

# Prevalence of Bacterial Agents in Children with Acute Gastroenteritis in the Pediatric Emergency Department of Ege University School of Medicine

Ege Üniversitesi Tıp Fakültesi Çocuk Acil Servisine Başvuran Akut Gastroenteritli Çocuklarda Bakteriyel Etkenlerin Prevelansı

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## Abstract

**Objective:** Acute gastroenteritis is one of the main causes of morbidity and mortality in childhood. Although rotavirus is reported to be the most common agent in diarrhea, infectious gastroenteritis due to enteric pathogenic bacteria is still widely seen in developing and underdeveloped countries. In this study, we aimed to determine the incidence of bacterial agents in children with acute gastroenteritis in Pediatric Emergency Department of Ege University School of Medicine.

**Material and Methods:** Data of patients aged 0-18 years with acute gastroenteritis, who were admitted to our hospital between September 2013 to August 2014 were analyzed retrospectively. Frequency of bacterial gastroenteritis, antimicrobial susceptibility and seasonal variations were investigated.

**Results:** Bacterial pathogens were detected in 143 (16.3%) of 875 stool cultures. Among them, *Campylobacter* spp. was detected in 88 (10.1%) cases, *Salmonella* spp. in 43 (4.9%) cases and *Shigella* spp. in 12 (1.4%) cases. No cases of *Vibrio* spp. and *E. coli* were isolated. *Campylobacter* spp. were isolated in all months of the year with a slight increase in frequency in spring while *Salmonella* spp. and *Shigella* spp. were the most common agents in summer. *Salmonella* spp. were the least resistant isolates. We determined increased resistance to quinolones in *Campylobacter* isolates. Majority of isolated *Shigella* spp. were resistant to co-trimoxazole. Resistance to 3<sup>rd</sup> generation cephalosporins was detected in three cases of *S. sonnei* gastroenteritis.

**Conclusion:** The most common bacterial enteropathogen in acute gastroenteritis was *C. jejuni* in our region and quinolone resistance was higher than expected. In the light of these findings, azithromycin may be a good option in the empirical treatment of acute diarrhea when antibiotics are indicated.

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**Keywords:** Acute gastroenteritis, etiology, bacteria, *Campylobacter*, child

## Özet

**Amaç:** Akut gastroenterit çocukluk çağında morbidite ve mortalitenin en önemli nedenlerinden biridir. Rotavirüs en sık ishal etkeni olarak bildirilmesine karşın enterik patojen bakteriler ile gelişen enfeksiyöz ishaller gelişmemiş ve gelişmekte olan ülkelerde halen yaygın olarak görülmektedir. Bu çalışmada Ege Üniversitesi Tıp Fakültesi Çocuk Acil Servisine başvuran akut gastroenteritli olgulardaki bakteriyel etkenlerin sıklığının belirlenmesi amaçlanmıştır.

**Gereç ve Yöntemler:** Hastanemize Eylül 2013-Ağustos 2014 tarihleri arasında başvuran 0-18 yaş grubundaki akut gastroenteritli olguların verileri geriye dönük olarak incelendi. Bakteriyel gastroenteritlerin sıklığı, antibiyotik duyarlılıkları ve mevsimsel değişiklik araştırıldı.

**Bulgular:** Toplam 875 dışkı kültürünün 143'ünde (%16,3) bakteriyel patojen saptandı. Bunlar arasında, *Campylobacter* spp. 88 (%10,1) olguda, *Salmonella* spp. 43 (%4,9) olguda ve *Shigella* spp. 12 (%1,4) olguda tespit edildi. *Vibrio* spp. ve *E. Coli* hiçbir olguda izole edilmedi. İlbaharda hafif bir artış olmakla birlikte *Campylobacter* spp.'nin sıklığı yılın tüm aylarında benzer bulundu. *Salmonella* spp. ve *Shigella* spp. yaz aylarında görülen en sık ajanlar idi. *Salmonella* spp. en az dirençli olanlar idi. *Campylobacter* spp.'de kinolon direncinde artış olduğu belirlendi. *Shigella* spp.'lerin çoğunluğu kotrimoksazol dirençli idi. *S. sonnei* sonucu gastroenterit gelişen 3 olguda 3. jenerasyon sefalosporinlere karşı direnç tespit edildi.

**Sonuç:** Bizim bölgemizde akut gastroenteritlerdeki en sık bakteriyel enteropatojen *C. jejuni* idi ve kinolon direnci beklenenden daha yüksek bulundu. Bu bulgular ışığında, akut gastroenteritlerde ampirik antibiyotik tedavisinde azitromisin iyi bir seçenek olabileceği düşünüldü. (*J Pediatr Inf* 2016; 10: 49-53)

**Anahtar kelimeler:** Akut gastroenterit, etyoloji, bakteri, *Campylobacter*, çocuk

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## Introduction

Infectious gastroenteritis still remains a major cause of morbidity and mortality worldwide (1). Although it is observed in all age groups, children under the age of five years are the most affected. Acute gastroenteritis (AGE) constitutes 8.4% of the diseases causing mortality in the 0–14-year-old age group in Turkey (2). A variety of pathogens can cause AGE depending on seasonal and geographical differences, socioeconomic status, and host immunity. Viruses are the most common agents of AGE. Gastroenteritis due to bacteria and parasites has become less frequent in industrial nations, but it continues to be an important reason for hospitalization in underdeveloped and developing countries. Bacterial agents are detected in 1.5% to 5.6% of cases with acute diarrhea (3). Bacterial agent distribution shows regional variations. *Salmonella* spp. and *Campylobacter* spp. are the most frequent bacterial causative agents (4). Bacterial and viral gastroenteritis present with different clinical features. Vomiting and watery diarrhea without mucus and blood are usually observed in patients with viral AGE, while bloody diarrhea and presence of mucus and leukocytes in stools are typically seen in bacterial AGE. Most cases of acute bacterial diarrhea are self-limiting. Treatment includes rehydration and, in some cases, antibiotics. Antibiotics are recommended in cases of febrile diarrhea, which is thought to cause an invasive disease (5).

In our hospital, we observed an increase in bacterial gastroenteritis, and the majority of them were bloody. A small number of patients required hospitalization, but several antibiotics were given to outpatients. This is the reason why we aimed to find the frequency of bacterial gastroenteritis, the most common enteropathogens, antimicrobial resistance, and seasonal variations in our region for a proper management.

## Material and Methods

Patients aged 0–18 years who were admitted to the Pediatric Emergency Department of Ege University School of Medicine between September 2013 and August 2014 and who were diagnosed with AGE (diarrhea lasting for less than 14 days) and had bacteria in stool cultures were included. Demographic data of patients and data on the season when they were admitted to the hospital, bacterial pathogens in the stool culture, and antibiotic susceptibilities were retrospectively investigated from medical records. Because this was a retrospective study, there was no requirement for ethical approval and patient consent.

Bacterial agents were identified using conventional culture methods in our hospital (Ege University Medical School Hospital, Department of Clinical Microbiology, Bacteriology Laboratory). Stool samples were investigat-

ed for the presence of bacteria, including *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Escherichia coli*, and *Vibrio* spp. using standard microbiological procedures; this was followed by antibiotic susceptibility testing.

### Laboratory procedures

*Salmonella* spp. and *Shigella* spp. were routinely investigated in all stool samples. *Campylobacter* spp. and enterohemorrhagic *E. coli* were investigated in patients in whom fecal leukocytes and erythrocytes had been respectively detected. *Yersinia* and *Vibrio* spp. were investigated on special request.

Stool samples were plated on Skirrow agar (bioMérieux, France) for *Campylobacter* spp., and plates were incubated at 42°C for 72 h in a microaerophilic environment provided in anaerobic jars with a Campy-Gen (Oxoid, England) kit. Identification was performed with VITEK 2 and VITEK MS (bioMérieux, France). Etest (AB Biodisk-bioMérieux, France) was used for antibiotic susceptibility.

Stool samples were plated on sorbitol MacConkey agar (bioMérieux, France) for *E. coli* O157:H7 isolation, and identification was made with VITEK 2 and VITEK MS (bioMérieux, France) and latex agglutination (Wellcolex *E. coli* O157:H7, Remel Europe Ltd., UK).

For *Salmonella* and *Shigella* spp., samples were plated on eosin–methylene blue agar (bioMérieux, France) and Hector the enteric agar (bioMérieux, France). Biochemical tests were performed for colonies suspected to be pathogenic after the recommended temperature was reached and time had lapsed and when necessary. Identification was made with VITEK 2 and VITEK MS (bioMérieux, France). *Salmonella* and *Shigella* serotypes were determined by slide agglutination (Selke Denka Co., Japan). The antibiotic susceptibilities of all *Salmonella* and *Shigella* species for ciprofloxacin (5 µg), ampicillin (10 µg), cefotaxime (30 µg), and trimethoprim/sulfamethoxazole (25 µg) (Oxoid, England) were examined with the Kirby–Bauer disk diffusion method according to the recommendation of the Clinical and Laboratory Standards Institute.

### Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences version 16.0 (SPSS, Inc.; Chicago, IL, USA).

## Results

During the study period, 3,762 patients were diagnosed with AGE in the Pediatric Emergency Department. Stool samples from 875 patients were sent for culture. Bacterial pathogens were detected in 143 (16.3%) stool cultures. We identified *Campylobacter* spp. in 88 (10.1%) cultures, *Salmonella* spp. in 43 (4.9%), and *Shigella* spp. in 12 (1.4%)

(Table 1). *Vibrio* spp. and *E. coli* were not isolated in any culture. In total, 378 patients (43.2%) were females and 497 (56.8%) were males. The median age was 28 months (2–204 months), while 46.1% of patients were two years old and three patients with *Campylobacter jejuni* in their stool cultures were under six months old. Around a quarter (27.8%) of the patients were admitted to the hospital in spring, 25.8% in autumn, 24.3% in summer, and 22.8% in winter. There was a peak in *Salmonella* spp. (53.5%) and *Shigella* spp. (83.3%) in summer, but majority of cases with *C. jejuni* (36.4%) were detected in spring (Figure 1). Bloody diarrhea was identified in 32 (23.8%) cultures. *C. jejuni* (56.2%) was the most frequent agent among children with bloody diarrhea. Totally, 131 patients (91.6%) were treated as outpatients, while 12 (8.3%) were hospitalized. Eighty-three (97.7%) *C. jejuni* strains were susceptible to erythromycin, and 52 (79.5%) of them were found to be resistant to ciprofloxacin. All *Salmonella* spp. isolates were susceptible to ampicillin. More than half of all *Shigella* spp. (58.3%) isolates were resistant to ampicillin, and 25% of them were resistant to third-generation cephalosporins (Table 2). All hospitalized patients had fever. *Salmonella enteritidis* was identified in seven and *C. jejuni* in four hospitalized patients. *Shigella sonnei* was detected in a 15-year-old who developed hypovolemic shock and who was followed in the pediatric intensive care department. Half of the patients received third-generation cephalosporins, three of them received metronidazole, and the remaining three received azithromycin. The median length of stay was five days (3–10 days). We have insufficient data on the antibiotic treatment of outpatients.

## Discussion

Acute gastroenteritis takes the second place in terms of morbidity among infections during childhood. More than

1.8 million children under the age of five years die due to diarrheal diseases every year (6). The causes of gastroenteritis vary depending on age, season, and geographical features. Bacteria, viruses, parasites, and amoebae play a role in etiology. Rotaviruses are the principal etiological agents in hospitalized children under the age of five years affected by AGE. Bacterial pathogens only account for 2–10% of cases in developed countries, but they are more frequent in underdeveloped countries (7).

In a study in Spain, the incidences of rotaviruses, *C. jejuni*, and *Salmonella* spp. were 22%, 7%, and 4%, respectively (8). Wiegeling et al. (9) reported rotaviruses as the main causative agent, while *Salmonella* was detected in 7.9% of the stool samples in Germany. Similarly, the incidence of bacterial gastroenteritis was reported to be 8.8%, and *Salmonella enterica* was the predominant pathogen in the long-term study by Maraki et al. (10) in Crete.

In our study, stool samples were examined for *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, and *E. coli*. *C. jejuni* was the predominant bacterial agent of infectious diarrhea in our region, with an incidence of 10.1%, and was responsible for more than half of the bloody diarrhea. It was self-resolving in majority of the cases. *Salmonella* spp. were the second most causative agent, and *Shigella* spp. were rarely detected (1.4%).

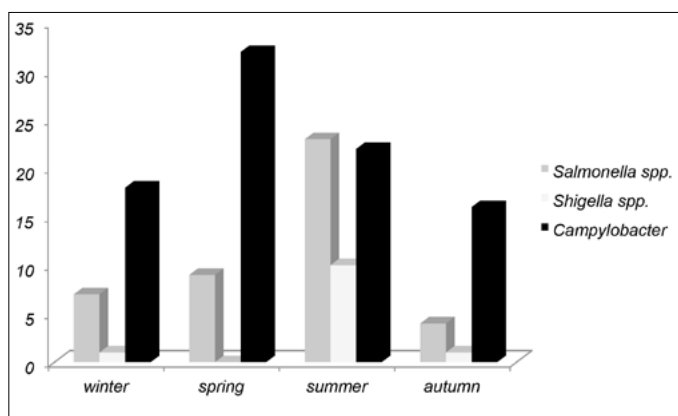
*Campylobacter* is a small gram-negative microaerophilic bacterium and is the leading cause of bacterial enteritis in industrialized countries these days (10, 11). This can be associated with food consumption in restaurants because the most common sources of infection are raw or undercooked meat, particularly poultry products. Contact with pets may cause infection, and outbreaks can be seen with the usage of untreated water and unpasteurized milk (11, 12). As in our region, increasing tempera-

**Table 1.** Prevalence of bacterial pathogens and age distribution of patients in those with acute gastroenteritis

Bacterial agents	Number of bacterial agents and frequency	0-6 months	6 month-2 years	2-5 years	> 5 years
<i>Campylobacter jejuni</i>	88 (10.1%)	3 (3.4%)	30 (34%)	22 (25.1%)	33 (37.5%)
<i>Salmonella</i> spp.	43 (4.9%)	0	11 (25.5%)	20 (46.5%)	12 (27.9%)
<i>Shigella</i> spp.	12 (1.4%)	0	1 (8.3%)	5 (41.6%)	6 (50%)

**Table 2.** Antimicrobial resistance of bacterial specimens isolated from stools

	<i>S. enteritidis</i> n=43 (%)	<i>S. sonnei</i> n=9 (%)	<i>S. flexneri</i> n=2 (%)	<i>S. dysenteriae</i> n=1 (%)	<i>C. jejuni</i> n=85 (%)	<i>C. coli</i> n=3 (%)
Ampicillin	0	4 (44.4)	1 (50)	0	--	--
Co-trimoxazole	0	7 (77.7)	1 (50)	1 (100)	--	--
Ciprofloxacin	0	0	0	0	52 (61.1)	3 (100)
Cefotaxime	--	3 (33.3)	0	0	--	--
Erythromycin	--	--	--	--	2 (2.3)	0



**Figure 1.** Seasonal distribution of bacterial pathogens in acute gastroenteritis

tures are more suitable for the survival of *Campylobacter*. Maragkoudakis et al. (3) found an increased incidence of *C. enteritis* in adult patients over the years (particularly after the tap water outbreak) in Greece, which has similar characteristics to our region.

*Campylobacter* spp. were isolated in all months of the year, but an increase in their incidence was observed in spring months. There was a peak in *Salmonella* spp. and *Shigella* spp. in summer, and *C. jejuni* gastroenteritis was also frequently detected in this season. This may be related to the positive effect of higher temperature on the proliferation of these pathogens in food and water. *C. jejuni* (15.7%) was the most common enteropathogen in infants and preschoolers in Western Mexico in the study by Larrosa-Haro et al. They reported higher summer rates of *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. (12).

Rehydration is the main treatment for AGE. Antimicrobials are frequently used inappropriately for the treatment of acute enteric infections; therefore, adverse outcomes, increased treatment costs, and antimicrobial resistance can occur (13). In cases where empirical antibiotic therapy is necessary, knowledge on local epidemiology and local patterns of susceptibility can be useful. Antibiotics are recommended for severe cases of *Salmonella* gastroenteritis (also in high-risk patients), in invasive cases, in cases with proven or strong suspicion of shigellosis, and in cases of early diagnosis of diarrhea due to *C. jejuni* (14).

*Campylobacter* infection is usually mild and self-limited. The estimated mortality from symptomatic infection in the United States is reported to be 2.4 per 1000 culture-confirmed cases (15, 16). Antimicrobial therapy is warranted only in patients with severe disease (with bloody stools, high fever, extraintestinal infection, worsening or relapsing symptoms, or symptoms lasting longer than one week) and in risk groups (immunocompromised) (15). First-line agents for the treatment of *Campylobacter* gastroenteritis include quinolones (if sensitive) or azithromycin. The rate of macrolide-resistance among *Campylobacter* spp. has

remained stable at <5% in most parts of the world, but quinolone resistance is increasing (17-19).

Antibiotic resistance has been changing over the years with the effect of regional differences and empirical antibiotic treatments. In a study conducted in Niger, among children under the age of five years, enteropathogenic *E. coli*, *Salmonella*, and *Campylobacter* spp. were frequent in watery diarrhea, and *Shigella* spp. were the most frequent in bloody diarrhea. More than half of all *Enterobacteriaceae* were resistant to amoxicillin and co-trimoxazole. They found that 13% of *Salmonella* cultures exhibited an extended-spectrum beta-lactamase phenotype (5). Randrairina et al. (20) reported that resistance to penicillin and co-trimoxazole was highly prevalent among *Shigella* and *E. coli* (60%–80%), but the resistance was less prevalent among *Salmonella* spp. in Madagascar. Resistance to third-generation cephalosporins was found only in 1.2% of *Salmonella* and 3.1% of *E. coli* cultures. Ampicillin resistance was the most common among *Campylobacter* spp., while most of them were susceptible to erythromycin and ciprofloxacin.

We determined an increased resistance to quinolones in 61.1% of *C. jejuni* and 100% of *E. coli* cultures. Almost all of them were susceptible to erythromycin. *Salmonella* spp. were the least resistant isolates. All *Salmonella* spp. were susceptible to ampicillin. Majority of isolated *Shigella* (*S. sonnei*, 77.7%; *S. flexneri*, 50%; and *S. dysenteriae*, 100%) were resistant to co-trimoxazole. Ampicillin resistance was found in 44.4% of *S. sonnei* and 50% of *S. flexneri* cultures, and 33% of *S. sonnei* cultures were resistant to cefotaxime. All *Shigella* isolates were susceptible to ciprofloxacin. In our hospital, the increased resistance to quinolones and co-trimoxazole may have been due to the widespread use of these antibiotics in empirical treatment, particularly in patients with bloody diarrhea.

In conclusion, the frequency of bacterial pathogens in AGE may show regional differences. Therefore, knowledge on local epidemiological differences is important for proper treatment. We showed the predominance of *C. jejuni* during childhood acute diarrhea in our region; *C. jejuni* was responsible for most bloody diarrhea cases and was found to be frequently resistant to quinolones. Therefore, we think that if antibiotics are indicated, azithromycin may be a good option for the empirical treatment of acute diarrhea in areas where *Campylobacter* is the most common agent.

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