



Original Article

Isolation of Bacteria from Diarrhoeal Samples Collected from Hospitals of Dehradun and Paonta Sahib

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The present study was designed to isolate and identify bacteria from diarrhoeal samples collected from hospitals of Dehradun and Paonta Sahib. Isolated colonies appear after broth was spread over the nutrient agar plates and kept for one day incubation. Identification of colonies on selective media was done by Gram staining and colour morphology. Different pathogenic bacteria were identified from diarrheal samples. The main resulting bacteria were *Salmonella*, *Pseudomonas* and *E.coli*. The results obtained clearly showed that the bacterial load of some specific bacteria in diarrhoeal samples is more than compared to the others. Further it is concluded that the mainly isolated bacteria are main causative agent for diarrhoea and can be pathogenic as well as harmful for the individual.

Keywords: Hospitals, Diarrhoea, Pathogens, Nutrient Broth.

1. INTRODUCTION

Diarrhoea is the condition of having three or more loose or liquid bowel motions each day¹⁶. Introduction of the concept is commonly given to Nobel Prize awardee Eli Metchnikoff, who in 1907 suggested that the dependence in the elderly, especially for the people who have been cured with antibiotics for unconcerned infections, a toxin produced by *Clostridium difficile* often causes severe¹⁰. Another cause of diarrhoea is gastroenteritis also. The loss of fluids through diarrhoea can cause dehydration and electrolyte

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disturbance. The first line treatment for diarrhoea is Oral rehydration solutions ³. Drugs are any chemical substances capable of affecting the body. The modest amounts of salts and zinc tablets are also given but, Oral rehydration solutions (ORS) are the treatment of choice and is very effective⁴. There are certain antibiotics available in the market that can be used for treatment of diarrhoea however this ought to cause hike in the cases of microbial resistance. With recent studied it has been found that probiotics can be safe and positive treatment for diarrheal patients ⁵. The organisms isolated from the diarrhoeal samples are-*E.coli*, *Staphylococcus* & *Pseudomonas*. *Klebsiella*, *Salmonella*. *E. coli* is gram -ve, rod shaped bacteria ¹². It is commonly found in the lower intestine of warm blooded animals and is isolated on EMB & Mac-Conkey agar ¹⁴. When the sample is streaked on Selective media-EMB and Mac-Conkey Agar plates, it gives Metallic green colonies on EMB & pink coloured colonies on Mac-Conkey agar ⁸. *Pseudomonas* is a gram -ve, rod shaped bacteria found in environment like soil, water and is isolated on PIA agar ¹¹. It gives green colour colonies on PIA agar & white colourless colonies on King's medium. *Staphylococcus* is a gram +ve, cocci bacteria and is found in Human Respiratory Tract & on the skin. It is isolated on MSA agar. There are two species of *Staphylococcus*: *S.aureus* gives yellow coloured colonies & *S. epidermidis* gives pink coloured colonies on MSA agar ².

2. MATERIALS AND METHODS

Sample Collection

Approximately 450 samples of diarrhoeal patients were collected from various hospitals in Paonta Sahib and nearby regions diagnosed by the doctors. Samples were collected as saliva, vomit and stool in sterile plastic containers.

Sample Isolation

Enrichment of 1ml of sample in 10ml of buffer peptone water aseptically was done and incubated at 37 °C for 24h. Next day this sample was poured in nutrient broth and incubated for 24h. Presence of turbidity in the broth showed the presence of bacteria. 100µl sample is spread over the nutrient agar plates and kept for one day incubation. Next day isolated bacterial colonies were seen on plate, these isolated colonies were picked and streaked on selective media plates such as Blood Agar, XLD, MSA, SM Agar, EMB Agar, and Mac-Conkey Agar ⁶. Incubation was given at 37 °C for 24h and on the basis of colour of the colony grown aerobically on the selective media micro-organisms were identified.

Identification of Species

Growth of bacteria occurs as creamish mucoid colonies as shown below in Fig 2,3,4. Different bacteria give different colours on selective media. They are identified on the basis of their colour & morphology ⁹ as shown in Table 1 in results.

3. RESULTS & DISCUSSION

All isolates have their own morphological characters. A total out of 450 samples, 350 were found out to be positive out of which 140 isolates were of *E.coli*, 120 *S.aureus*, 90 *Pseudomonas* and rest present were *Salmonella*, *Klebsiellae.t.c*. *E.coli* appears as in metallic green shine coloured colony on eosin methylene blue agar and gives pink coloured colony on MacConkey agar. *Staphylococcus* appears as yellow coloured colony on Mannitol Salt Agar medium. Similarly, *Salmonella* gives yellow with black centre colony on Xylose Lysine deoxycholate Agar and black with metallic sheen coloured colony on Bovine Serum Albumin media. *Pseudomonas* gives colourless colony on King's medium and green coloured colony on Pseudomonas Isolation Agar as shown in Table.1. as well as Fig 2-9 shows identification on the basis of colour morphology on their selective media. The isolation rate of diarrhoea was 350/450 (77.7%) with three main species being isolated: *Escherichia coli* (140/450; 33%), *Staphylococcus* (120/450; 26.6%), and *Pseudomonas* (90/450; 20%) as shown in Table.2. Growth of isolated bacteria on nutrient agar by serial dilution technique is shown in Fig.1. Identification of bacterial species is done on selective media as according to their colour morphology as shown in Fig 2-9. The colony & gram staining characteristics of various microbial colonies isolated from the media plates. The media plates being EMB, XLD & MacConkey. It was found that most of the organisms were Gram negative bacilli. This data is supported by ¹³. Occurrence of different types of microbes in stool sample and microorganisms found in the stool sample are *E Coli*, *Pseudomonas* and *Staphylococcus*. *E Coli* was found in larger proportion (66.66%) as supported by (Bardhan PK *et al.*, 1998)⁷. The total viable bacterial count as well as total coli form count is shown in Table.1.

Table 1: Colony characteristics of Isolated Bacteria on Selective media

S.No.	Media	Bacteria	Colony colour
1	EMB	E.coli	Metallic green
2	MacConkey	E.coli	Pink Colour
3	MSA	Staph	Yellow Colour
4	KIA	Klebsiella	Creamy Colour
5	MacConkey	Klebsiella	Mucoid pink Colour
6	XLD	Salmonella	Yellow with black centre
7	BSA	Salmonella	Black with Metallic sheen
8	PIA	Pseudomonas	Green Colour
9	King's medium	Pseudomonas	Colourless

Table 2: Prevalence of Isolated Bacteria from Clinical Samples

Isolated Bacteria	Number isolates	Percentages (%) of isolated bacteria
<i>Escherichia coli</i>	140	33.33%
<i>Salmonella sp.</i>	120	26.66%
<i>Pseudomonas Sp</i>	90	20%

Growth of Bacteria on Nutrient Agar by Serial Dilutions

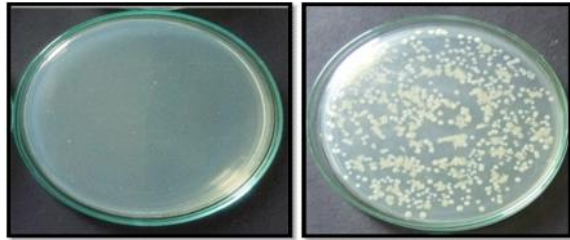


Fig 1: CONTROL

Fig 1.1: Direct 10⁻¹

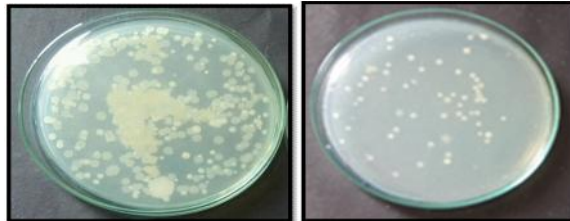


Fig 1.2: 10⁻²

Fig 1.3: 10⁻³

Identification of Bacterial species on basis of Colour Morphology

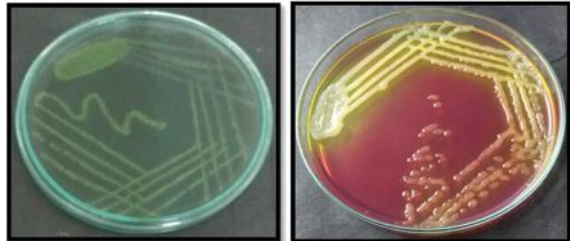


Fig 2: *E.coli* on EMB

Fig 3: *Staphylococcus* on MSA.

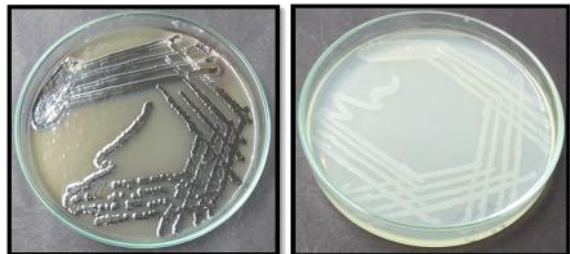


Fig 4: *Salmonella* on BSA.

Fig 5: *Pseudomonas* on King's medium

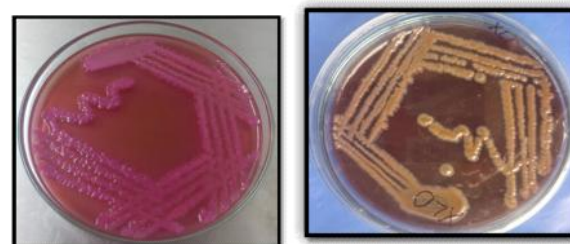


Fig 6: *Klebsiella* on Mac Conkey Fig 7: *Salmonella* on XLD.



Fig 8: *Klebsiella* on KIA.

Fig 9: *Pseudomonas* on PIA.

Table 3: Total Colony Forming Unit count of Isolated Bacteria

Organisms	Total count (CFU/ml)
<i>E.coli</i>	87 × 10 ²
<i>Salmonella</i>	4 × 10 ²
<i>Pseudomonas</i>	6 × 10 ²

4. CONCLUSION

As the present study showed, three bacterial species *E.coli*, *Pseudomonas* and *Staphylococcus* were main isolates from diarrhoeal children's samples collected from various regions of Dehradun and Paonta Sahib. Although there are geographical differences in the spectrum of bacteria incriminated in childhood diarrhea *E. coli*, *Proteus sp.* and *Salmonella* were isolated at a relatively high rate with *E. coli* being the most predominant¹. From this study it is concluded that isolated bacteria's are main causative agent for diarrhoea and can be pathogenic as well as harmful for the individual¹⁵. The results concluded that there are no. of different pathogenic bacteria are present in the diarrhoeal samples. In this research, it was revealed that, out of 450 samples, 140 *E. coli*, 120 *S. aureus*, 90 *Pseudomonas* & others are also present. The total viable count of bacteria was in range of 1.0×10⁻¹ to 6.0×10⁻⁴CFU/ml. The bacterial pathogens thus identified include *E.coli*, *Staphylococcus aureus*, *Pseudomonas* that have reported to occur most frequent in samples. It was observed from this study that *E. coli* is the predominant entero pathogens amongst other entero pathogens causing diarrhea in children less than 6 month. It is advised therefore, that appropriate measures should be taken by parents and caregivers to prevent the contamination of food and water by these entero pathogens to avoid occurrence of diarrhea. As out of 450 patients of diarrhoea, 350 were found to be positive, thus the diagnosis of diarrhoea was established in 77.7% of the cases examined. In present investigation, it was concluded that among confirmed cases of diarrhoea, children were more prone to diarrhoea than adults. The possible outcomes of diarrheal case among children may be due to poor sanitation and contaminated water. The maximum number of diarrheal patients was found from rural areas of Dehradun and Paonta Sahib. The maximum number of children belongs to nearby slum areas of Dehradun. It is concluded that the bacterial load of some specific bacteria in diarrhoeal samples is more

than compared to the others. This work done further concludes that these bacteria are causative organism for the diarrhoeal outbreak which might be due to poor hygienic conditions.

5. REFERENCES

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