ganguly, S., & Sasmal, S. (2025). Morphological and Textural Properties of Feta Cheese Made of Vegetable Protease. *Food Bioengineering*, 4(2), 142-154.
35. Taviani, E., & Pedro, O. (2022). Impact of the aquatic pathobiome in low-income and middle-income countries (LMICs) quest for safe water and sanitation practices. *Current Opinion in Biotechnology*, 73, 220-224.
36. Tilahun, M., Belete, M. A., Gedefie, A., Debash, H., Alemayehu, E., Weldehana, D. G., & Shibabaw, A. (2025). Prevalence of *Salmonella* and *Shigella* species and their multidrug resistance patterns among pediatric populations in Ethiopia: a systematic review and meta-analysis. *BMC Infectious Diseases*, 25(1), 52.
37. Tille, P.M. (2017). *Bailey and Scott's Diagnostic Microbiology* (14th ed.). Elsevier.
38. Viana, G. G. F., Cardozo, M. V., Pereira, J. G., & Rossi, G. A. M. (2025). Antimicrobial resistant *Staphylococcus* spp., *Escherichia coli*, and *Salmonella* spp. in food handlers: a global review of persistence, transmission, and mitigation challenges. *Pathogens*, 14(5), 496.
39. Vildan Akdeniz, Melisa Ünal, & Nayil Dinkç. (2023). The importance and health benefits of whey proteins. *Journal of Hygienic Engineering and Design*. Review paper UDC 637.344.045:613.292
40. World Health Organisation (WHO). (2024). Diarrhoeal Diseases.
<https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease>
41. Xu, J., Hei, X., Zheng, M., Wang, Y., Xie, N., Li, X., & Zhang, S. (2025). Effect of heat-resistant protease from psychrotrophic bacteria in raw milk on shelf-life quality of UHT milk. *Food Control*, 111927.
42. Zelelie, T. Z., Eguale, T., Yitayew, B., Abeje, D., Alemu, A., Seman, A., & Abebe, T. (2023). Molecular epidemiology and antimicrobial susceptibility of diarrheagenic *Escherichia coli* isolated from children under age five with and without diarrhea in Central Ethiopia. *PLoS One*, 18(7), e0288517.
43. Zulfkar, Q., Humaira, A., Mohd, A. D., & Afshana, Q. (2025). The growing threat of antibiotic resistance: Mechanisms, causes, consequences, and solutions. *International Journal of Cognitive Neuroscience and Psychology*, 3(3), 28-36.



© 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).