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Antimicrobial susceptibility profile of enterotoxigenic and enteropathogenic *Escherichia coli* isolates obtained from fecal specimens of children with acute diarrhea

Perfil de suscetibilidade a drogas antimicrobianas de amostras de Escherichia coli *enterotoxigênica e enteropatogênica isoladas de espécimes fecais de crianças com doença diarreica aguda*

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ABSTRACT

Among the diseases which etiopathogenesis is associated with *Escherichia coli*, acute diarrhea stands out. Studies on the characterization of the antimicrobial susceptibility profile contribute to the selection of appropriate empirical antimicrobial therapy. In this study, the antimicrobial susceptibility profile of 98 enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) strains isolated from fecal specimens of children with acute diarrhea was evaluated. The resistance rates to ampicillin, sulfamethoxazole/trimethoprim, amoxicillin/clavulanate, and nalidixic acid were high, ranging from 34.7% to 10.2%. The result of this research recommends the use of cefotaxime and ceftriaxone for the empirical treatment of children with acute diarrhea which the etiology suggested is ETEC or EPEC.

Key words: *Escherichia coli*; enteropathogenic *Escherichia coli*; enterotoxigenic *Escherichia coli*; diarrhea; antimicrobial resistance.

INTRODUCTION

Acute diarrhea is still a serious public health issue. It mainly affects children from developing countries, where poor hygiene and lack of access to basic sanitation allow their dissemination[1]. It is estimated that about 10% of deaths of children under five years of age are due to the disease[2].

The *Escherichia coli* species harbors extremely diversified microorganisms. Among the diarrheagenic *E. coli* pathotypes, enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) stand out. EPEC is associated with the development of a characteristic intestinal histopathological lesion, known as attaching and effacing (A/E), which generates a condition of watery diarrhea in the affected patient. ETEC, also an agent of watery diarrhea, colonizes the epithelium of the small intestine and produces enterotoxins, heat-labile (LT) and/or heat-stable (ST), which interfere with intestinal absorption and secretion processes[3].

The relevance of the use of antimicrobials, when indicated, is unquestionable. However, the incidence of infectious diseases associated with multidrug-resistant (MDR) bacteria and of cases for which effective antibiotic therapy is not available, has been increasing dramatically[4]. Multidrug-resistant microorganisms were much more frequently observed in health services, where the use of antimicrobials is broader. More recently, the occurrence of resistant bacteria in the community has spread, reaching high levels, for example, in urinary, respiratory and intestinal infections[5,6].

Considering the increasing challenge of the control of antimicrobial resistance in bacteria, the monitoring of the susceptibility profile to antimicrobial drugs of etiological agents of infectious diseases, highlighting the need to obtain regional data, is of great relevance. This is an important tool to subsidize health professionals in the choice of appropriate antibiotic therapy, especially when empirical treatment is ordinarily adopted, as is

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the case of acute diarrhea for specific groups of patients. The high prevalence of *E. coli* as a diarrheagenic agent, predominantly the ETEC and EPEC pathotypes, supports the choice of the organism. Therefore the aim of this study was to evaluate the antimicrobial susceptibility profile of ETEC and EPEC samples isolated from fecal specimens of children with acute diarrhea, in order to contribute to the establishment of a more effective empirical antibiotic therapy for the disease.

MATERIALS AND METHODS

We included in the study 98 samples of diarrheagenic *E. coli* isolated between 2004 and 2007, among them, 57 ETEC samples and 41 EPEC samples recovered from fecal specimens from children with acute diarrhea. The project was approved by the Research Ethics Committee of the Universidade Federal de Minas Gerais (UFMG) (opinion no. ETIC 047/03) and the parents or guardians of the children recruited signed the free and informed consent form. The children were of low socioeconomic level, were up to 69 months of age, had not used antimicrobial drugs in the last 15 days, and were attended at the Hospital Infantil João Paulo II, in Belo Horizonte (MG). Bacterial samples were identified through biochemical and physiological tests and the different diarrheagenic *E. coli* pathotypes were characterized by genotypic virulence markers.

The investigation of the susceptibility profile to antimicrobial drugs was performed by the agar diffusion method, according to the specifications of the Clinical and Laboratory Standards Institute (CLSI) (2015)[7]. Antimicrobials ampicillin, amoxicillin/clavulanate, cefotaxime, ceftriaxone, cefoxitin, amikacin, ciprofloxacin, sulfamethoxazole/trimethoprim and nalidixic acid were tested. The selection of the antimicrobial drugs considered their use in our region for empiric treatment of acute infectious diarrhea. As a control, the reference sample *E. coli* ATCC 25922 was used.

RESULTS AND DISCUSSION

The criteria for interpretation of the results considered the fact that, according to the CLSI (2015)[7], the "intermediate" category (or "intermediate resistance", term used in this study), is assigned to the relationship between antimicrobials and bacterial samples, for which the response rates in blood and tissue levels may be lower than those presented by susceptible samples. Therefore, since from the clinical view there is little distinction between the "intermediate" and the "resistant" categories, in this study, both are treated as resistant.

Considering all the bacterial samples and the antimicrobial drugs used, 882 antibiogram tests were performed. The resistance rate for ETEC was 15.2% [resistance (R) = 12.3% and intermediate resistance (IR) = 2.9%]. For EPEC, we observed a rate of 5.1% (R = 4.3% and IR = 0.8%).

As regards the ETEC samples, the highest resistance rates were observed for ampicillin (49.1%; R = 45.6% and IR = 3.5%), amoxicillin/clavulanate (28.1%; R = 7 and IR = 21.1%), sulfamethoxazole/trimethoprim (26.3%; all R), nalidixic acid (14%; all R) and ciprofloxacin (12.3%; R = 10.5% and IR = 1.8%). Regarding the EPEC samples, the highest resistance rates were observed for sulfamethoxazole/trimethoprim (19.5%; R = 17.1% and IR = 2.4%) and ampicillin (14.6%; all R) (**Figure**).

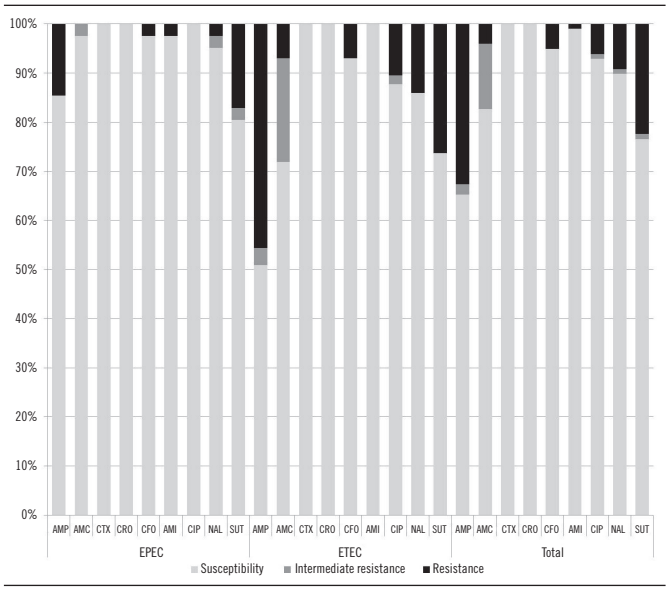

FIGURE – *Antimicrobial resistance susceptibility profile of ETEC and EPEC samples isolated from fecal specimens of children with acute diarrhea*

EPEC: enteropathogenic E. coli*; ETEC: enterotoxigenic* E. coli*; AMP: ampicillin; AMC: amoxicillin/clavulanate; CTX: cefotaxime; CRO: ceftriaxone; CFO: cefoxitin; AMI: amikacin; CIP: ciprofloxacin; NAL: nalidixic acid; SUT: sulfamethoxazole/trimethoprim.*

When the analysis considers the total of samples studied, it is possible to observe that the highest resistance rates were detected for ampicillin (34.7%; R = 32.6% and IR = 2.9%), sulfamethoxazole/trimethoprim (23.5%; R = 22.4 and IR = 1%), amoxicillin/clavulanate (17.3%; R = 4.1% and IR = 13.3%) and nalidixic acid (10.2%; R = 9.2% and IR = 1%). All samples showed susceptibility to cefotaxime and ceftriaxone.

Antimicrobial resistance is an emerging major worldwide problem observed in several bacterial groups, including *E. coli*. The species has high rates of resistance to antimicrobial drugs,

such as ampicillin, sulfamethoxazole/trimethoprim, tetracycline, chloramphenicol and nalidixic acid, which makes it difficult to establish an effective antibiotic therapy when necessary[8, 9].

Diarrheal disease is, as a rule, self-limiting, and rehydration therapy is the basis of the treatment. The indication of antibiotic therapy depends on several factors, such as the severity of the condition, the patient's immunological conditions and likelihood of infection transmission. Studies have shown that the use of antimicrobials may reduce the period of manifestation of some types of diarrhea[10]. The antibiotic therapy scheme, when indicated, is usually established empirically, which allows the immediate onset of treatment, which is essential in severe cases of the disease. Thus, studies aimed at assessing the susceptibility profile of diarrheagenic bacteria to antimicrobials are important, and may support the establishment of effective empirical antibiotic therapy.

The data obtained show resistance rates to ampicillin, sulfamethoxazole/trimethoprim, amoxiline/clavulanate, nalidixic acid and ciprofloxacin for ETEC, EPEC or both pathotypes considered high. These data are in agreement with previous reports[8, 9], which discourage the use of these antimicrobials. Thus, although these antimicrobial drugs have a history of indication for the treatment of diarrheal disease in our country, they should no longer be used due to the greater possibility of therapeutic failure resulting from antimicrobial resistance and their participation in the increase of resistance rates to them.

Our results suggest cefotaxime and ceftriaxone as the drugs of choice for the treatment of children with watery acute diarrhea, possibly associated with ETEC or EPEC. Third-generation cephalosporins have been considered appropriate for the empiric treatment of severe acute diarrhea in children[8].

Taking together, the data reinforce the need for constant monitoring of bacterial resistance and the adoption of procedures aimed at controlling the increase of antimicrobial resistance rates.

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RESUMO

Entre as doenças cuja etiopatogenia está associada à Escherichia coli*, destaca-se a doença diarreica aguda. Estudos que visam à caracterização do perfil de suscetibilidade antimicrobiana contribuem para o delineamento de antibioticoterapia empírica eficaz. Neste estudo, foi avaliado o perfil de suscetibilidade a antimicrobianos de 98 amostras de* E. coli *enterotoxigênica (ETEC) e* E. coli *enteropatogênica (EPEC) isoladas de crianças com doença diarreica. As frequências de resistência a ampicilina, sulfametoxazol-trimetoprima, amoxicilina-clavulanato e ácido nalidíxico foram elevadas, variando entre 34,7% e 10,2%. Esta pesquisa recomenda o emprego de cefotaxima e ceftriaxona para o tratamento empírico de crianças com quadro de diarreia cuja etiologia sugerida seja ETEC ou EPEC.*

Unitermos: Escherichia coli*;* Escherichia coli *enteropatogênica;* Escherichia coli *enterotoxigênica; diarreia; resistência microbiana a medicamentos.*

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