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Short Communication

Resistance To Beta-Lactamics in Gastroenteric Processes in the Brazilian Amazon

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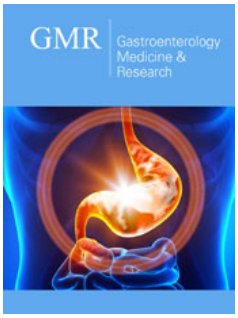

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Abstract

In recent years there has been a rapid increase in bacterial resistance in Brazil and worldwide. Among the mechanisms described, Extended-Spectrum ß-Lactamases (ESBL) are of great importance. The continuous assessment of bacterial resistance have fundamental importance in preventing the occurrence of outbreaks and the spread of resistance, especially in the Amazon due to the relative isolation of cities and difficulties in epidemiological surveillance in the region. In the present work we approach the use of phenotypic and genotypic characterization in *Enterobacterial* isolates by determining the resistance profile to β-lactam antimicrobials by antibiogram and the search for blaCTX-M, blaTEM, blaSHV genes associated with β-lactam resistance. Thus, 88 samples from outpatients from 14 regions in northern Brazil, belonging to the Brazilian Amazon, were analyzed from 2012 to 2014. Of these, *Salmonella Typhi* (57/64.77%), *Salmonella* spp. (26/29.54%), *Shigella flexneri* (2/2.27%), *Shigella dysenterie* (1/1.14%), *Shigella sonnei* (1/1.14%) and diarrhogenic *Escherichia coli* (1/1.14%). As for the resistance profile, only *Escherichia coli* showed resistance to first, second, third and fourth generation penicillins and cephalosporins and the aztreonam monobactamide, the other isolates did not show resistance profile according to the Clinical and Laboratory Standards (CLSI) standards. The evaluation of the blaCTX-M, blaTEM and blaSHV genes showed positive results only for the blaCTX-M gene in the *Escherichia coli* sample, with no amplifications being observed in the other analyzed samples. Although resistance was observed in only one sample, the data suggest the circulation of β-lactam resistance genes in *Enterobacteria* and, therefore, we suggest that continuous resistance evaluation can prevent the spread of β-lactamase-producing *Enterobacteria*, evidencing the importance of continuous monitoring and efficient strategies to reduce the spread of these agents by preventing outbreaks by resistant strains.

Keywords: β-lactamases; ESBLs; Antimicrobial resistance; *Enterobacteria*, PCR

Introduction

In recent years there has been a rapid increase in bacterial resistance in Brazil and worldwide, the occurrence of this phenomenon in regions such as the Brazilian Amazon is still poorly known, but they are already observed in populations in northern South America in hospital cases and in localized outbreaks [1,2]. The production of Extended Spectrum B-Lactamase (ESBLs) is an important mechanism of resistance in *Enterobacteria*. The emergence and spread of ESBL among family members has been described worldwide as a point of clinical urgency due to the high incidence of these isolates in infections related to health care, such as urinary and intestinal infections, pneumonia, septicemia, meningitis, among others [3,4]. The main genera producing ESBLs, among the *Enterobacteria*, are *Escherichia coli* and Klebisiella pneumoniae [5]. Currently, increasing resistance to antimicrobials is a highly complex problem for global health that is often associated with the widespread and sometimes indiscriminate use of antimicrobials that work in the selection and dissemination of resistance [6-8]. Resistance to the vast majority of beta-lactams, including carbapenemics in ESBL-producing and KPC-type bacteria, is often concomitantly associated with resistance to other classes of antimicrobials, such as aminoglycosides and fluorquinolones. Because of this, the rapid identification of strains that produce these enzymes is of fundamental importance in the selection of the appropriate antimicrobial for the treatment [9-11].

The need for speedy identification of antimicrobial therapy is due to the fact that bacteria have a great capacity to conserve and transmit plasmids, as well as other mobile elements that contain resistance genes, such as beta-lactamase genes, thus facilitating the spread of Anti Microbial Resistance (AMR). The presence of these resistance mechanisms has a direct interference in the treatment of the patient, reducing the therapeutic alternatives available,

arising the need to resort to the latest generation of antimicrobials, which increases the selective pressure and can cause the appearance of new types of resistance, restricting increasingly possible treatment options [12-16].

Methods

88 pathogenic strains isolated from species belonging to the Enterobacteriaceae family were analyzed, collected from 2012 to 2014 (2012-27 samples; 2013-19 samples and 2014-42 samples) from clinical cases treated in the Brazilian Amazon from 14 municipalities outpatients infected with pathogenic Enterobacteria. CAAE Ethics Committee: 52530916.0.0000.0019 Instituto Evandro Chagas (CEP/IEC/SVS/MS). The susceptibility to antimicrobial agents was assessed by the disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute- (CLSI, 2012) [17] and by the automated system Vitek 2 (BioMèrieux) following the manufacturer's recommendations. As controls for the susceptibility tests to antimicrobials, the strains American Type Culture Collection (ATCC), *K. pneumoniae* ATCC 700603 producer of ESBL and *E. coli* ATCC 25922 were used. For the extraction of bacterial DNA, the DNA IQ kit (Promega) was used, following the manufacturer's recommendations. For the amplification of the blaCTX-M, blaTEM, blaSHV genes, the primers described by Schmitt et al. [18], Nasehi et al. [19] and Edelstein et al. [20] were used. Each amplification reaction had a final volume 25µL, containing 20ng of DNA, 10Mm Tris-HCl, pH 8.5, 50mMKCl, 1.5/µM MgCl2, 1.25mM of each dNTP, 1.25mM of each primer and 0.5 unit of Taq DNA polymerase Platinum (Invitrogen). The amplifications were performed in a Vereti ™ 96-Well Thermal Cycler thermocycler (Applied Biosystems-US). After amplification, the samples were applied to a 2% agarose gel, observed in an ultraviolet transluminator and recorded in Bioimaging Systems (UPV, USA) detection systems, the molecular weight of the amplified fragments was measured using a 1Kb molecular weight marker, along with the positive and negative controls.

Results

It was observed that the occurrence of cases had a higher percentage distribution for males (57.35%) and the prevalent frequence among adults (67.04%) (Table 1). Among the isolates, the genus *Salmonella* was predominantly observed in 83(94.32%) of the 88 isolates belonging to this genus. Of these 57(64.77%) were identified as *Salmonella Typhi* and 26(29.54%) as *Salmonella spp* (Table 2). The results of the antimicrobial susceptibility profile showed that *Escherichia coli* was the only microorganism that showed resistance to penicillins and first, second, third and fourth generation cephalosporins and the monobactamide aztreonam. While *Salmonella Typhi*, *Salmonella spp*, *Shigella flexneri*, *Shigella dysenterie* and *Shigella sonnei*, did not present a resistance profile according to the CLSI (2012) [17] (Table 3). After PCR amplification to assess the presence of the ESBLs encoding genes blaCTX-M, blaTEM and blaSHV, amplifications were observed exclusively for the CTX-M gene and only in *Escherichia coli* (Figure 1), with no amplifications for the blaTEM and blaSHV investigated. All other samples analyzed did not show amplifications for the genes encoding ESBLs (blaCTX-M, blaTEM and blaSHV) analyzed.

Table 1: Age/gender distribution of patients infected with *Enterobacteria* between January 2012 and December 2014.

Groups	Age Category	Male		Female		Total	
		Nº*	(%)**	Nº*	(%)**	Nº*	(%)**
Children	*01-12*	10	11,36%	9	10,22%	19	21,59%
Adolescents	*13-18*	5	5,68%	0	0%	5	5,68%
Adults	*19-80*	35	39,77%	24	27,27%	59	67,04%
	N.I.	4	4,54%	1	1,13%	5	5,69%
Subtotals		54	57,35%	34	38,62%	88	100%

N.I= Not Informed

Table 2: Frequency of isolated microorganisms belonging to the Enterobacteriaceae family from 2012 to 2014.

Isolated *Enterobacteria*	Nº	(%)
Salmonella Typhi	*57*	64,77%
Salmonella spp.	*26*	29,54%
Shigella flexneri	*2*	2,27%
Shigella dysenterie	*1*	1,14%
Shiegella sonnei	*1*	1,14%
Diarrheagenic *Escherichia coli*	*1*	1,14%
TOTAL	*88*	100%

Table 3: Profile of susceptibility to antimicrobials among samples belonging to the Enterobacteriaceae family.

Antibiotics	*Salmonella Typhi*	*Salmonella Spp*	*Shigella Flexneri*	*Shigella Dysenterie*	*Shigella Sonnei*	*Escherichia Coli*
AMP	*S*	S	R	S	S	R
ASB	*S*	S	R	S	S	R
PPT	*S*	S	S	S	S	S
CA	*R*	R	R	R	R	R
CRX ACETIL	*R*	R	R	R	R	R
CAZ	*S*	S	S	S	S	I
CFO	*R*	R	R	R	R	S
CRO	*S*	S	S	S	S	R
CPM	*S*	S	S	S	S	R
ERT	*S*	S	S	S	S	S
IPM	*S*	S	S	S	S	S
MER	*S*	S	S	S	S	S
AM	*R*	R	R	R	R	S
GEN	*R*	R	R	R	R	R
CIP	*S*	S	S	S	S	R
TIG	*S*	S	S	S	S	S
COL	*S*	S	S	S	S	S
CFL	*S*	S	S	S	I	R
NAL	*S*	-	S	S	S	-
ATM	*S*	S	S	S	S	R
AMC	*S*	S	S	S	S	I
SUT	*S*	-	-	-	-	S
NIT	*R*	-	-	-	-	S
NOR	*S*	-	-	-	-	R

S=Sensitive; R=Resistant; AMP=Ampicillin; ASB=Ampicillin + Sulbactam; PPT=Piperacillin + Tazobactam; CRX=Cefuroxime; CA=Cefuroxime axetil; CAZ=Ceftazidime; CFO=Cefoxitin; CRO=Ceftriaxone; CPM=Cefepime; ERT=Ertapenem; IPM=Imipenem; MER=Meropenem; AM=Amikacin; GEN=Gentamycin; CIP=Ciprofloxacin; TIG=Tigecycline; COL=Colistin; CFL=Cephalothin; NAL=Nalidixic acid; ATM=Aztreonam; AMC=Amoxacillin + Clavulanic Acid; SUT=Sulfamethoxazole + Trimetroprim; NIT=Nitrofurantoin; NOR=Norfloxacin.

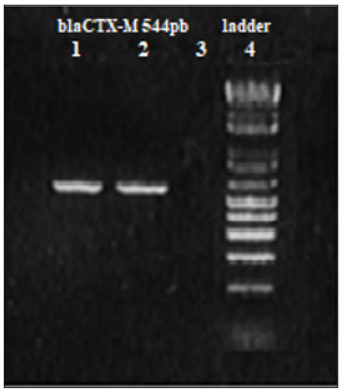

Figure 1: -2% agarose gel, blaCTX-M amplification in *Escherichia coli.*

A. Line 1: Positive control.

B. Line 2: blaCTX-M in *E. coli.*

C. Line 3: negative control.

D. Line 4: DNA Ladder 1Kb.

Discussion

Diarrheal cases are frequent in the Brazilian Amazon, especially in rainy periods [21], monitoring these cases is crucial to determine mitigation measures in the populations in order to prevent outbreaks [6]. Although samples of *Salmonella Typhi*, *Salmonella spp*, *Shigella flexneri*, *Shigella dysenterie*, *Shigella sonnei* have been shown to be sensitive to first and second generation cephalosporins and aminoglycosides, according to the CLSI (2012) [17]. For periodic analysis the phenotypic and molecular profile of antimicrobial resistance genes are crucial for the early detection of the horizontal transmission of the genetic components encoding ESBLs. [22-24]. Similar to the evidence in the early 2000s, where a study carried out on *Shigella* strains demonstrated resistance to tetracycline (93.4%) followed by chloramphenicol (63.9%), trimethoprim/sulfamethoxazole (63.1) and ampicillin (43.4%) [25] that previously identified allowed a quick and concrete action in the population. Diarrheal cases caused by diarrheal *E. coli* are often associated with children under 5 years old [26-28] and are a serious health problem in the Amazon. The observation of a sample of *Escherichia coli* producing ESBL by the blaCTX-M gene, with resistance to first to fourth generation cephalosporins, penicillin, aminoglycosides, fluoroquinolones, quinolones and aztreonam demonstrates the circulation of this gene in samples in the studied population. This shows that the detected *E. coli* has high levels of resistance to antibiotics currently prescribed, which leads to the therapeutic choice of carbapenem antibiotics. In a study involving 354 clinical isolates of *E. coli*, from five outpatient and hospital units, from October 2002 to May 2003, in Rio de Janeiro, 8 isolates producing ESBL were found, that is, in 2.2 % of those [29]. Nogueira et al. [30] studied 498 isolates from patients at a university hospital in Curitiba, from 2003 to 2004, and determined that 7.2% were *E. coli* producing ESBL, that is, 2.2% of these. In the same sense, Lago et al. [3] in their study of 838 bacterial isolates, from patients hospitalized in Passo Fundo, RS, from July to December 2007, 96 *E. coli* were identified and 11.4% of these were in our studies, in the molecular detection of the genes encoding the enzymes ESBL, the PCR technique revealed a gene of the researched ESBLs of the CTX-M type. Enzymes belonging to the CTX-M family have been predominant in South America, as well as in Spain and Eastern Europe [31]. The data show an increase in the percentage of isolation of ESBL-producing *E. coli* in Brazil, and the same can occur in the Brazilian Amazon, but the lack of studies in populations and the great distances associated with the isolation of cities makes surveillance difficult or unviable, however, monitoring as proposed in the present study can ensure a minimum of surveillance to determine the need for more concrete actions in the care of outbreaks as well as sporadic cases.

Conclusion

The results obtained through this study demonstrated the permanence and the spread of *Enterobacteria* that cause gastroenteritis, showing the importance of a continuous evaluation and efficient strategies to reduce the spread of these agents. The production of extended-spectrum beta-lactamases was evidenced, even if in a low proportion, this data is of concern, since in the clinic the production of ESBLs limits the effectiveness of beta-lactam antibiotics including extended-spectrum cephalosporins.

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