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RESEARCH ARTICLE
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COMPARATIVE STUDY OF DETECTION OF DIARRHEAGENIC ESCHERICHIA COLI (EHEC) IN CHILDREN (LESS THAN 10 YEARS) BY STOOL CULTURE AND ANTIGEN DETECTION BY RAPID CARD AT A TERTIARY CARE HOSPITAL IN KANPUR

Mansi Srivastav[1], Deepak Sameer[2], R. Sujatha[*], Suneet Yadav[3]

PG student[1], Department of Microbiology Rama Medical College and Hospital & Research Centre Kanpur.
Tutor[2], Department of Microbiology Rama Medical College and Hospital & Research Centre Kanpur.
Professor and Head[*], Department of Microbiology Rama Medical College Hospital & Research Centre Kanpur.
Associate Professor[3], Department of Microbiology Rama Medical College Hospital & Research Centre Kanpur.

***Corresponding author:-** Dr R Sujatha
Mail. Id drsujatha152@gmail.com Mob no. 7892792526)

ABSTRACT

Background: Escherichia coli is a major public health concern that causes diarrhoea. Diarrheagenic Escherichia coli (DEC), is one of the most common species causing gastrointestinal illnesses worldwide, and causes hemorrhagic colitis, diarrhoea, and haemolytic-uremic syndrome (HUS)in children.

Objective: A comparative study of detection of diarrheagenic Escherichia coli (EHEC) in children (less than 10 years) by stool culture and antigen detection by rapid card was done at a tertiary care hospital in Kanpur.

Methods: The present study is a cross sectional observational study conducted in Department of Microbiology and Paediatrics Rama Medical College Hospital and Research Centre between April 2023 to March 2024. 45 stool samples were collected in a sterile container. The sample was cultured on Sorbitol MacConkey agar (SMAC) and standard guidelines were followed for identification and AST was performed according to CLSI guidelines. The rapid card antigen detection was also done for the detection of diarrhogenic EHEC.

Results: Out of 45 stool samples studied 24 were male and 21 were female children. In the age group 1-2 years two were positive by rapid card E.coli O157:H7 and one were positive by SMAC. The sensitivity and specificity of Rapid card is 100% and 97.72% EHEC E.coli was 100% sensitive to only Meropenem, Ciprofloxacin, Imipenem, Gentamicin and resistant was 100% to Tobramycin, Amikacin, Amplicillin sulbactam.

Conclusion: In our study Rapid card E.coli O157:H7 is more effective method of diagnosing EHEC when compared to SMAC with higher sensitivity and specificity value. Hence Rapid card is rapid and relevant method for detection of EHEC E.coli.

Keywords: EHEC, Sorbitol MacConkey agar, Rapid card O157:H7.

INTRODUCTION

In developing nations, acute diarrhea is a common cause of mortality and ranks second globally in infant mortality [1]. According to estimates, children under the age of five experience 1.3 billion episodes of diarrhea, which results in almost 760,000 fatalities annually [2]. E coli is a gram negative bacteria that are known to cause infections in the gastrointestinal tract are called enteric pathogens. Another name for stomach illness is diarrhoea that is excessively fluid and painful in the stomach. Symptoms of gastroenteritis can be caused by viruses, bacteria or parasites in which E.coli being the most common cause'shere are at least six well-characterized classes or pathotypes of E. coli that can cause intestinal infections in humans: Enteropathogenic E. coli (EPEC), Enterohemmorhagic E.coli (EHEC), Enterotoxigenic E. coli (ETEC), Enteroinvasive E.coli (EIEC), Entero-aggregative E. coli (EAEC), and diffusely adherent E. coli (DAEC) [3,4]. Hemorrhagic colitis, diarrhea, and Hhemolytic-uremic syndrome (HUS) are all caused on by Escherichia coli O157:H7 [5,6]. There are different techniques to identify the EHEC that are SMAC, rapid card and PCR but PCR is expensive and every lab doesn't have it . The main aim of this study is to compare the detection of diarrheagenic Escherichia coli (EHEC) in children (less than 10 years) by stool culture and antigen detection by rapid card, to find out an simple , reliable, cost effective and a highly sensitive test.

MATERIAL AND METHODS

An observational cross- sectional study was carried out between April 2023 to March 2024 at Department of Microbiology and Pediatrics Rama Medical College Hospital and Research Centre in Kanpur, Uttar Pradesh India. A total of 45 stool samples from diarrhoea patients (aged below 10 years showing sign of diarrhoea, abdominal pain, fever, vomiting, was included in the study). Childrens already on antibiotics and other cause of diarrhea were excluded from the study. Institutianal ethical clearance was taken for the study.

Sample collection: After obtaining informed consent from the guardians of the children suffering from diarrhoea, stool samples were collected in a sterile container, and stored in the refrigerator.

Testing methods: Detection of EHEC in stool samples are done by using two methods, culture on SMAC and Rapid card test.

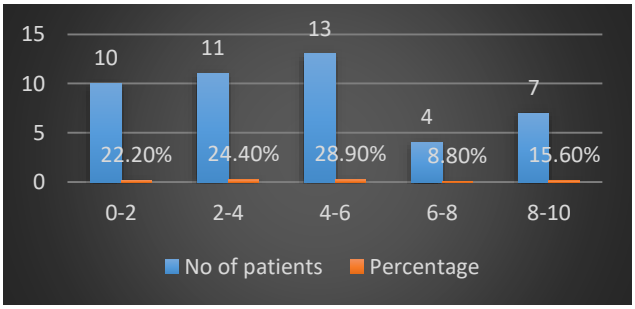
Procedure of SMAC: MacConkey Sorbitol Agar is based on the formulation described by Rappaport and Henigh . This medium is recommended for isolation of enteropathogenic Escherichia coli O157: H7, which ferments lactose but does not ferment sorbitol, hence produces colourless colonies. This organism has been recognized as a cause of hemorrhagic colitis . E.coli O157: H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT) . On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting E.coli. In MacConkey Sorbitol Agar Base, lactose is replaced by sorbitol. Unlike most E.coli strains, E.coli O157:H7 ferments sorbitol slowly or not at all . The growth of E.coli O157:H7 on MacConkey Agar with Sorbitol shows colourless colonies and most of the fecal flora ferment sorbitol and appear pink. MacConkey Agar with Sorbitol therefore permits ready recognition of E.coli O157:H7. Peptone and proteose peptone supply necessary nutrients like nitrogenous and carbonaceous compounds, long chain amino acids, minerals, vitamins and trace ingredients for the growth of organisms. Crystal violet and bile salt mixture present in the medium inhibit growth of gram-positive bacteria. Sodium chloride maintains osmotic equilibrium. Neutral red is an indicator. D-Sorbitol is the fermentable carbohydrate.

Test principle of rapid card: CerTest E coli 0157:H7 is based on the principle of a qualitative immunochromatographic assay for the determination of Escherichia coli 0157:H7 in stool samples and E. coli O157:H7 suspected colonies in stool culture. The strip consists of a nitrocellulose membrane pre-coated with antibodies on the test line (T), in the results window, against Escherichia coli 0157:H7 and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (antibodies anti-Escherichia coli O157:H7) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming coloured conjugate complexes.

Test method: Allow tests, samples and controls to reach room temperature (15-30°C) prior to testing. Do not open pouches until the performance of the assay.
1. Proceed to shake the collection tube in order to assure good sample dispersion.
2. Remove the CerTest E. coli O157:H7 card test from its sealed bag just before using it.
3. Take the collection tube, cut the end of the cap and dispense 4 drops in the circular window marked with the letter S.
4. Read the results at 10 minutes. Do not read the test result later than 10 minutes.
If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn't work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

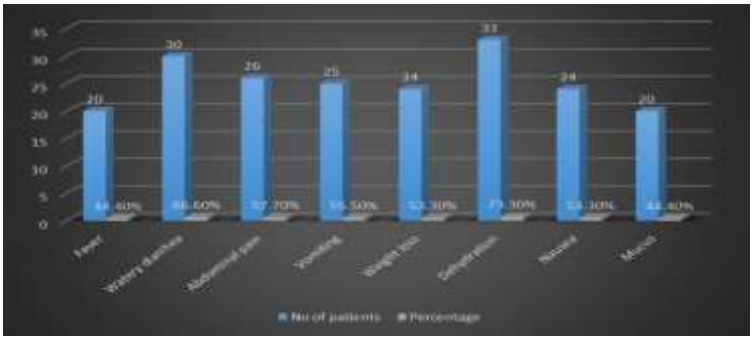
Interpretation of Results
Negative: Only the GREEN line appears across the results window in the site marked with the letter C (control line).
Positive: In addition to the GREEN control line, a RED line also appears in the site marked with the letter T (test line) in the results window.
Invalid: Total absence of the control coloured line (GREEN) regardless the appearance or not of the test line (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persist, discontinue using the test kit and contact your local distributor.
Statistical analysis: Data recorded on the case report from and structured proforma were subsequently entered into a spreadsheet. Data management and analysis were performed using Microsoft Excel.

RESULTS
A total of 45 stool samples were collected from children with diarrhoea in which EHEC suspected cases were studied, between the age group of under 0-10 years as shown in graph no 1 where the maximum number of suspected cases in 4-6 years of age group .



Graph no.1 Age wise distribution of diarrhogenic cases.

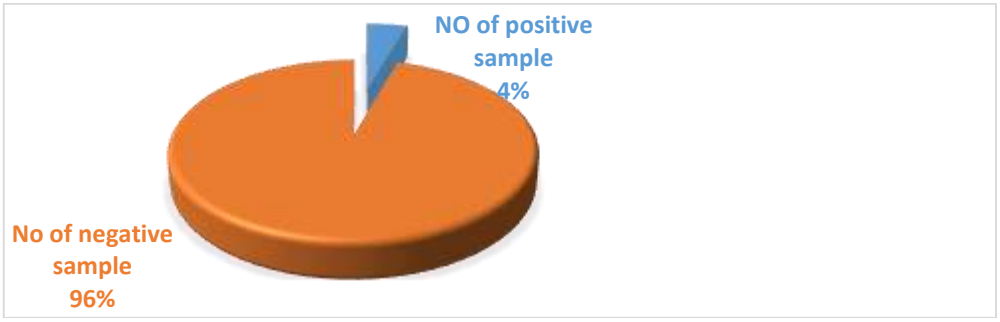
Out of total, maximum suspected cases were male (53.33%) and female (46.66%) . The distribution of suspected cases were done according to sign & symptoms, in which dehydration is most common followed by watery diarrhoea as shown in graph [2].



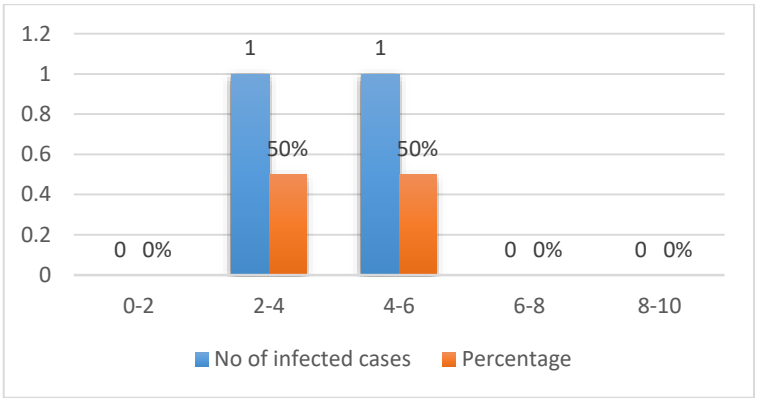
Graph no. 2: Distribution of suspected cases according to signs and symptoms.

Among all the suspected cases 69% cases were from rural area and 31% cases were from urban area. Out of which maximum patients were from IPD ward; 51% and 49%; were from OPD.

Incidence of EHEC among the infected cases is 4% as shown in graph [3].



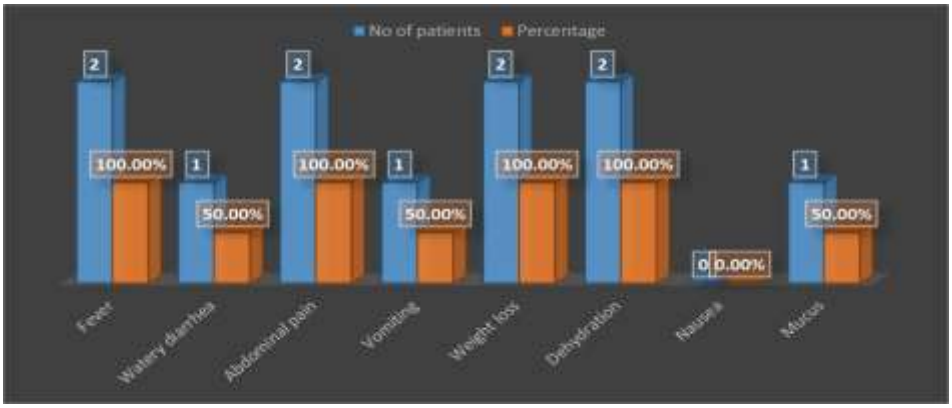
Graph No.3: Incidence of EHEC infected cases.



Graph No. 4: Age wise distribution of EHEC infected cases.

In EHEC infected cases females are 100% infectedand the demographic profile of the EHEC infected were 50% cases were from the rural area and 50% cases were from the urban area.

Among all the infected cases dehydration and abdominal pain are the most common symptoms followed by watery diarrhea as shown in graph [5].



Graph No. 5: Distribution of EHEC infected patients according to Signs and symptoms.

In our study PTZ, Gen, MRP, IPM, FOS, PB, CL, are 100% sensitive and AMP, A/S, LE, TOB, AK are 100% resistant to EHEC.
In our study out of 45 samples, 2 were positive by ICT and 1 were found to be positive by SMAC as shown in Table 1.

ICT (Gold standard test)	SMAC		TOTAL
	Positive	Negative	
Positive	1	1	2
Negative	0	43	43
Total	1	44	45

Table no 1: Shows comparison of SMAC and ICT.

The sensitivity and specificity of ICT is 100% & 97.72% respectively. The PPV and NPV of ICT is 50 % & 100% respectively as shown in table no 2.

Sensitivity	Specificity	PPV	NPV	Incidence
100%	97.72%	50%	100%	4%

Table no. 2: Ssensitivity, specificity, PPV, NPV of ICT for the detection of EHEC.

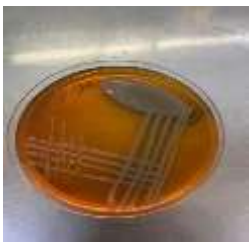


Fig 1: SMAC agar **Fig 2:** CERTEST E.coli 0157:H7 Negative **Fig 3:** CERTEST E.coli 0157:H7 Positive card

DISCUSSION

In present study paediatric age group patients stool samples was included for the study.
We observed that out of 45 samples 53.33% were male and 46.66% were female which is similar to the study conducted by Mrudul Lanjewar et al [7] (male 55.81% and female 44.19%).

In our study, most of the cases belonged to the age group is 2-4 &4-6 year which is similar to the study conducted by Madhu yadav et al.,[8]in this common age group was2-3 years and 4-5 years.In our study, the incidence of EHEC was observed to be 4.4% in diarrhoea patients. The finding was higher than the 2.6% obtained by Yilgwan and Okola[9] and lower than the43.1% obtained by Ifeanyi et al.[10]

Out of total 45 sample only 2 were infected by EHEC and these 2 were females which is similar to the study conducted by Madhu yadav et al.,[8]in this also only female were affected (100%).

In the present study there are equal no of children's are infected from EHEC from rural and urban area because children's of this age group have higher risk of diarrhoea due to the fact that they own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiment of adherence to aseptic or hygienic practices.

In our study most common symptoms of infected patients are abdominal pain followed by watery diarrhoea. In previous study common symptoms are abdominal pain followed by bloody diarrhoea. [11]

In our study we found that 2 were positive by IC-STAT and out of it only 1 is positive by SMAC. Direct IC-STAT testing of stools could be useful in E. coli O157:H7 outbreaks, in which the early and accurate identification of probable infected patients can accelerate outbreak investigations. If a patient presents late in the course of illness, when cultures are frequently negative and E. coli O157:H7 was not previously sought, the use of the IC-STAT test might increase somewhat the ability to determine the etiologic agent. [12]

The sensitivity, specificity, PPV and NPV of ICT vs. culture in our study is 100%, 97.72%, 50% and 100% which is similar to the study conducted by Thompson J et al in which the sensitivity, specificity, PPV and NPV is >99%, 85%, 70% and >99%.[13]

The IC-STAT test is highly specific, but we nevertheless urge that microbiologists attempt to isolate the infecting organism from the stool when the IC-STAT test is positive. In fact, such recovery efforts should be enhanced when the IC-STAT test is positive. Public health surveillance and control measures for this infection depend critically on routine molecular sub typing of the recovered organism. [14]

In our study we found 2 EHEC belonged to 0157:H7 serotype. In previous studies it is found that EHEC was less frequently isolated than other of diarrheagenic E coli. [15]

In our study the rate of antibiotic- resistant is high in isolates of EHEC was seen to Ampicillin, Cotrimozole, Tobramycin, are 100% which is similar to the Madhu Yadav et al [8] in this study Ampicillin and Cotrimoxazole are 100 % resistant.

CONCLUSION

In our study Rapid card E.coli O157:H7 is more effective method of diagnosing EHEC when compared to SMAC with higher sensitivity and specificity value. Hence Rapid card is rapid and relevant method for detection of EHEC E.coli.

LIMITATION

In our study, sample size was 45 were studied due to cost constraints.

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