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Original Article

Paediatrics Section

Recurrent Paediatric Abdominal Pain Associated with ESBL-producing *Escherichia coli* Attributable to Unscreened use of Empirical Antibiotics: A Cross-sectional Study

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ABSTRACT

Introduction: Recurrent Abdominal Pain (RAP) has a devastating impact on children's activities and mental health. In this context, physicians often empirically start antibiotics with each visit. As a result, the normal commensal *Escherichia coli (E. coli)*, which protects the gut through immunophysiological functions, decreases in number, becomes pathogenic and develops resistance to antibiotics. This imbalance between the host and the gut microbiome presents as abdominal discomfort.

Aim: To investigate the response of normal gut flora *E. coli* to commonly used empirical antibiotics and its association with persistent cases of RAP in children.

Materials and Methods: A cross-sectional study was conducted on 64 children under 10 years of age with RAP over a period from March 2021 to February 2022 at the Gayatri Vidya Parisad (GVP) Medical College and Hospital, Visakhapatnam, Andhra Pradesh, India. Stool culture and sensitivity, as well as the empirical use of antibiotics, were recorded. The bacteriological profile and their sensitivity were analysed. The major bacteria, *E. coli*, that were resistant to third-generation cephalosporins were further processed phenotypically using the disc diffusion method to detect their Extended Spectrum Beta-Lactamase (ESBL) production. Data were analysed using Statistical Package for Social Sciences (SPSS) software version 20.0.

Results: Out of 64 children with RAP, 36 (56.3%) cases were persistent in nature, and 40 (62.5%) were under five years of age, with a predominance of girls 36 (56.3%). Among these 36 (56.3%) RAP cases, 16 (25%) were found mostly around the epigastrium with a periumbilical area. The major bacteria isolated were *E coli* 27 (87.1%) found in 31 (73.8%) positive stool culture from persistent RAP cases on empirical antibiotics with 22 (81.5%) being resistant to third-generation cephalosporins (Cefotaxime 17 (62.9%), Ceftazidime 12 (44.5%), Ceftriaxone 8 (29.6%)). Out of 22 (81.5%) third-generation cephalosporin-resistant *E. coli*, 15 (68.2%) were ESBL producers.

Conclusion: ESBL-producing *E. coli* were one of the common causes of persistent cases of RAP, attributed to the empirical overuse of third-generation cephalosporins by health professionals. There is a need for the rational use of antibiotics.

Keywords: Antimicrobial resistant, Cephalosporin, Gut microbiome, Pathogens

INTRODUCTION

The RAP is a term used to describe at least three episodes of pain that last for at least three months, affecting 10-20% of school-going children who require symptomatic treatment in most non organic cases. It is challenging to treat in children and has a devastating impact on their school performance, daily activities and mental health [1]. Over the years, despite the development of new advanced technologies to understand the pathophysiology of RAP and the identification of more organic causes, the most common cause of RAP remains functional in origin.

Several difficulties interfere with management, such as parental anxiety, limited information from parents of young children needed for diagnosis, and the difficulty of conducting abdominal examinations in children with abdominal pain. Additionally, in young children, the localisation of abdominal pain is often vague, and its severity and frequency bear no relation to aetiology. There are several red flag signs that indicate organic aetiology. Furthermore, when history and clinical examination are unable to define the aetiology, and when screening lab tests are unavailable in rural areas, it becomes an obstacle for managing these children.

In this context, physicians often start antibiotics early, empirically and without screening, primarily using third-generation cephalosporins and fluoroquinolones in all cases of abdominal pain, which are not specific to organic illnesses [2]. At the same time, because of non compliance with medication by worried parents, the normal majority of commensal *E. coli*, which protect the gut through immunological functions, decreases in number, becomes pathogenic and develops resistance to antibiotics [3].

Antibiotics alter the gut microbiome (*E. coli, Klebsiella* spp.) and may subsequently affect the corresponding translocation process, resulting in an imbalance between intestinal microbiota and the host. As reported by Jesús RB et al., ESBL-producing *E. coli* is a notable cause of community-acquired infections [4]. This imbalanced state can present various signs and symptoms in children. Although abdominal pain is recurrent, some cases are relieved gradually, while others persist for an extended duration upon follow-up. To date, few studies have been conducted on the role of ESBL-producing *E. coli* related to community-acquired infections [4-6], but not specifically in persistent cases of RAP in children.

The aim was to investigate the association and role of *E. coli* in the frequent empirical use of antimicrobials in persistent cases of RAP in children, as well as the relationship between recurrence and the site of pain with the involvement of mesenteric lymph nodes.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of one year, from March 2021 to February 2022, in the Department of Paediatrics at GVPMC, Visakhapatnam, Andhra Pradesh, India. The study commenced after obtaining institutional ethical approval (IEC no: VPIHCMT/IEC/20221213/01).

Inclusion criteria: A total of 64 patients aged under 10 years who attended the Outpatient Department (OPD) and had at least three episodes of pain over a three-month period were included in the study, having been diagnosed with RAP cases (according to Apley's definition) [5]. Children with RAP of non organic origin, based on their history, physical examination and imaging studies, as well as those who frequently received antibiotics for abdominal pain without screening, were included in the study.

Exclusion criteria: RAP cases who were relieved of pain within a six-month period were excluded from the study.

Study Procedure

In all cases, the patients' demographic profiles (name, age, sex), clinical data (symptoms, rashes, icterus, pallor, weight loss, etc.), physical examinations, and systemic examinations were recorded. All relevant investigations, such as complete blood count, urine analysis and stool culture with sensitivity testing, were evaluated. An abdominal ultrasonography was performed to detect the site, size and number of mesenteric lymph nodes related to cases of RAP. The use and type of empirical antibiotics administered to the patients were noted. The bacteriological profiles from stool cultures were extracted, and third-generation cephalosporin-resistant *E. coli* were further processed phenotypically by the disc diffusion method to detect their ESBL production.

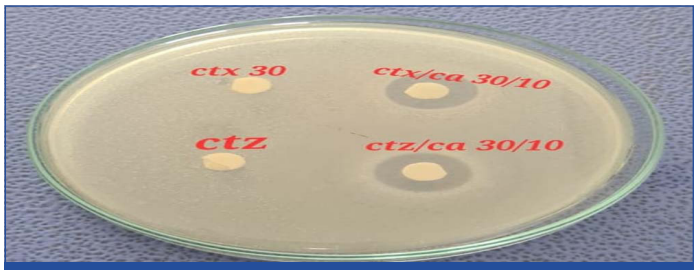
All patients were followed-up for a duration of six months. Cases with a prolonged period of pain (each episode lasting 18 to 24 hours) that were continuously not relieved (this type of pain is progressive in nature, present for weeks, months, or even years and steadily worsens over time) were classified as persistent cases of RAP [7-10]. Relieved cases were defined as those with no pain at all or with minimal pain that did not affect their daily activities, which could be attributed to changes in their diet, supportive care, or medication.

Sample Collection and Isolation of Microorganism

Stool samples were collected as the preferred method to investigate the bacterial enteropathogens present, using sterile stool containers provided to the children. The samples were held at room temperature for up to 24 hours after collection and processed within two hours for stool culture and sensitivity testing. Samples that could not be processed within two hours of collection were stored in Cary-Blair medium for later processing. The samples were diluted two- to four-fold to obtain distinct, non overlapping colonies on MacConkey agar plates. For culture, 0.1 ml of the liquid sample or a loopful of the specimen was placed using a flame sterilised loop over a small area of each plate, and streaking was performed over the entire plate from the inoculated area. All plates were incubated for 18 hours at 37ºC, and the number of colony-forming units per gram of wet weight of stool was counted. All bacterial colonies (e.g., large flat colonies of *E. coli* with a pink colour) were isolated based on their appearance and colour on the agar plates. The organisms were identified based on their biochemical reactions. The major bacteria found were gram-negative *E. coli*, determined by the number and size of the colonies.

Antibiotics susceptibility test: Antibiotic susceptibility tests were performed for all bacteria isolated in the culture, with the major bacterium, *E. coli*, being determined by the standard Kirby-Bauer disk diffusion technique, following the Clinical and Laboratory Standards Institute (CLSI) guidelines from 2020 [11]. The antibiotics used for this study were amikacin (20 mg), gentamicin (20 mg), amoxicillin-clavulanic acid (22 mg), cefepime (28 mg), cefotaxime (30 mg), ceftriaxone (30 mg), ceftazidime (30 mg), ciprofloxacin (28 mg), piperacillin-tazobactam (26 mg), meropenem (30 mg), and levofloxacin (22 mg). In each case, the zone of inhibition was measured, and the sensitivity of the isolates was classified as susceptible, intermediate, or resistant according to the interpretation guidelines provided by CLSI in 2020. Gram-negative bacilli resistant to third-generation cephalosporins were screened for ESBL production.

Test for ESBL: The characteristics of ESBL isolates were determined using a screening method with cefotaxime (30 µg) and ceftazidime (30 µg) discs. Isolates with zone diameters ≤27 mm for cefotaxime and ≤22 mm for ceftazidime were confirmed for ESBL production. Phenotypically, ESBL confirmation was performed using a disc diffusion test as recommended by CLSI 2020 [11]. This test utilised both cefotaxime (30 µg) and ceftazidime (30 µg) alone, as well as in combination with clavulanic acid. The zone of inhibition of each individual antibiotic was compared to the zone of inhibition in combination with clavulanic acid. An increased zone of inhibition was observed around cefotaxime and ceftazidime, and a >5 mm increase in zone diameter was considered positive for ESBL production [Table/Fig-1].



[Table/Fig-1]: Detection of ESBL production by disc diffusion test.

STATISTICAL ANALYSIS

All demographic and clinical data were presented as frequencies and percentages. These were entered and analysed using SPSS version 20.0.

RESULTS

During the study period, a total of 92 children attended the paediatric OPD for abdominal pain, and 64 (69.6%) children with RAP, who fulfilled the inclusion criteria, were enrolled in the study.

Out of the 64 enrolled cases, 28 (43.7%) were boys and 36 (56.3%) were girls with RAP. Most of the children with RAP, 40 (62.5%), were in the under-five years age group [Table/Fig-2].

Age (years)	Sex		Total {n=64 (69.6%)}
	Boys 28 (43.7%)	Girls 36 (56.3%)	
0-5	18 (64.3%)	22 (61.1%)	40 (62.5%)
5-10	10 (35.7%)	14 (38.9%)	24 (37.5%)

[Table/Fig-2]: Demographic data of children with RAP.

According to the site of recurrent pain, the majority of the persistent cases, 36 (56.3%), were around the periumbilical area, followed by the right iliac fossa and epigastric area. Among these, 16 (25%) cases were in the periumbilical and epigastric areas, where the pain was crampy and colicky in nature. All persistent cases lasted four to six months, with prolonged periods of pain (each episode lasting 18 to 24 hours). In these patients, enlarged mesenteric lymph nodes larger than 8 mm were found by ultrasonography, primarily in the periumbilical area, followed by the right iliac fossa. All 28 (43.7%) cases with relieved pain were acute cases, characterised by crampy or dull aching pain, with a duration of less than three months [Table/Fig-3].

Site of pain	Type of pain	Duration of each episode (hrs)	Frequency (week)	MLN size (mm)	Persistent/ Relieved	Number (%)	Total no (64) Persistent (P) Relieved (R)
Epigastric+Periumbilical+Hypogastrium	Dull aching	2	1	>8	Persistent	05 (7.8)	P=36 (56.3%) R=28 (43.7%)
					Relieved	06 (9.4)	
Periumbilical+Right iliac fossa	Crampy / Colicky	18	3	>8	Persistent	12 (18.8)	
					Relieved	08 (12.5)	
Periumbilical+Right iliac fossa+Left Iliac fossa	Sharp/ Dull	8	1	>8	Persistent	03 (4.7)	
					Relieved	4 (6.3)	
Periumbilical+Epigastrium	Crampy	24	3	>8	Persistent	16 (25)	
					Relieved	10 (15.6)	

[Table/Fig-3]: Profile of RAP in relation to local mesenteric lymphnode.

The pattern of microbial growth in stool culture positive RAP cases showed 42 (65.6%) microorganism of which 32 (76.2%) were *E. coli*, followed by 1 (2.4%) each *Enterococcus, Enterobacter* and *Klebsiella*, 3 (7.1%) each *Salmonella* and *S. aureus*. The commensal Gram-negative bacteria *E. coli* were predominantly found in both persistent cases (27 [87.1%] of a total of 31 [73.8%]) and relieved cases (5 [45.5%] of a total of 11 [26.2%]) of RAP isolated in stool culture [Table/Fig-4]. The sensitivity pattern of *E. coli* (27 [87.1%]) in conventional aerobic culture to antimicrobials showed that the bacteria were mostly resistant to third-generation cephalosporins: cefotaxime (17 [62.9%]), ceftazidime (12 [44.5%]), and ceftriaxone (8 [29.6%]), followed by amikacin (5 [18.5%]), levofloxacin (3 [11.1%]), and ciprofloxacin (2 [7.4%]) [Table/Fig-5].

Micro-organism	Culture positive RAP		Number (%) 42 (65.6%)
	Persistent 31 (73.8%)	Relieved 11 (26.2%)	
E. coli	27 (87.1)	05 (45.5)	32 (76.2)
Enterococcus	01 (3.2)	0	01 (2.4)
Enterobacter	0	01 (9.1)	01 (2.4)
S. aureus	02 (6.4)	01 (9.1)	03 (7.1)
Klebsiella	0	01 (9.1)	01 (2.4)
Salmonella	01 (3.2)	02 (18.2)	03 (7.1)
Clostridium spp	0	01 (9.1)	01 (2.4)

[Table/Fig-4]: Bacteriological profile in stool culture positive Recurrent Abdominal Pain (RAP) cases.

DISCUSSION

The RAP is a pain that intermittently persists for more than three months [12]. It is a frequent complaint in childhood, and during certain periods of school age, children may experience pain that is mild and transient in nature but comes to the parents' notice when it affects the child's daily activities. Its incidence in school-going children was up to 10% [13]. The prevalence was found to differ, ranging from 11 to 45% in subsequent studies using Apley's criteria [14-17]. This range has been suggested in these studies due to differences in age, geographical area, and social factors. In this study, the prevalence was found to be 69.6%. Most of the children (40, or 62.5%) were under the age of five. According to gender, girls (36, or 56.3%) were affected more than boys in this study.

Hyman PE, reported that one in ten children presented with chronic or recurrent abdominal pain in his reviewed study on chronic and recurrent abdominal pain in children in 2016 [18]. It is very difficult to screen these children quickly. In such situations, the duration and persistence of each episode were considered for diagnosis. The site of abdominal pain also provides clues to the diagnosis, indicating whether it is likely to resolve or recur and persist for a long time. Although the pains were recurrent in nature, some were relieved gradually over time, while others persisted for a long duration upon follow-up. In a review of the literature, it was found that RAP persisted for a long time in 29.1% of cases [19,20], whereas in present study, persistent cases of RAP accounted for 36 (56.3).

Antibiotic sensitivity of *E. coli* (n=27 (87.1%))				Zone diameter (mm) Antibiotics interpretation criteria		
Antimicrobial agents	Zone (mm)	Interpretation	Number (%)	Susceptible	Intermediate	Resistant
Amikacin	20	R	5 (18.5)	>17	14-16	<13
Gentamicin	20	S	2 (7.4)	>15	13-14	<12
Amoxycillin-clavulanic acid	22	S	2 (7.4)	>18	14-17	<13
Piperacillin-tazobactam	26	S	3 (8.3)	>25	21-24	<20
Cefepime	28	S	2 (7.4)	>25	19-24	<18
Cefotaxime	30	R	17 (62.9)	>26	23-25	<22
Ceftriaxone	30	R	8 (29.6)	>24	23-24	<22
Levofloxacin	22	R	3 (11.1)	>21	17-20	<16
Meropenem	30	S	1 (2.7)	>23	20-22	<19
Cefoperazone	30	S	3 (11.1)	>25	20-24	<19
Ciprofloxacin	28	R	2 (7.4)	>26	22-25	<21
Ceftazidime	30	R	12 (44.5)	>21	18-20	<17

[Table/Fig-5]: Sensitivity pattern of *E. coli* to antimicrobials by Kirby-Bauer disk diffusion method in persistent case of RAP.

The ESBL-producing *E. coli* bacteria resistant to the 3rd generation cephalosporin antimicrobial group (22 [81.5%]) were tested by the combination disk test (PCDDT) method. Among them, confirmed cases of ESBL-producing *E. coli* were 15 (68.2%), while non producers accounted for 7 (31.8%) cases [Table/Fig-6].

In the present study, an association between the site and size of mesenteric lymph nodes as assessed by ultrasonography and RAP in children was analysed. It was found that lymph nodes larger than 8 mm in the periumbilical, right iliac fossa, and epigastric areas were present in persistent cases of RAP in children.

3rd GC resistant *E. coli* Number (n=22(81.5%))	Phenotypic Confirmatory Disk Diffusion Test (PCDDT)
ESBL Producers	15 (68.2%)
Non ESBL producers	7 (31.8%)

[Table/Fig-6]: ESBL producer *E. coli* by Phenotypic Confirmatory Disk Diffusion Test (PCDDT) in persistent case of RAP.

It is common practice and knowledge among our physicians to explore the status of the Gastrointestinal (GI) tract, and stool tests are a valuable medium for this. Therefore, in all cases of RAP, routine stool examination and culture sensitivity are mandatory investigations for us. Previous studies on stool culture for bacterial pathogens found positivity rates ranging from 2.4 to 32% [21]; in comparison, this study found a positivity incidence of 65.6%.

Common antimicrobials, specifically third-generation cephalosporins, were used by physicians during each visit of these RAP children for treatment without screening. The normal flora of the GI tract is composed of various bacteria and fungi that play a vital role in the digestion of food. They also help restrict the growth of pathogenic organisms. The use of broad-spectrum antibiotics may alter the balance of the normal flora, inhibiting the growth of beneficial bacteria and allowing antibiotic-resistant bacteria to persist and overgrow [22]. Previous studies have found a positive stool culture for bacterial pathogens, suggesting that factors such as young age, unformed stool, and low serum bicarbonate levels might contribute to positive stool cultures [23].

In this study, the bacteriological profile of positive stool culture cases included *E. coli* (32, 76.2%), *Enterococcus* (1, 2.4%), *Enterobacter* (1, 2.4%), *Salmonella* (3, 7.1%), *Staphylococcus aureus* (3, 7.1%), and *Klebsiella* (1, 2.4%). Oliver WM and Musa ON reported that *E. coli* was the major bacterium among the 11 genera isolated in the stools of children under five years [24]. Additionally, the study by Sapkota B et al., showed the predominance of *E. coli* in stool cultures of Nepalese patients [25].

In this study, the sensitivity pattern of these bacteria was determined using the Kirby-Bauer disk diffusion method. The major Gram-negative bacterium, *E. coli* (27, 87.1%), was found to be resistant to most third-generation cephalosporins: cefotaxime (17, 62.9%), ceftazidime (12, 44.5%), and ceftriaxone (8, 29.6%), followed by amikacin (5, 18.5%), levofloxacin (3, 11.1%), and ciprofloxacin (2, 7.4%). The number of ESBL-producing *E. coli* tested using the combination disk method was 15 (68.2%), compared to the study by Sapkota B et al., where it was 66 (95.7%) and *Klebsiella* (3, 4.3%) [21].

In another study by Saleem AF, on the gut of healthy infants in the community, 43% of infant stool cultures yielded ESBL-producing Enterobacteriaceae, 84% of which were *E. coli*, and 10% were *Klebsiella*. Among *E. coli* isolates, 80% were ESBL producers [26]. The lower prevalence of ESBL-producing *E. coli* in present study may be attributed to fewer stool samples collected according to the inclusion criteria, as well as the quality of the laboratory methods used to detect ESBL.

Limitation(s)

The sample size of this study, based on the prevalence of RAP in children of a specific age group, was small. Additionally, a genotypic test for *E. coli* was not conducted to confirm the disparity of the gene responsible for resistance.

CONCLUSION(S)

The prevalence of ESBL-producing *E. coli* in the present study was found to be high (68.2%) among all persistent cases of RAP in children who frequently received antibiotics in the community. This frequent use of empirical antibiotics, without screening all cases of abdominal pain in children by physicians in their busy clinics, results in a decrease in the number of cases and the development of resistance among ESBL-producing strains of the common gut commensal *E. coli* to commonly used empirical third-generation cephalosporins compared to other preferred antibiotics. This imbalanced state between the intestinal microbiota and the host manifests as persistent cases of RAP in children. Authors recommend that more studies with larger samples be conducted at the community level to determine the exact prevalence. Additionally, there should be guidelines for the rapid detection of ESBL producers that are available at low cost, as well as an antibiotic policy specific to non organic causes, to assist physicians at the community level in their rational use of antibiotics.

Authors' contributions

MS: Literature search, Draft preparation, Review of the manuscript and sharing of critical viewpoints; UKR: Literature search; SKD: Review of the manuscript and sharing of critical viewpoints, Project supervision, Writing of the manuscript; MP: Draft preparation, Writing of the manuscript.

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