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Brief Original Article

Genetic determinants of pathogenicity of *Escherichia coli* isolated from children with acute diarrhea in Maputo, Mozambique

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Abstract

Introduction: Diarrheagenic *Escherichia coli* (DEC) represents one of the leading cause of diarrhoea in developing countries. In this study a molecular approach was applied for the detection of diarrheagenic *Escherichia coli* (DEC) circulating in Maputo, Mozambique.
Methodology: All isolates were PCR tested for species-specific genes and for 11 molecular markers: s*tx1, stx2, eae, bfpA, lt, st, ipaH, aap, aggR CVD432* and *daaE.*
Results: Of the 80 *E. coli* isolated, 74% were potential DEC: 21% EIEC, 19% EPEC, 15% EAEC, 13% ETEC, 5% DAEC and 1% hybrids.
Conclusion: This study revealed the complexity of the etiology of diarrhea caused by pathogenic *E. coli* in Mozambique, and the risk of the emergence of new pathogenic variants due to the horizontal transmission of pathogenicity factors.

Key words: Pathogenicity determinants; diarrheagenic *Escherichia coli;* DEC.

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Introduction

Infections caused by diarrheagenic *Escherichia coli* (DEC) constitute a major human health issue, especially in developing countries like Mozambique, where they represent a significant cause of morbidity and mortality [1,2]. Transmission occurs through contaminated food and water; moreover, environmental and climatic factors contribute to increased infection rates in several countries [3].

E. coli is a naturally occurring commensal of the human gastrointestinal tract, and plays an important role in the composition of intestinal flora [4]. However, pathogenic strains arise by acquisition through horizontal gene transfer of genetic determinants; these allow them to adhere, to, colonize and invade the gastrointestinal epithelial cells [5]. Several pathogenic *E. coli* are currently recognized and their classification depends upon mechanisms of pathogenicity and clinical and epidemiological manifestations: enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorragic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC), diffusely adherent (DAEC), and cytolethal distending toxin producing (CDTEC) [4].

Molecular characterization of *E. coli* pathotypes is essential for epidemiological surveillance with the aim of monitoring the insurgence of new potentially virulent variants. A surveillance based solely on biochemical criteria is insufficient since pathotypes are indistinguishable from their non-pathogenic commensal *E. coli* commonly found in the intestinal flora. Even serotyping of O and H antigens has limitation since not all isolates of a serotype are associated to pathogenicity [6].

Therefore, in this study, the presence of DEC in stool samples was screened using a molecular approach to identify 11 pathogenicity determinants characterizing each DEC. These were the "bundle-forming pili" (*bfpA*), and the intimin (*eae*) genes residing on the EAF plasmid and responsible for the attaching and effacing phenotype typical of EPEC; the Shiga-like toxins 1 and 2 (*stx1*, *stx2*) and the intimin

(*eae*) gene for EHEC; the thermolabile (LT) and thermostable (ST) enterotoxins for ETEC; the virulence plasmid *pInv*, required for invasion and dissemination in the host (*ipaH*) for EIEC; the dispersin (*aap/CVD432)* and regulatory (*aggR)* genes associated with EAEC and *daaE* designated fimbriae for DAEC [5].

The epidemiological impact of each pathotype of *E. coli* in childhood diarrhea varies with the geographic area, specifically in Africa [2,6-9]. In Mozambique, diarrhea is the third leading cause of death in children under 5 years of age, causing more than 13,000 annual deaths. However, there is a paucity of information at national level regarding the main specific agents causing diarrhea, and, to date, only three studies have been conducted on the molecular characterization of *E. coli* from children with diarrhea in Maputo (1998-1999) and Manica (2000-2001) [7,9-10].

Methodology

Stool samples were collected at Maputo Central Hospital (HCM) and transported for laboratory analysis to the Faculty of Medicine, University E. Mondlane (UEM), Maputo, Mozambique.

Faecal samples were cultured on MacConkey and Sorbitol MacConkey agar (Oxoid, Basingstoke, UK) for preliminary tests: Simmons citrate, indole, and urease Kligler and subsequently identified with the Enterotube (BBL ENTEROTUBE II). DNA extraction was performed using the alkaline lysis method [9].

Results

A total of 121 stool samples were collected from children with acute diarrhea treated at the pediatric service of Maputo Central Hospital between June and September 2012; as a result, 104 bacterial strains with microbiological characteristics of *E. coli* were isolated. Of these, 80 (66.9%) were confirmed to be *E. coli* by positive PCR amplification of *uidA* gene (Table 2).

The investigation of the pathogenicity determinants revealed a total of 59 (74%) DECs including 17 EIEC (21%), 15 EPEC (19%), 12 EAEC (15%), 10 ETEC (13%), and 4 DAEC (5%) (Table 2). In addition, 1 indefinite pathotype harboured a plurality of pathogenicity determinants (*aap/agg/CVD432/daaC-aafF*) normally found in pathotypes DAEC and EAEC (Table 2).

Table 1. Primers used to test for genetic determinants of pathogenicity associated with DEC.

DEC	Primers	target	Size (pb)	Reference
E. coli	F: CCAAAAGCCAGACAGAGT R: GCACAGCACATCAAAGAG	*uidA*	623	Rappelli *et al.*, 2005.
EHEC (STEC	F: GAAGAGTCCGTGGGATAACG R: AGCGATGCAGCTATTAATAA	*stx1*	130	Rappelli et al., 2005..
	F: GGGTACTGTGTGCCTGTTACTGG R: GCTCTGGATGCATCTCTGGT	*stx2*	510	Rappelli *et al.*, 2005..
	F: TGATAAGCTGCAGTCGAATCC R: CTGAACCAGATCGTAACGGC	*eae*	229	Rappelli et al., 2005..
EPEC	F: CACCGTTACCGCAGGTGTGA R: GTTGCCGCTTCAGCAGGAGT	*bfpA*	450	Rappelli *et al.*, 2005.
	F: TGATAAGCTGCAGTCGAATCC R: CTGAACCAGATCGTAACGGC	*eae*	229	Rappelli *et al.*, 2005.
ETEC	F: TCTCTATGTGCACACGGAGC R: CCATACTGATTGCCGCAAT	*lt*	322	Rappelli *et al.*, 2005.
	F: TCTTTCCCCTCTTTTAGTCAGTC R: CCAGCACAGGCAGGATTAC	*st*	170	Rappelli *et al*., 2005.
EIEC	F: GCTGGAAAAACTCAGTGCCT R: CCAGTCCGTAAATTCATTCT	*ipaH*	424	Rappelli *et al.*, 2005.
EAEC	aapF: CTTGGGTATCAGCCTGAATG aapR: AACCCATTCGGTTAGAGCAC	*aap*	310	Cerna *et al.*, 2003.
	aggRF: CTAATTGTACAATCGATGTA aggRR: AGAGTCCATCTCTTTGATAAG	*aggR*	457	Cerna *et al*., 2003.
	F: CTGGCGAAAGACTGTATCAT R: CAATGTATAGAAATCCGCTGTT	*CVD432*	630	Cerna *et al.*, 2003.
DAEC	daaCaafF: CCTGCGGGATGTTACT daaCaafR: GCCATCACATCAAAAA	*daaE*	333	Cerna *et al.*, 2003.

Table 2. Frequency of pathogenic *E. coli* strains isolated from fecal samples from children with acute diarrhea attended at the Central Hospital of Maputo, Mozambique.

	Count	%
Stool Samples	121	
E. coli	80	100
Non-DEC	21	26
DEC	59	74
EPEC	15	19
EAEC	12	15
STEC	0	0
ETEC	10	13
EIEC	17	21
DAEC	4	5
Indef.	1	1

Discussion

The aim of our investigation was the molecular characterization of DECs isolated from children with diarrhea in one of the largest hospital in Maputo.

Molecular analysis revealed the presence of a large proportion of pathogenic DEC accounting for 74% of *E. coli* isolated.

From this study, it appeared that the EIEC (21%) was the prevalent DEC identified with higher frequency when compared with two other studies in children in Mozambique [7,10], but comparable to prevalence reported from other countries in Africa and South America [12,13]. EPEC was the second most common DEC with a frequency of 19%, higher than previously reported from Mozambique [7,9-10]. However, studies in Brazil and Mexico have shown that 30%-40% of childhood diarrhea was attributed to EPEC [14]. Most EPEC strains isolated in this study were typical (*eae*$^+$, *bfpA*$^+$), while only two were atypical (*eae*$^+$, *bfpA*$^-$) confirming that atypical EPEC are consistently lower than typical in sub-Saharan countries, contrarily to recent observations in epidemiological studies from Mozambique [10,15,16].

The EAEC is an emerging enteropathogen especially in developing countries. In this study, we detected 12 (15%) of EAEC pathotype. Variable prevalence of EAEC in Mozambique has been reported in studies conducted by Mandomando (9.6%) [9] and Rappelli (22.8%) [7] in Manica and Maputo, respectively. Another study in children affected by diarrhea in Nigeria revealed a percentage of 7.84% of this DEC [3].

The prevalence of identified ETEC was relatively low (13%). This result is consistent with several studies, since this DEC usually affects foreign travelers moving to endemic areas [17,18]. Previous studies conducted in Mozambique reported an incidence of 4.5% and 6.8%, in 1998-1999, and 2000-2001, respectively [9-10].

The low prevalence of DAEC (5%) observed in this study may be related to this strain being an emerging DEC or to the lack of phenotypic tests to verify the pattern of adherence to HEp-2 or HeLa cells. *In vitro* analysis revealed a much higher prevalence (22.8%) of diffuse pattern of adherence of DAEC in a previous study from Maputo [7].

Consistently with previous studies in sub-Saharan Africa, our study revealed the absence of EHEC in the population tested [2,3,7]. This can be explained since in Africa EHEC pathotype is commonly found more in adults than in children [7].

Interestingly, our study disclosed an *E. coli* strain that could not be categorized in any known pathotype due to the presence of genes *aap/agg/CVD432* belonging to the AA plasmid of EAEC and genes *daaC-aafF* of the *afa* operon responsible for the biosynthesis of adhesins typical of the DAEC. This finding confirms the great adaptability of certain types of *E. coli* based on the genomic plasticity of this species. The ability to horizontally acquire genetic elements favours the establishment of new virulence properties, causing a broad spectrum of diseases and evolution of new pathotypes [4].

In conclusion, this study revealed the complexity of the etiology of diarrhea caused by pathogenic *E. coli* in children of Maputo and highlights the need for continued studies as a way to control the emergence of new pathogenic varieties as a basis for further epidemiological surveillance.

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