

Original Research Article

EMERGING PATTERNS OF ENTEROPATHOGENIC E. COLI IN PAEDIATRIC ACUTE DIARRHEA: INSIGHTS FROM A TERTIARY CARE HOSPITAL

Gayathridevi Durairaj¹, Ramani Chellappan Parvathy², Mangala Adishesh³

¹Assistant Professor, Department of Microbiology, Stanley Medical College, Chennai, Tamil Nadu, India.

²Professor, Institute of Microbiology, Madras Medical College, Chennai, Tamil Nadu, India.

³Former Director, Department of microbiology, Madurai Medical College, Tamil Nadu, India.

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Corresponding Author:

Dr. Gayathridevi Durairaj,
Assistant Professor, Department of
Microbiology, Stanley Medical
College, Chennai, Tamil Nadu, India.
Email: gayathridevidurairaj@gmail.com

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ABSTRACT

Background: Diarrhea is a leading cause of mortality in infants and children, second only to acute respiratory infections. In developing countries like India, bacterial and parasitic infections predominate over viral causes. Among bacterial pathogens, Escherichia coli (E. coli), Shigella, Salmonella, and Vibrio cholerae contribute significantly to disease burden. Poor sanitation, unsafe water, and inadequate hygiene, especially in rural and urban slum areas, increase morbidity and mortality. Seasonal factors like monsoons and floods further exacerbate outbreaks. **Objective:** This study investigates the epidemiological trends, clinical manifestations, and antibiotic resistance profiles of bacterial strains isolated from pediatric patients with acute diarrhea at a tertiary care hospital.

Materials and Methods: A total of 150 children under five years of age with acute diarrhea (<1 week duration) were enrolled. Stool samples underwent microbiological analysis for pathogen identification, serotyping, and antibiotic susceptibility testing. Genotyping for the bundle-forming pilus was performed to confirm Enteropathogenic E. coli (EPEC).

Results: Among the 150 samples, E. coli was identified in 40% of cases. Serotyping revealed EPEC as the predominant strain, with a few Enteroaggregative and Enterohemorrhagic E. coli species. Antibiotic susceptibility testing showed high resistance to cotrimoxazole and ampicillin due to empirical antibiotic use. **Conclusion:** Despite oral rehydration therapy (ORT), diarrhea remains a major health concern, contributing to malnutrition and poor growth. The high prevalence of antibiotic-resistant E. coli highlights the need for rational antibiotic use and better preventive strategies.

Keywords: Enteropathogenic Escherichia coli, paediatric diarrhoea, antibiotic resistance, tertiary care hospital, epidemiology.

INTRODUCTION

Diarrhoea is defined as the passage of loose or watery stools more than three times per day within 24 hours duration often accompanied by fever or vomiting. It is a significant health issue worldwide, particularly affecting children under 5 years old, especially in developing countries like India.^[1] Acute diarrhoea is typically defined as lasting less than 14 days. The World Health Organization (WHO) reports that a substantial proportion of diarrheal deaths in this age group occur in countries such as India, Nigeria, Pakistan, and Ethiopia.^[2]

Diarrhoea has long term morbidity among children like malabsorption, poor growth and vit A deficiency. Poor hygienic practices, malnutrition, and lower socio-economic status exacerbate the impact of diarrhoea in developing nations. Bacterial pathogens such as Escherichia coli, Shigella, Salmonella, and Vibrio cholerae are common causes of bacterial diarrhoea. Among various types of E. coli, Enteropathogenic Escherichia coli (EPEC) is predominant among children under 5 years old.^[3] Epidemiologically, diarrhoea outbreaks are more frequent during winter and rainy seasons, facilitating rapid community spread and occasional epidemics.

Key risk factors include inadequate water supply, poor personal hygiene, and suboptimal household sanitation.^[4] Transmission occurs primarily via the faeco-oral route, through contaminated drinking water and food. The clinical consequences of diarrhoea can be severe, leading to dehydration and potentially death if not promptly treated.^[5] Dehydration is managed primarily through rehydration therapy, which has been instrumental in reducing mortality rates associated with diarrheal diseases.^[6] Most cases of diarrhoea are self-limiting, and mild cases can be managed conservatively at home with oral rehydration solutions. Antibiotics are sometimes used in bacterial diarrhoea to shorten the duration and severity of symptoms, although their routine use is debated due to concerns about antimicrobial resistance and the self-limiting nature of many cases.^[7] Despite the preventable and treatable nature of diarrheal diseases, effective management remains a challenge in many resource-limited settings. Integrated efforts involving healthcare systems and community-level interventions are crucial for reducing the burden of diarrheal illnesses globally.^[8] This manuscript aims to address the current gap in knowledge regarding the epidemiology, clinical features, and antibiotic resistance patterns of bacterial isolates in paediatric patients with acute diarrhoea, based on a detailed analysis conducted at our tertiary care hospital.

MATERIALS AND METHODS

The primary objective of this study was to determine the prevalence of acute diarrheal diseases among children under 5 years of age and to investigate the antibiotic susceptibility patterns, antibiotic resistance profiles, genotype, and phenotype of Enteropathogenic *Escherichia coli* (EPEC) strains causing acute diarrhea at a tertiary care hospital. The study period spanned from April 2016 to March 2017.

Study Design and Participants

This study enrolled children aged 1 month to 5 years who were admitted to the Institute of Child Health, Madras Medical College, Egmore, Chennai, with acute diarrheal diseases. Stool samples were collected from eligible participants, while children with known chronic gastrointestinal disorders and those older than 5 years were excluded. Informed written consent was obtained from the mothers in both regional language and English. Ethical approval was obtained from the Institutional Ethical Committee to ensure compliance with ethical standards. All data were handled confidentially and anonymously.

Microbiology Methodology

Stool samples were collected by the child's mother using sterile screw-capped containers and transported to the laboratory. In the laboratory, samples were enriched in selective broths such as selenite F, alkaline peptone water, and tetra thionate

broth, and incubated for 6-12 hours. Subsequently, cultures were plated onto differential media like MacConkey agar and incubated for 16-18 hours. Isolated colonies were subjected to a battery of biochemical tests including catalase, oxidase, indole, triple sugar iron (TSI), citrate utilization, urease, methyl red, and Voges-Proskauer tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. *E. coli* isolates were confirmed by subculturing on sorbitol MacConkey agar to detect Enterohemorrhagic *E. coli* species. Polymerase chain reaction (PCR) tests were conducted to detect the presence of bundle-forming pilus (*bfpA*) genes in *E. coli* isolates. Serotyping of *E. coli* isolates was conducted using commercial antisera (Antisera 1: O1, O26, O86a, O111, O128, O127a; Antisera 2: O44, O55, O125, O126, O146, O166). A subset of isolates was sent to the Central Laboratory Institute, Kausali, for confirmation of serotyping results.

Statistical Analysis

Statistical analysis was performed using SPSS Software version 17. Differences between groups were assessed using the Chi-Square method to determine significance.

RESULTS

This study was conducted in 150 children aged under 5 years admitted with acute diarrhea at the Institute of Child Health, Egmore. The isolation rate of the colonies was only 41% due to the administration of antibiotics before admission at the hospital. Male children (51%) were more affected than female children (49%) and Acute Diarrheal Diseases was more seen in children in the age group between 7 months to 3years. Acute Diarrheal Disease was more among children living in urbanized metropolitan cities using water tank as the water source and regarding seasonal variation bacterial diarrhea occurs throughout the year, more prevalence in summer season. On analyzing breastfed children, diarrhea was more common during the weaning period than during on exclusively breastfed period. *Escherichia coli* infection has equal distribution in age groups of 0 - 6 months (18.03%) and between children aged above 3 to 5 years (14.75%). Among children aged between 7months to 1year, female children (38.24%) were more infected with *E. coli* compared to male children (33.33%) of the same age group. Among the children presented with acute watery diarrhea, 90% presented with diarrhea of less than 5days duration and with mild dehydration and only 5% percent of patients presented with severe dehydration. 25%percent among them presented with fever and 16% of patients with vomiting.10% percent of patients with blood and mucus instool.70% presented with mild dehydration, 28%

has no dehydration, one with moderate dehydration but none of the patient with severe dehydration. Antibiotic use prior to the admission has reduced the isolation rate of E. coli to 26%. There was high degree of resistance to Cotrimoxazole, Ampicilin and moderate degree of resistance to ciprofloxacin (52.5%), Gentamicin (45.9%). Amikacin (90.2%), Cefotaxime (68.9%) and Chloramphenicol (65.6%) were sensitive. 19 isolates were found to ESBL producers. Among the E. coli isolates 19 belong to polyvalent 1 and 20 belong to polyvalent 2 and 22 were negative to both. genotyping of few E. coli isolates done by Polymerase chain reaction showed Bundle Forming Pilus Gene (bfpA gene) was present, which confirmed Enteropathogenic E. coli was the causative agent of diarrhea in children.

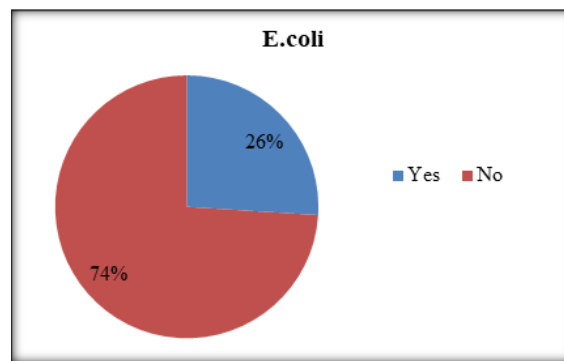


Figure (a and b) denotes that the study was conducted in 150 children aged under 5 years admitted with acute diarrhea. The isolation rate of the colonies was only 40% due to the administration of antibiotics

Table 1: Bacterial Pathogens isolated by stool culture in children with acute diarrheal disease

| S.No | Organism | No of Isolates (n=150) | Percentage (% of Growth) |
|------|-----------------|------------------------|--------------------------|
| 1 | E.Coli | 61 | 41 |
| 2 | Shigella sp | - | - |
| 3 | Salmonella Sp | - | - |
| 4 | Vibrio cholerae | - | - |

Table 2: Antibiotic administration prior to hospitalisation in children with diarrhea

| Antibiotic Use Prior To Admission | | ORGANISM | | | p VALUE |
|-----------------------------------|--|----------|-----------|---------|---------|
| | | E. coli | No Growth | Total | |
| YES | N | 16 | 64 | 80 | <0.001 |
| | % with antibiotic use prior to admission | 20.00% | 80.00% | 100.00% | |
| | % within organism | 26.20% | 71.90% | 53.30% | |
| NO | N | 45 | 25 | 70 | |
| | % within antibiotic use prior to admission | 64.30% | 35.70% | 100.00% | |
| | % within ORGANISM | 73.80% | 28.10% | 46.70% | |
| | N | 61 | 89 | 150 | |
| | % within antibiotic use prior to admission | 40.70% | 59.30% | 100.00% | |
| | % within organism | 100.00% | 100.00% | 100.00% | |

Table 3: Gender distribution among diarrhoea cases

| GENDER | NUMBERS | PERCENTAGE |
|--------|---------|------------|
| MALE | 76 | 51% |
| FEMALE | 74 | 49% |
| TOTAL | 150 | 100% |

Table 4: Age distribution among diarrhoea cases

| AGE | Male | Female | Total | p VALUE |
|---------------|--------|--------|--------|---------|
| 0 - 6 Months | 7 | 14 | 21 | 0.695 |
| | 9.21% | 18.92% | 14.00% | |
| 7 - 12 Months | 28 | 22 | 50 | |
| | 36.84% | 29.73% | 33.33% | |
| 1 - 3 Years | 24 | 26 | 50 | |
| | 31.58% | 35.14% | 33.33% | |
| 3 - 5 Years | 17 | 12 | 29 | |
| | 22.37% | 16.22% | 19.33% | |

Table 5: Association of breast feeding and diarrhoea in children

| BREAST FED | NO. OF CHILDREN |
|-------------------------|-----------------|
| EXCLUSIVELY BREAST FED | 22 |
| ALONG WITH WEANING FOOD | 58 |

Table 6: Clinical features of e.coli diarrhea

| ORGANISM | | Culture positive for E.coli | | Culture negative for E.coli | | p Value | TOTAL | |
|-----------------------|----------|-----------------------------|--------|-----------------------------|--------|---------|-------|--------|
| | | n | n% | n | n% | | n | n% |
| FEVER | YES | 15 | 24.60% | 19 | 21.30% | 0.63 | 34 | 22.70% |
| | NO | 46 | 75.40% | 70 | 78.70% | | 116 | 77.30% |
| VOMIT | YES | 10 | 16.40% | 5 | 5.60% | 0.03 | 15 | 10.00% |
| | NO | 51 | 83.60% | 84 | 94.40% | | 135 | 90.00% |
| DURATION OF DIARRHOEA | 1 | 5 | 8.20% | 13 | 15.10% | 0.670 | 18 | 12.20% |
| | 2 | 24 | 39.30% | 35 | 40.70% | | 59 | 40.10% |
| | 3 | 16 | 26.20% | 19 | 22.10% | | 35 | 23.80% |
| | 4 | 9 | 14.80% | 9 | 10.50% | | 18 | 12.20% |
| | > 5 | 7 | 11% | 10 | 12% | | 17 | 12% |
| BLOOD | YES | 6 | 9.80% | 1 | 1.10% | 0.01 | 7 | 4.70% |
| | NO | 55 | 90.20% | 88 | 98.90% | | 143 | 95.30% |
| MUCUS | YES | 6 | 9.80% | 1 | 1.10% | 0.01 | 7 | 4.70% |
| | NO | 55 | 90.20% | 88 | 98.90% | | 143 | 95.30% |
| DEHYDRATION | MILD | 42 | 68.90% | 16 | 18.00% | <0.001 | 58 | 38.70% |
| | MODERATE | 1 | 1.60% | 0 | 0.00% | | 1 | 0.70% |
| | NO | 18 | 29.50% | 73 | 82.00% | | 91 | 60.70% |

Table 7: Antibiotic susceptibility pattern of e.coli isolates (n=61)

| ANTIBIOTICS | | E.coli Isolates (n=61) | |
|-----------------|---|------------------------|--------|
| | | n | n% |
| AMPICILIN | R | 36 | 59.00% |
| | S | 25 | 41.00% |
| AMIKACIN | R | 6 | 9.80% |
| | S | 55 | 90.20% |
| GENTAMICIN | R | 32 | 52.50% |
| | S | 28 | 45.90% |
| COTRIMOXAZOLE | R | 43 | 70.50% |
| | S | 18 | 29.50% |
| CIPROFLOXACIN | R | 29 | 47.50% |
| | S | 32 | 52.50% |
| TETRACYCLINE | R | 31 | 50.80% |
| | S | 30 | 49.20% |
| CEFOTAXIME | R | 19 | 31.10% |
| | S | 42 | 68.90% |
| CHLORAMPHENICOL | R | 21 | 34.40% |
| | S | 40 | 65.60% |

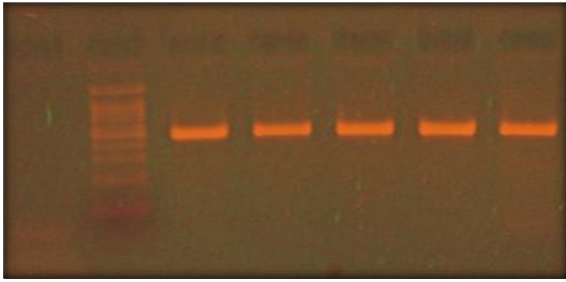
Table 8: Serotyping of e.coli isolates (n=61)

| SEROTYPES | E.coli Isolates (n=61) | |
|--------------|------------------------|------------|
| | Count | Column N % |
| POLYVALENT 1 | 19 | 31.10% |
| POLYVALENT 2 | 20 | 32.80% |
| NEGATIVE | 22 | 36.10% |

Table 9: Genotyping

| Lab ID | Serial no | Organism | Tested Serotypes |
|----------|-----------|----------|------------------|
| E-984/17 | 1 | E.coli | O8 |
| E-985/17 | 2 | E.coli | O157 |
| E-986/17 | 3 | E.coli | O126 |
| E-987/17 | 4 | E.coli | N.A |
| E-988/17 | 5 | E.coli | N.A |
| E-989/17 | 6 | E.coli | O149 |
| E-990/17 | 7 | E.coli | O114 |
| E-991/17 | 8 | E.coli | O11 |
| E-992/17 | 9 | E.coli | O8 |

Some of the isolates were sent and tested at Kausali lab which again confirms the serotyping of the isolates



GENOTYPING OF few E.coli isolates done by POLYMERASE CHAIN REACTION showed Bundle Forming Pilus Gene (bfpA gene) was present, which confirmed Enteropathogenic E.coli was the causative agent of diarrhoea in children

DISCUSSION

The findings underscore the significant burden of EPEC in pediatric acute diarrhea cases at our tertiary care hospital. The study identifies key epidemiological trends, clinical manifestations, and antibiotic resistance profiles of EPEC strains, emphasizing the evolving landscape of pediatric diarrheal diseases. Implications for clinical practice include the need for targeted diagnostic strategies and judicious antibiotic use to mitigate resistance emergence.

In the present study *Escherichia coli* was the predominant pathogen of diarrhea in children. There was no bacterial growth in 59% of stool samples. This could be probably due to etiological agents other than bacteria. The study was supported by Korie et al and colleagues by the study conducted at Enugu, Nigeria.^[9] In this study there was reduced isolation of bacteria in culture could be due to administration of antibiotics prior to collection of stool samples (P value < 0.001). This was supported by the study conducted in Enugu, Nigeria.^[9,10] In our study higher distribution of acute diarrhea in male children when compared to female children less than 5 years. More number of male children are brought for the hospital care due gender preponderance in the community of people. Similar findings were observed in the study conducted by Vyas kumar Rathur et.al.^[11] In the present study, there was an increase in incidence of diarrhea during and after weaning period. This is due to the disappearance of protective maternal antibodies and during development, children are exposed to the harmful environment of pathogens. Regarding seasonal variations, our study showed bacterial diarrhea occurs throughout the year with increased incidence in summer because *E. coli* diarrhea spreads in the community due to increased ground water depth, scarcity of water supply, promoting contamination of drinking water leading to the outbreak of water borne diseases including acute diarrheal diseases in summer season especially of *E.coli* which occurs naturally in human and animal feces contaminating the soil and so the ground water gets contaminated facilitated more during summer season.

In our study diarrhea is more common in urban metropolitan cities than in the rural areas due to reduce per capita income of the family, reduced standard of living and reduced living area along with poor personal hygienic practices of the family members and the environmental hygiene makes the urbanized slum children more prone to get infected and dehydrated by harmful pathogens, also middle & low economic families using water tank as a source of water for drinking, cooking and other purposes, their children has more incidence of diarrhea because of drinking unboiled water, improper chlorination, water tank not covered or left open. But in the study conducted by Vyaskumar Rathur et.al., and colleagues,^[11] which showed higher incidence of diarrhea among children living in rural areas. Among the patients who tested culture positive, 25% of children are presented with fever and 16% of patients vomiting and 10% with passage of blood and mucus or dysentery-like symptoms. In this study 24.60% of children presented with fever have positive blood culture and 54% culture was negative. This contrast Vyas kumar et.al., where 93% presented with fever, may be due to prior administration of antibiotics before admission at the hospital or the etiological agent may be other than bacterial causes. 16.40% of children with vomiting had positive stool culture and 5.60% culture was negative (P value 0.03), supported by study conducted Vyas kumar Rathur et.al,^[11] reported less no of stool culture positives in patients with vomiting. 86% of children with blood and mucus in stool has positive blood culture and 14% have negative stool culture. The negative stool culture in this 14% because of intestinal parasitic infections, small anal tears due to passage of hard stools, small passage of mucus may be a normal habit, some food reactions may cause enterocolitis example milk, soyabean and rarely intussusceptions, where 100% of patients with blood and mucus in stool has positive blood culture. In this study duration of diarrhea in culture positive patients 93% with diarrhea of less than 5 days and 2% with up to 10 days and 1% presented with persistent diarrhea of 20 days duration. In our study 29.5% of patients with culture positivity did not present with any dehydration and 68.9% with mild dehydration 1.06% and with moderate dehydration and none with severe dehydration. Children are treated with probiotics like bifilac and oral rehydration solutions and with antibiotics like septran and cefepime for 5-7 days are given only after culture reports and for mild and moderate dehydration and IV fluids are additionally needed for rehydration are needed only in severely dehydrated children. In the present study, antimicrobial susceptibility pattern of the isolates showed there was increasing degree of resistance to commonly used drugs. 90.2% of isolates were sensitive to Amikacin and 68.90% are sensitive to Cefotaxime and 31% of Extended Spectrum beta lactamases producers. ESBL producers were sensitive to cefotaxime plus

clavulanic acid. There was a higher degree of resistance to Cotrimoxazole 70.5% and Ampicillin 59%. Cotrimoxazole was the commonly used drug in children for diarrhea in peripheral hospitals. There was increasing resistance to this drug due to the empirical, widespread and irrational use of this antibiotic. There was moderate degree of resistance to Gentamicin 52.50%, Tetracycline 50.80%, and Ciprofloxacin 47.50%. Similar findings were observed in the study conducted by Willie Kipkemboi Sang et.al.^[12] The study conducted by Amir Saeed et.al.^[13] showed isolates were highly sensitive to Chloramphenicol, Gentamicin, tetracycline, and ciprofloxacin. In our study serotyping of *E. coli* isolates, 19 isolates were positive to polyvalent 1 (O1, O26, O86a, O111, O119, O127a, O128) and 20 isolates were positive to polyvalent 2 (O44, O55, O125, O126, O146, O166). 22 isolates tested were negative to both polyvalent 1 and polyvalent 2 antisera confirming Enteropathogenic *E. coli* is the major cause of pediatric diarrhea followed by enterohaemorrhagic and enteroaggregative isolates. This favors study conducted by,^[14] Mustafa Mohamed Osman et al on study of bacterial etiology of acute diarrheal disease in Ombada hospital, Sudan. In the present study, the genotyping of a few *E. coli* isolates tested by polymerase reaction showed Bundle Forming Pilus gene (bfpA) positive. Similar findings were reported in the study conducted by Gunzburg et al,^[15] on identification of Enter pathogenic *E. coli* by PCR based detection of Bundle Forming Pilus Gene and by the study of Orn-Anong Ratchrachenchai et al,^[16] on prevalence of childhood diarrhea associated *E. coli*.

CONCLUSION

Acute diarrheal diseases remain a leading cause of morbidity and mortality among children under five years of age in developing countries. This study was conducted at the Institute of Microbiology, Madras Medical College (MMC), Chennai, and the Institute of Child Health, Egmore, among 150 children aged 1 month to 5 years admitted with acute diarrheal disease.

The prevalence of diarrhea was higher in male children compared to females, with an increased occurrence in the weaning period (6–12 months). This is likely due to inadequate parental attention to feeding practices and increased child mobility, leading to higher exposure to contaminants. Children from urban slum areas had a higher prevalence than those from rural areas due to poor hygiene, lower per capita income, and reduced living standards. Socioeconomic status also played a role, with children from low-income families being more susceptible. Exclusively breastfed children had a lower prevalence due to the protective immunity provided by breast milk immunoglobulins.

Bacterial diarrhea was endemic throughout the year, with a peak in summer, likely due to water scarcity leading to food and water contamination. *Escherichia coli* was the predominant pathogen, with fever present in one-fourth of cases and vomiting in 6% of children with *E. coli* infection. Most cases involved diarrhea lasting less than five days with mild dehydration, while moderate dehydration was observed in 1% of patients.

Antimicrobial susceptibility testing showed the highest sensitivity to amikacin, cefotaxime, and chloramphenicol, with moderate sensitivity to ciprofloxacin, tetracycline, and gentamicin. However, there was emerging resistance to cotrimoxazole and ampicillin due to empirical and irrational antibiotic use.

Serotyping confirmed Enteropathogenic *E. coli* (EPEC) as the leading cause of pediatric diarrhea, followed by Enterohemorrhagic *E. coli* (EHEC) and Enteroaggregative *E. coli* (EAEC). Genotyping using polymerase chain reaction (PCR) further confirmed EPEC as a major causative agent, with the bfpA gene detected in some isolates.

This study highlights the urgent need for improved hygiene, better feeding practices, and rational antibiotic use to reduce the burden of diarrheal diseases in young children.

Declarations:

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Authors' Contribution: All author equally contributed.

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Ethics Statement: Ethical approval was obtained from the Institutional Ethical Committee to ensure compliance with ethical standards. Ethics approval number: 19032016 approved by IEC – Madras Medical College, Chennai

Informed Consent: The informed consent was obtained for the study.

Data Availability: Not Applicable

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