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EXPLORING THE DIVERSITY OF ENTEROPATHOGENS IN NEONATES

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ABSTRACT: Diarrhea alters the movement of ions and water that follows an osmotic gradient and leads to Loose, watery stools, abdominal cramps, abdominal pain, fever, bloating, blood, and mucus in the stool. Numerous critical cases have been observed in both infants and adults. The main objective of this study is based on an exploration of risk factors of diarrheal infection caused in neonates by entering pathogenic Bactria. The enteric infections responsible for diarrhea are the major cause of morbidity and mortality worldwide, while 2–4 billion cases of diarrheal infections in infants occur worldwide every year. The major microbes associated with diarrheal infection belong to all the major groups, including viruses, bacteria and protozoans. Amongst various pathogenic factors, preferred bacterial pathogens have been considered as classical organisms for the study of diarrhea *viz* the strains of Escherichia coli for ion absorption mechanisms, *Clostridium difficile* and *Shigella spp*. as inflammatory diarrhea and *Vibrio cholera* for secretory diarrhea. The current study is focused on the members of Enterobacteriaceae, including the strain of *E. coli* O157: H7 (enterohemorrhagic). In the current investigation, 35 fecal samples were collected from hospitals belonging to diverse age groups and various biochemical tests were performed to analyze the pathogen city and property of isolates.

INTRODUCTION: Diarrhea is the second leading cause of morbidity and mortality every year all over the world. Among all ages, particularly prevalent in children under the age of 5 may lead to malnutrition and severe complications [1]. Diarrhea is typically a symptom of an infection in the intestinal tract, which can be spread through contaminated food or drinking water or become contagious in nature because of poor hygiene [25].

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This pathological condition may lead to severe gastrointestinal complications in children [2]. In clinical practice, there are three major types of diarrheas reported. One is acute watery diarrhea, which lasts several hours or days and includes cholera. The second is acute bloody diarrhea, also known as dysentery. The third is persistent diarrhea, which can be continued for more than 14 days [5].

Acute diarrhea of infectious etiological is also referred to as gastroenteritis. Some of these infections may present predominantly and may cause nausea and vomiting. Additional symptoms include abdominal distention, abdominal pain, borborygmus, dehydration, flatulence, halitosis, melena, hematochezia, polydipsia and tenesmus,

vomiting, and weight loss [3]. Diarrhea has been assessed on global epidemiology periodically since 1982. However, there is a significant difference between developed and developing countries in terms of morbidity and mortality rates in case of diarrhea worldwide [1]. In developing countries [24], rotavirus is thought to be responsible for 60% of all diarrheal illnesses.

The microbiologic causes of protracted diarrhea include detectable parasitic (*e.g., Giardia, cryptosporidium*) and bacterial (*e.g.,* enter aggregative *Escherichia coli, Shigella*) pathogens [4]. In the case of bacterial sources, most importantly pathogenic *E. coli*, nonetheless also *Campylobacter*, *Yersinia* and *Salmonella* spp. are common, with *Shigella* spp. Causes bacterial dysentery, contributing up to 15% of mortality attributable to diarrheal illness [3].

MATERIALS AND METHODS:

Collection of Samples: Fecal samples were procured from various hospitals of Meerut and NCR **Table 1**. The samples immediately after collection were transported to the laboratory at 4 ºC.

TABLE 1: DETAILS OF THE SAMPLES COLLECTED

S. no.	Location	Sample code
1	Chiranjeev Child Hospital, Hapur Road, Meerut-250001.	CCH-SS1-8
2	Yogi Nursing Home, Garh Road, Meerut-250002.	YNH-SS9-14
3	LLR Medical College, Garh Road, Meerut.	LLRM- SS15-24
4	Metro Hospital, Noida.	MH-SS25-35

Processing of Samples: All stool samples were processed for the presence of lactose fermenting (*Escherichia coli*) and non-lactose fermenting (*Salmonella* spp. and *Shigella* spp.) as follows:

Spreading on MacConkey's Agar: 1 mL of the water sample was spread on MacConkey and nutrient agar using the spread plate technique and incubated at 37ºC for 24 h [4]. Pink colonies on MacConkey agar were considered as lactose fermenters most likely to be *E. coli* and stored at 4ºC. Colonies on nutrient agar were used to calculate the total microbial load in the water [23].

Spreading on XLD Agar: The isolated colonies were sub-cultured on XLD agar. The plates were observed for the color of colonies as pink, most likely to be *E. coli*, yellow *Shigella* spp., and blackish *Salmonella* spp., after an incubation of 24h at 37°C. The selected colonies were subsequently transferred on nutrient agar at 4°C and as glycerol stock at -20°C till further characterization[24].

Characterization of Isolates:

Gram's Staining: Thin smears were prepared on clean glass slides and heat-fixed. The smears were flooded with crystal violet for 1 min and rinsed with water. A few drops of mordant (Gram's iodine solution) were added to the smear and left for 1 min. After rinsing with water, the decolorizer (70% ethanol) was added drop-wise for 10-15 sec. The smear was then counter-stained with safranin for 30 sec, rinsed with water, air-dried, and observed under the microscope in oil immersion [25].

Biochemical Characterization of Isolates: The isolates were further characterized based on their biochemical properties. Classical biochemical tests were performed, including Indole, Methyl red, Voges-Proskauer and Citrate utilization tests, catalase test, and production of H_2S on motility on TSI agar for their identification.

Indole Production or Tryptophanase: Indole is one of the metabolic degradation products of the amino acid tryptophan. Tryptophan broth was inoculated with test organisms and incubated at 37°C, for 24 h.

At the end of incubation, 15 drops of Kovac's reagent were added to each tube. The formation of the cherry red-colored ring at the interface of broth and reagent within seconds indicated indole production [23].

Methyl-Red (MR) test: MR broth (gL-1: peptone, 7.0; glucose, 5.0; NaCl, 5.0; pH, 6.5) was inoculated and incubated at 37°C for 24 h, and 5 drops of methyl red solution (0.1 g methyl red in 300 mL of 95% amyl alcohol and 200 mL of distilled water) were added. The red color of the solution indicated positive while yellow was regarded as negative [26].

Voges-Proskauer (V-P) Test: The production of acetylmethylcarbinol (acetoin or 2, 3-butanediol or diacetyl) was tested by growing the strain in V-P broth (gL-1: peptone, 7.0; glucose, 5.0; NaCl, 5.0; K_2HPO_4, 5.0; pH, 6.9) for 24 h at 37 °C.

Three mL of NaOH (40%) and 2-3 drops of creatine solution (0.3% w/v) were added to the culture broth. The development of cherry red color after 30-60 min at room temperature was recorded as positive.

Citrate Utilization: Citrate agar [gL-1: trisodium citrate, 1.0; NaCl, 5.0; $MgSO_4$, 0.2; $NH_4H_2PO_4$, 1.0; K_2HPO_4, 1.0; bromothymol blue 0.08; Agar, 25.0; pH, 7) slants were inoculated and incubated for 24 h at 37 °C. The citrate utilization was observed by the change in color from green to blue.

Catalase Test: A loop full of bacterial culture was taken on the glass slide, flooded with 10% H_2O_2, and observed for the formation of effervescence.

Triple Sugar Iron (TSI) test: TSI agar slants (gL-1: beef extract, 8.0; yeast extract, 3.0; peptone, 20.0; glucose, 1.0 lactose, 10.0; sucrose, 60.0; $FeSO_4$. $7H_2O$, 0.20; NaCl, 5.0 $Na_2S_2O_3$. $5H_2O$, 0.3; phenol red, 0.018; agar 25.0) were inoculated and incubated at 37 °C for 24h. After 24h of incubation the slants were observed for:

- ✓ Acid production (by color change)
- ✓ Gas production
- ✓ H_2S production (by blackening of the medium)

RESULT AND DISCUSSION: Globally, > 1.1 billion people drink unsafe water. Most diarrheal diseases are attributable to unsafe water, sanitation, and hygiene[6]. In India, a large population depends on processed surface waters for drinking. Water-borne and food-borne diseases are common in summers and monsoons in India [7]. A vast majority of diarrheal diseases are attributable to unsafe water, sanitation, and hygiene. The 35 fecal samples used in the present study were procured from four hospitals of Meerut and NCR and subjected to the isolation of coliforms and their antibiotic-resistant profile [8].

Analysis for coliforms provides a sensitive, although not the most rapid, an indication of all pathogens in fecal matter [9]. The colonies were counted on MCA and NA plates using a colony counter. And for this, we were using various differential media such as MacConkey Agar, Nutrient Agar, Pure culture of selected bacteria on Nutrient Agar, Sub Culturing of Lactose Fermenting Colony on Eosin Methylene Blue Agar (EMB Agar), Sub Culturing of Lactose Fermenting colony on MacConkey's Agar (MCA) which shown in **Fig. 1**.

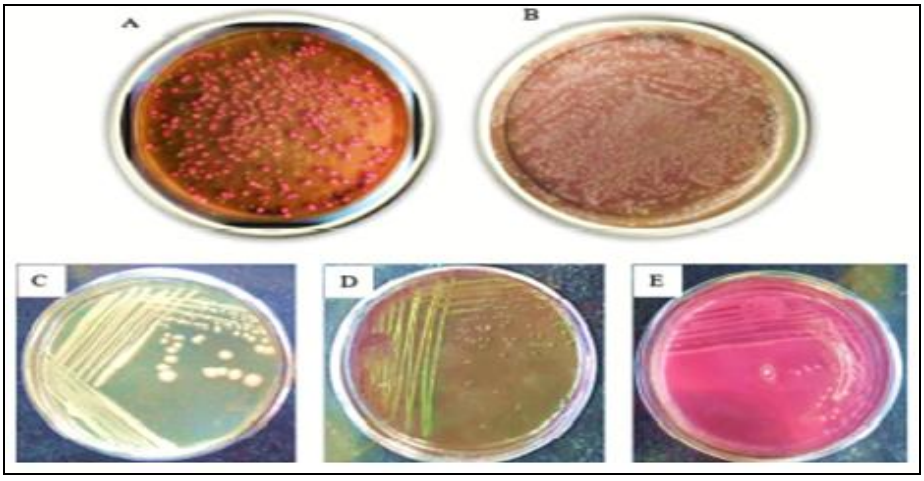

FIG. 1: SAMPLE PROCESSING ON VARIOUS GROWTH MEDIUMS

Sample wise detail of lactose fermenting isolates are shown in table 2. nutrient agar plates were used to estimate the total count of bacteria in the fecal sample [10]. The total colony count ranged from 6.1×10^5 - 7.5×10^9 CFU/gm of a sample, while the count of lactose fermenting ranges from 1.6×10^2 - 3.3×10^6 CFU/gm of sample and non-lactose fermenting ranges from 2.3×10^3 - 5.2×10^7 CFU/gm of a sample **Table 2.**

TABLE 2: DETAILS OF BACTERIAL LOAD IN FAECAL SAMPLES PROCESSED ON VARIOUS GROWTH MEDIUMS

Sample No.	Lactose Fermenting (MacConkey Agar) CFU/mL	Non-Lactose Fermenting (MacConkey Agar) CFU/mL	Total Count (Nutrient Agar) CFU/mL
CCH-SS1	6.2×10^{5}	2.3×10^{4}	4.1×10^{7}
CCH-SS2	4.4×10^{4}	4.6×10^{3}	6.1×10^{5}
CCH-SS3	4.6×10^{5}	5.6×10^{4}	2.4×10^{8}
CCH-SS4	5.5×10^{5}	5.5×10^{5}	2.3×10^{7}
CCH-SS5	8.2×10^{4}	8.2×10^{5}	4.5×10^{6}
CCH-SS6	4.2×10^{4}	6.4×10^{5}	1.9×10^{8}
CCH-SS7	2.4×10^{3}	3.8×10^{4}	7.1×10^{8}
CCH-SS8	5.8×10^{2}	4.4×10^{4}	3.5×10^{7}
YNH-SS9	1.5×10^{3}	5.5×10^{5}	6.2×10^{7}
YNH-SS10	3.9×10^{2}	8.2×10^{4}	4.3×10^{6}
YNH-SS11	7.8×10^{3}	6.4×10^{5}	8.5×10^{8}
YNH-SS12	4.8×10^{2}	4.8×10^{6}	3.7×10^{8}
YNH-SS13	8.1×10^{4}	6.6×10^{5}	2.9×10^{9}
YNH-SS14	2.3×10^{2}	3.2×10^{5}	4.3×10^{7}
LLRM- SS15	4.3×10^{2}	2.4×10^{3}	3.9×10^{8}
LLRM- SS16	2.1×10^{3}	6.1×10^{5}	6.5×10^{9}
LLRM- SS17	1.6×10^{2}	2.4×10^{5}	2.1×10^{7}
LLRM- SS18	2.5×10^{3}	8.2×10^{5}	3.8×10^{9}
LLRM- SS19	3.3×10^{6}	5.2×10^{7}	5.8×10^{9}
LLRM- SS20	5.3×10^{4}	7.1×10^{5}	7.5×10^{9}
LLRM- SS21	1.8×10^{2}	1.5×10^{4}	4.7×10^{7}
LLRM- SS22	8.9×10^{3}	7.2×10^{5}	2.5×10^{7}
LLRM- SS23	4.8×10^{2}	4.9×10^{4}	5.4×10^{8}
LLRM- SS24	3.4×10^{2}	3.6×10^{3}	4.3×10^{8}
MH-SS25	4.6×10^{2}	2.3×10^{4}	5.1×10^{9}
MH-SS26	4.2×10^{2}	4.8×10^{6}	6.2×10^{7}
MH-SS27	4.4×10^{2}	5.0×10^{6}	7.5×10^{8}
MH-SS28	4.6×10^{2}	3.6×10^{3}	7.2×10^{7}
MH-SS29	6.1×10^{2}	7.2×10^{4}	1.7×10^{9}
MH-SS30	6.8×10^{2}	3.5×10^{6}	1.8×10^{9}
MH-SS31	3.9×10^{4}	8.2×10^{5}	4.0×10^{9}
MH-SS32	7.9×10^{2}	2.6×10^{4}	6.8×10^{7}
MH-SS33	3.7×10^{4}	7.4×10^{5}	7.5×10^{8}
MH-SS34	6.2×10^{4}	5.2×10^{5}	4.9×10^{7}
MH-SS35	2.3×10^{3}	2.3×10^{3}	6.1×10^{9}

The high amount of coliform was according to the expectation and similar to the other finding[11].Microscopic analysis of pure culture smear using Gram's staining under oil immersion objective various shapes with gram reaction was observed. Among 121 isolates selected based on the colony, morphology was revealed 6.6% of isolates were found to be positive cocci while 2.4% were positive rods, while 95% were given Gram's negative reaction [28]. Details of isolates are shown in **Table 3**. The result indicates that some Gram reaction positive cultures were also able to grow on MacConkey's agar, although their ratio was very small, which was 6.6% for Gram reaction positive cocci and only 2.4% were Gram reaction positive rods [12]. In this study, out of 144 specimens, enter pathogens were found in 89 (61.8%) while 55 (38.2%) cases yielded negative results. Out of 89 enter pathogens, 48 (53.9%) were bacterial; the result was different from the current investigation. Approximately 500 stool samples were studied[15]; the stool samples were collected from Diarrhea patients, including infants and children under ten years of age admitted to the Pediatric and Maternity Hospital in Erbil City. Surprisingly, they found infectious agents in 75 (15%) samples, which contradicts the present study's result. In the study reported by Nair *et al.* [16] in Kolkata, India, where 27.9% of the Diarrhea patients had no potential pathogen, which was also different from the results presented in the current study [27, 32]. The classical biochemical test series, commonly known as IMViC were used to further characterize isolates. Details of the results are shown in **Table 3** and **Fig.**

3. For further study, the isolates giving a clear test for Klebsiella sp., E. coli, Citrobacter freundii and Proteus Vulgaris were selected. Among 110 isolates characterized biochemically, approximately 16% were indicative to be *E. coli*; 3.6% *Klebsiella* sp., 9% *Citrobacter freundii*, and 2.7% *Proteus Vulgaris* [14, 31].In one similar study on patients suffering from Diarrhea, the most commonly isolated pathogens were *Aeromonas* spp. (33, 16.3%), NLF *E.coli* (19, 9.4%), *Proteus mirabilis* (14, 6.9%), Other *Salmonella* spp. (6, 3.0%), *Edwardsiella* spp. (4, 2.0%), *Shigella* spp. (2, 1.0%), *Proteus vulgaris* (2, 0.7%), *Salmonella Typhi* (1, 0.5%), *Salmonella Paratyphi* (1, 0.5%), *Citrobacter* spp. (1, 0.5%), *Plesiomonas shigelloides* (1, 0.5%) [17].

Taneja *et al.* [17] in another study conducted in PGIMER, Chandigarh from January 2000 to September 2002 in which 1802 fecal samples were analyzed from the same number of patients suffering from diarrhea submitted to the Department of Medical Microbiology, in which they reported *Aeromonas* spp. (14), *Salmonellatyphi* (2) and *Salmonella paratyphi* (2). In the study carried out by Sherchand *et al.*[18]; the higher incidence of *Shigella* spp. (36.8%) and *Salmonella* spp (14.03%). Similarly, in a study carried out by Okon *et al.* [13, 30] *Salmonella* spp. accounts for 1 (0.4%). In another study carried out by Kumar *et al.* [19] *Shigella* (7.5%), *Salmonella* (7.5%), *Proteus* (5.5%), were isolated, which was a bit comparable with the current study.

The variation in the number and type of isolates in different studies might be due to the variation in place, time and season pattern of feeding and socio-economic status of the patients from which the sample was collected [20].

Characterization of Isolates: When examining thin smears of pure culture under the microscope in oil immersion with Gram's staining, the different shapes and colorations of bacteria were detected. (a) gram's negative rod with capsules (b) mucoid creamish yellow *klebsiella* sp. (c) gram's negative rods (d) colony of *E. Coli*. The 6.6% isolates were found to be positive cocci while 2.4% were positive rods, on the other hand, 95% were given Gram's negative reaction [21]. Details of isolates are shown in **Table 3** and **Fig. 2.**

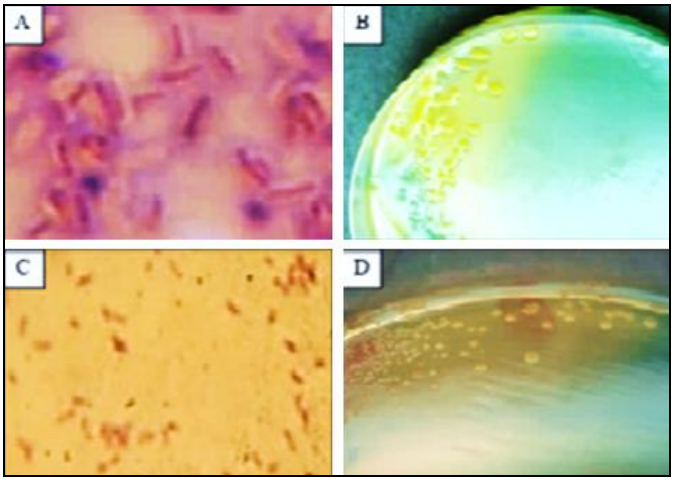

FIG. 2: MICROSCOPIC IMAGE OF ISOLATES AND COLONY MORPHOLOGY

TABLE 3: DETAILS OF BACTERIAL ISOLATES OBTAINED FROM VARIOUS GROWTHS MEDIUM

S. no.	Sample No.	Isolate Code	Colony morphology	Gram+/-, Shape
1.	CCH-SS1	CCH-IS1; CCH-IS 2; CCH-IS 3	Colorless; Pink, round; colorless opaque	(+), cocci; (-), rod; (-), rod
2.	CCH-SS2	CCH-IS 4; CCH-IS 5;	Pink mucoid; Pink, round	(-), rod; (-) rod
3.	CCH-SS3	CCH-IS 6; CCH-IS 7; CCH-IS 8	Colorless; Pink mucoid; Colorless-mucoid	(-), rod; (-), rod; (+), cocci
4.	CCH-SS4	CCH-IS 9; CCH-IS 10; CCH-IS 11	Colorless; Pink; Colorless irregular	(-), rod; (-), rod; (-), rod
5.	CCH-SS5	CCH-IS 12; CCH-IS 13; CCH-IS 14	Pink mucoid; Colorless bulging; Colorless small	(-), rod; (-), rod; (-), rod
6.	CCH-SS6	CCH-IS 15; CCH-IS 16; CCH-IS 17; CCH-IS 18	Colorless; Off-white; Transparent white; White	(-), rod; (-), rod; (-), rod; (+), cocci
7.	CCH-SS7	CCH-IS 19; CCH-IS 20; CCH-IS 21	Mucoid transparent; Yellow white; Colorless	(-), rod; (+), cocci; (-), rod
8.	CCH-SS8	CCH-IS 22; CCH-IS 23; CCH-IS 24	Pink, mucoid; Irregular-Pink; Transparent	(-), rod; (-), rod; (+), rod
9.	CCH-SS9	CCH-IS 25; CCH-IS 26	Pink-mucoid; Off-white transparent	(-), rod; (-), rod
10.	YNH-SS10	YNH-IS 27; YNH-IS 28; YNH-IS 29; YNH-IS 30	Small, transparent; Pink, round; Large Pink; Small pink	(-), rod; (-), rod; (-), rod; (-), rod

11.	YNH-SS11	YNH-IS 31; YNH-IS 32; YNH-IS 33; YNH-IS 34	White; Yellow; Pink whitish; Black pigment round	(-), rod; (+), cocci; (-), rod; (-), rod
12.	YNH-SS12	YNH-IS 35; YNH-IS 36; YNH-IS 37	Transparent, round; Transparent, irregular; Greenish grey	(-), rod (-), rod; (-), rod
13.	YNH-SS13	YNH-IS 38; YNH-IS 39; YNH-IS 40; YNH-IS 41	Pink, round; Yellow, round; Small transparent; Small translucent	(-), rod; (+), cocci; (-), rod; (-), rod
14.	YNH-SS14	YNH-IS 42; YNH-IS 43; YNH-IS 44; YNH-IS 45	Pink, round; Small transparent; Pink, round; Transparent, large	(-), rod; (-), rod; (-), rod; (-), rod
15.	LLRM-SS15	LLRM-IS 46; LLRM-IS 47; LLRM-IS 48; LLRM-IS 49; LLRM-IS 50; LLRM-IS 51	Pink mucoid; Greenish grey; Transparent, small; Yellow mucoid; Off-white mucoid; Pink rough	(-), rod; (-), rod; (-), rod; (-), rod; (-), rod; (-), rod
16.	LLRM-SS16	LLRM-IS 52; LLRM-IS 53; LLRM-IS 54; LLRM-IS 55	Off-white round; Mucoid large; Pink mucoid large; Transparent round	(-), coccobacilli; (-), rod; (-), rod; (+), rod
17.	LLRM-SS17	LLRM-IS 56; LLRM-IS 57; LLRM-IS 58	Round dry; Transparent Mucoid small; Pink mucoid	(-), rod; (-), rod; (-), rod
18.	LLRM-SS18	LLRM-IS 59; LLRM-IS 60; LLRM-IS 6; LLRM-IS 62; LLRM-IS 63	Transparent round; Off-white round; White mucoid; small Mucoid irregular; Green-white round	(+), rod; (-), rod; (-), rod; (-), rod; (-), rod
19.	LLRM-SS19	LLRM-IS 64; LLRM-IS 65; LLRM-IS 66; LLRM-IS 67	Transparent round; Pink; round Pink large; Pink round dry	(-), rod; (-), rod; (-), rod; (-), rod
20.	LLRM-SS20	LLRM-IS 68; LLRM-IS 69; LLRM-IS 70; LLRM-IS 71; LLRM-IS 72	Transparent round small; Pink round; Transparent round; Pink round dry; Transparent round mucoid	(-), rod; (-), rod; (-), rod; (-), rod; (-), rod
21.	LLRM-SS21	LLRM-IS 73; LLRM-IS 74; LLRM-IS 75; LLRM-IS 76	Pink round; Pink mucoid; Pink round; Transparent round	(-), rod; (-), rod; (-), rod; (-), rod
22.	LLRM-SS22	LLRM-IS 77; LLRM-IS 78; LLRM-IS 79; LLRM-IS 80	Greenish white; Pink mucoid; Off-white; Reddish pink	(-), rod; (-), rod; (-), rod; (+), cocci
23.	LLRM-SS23	LLRM-IS 81; LLRM-IS 82; LLRM-IS 83; LLRM-IS 84; LLRM-IS 85	Transparent small; Pink mucoid; Transparent small; Pink, small; Off-white	(-), rod; (-), rod; (-), rod; (-), rod; (-), rod
24.	LLRM-SS24	LLRM-IS 86; LLRM-IS 87; LLRM-IS 88	Pink round; Transparent, round; Pink small	(-), rod; (-), rod; (+), cocci
25.	MH-SS25	MH-IS 89; MH-IS 90; MH-IS 91; MH-IS 92; MH-IS 93; MH-IS 94	Pink round; Greyish; Transparent, small; Round pink; Pink, round; Black pigment producing round	(-), rod; (-), rod; (-), rod; (-), rod; (-), rod; (-), rod
26.	MH-SS26	MH-IS 95; MH-IS 96; MH-IS 97; MH-IS 98	Transparent small; Pink round; Pink round small; Green transparent	(-), rod (-), rod; (-), rod; -
27.	MH-SS27	MH-IS 99; MH-IS 100; MH-IS 101	Pink round; Pink transparent irregular margin; Pink, round	(-), rod; (-), rod; (-), rod
28.	MH-SS28	MH-IS 102; MH-IS 103; MH-IS 104	Pink transparent large; Pink transparent small; Transparent irregular margin	(-), rod; (-), rod; (-), rod
29.	MH-SS29	MH-IS 105; MH-IS 106; MH-IS 107	White, round; Transparent, small; Transparent	(-), rod (-); rod; (-); rod
30.	MH-SS30	MH-IS 108; MH-IS 109; MH-IS 110	Transparent round small; Transparent bulging round; Transparent round	(-), rod; (-), rod; (-), rod
31.	MH-SS31	MH-IS 11; MH-IS 112	Pink, round; Transparent, small	(-), rod; (-), rod
32.	MH-SS32	MH-IS 113; MH-IS 114; MH-IS 115	Transparent small; Pink, round; Pink mucoid;	(-), rod; (-), rod; (-), rod
33.	MH-SS33	MH-IS 116; MH-IS 117	Small transparent; Pink mucoid	(-), rod; (-), rod
34.	MH-SS34	MH-IS 118; MH-IS 119	Transparent small; Pink, round	(-), rod; (-), rod
35.	MH-SS35	MH-IS 120; MH-IS 121	White round; Pink transparent irregular margin	(-), rod; (-), rod

Biochemical Characterization of Isolates: Isolates were further characterized using the traditional biochemical sequence known as IMViC. The isolates that passed the *Klebsiella sp.*, *E. coli*, and *Salmonella sp.* test. *E. coli*, *Citrobacter freundii* and *Proteus vulgaris* were chosen for more research. (a) biochemical profile for *E. Coli* and (b) biochemical profile for *klebsiella sp.* A total of 110 isolates were biochemically characterized [22, 29]. The findings are mentioned in detail **Table 4 and Fig 3.**

TABLE 4: DETAILS OF BIOCHEMICAL PROFILE OF BACTERIAL ISOLATES OBTAINED FROM VARIOUS GROWTH MEDIUMS

S. no.	Isolate code	Biochemical tests				Suspected bacteria
		Indole	Methyl Red	Voges Proskauer	Citrate	
1.	CCH-IS 3	+	+	-	-	*E. coli*
2.	CCH-IS 5	+	+	-	-	*E. coli*
3.	CCH-IS 6	+	+	-	-	*E. coli*
4.	CCH-IS 9	+	+	-	-	*E. coli*
5.	CCH-IS 13	-	-	+	+	*Klebsiella* sp.
6.	CCH-IS 11	+	+	-	-	*E. coli*
7.	CCH-IS 14	-	+	-	+	*Citrobacter freundii*
8.	CCH-IS 16	-	+	-	+	*Citrobacter freundii*
9.	CCH-IS 17	-	+	-	+	*Citrobacter freundii*
10.	CCH-IS 21	+	+	-	-	*E. coli*
11.	CCH-IS 26	+	+	-	-	*E. coli*
12.	YNH-IS 31	-	+	-	+	*Citrobacter freundii*
13.	YNH-IS 35	+	+	-	-	*Proteus Vulgaris*
14.	YNH-IS 36	+	+	-	-	*E. coli*
15.	YNH-IS 40	+	+	-	-	*Proteus vulgaris*
16.	YNH-IS 41	-	+	-	+	*Citrobacter freundii*
17.	YNH-IS 43	-	+	-	+	*Citrobacter freundii*
18.	LLRM-IS 48	-	+	-	+	*Citrobacter freundii*
19.	LLRM-IS 53	-	-	+	+	*Klebsiella* sp.
20.	LLRM-IS 57	-	-	+	+	*Klebsiella* sp.
21.	LLRM-IS 61	-	-	+	+	*Klebsiella* sp.
22.	LLRM-IS 64	+	+	-	-	*E. coli*
23.	LLRM-IS 76	+	+	-	-	*E. coli*
24.	LLRM-IS 79	+	+	-	-	*E. coli*
25.	LLRM-IS 85	+	+	-	-	*E. coli*
26.	LLRM-IS 87	+	+	-	-	*E. coli*
27.	MH-IS 94	+	+	-	-	*E. coli*
28.	MH-IS 95	+	+	-	-	*E. coli*
29.	MH-IS 106	+	+	-	-	*E. coli*
30.	MH-IS 107	-	+	-	+	*Citrobacter freundii*
31.	MH-IS 108	-	+	-	+	*Citrobacter freundii*
32.	MH-IS 116	+	+	-	-	*Proteus vulgaris*
33.	MH-IS 120	+	+	-	-	*E. coli*
34.	MH-IS 121	-	+	-	+	*Citrobacter freundii*

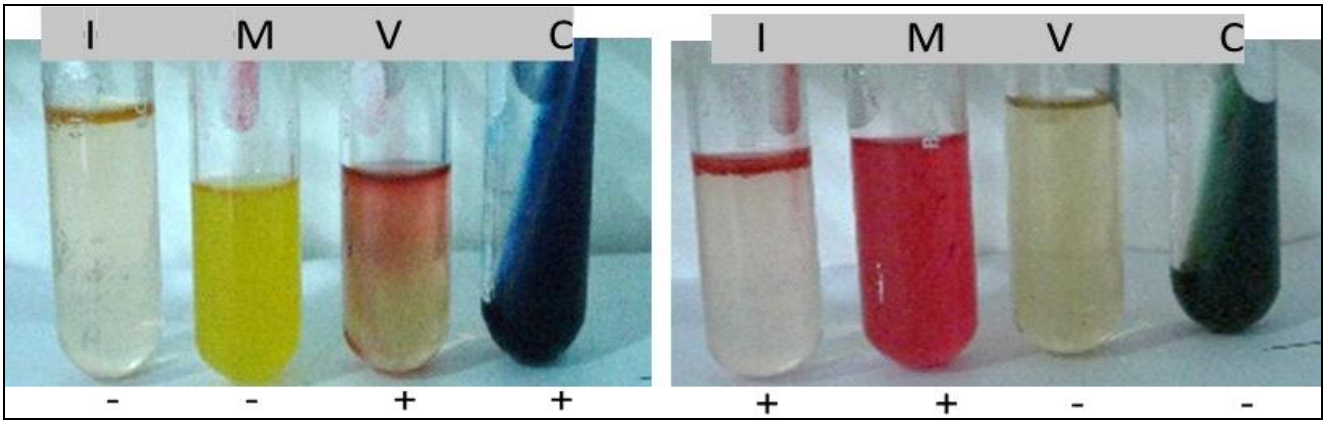

FIG. 3: BIOCHEMICAL EVALUATION OF ISOLATE

Approximately 16% were indicative of being *E. coli*; 3.6% *Klebsiella sp*., 9% *Citrobacter freundii*, and 2.7% *Proteus vulgaris*. The isolates were subjected to further characterization.

CONCLUSION: Diarrhea is the world's second-highest cause of morbidity and mortality in infants as well as in adults. It affects people of all ages but is more common in children under the age of five, and it can lead to malnutrition and other various life-threatening problems. The current research focuses on Enterobacteriaceae members, especially the *E. coli* (Enterohemorrhagic). In this study, 35 fecal samples from hospitals of various ages were obtained, and various biochemical tests were done to determine the pathogenicity and properties of isolates. Finding of this study, the stool samples were collected from Diarrhea patients, including infants and children under ten years of age, admitted to the Pediatric and Maternity Hospital in NCR. Results show a high prevalence of coliform in processed samples.

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