ABSTRACT

Background: Antibiotics are frequently prescribed for the treatment of infectious diseases. Although antibiotic-associated diarrhea (AAD) is a common adverse event in children, few studies have investigated this issue. Objectives: To investigate the frequency, the antibiotics incriminated and the risk factors for developing Pseudomonas-associated diarrhea (PAAD) in children admitted to our hospital. Material and methods: The main anamnestic and demographic features, characteristics of diarrhea, therapeutic management, and clinical outcomes of children with PAAD were evaluated. We have considered a child diagnosed with PAAD if he had three or more stools a day of liquid consistency for at least two days from the start of antibiotic administration. Children with acute/chronic diarrhea, immunodeficiencies and no history of antibiotics before the diarrhea started were excluded. Results: Out of 55 children diagnosed with diarrhea, fifteen children (27.27%) had PAAD. The majority were infants and toddlers (66.67%). Antibiotic therapy was prescribed mainly for pulmonary infections (40%), neonatal sepsis (20%) and upper respiratory tract infections (13.33%). PAAD was associated with the use of Cephalosporins (40%), Amoxicillin/Clavulanate (15%) and Aminoglycosides (15%). The relative risk of onset of PAAD in a child receiving Cephalosporins was 1.83, respectively 1.5 when the child was younger than 2 years and 2.4 for patients admitted in intensive care units. Conclusions: The diaper-wearing group, the use of Cephalosporins and the admission to intensive care units are risk factors for PAAD. Further investigations on PAAD with larger cohort are required to confirm our results.

Key Words: children, Pseudomonas aeruginosa, antibiotic associated diarrhea

INTRODUCTION

Antibiotics are the most prescribed drugs in children, used to treat acute infectious diseases frequently seen in this age group. The demonstration that acute infections are primarily of viral origin has not reduced the use of antibiotics, nor has the fact that antibiotics afford only marginal alleviation of the clinical symptoms.

The most common complication of antimicrobial therapy is antibiotic-associated diarrhea (AAD). World Health Organization defines AAD as three or more abnormally loose bowel movements in a 24-hour period that occurs in association with the administration of antibiotics.
Definitions used in pediatric and adult studies varied from one to three abnormally loose stools per 24–48 hours. The incidence of AAD has been estimated to vary between 5% and 25% in adults and between 8% and 30% in children. The discrepancies in the incidence may have been attributable to differences in the definition of diarrhea, the antimicrobial agent used, the number of daily doses, the duration of the treatment and the time from previous antimicrobial treatments.

The onset of AAD may be rapid (while the patient is on antibiotics) or may be delayed for up to six weeks after the antibiotics have been discontinued. The severity of AAD may range from a brief, self-limiting disease to devastating diarrhea with serious complications including electrolyte disturbances, dehydration, pseudomembranous colitis, toxic megacolon or even death.

The mechanisms by which antibiotics lead to AAD are multiple. Antimicrobial treatment is responsible for the disturbance of the gastrointestinal microflora, reducing concentrations of the normal gut bacteria, causing pathogenic bacterial overgrowth or the lack of the nutrient competition. This gastrointestinal microflora acts as a barrier against colonization by facultative and obligative enteropathogens. The disruption of this barrier causes also functional disturbance of the normal intestinal carbohydrate and bile acid metabolism, resulting in osmotic diarrhea. The unveiling of toxin receptors or of the attachment sites caused by the disappearing of the normal flora is another theory of the etiology of AAD. Allergic, toxic and direct pharmacological motility effects on the gut can also be factors. For example, erythromycin acts as a motilin receptor agonist and accelerates the rate of gastrointestinal emptying. The clavulanate in amoxicillin–clavulanate appears to stimulate small-bowel motility and in rare instances, penicillins may cause segmental colitis.

Virtually all antibiotics have been implicated in the appearance of AAD. The antimicrobial spectrum of antibiotic (particularity the activity against anaerobic bacteria), pharmacokinetic properties (the rate of gastrointestinal absorption and enterohepatic circulation) and the fecal concentration of the antibiotic are probably important factors in the developing of diarrhea. Studies showed that, among all antibiotics, cephalosporins, clindamycin and ampicillin are responsible for the occurrence of AAD. Another study reported that a single dose of antibiotic might be sufficient to trigger diarrhea.

Most cases of AAD can be classified into two categories: cases in which a microorganism is responsible for the AAD, and cases in which no etiological agents are found (enigmatic AAD). Major infectious agent of AAD is toxin-producing Clostridium difficile, estimated to be responsible for about 10-20% of cases of AAD and almost all cases of pseudomembranous colitis. If this bacterium reaches a certain density, the enterotoxin it produces causes mucosal damage that subsequently leads to AAD. Other bacterial causes of AAD are rare, estimated to cause around 0.5% of cases. These include enteropathogens such as enterotoxin-producing Clostridium perfringens, multidrug-resistant Salmonella species, Klebsiella oxytoca, Staphylococcus aureus, Candida species or Pseudomonas aeruginosa.

OBJECTIVES

The objectives of our study were to investigate the frequency of PAAD, to establish the antibiotics incriminated and to evaluate the risk factors for developing PAAD in children admitted to our hospital.

MATERIAL AND METHODS

Our study was conducted at “Louis Turcanu” Children Emergency Hospital, Timisoara, from January 2008 to April 2009. Clinical chart, hospital records and microbiology results of children diagnosed with PAAD were reviewed. Inclusion criteria were: (1) age at hospitalization ≤ 18 years; (2) presence of antibiotic associated diarrhea, defined as three or more stools a day of liquid or semi-liquid consistency for at least two days from the start of antibiotic administration; and (3) positive stool cultures with Pseudomonas aeruginosa. A child was diagnosed with Candida albicans AAD if he had liquid or semi-liquid stools for at least two days from the start of antibiotic administration with positive stool cultures for Candida albicans on Sabouraud agar. We excluded from our study pediatric patients without a history of antibiotic use, those who discontinued using antibiotics two months before the diarrhea started, children with immunodeficiencies and those with a diagnosis of acute diarrhea on admission (infectious diarrhea), recurrent diarrhea, bowel pathology that could result in diarrhea or bowel surgery, inflammatory bowel disease, ischemic colitis and lactose intolerance.

Data were recorded in a specific reporting form made up of 5 sections: (1) demographic characteristics: gender, age of the child at hospital admission; (2) anamnesis: symptoms and complains responsible for antibiotic usage; (3) antibiotics prescribed: type, dosage and administration (oral or parenteral);
(4) characteristics of diarrhea: number of bowel movements, stool consistency, presence of blood and mucus in the stools, diarrhea duration and severity of dehydration; and (5) therapeutic management and clinical outcomes.

One or more stool specimen(s) were collected from children with diarrhea. Stool microscopy for red and white blood cells, parasitic cysts and ova examination were carried out, too. Inoculation of these samples was done on blood agar plates and Mac Conkey agar plates and then incubated at 37°C for 18–24 hours, in order to detect the etiological organism of diarrhea. Colonies were identified using conventional biochemical tests, while antibiotic sensibility of bacterial isolates was determined using the disk-diffusion method, according to the actual recommendations. The susceptibility of aerobic bacteria was determined for the following antibiotics: ampicilin, ciprofloxacin, trimethoprim/sulfamethoxazole, netilmicin, amikacin, ticarcillin/clavulanic acid, pipericillin/tazobactam, cefotaxime, ceftazidime, ceftiraxone, meropenem, imipenem, nalidixic acid, furazolidon and colistin. Quantitative cultures were carried out for Candida sp. Diluted stool sample was inoculated into Sabouraud agar and incubated at room temperature for 24–48 hours. Detection of enteric viruses was not done.

The study protocol was approved by the Ethics Committee of our institution and complied with the Declaration of Helsinki. Data analysis was performed using SPSS 17.0.

RESULTS

The clinical charts of all patients admitted in hospital in the study period were reviewed and 55 children were diagnosed with enterocolitis according to the results of microbiological analysis. The microorganisms responsible were Pseudomonas aeruginosa (72.72%), Yersinia enterocolitica (1.81%), Salmonella (1.81%), enteroinvasive Escherichia coli (1.81%) and Candida albicans (20%). Eleven pediatric patients were diagnosed with Candida albicans AAD, because they developed liquid stools with mucus after the administrations of different antibiotics. (Table 1) Such antibiotics were cephalosporins (45.45%), aminoglycosides (36.36%) and ciprofloxacin (18.18%).

Out of 40 children diagnosed with Pseudomonas aeruginosa diarrhea, 22 children (40%) were classified as having infectious diarrhea. In these cases, the stools were liquid with mucus and blood and were associated with the presence of abdominal cramps, vomiting and dehydration, fever and leukocytosis. A diagnosis of neutropenic enterocolitis with Pseudomonas aeruginosa was reported in 13 pediatric hematology-oncology patients (23.63%) with neutropenia more than one week after chemotherapy.

According to anamnesis, clinical findings and the results of microbiology studies, fifteen children (27.27%) received the diagnosis of PAAD, because they all fulfilled the inclusion criteria in the study lot. Demographic characteristics are presented in the Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Pseudomonas AAD</th>
<th>Candida albicans AAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Male</td>
<td>6 (40%)</td>
<td>7</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant</td>
<td>6 (40%)</td>
<td>1</td>
</tr>
<tr>
<td>Toddler</td>
<td>4 (26.67%)</td>
<td>3</td>
</tr>
<tr>
<td>3-18 years</td>
<td>5 (33.33%)</td>
<td>7</td>
</tr>
<tr>
<td>Mean age</td>
<td>3.41±1.37</td>
<td>8.5 ± 2.35</td>
</tr>
<tr>
<td>Hospital admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU admission</td>
<td>9 (60%)</td>
<td>1 (9.09%)</td>
</tr>
<tr>
<td>Other wards</td>
<td>6 (40%)</td>
<td>10 (90.90%)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>11 (73.33%)</td>
<td>5 (45.45%)</td>
</tr>
<tr>
<td>Other antibiotics</td>
<td>4 (26.67%)</td>
<td>6 (54.54%)</td>
</tr>
<tr>
<td>Administrations</td>
<td></td>
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</tbody>
</table>

All pediatric patients had a history of a medical condition other than PAAD before admission in hospital. The main reason for prescription of antibiotic treatment was lower respiratory tract infections (40%), neonatal sepsis (20%) and pharyngotonsillitis (13.33%). Other medical conditions presented (20%) were acute otitis media, meningitis, urinary tract infection and burn wound infection. They associated various underlying conditions such as prematurity, intraturner growth retardation, seizures, or congenital myopathy. We have to underline the fact that our patients had no concomitant infections caused by Pseudomonas other than diarrhea and no other potential or recognized enteropathogens were identified in the stool cultures. The median duration of hospitalization before the onset of PAAD was 10 (3-16) days and 80 % of them were admitted to pediatric and neonatal intensive care units.

The antibiotics prescribed for the their disorders and responsible for the developing of PAAD were 3rd and 4th generation cephalosporins (40%, 50-100mg/kg/day), aminoglycosides (15%, 4-6mg/kg/day), amoxicillin/clavulanate (15%, 30-50mg/kg/day), clarithromycin (10%, 15 mg/kg/day), clindamycin (5%, 30 mg/kg/day), ciprofloxacin (5%, 10mg/kg/
day), vancomycin (5%, 40mg/kg/day) and imipenem (5%, 50-60mg/kg/day). Therefore, we can state that PAAD have occurred after the use of broad-spectrum antibiotic therapy at the dosages frequently used in children. Twenty percent of children that developed PAAD received oral antibiotics (amoxicillin/clavulanate or clarithromycin), while 25% of them were treated with a combination of antibiotics (cephalosporins and aminoglycosides, fluoroquinolones or carbapenems). PAAD began 7.3±3.5 days after the start of antibiotic administration and the mean duration was 5±2 days, without differences between oral and parenteral administration of antibiotic therapy. The number of diarrhea per day ranged between 3-8 watery stools, without mucus or blood in the stools. A percentage of 53.33% patients were dehydrated and needed parenteral rehydration.

Diarrhea stopped two-three days after withdrawal of probable offending antibiotic without specific treatment in ten patients. The other five patients (33.34%) with continuous diarrhea despite withdrawal of probable responsible antibiotics were treated with anti-pseudomonal antibiotic according to antibiograms for two-four more days after resolution of diarrhea with a good evolution. Negative stool cultures obtained at the end of antibiotic therapy have demonstrated the eradication of *Pseudomonas aeruginosa*. No patient died of PAAD.

According to the results of antibiograms, *Pseudomonas aeruginosa* isolates were highly susceptible to colistin, carbapenems, and piperacillin/tazobactam as shown in Figure 1. No isolates of *Pseudomonas aeruginosa* from stool cultures were susceptible to previously given antibiotics. High rates of resistance were encountered in the most frequent antibiotics prescribed in our study such as last generations cephalosporins (36.37-37.5%), aminoglycosides (40-42%) or amoxicillin/clavulanate (73%).

The relative risk of onset of an episode of PAAD in a child receiving cephalosporins (cefotaxime, ceftazidime or ceftriaxone) was 1.83 (95% CI: 0.84-3.93) and 1.5 (95% CI: 0.72-4.64) when the child was aged less than 3 years. Admission in pediatric intensive care units was another risk factor for developing PAAD (RR=2.4, 95% CI: 1.27-3.02). In contrast, we could not prove that sex, duration of hospital stay or severe underlying disease are also risk factors for developing of PAAD.

**DISCUSSIONS**

*Pseudomonas aeruginosa* is an environmental organism (in water, soil and on plants). Although it is occasionally detected as part of the normal human microflora of healthy individuals, it can cause serious infections in immunocompromised and critically ill patients. Even though *Pseudomonas aeruginosa* is not generally considered as a cause of AAD, there are evidences that it can cause infections of gastrointestinal tract with diarrhea in children. It has only rarely been implicated as a cause of infectious diarrhea, with reported cases predominantly involving individuals suffering from hematological malignancies and neutropenia secondary to chemotherapy or epidemics in infants in the past. Studies presented *Pseudomonas septicemia* in infants as necrotizing bowel lesions with a history of diarrhea and complicated with colonic perforation, ecthyma gangrenosum and even death. Chronic colonization of the gastrointestinal mucosa is also known as an important component of *Pseudomonas aeruginosa* diarrhea and systemic disease. Other medical report showed *Pseudomonas aeruginosa* as the cause of hospital acquiring infection that developed after the use of antibiotics, particularly ceftriaxone. In his study, Cheng presented 20 patients without any gastrointestinal problems on hospital admission, from whom 17 developed PAAD after receiving antibiotics.

Although PAAD has been studied in adult hospitalized patients, there is little available information concerning the epidemiologic characteristics in children. The incidence rate of PAAD in our study was 27.27%, which is similar to the data quoted by the literature. We identified two risk factors responsible for the PAAD, namely the young age of the child and the type of antibiotic treatment received. In our study, two-thirds of children with PAAD were less than 2 years (RR=1.5), similar to the study mentioned, where AAD was more common in the diaper-wearing group (0-24
months). Certain antibiotics had a higher risk for PAAD as predicted. We found that the use of the last generations cephalosporins was significantly associated with PAAD (p =0.003, RR= 1.83). Different reports underlined the significantly increased risk of acquiring AAD in patients treated with the last generations of cephalosporins (OR, 1.82-3.5). In a report from Thailand, 6.2% of 225 children from the study lot developed AAD, amoxicillin and cloxacillin combination being the most commonly prescribed antibiotics. There was a trend towards a higher incidence of AAD associated with the prescription of amoxicillin/clavulanate, cephalosporins, amoxicillin or erythromycin, similar to our results.

Kelly et al classified antibiotics according to the risk of developing AAD in antibiotics with low risk - metronidazole and aminoglycosides (gentamicin, netilmicin, amikacin); with medium risk - tetracyclines (oxytetracycline), sulphonamides (trimetroprim/ sulfamethoxazole), macrolides (azithromycin, clarithromycin, erythromycin), fluoroquinolones (ciprofloxacin) and antibiotics with high risk - aminopenicillines (amoxicillin, benzylpenicillin, flucloxacillin), cephalosporins (cefalexin, ceftazidime, cefuroxime).

The possibility of AAD should be considered in all patients with unexplained diarrhea who are receiving or who have recently received antibiotics. The tests used for diagnosis will depend on the kinds of laboratory tests that are available. Effective treatment is generally limited to discontinuation or changing of the implicated antibiotic and supportive management with fluid and electrolytes, if required. It depends on the severity of symptoms, the probability of Clostridium difficile infection (fever, lower quadrant abdominal pain, leukocytes in stool and peripheral leukocytosis) and the need for further antibiotic therapy.

Most of the AAD would respond to only discontinuation or changing of the antibiotic. In some cases of AAD, withdrawal of the responsible antibiotic will lead to resolution of clinical signs in three days. In the cases in which diarrhea continuous despite withdrawal of responsible antibiotic, antipseudomonal antibiotics should be prescribed with care. George et al reported a high risk of emerging resistance during treatment with cefotaxime, imipenem and piperacillin/tazobactam in a study on the incidence of Pseudomonas aeruginosa resistance to beta-lactam antibiotics in ICU patients.

Multidrug resistant Pseudomonas aeruginosa strains result from convergence of different resistance mechanisms such as low outer membrane permeability, production of an AmpC beta-lactamase and the presence of numerous genes coding for different multidrug resistance efflux pumps as well as a high number of acquired resistance genes.

Prophylactic agents such as probiotics would be more beneficial, because these would decrease the medical complications and costs of treating the acute disease. Probiotics, defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host", include Streptococcus thermophilus, Enterococcus species, Saccharomyces species and various species of lactobacilli and bifidobacteria. Their effects consist in the normalization of unbalanced indigenous microflora, production of antimicrobial substances, local competition of adhesion receptors and nutrients and stimulation of antigen specific and nonspecific intestinal immune responses. The gut interacts with intestinal bacteria, both resident and ingested, to develop protective mechanisms (via improving gut barrier function and immune stimulation for defense) and appropriate, no exaggerated responses (via immune modulation and immune tolerance) to support host health. This is the reason why probiotics are efficient both in the treatment and in the prevention of disturbances in intestinal microflora and AAD.

Hand washing, isolation of the culprits and environmental decontamination are the factors that can prevent recurrences and reinfection. Avoiding usage of rectal thermometers from one infant to another, usage of vinyl gloves by medical stuff and hospital antibiotic policies are other factors that can help. The occurrence of AAD in hospitalized patients has been associated with increased length of stay, higher medical care cost, increased risk of developing another hospital acquired infections and increased mortality.

STUDY LIMITATIONS

First, the sample size was small; a larger study is required to confirm our results.

Second, a causal relationship between the presence of organisms in the stool and diarrhea is difficult to establish. In the context of PAAD, the question as to whether such “opportunistic” agents are innocent bystanders or true pathogens responsible for AAD is crucial.

Third, the possibility of PAAD associated with Clostridium difficile had not been considered in this study and no endoscopy and biopsy or detection of Clostridium difficile toxins by enzyme-linked immunosorbent assay (ELISA) were done.
CONCLUSIONS

Diarrhea represents a major condition responsible for pediatric mortality worldwide. The onset of diarrhea may rapidly lead to life threatening dehydration and malnutrition. Thus, early diagnosis and timely treatment are both crucial in its management. The list of diseases and mechanisms responsible for diarrhea in children is large and the number of possible etiologies is high. This study underlines the fact that PAAD can be one of them. It is a challenging clinical condition, with heterogeneous risk factors (young age, antibiotics administration, duration of hospital stay or severe underlying disease), hard to prevent and with possible severe outcomes. What can we do to prevent it? Almost nothing. Therefore, pediatricians should be aware of this problem and they should prescribe broad-spectrum antibiotics only when there is a real need for them.

The results of our investigation showed that this type of diarrhea is relatively rare, because it is frequently misdiagnosed. Therefore, we believe that this article will help pediatricians to recognize it and to prevent diarrhea from becoming a severe clinical condition.

This is the first study describing PAAD in pediatric patients from our country and it will open the way for new investigations in this area of diarrhea diseases. Multicenter, clinical and epidemiologic studies defining severity and etiology are needed in order to improve diagnostic and therapeutic approaches for AAD.

REFERENCES