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Detection of integron-associated gene cassettes and other antimicrobial resistance genes in enterotoxigenic *Bacteroides fragilis*

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ABSTRACT

Twenty seven Enterotoxigenic *Bacteroides fragilis* (ETBF) strains isolated from children in Kolkata, India, were tested for their antimicrobial resistance, presence of integrons and resistance encoding genes. Almost all the strains (>90%) were resistant to two or more antimicrobials. About 59–92% of the strains were resistant to ampicillin, amoxicillin, streptomycin, tetracycline, ciprofloxacin and norfloxacin. Most of these antimicrobial agents have been used in the treatment of diarrhea and other infectious diseases. In addition, about half a number of strains (48–55%) were resistant to clindamycin, cefotaxime, ceftazidime, ampicillin/sulbactam and trimethoprim/sulfamethoxazole. Moxifloxacin and metronidazole resistance ranged from 30 to 40%. All strains however, were found to be susceptible to chloramphenicol and imipenem. Class 1 integrase (*intI*1) was detected in seven and class 2 integrase (*intI*2) in one of the twenty seven ETBF strains. Resistance gene cassettes carried by these integrons had different alleles of *dfr* or *aad* genes. Beside these integron-borne genes, other genes encoding different antimicrobial resistance were also detected. Resistance genes such as *cep(A)* and *tet(Q)* were detected in most of the ETBF strains. To the best of our knowledge, this work constituted the first extensive report from India on the detection of integrons and antimicrobial resistance genes in ETBF.

1. Introduction

In developing countries, diarrhea is one of the major causes of morbidity and mortality of children under the age of five years. Several pathogroups of bacteria, viruses and parasites are responsible for the diarrheal infection. The *Bacteroides* is one of the predominant bacterial groups in the human commensal gut microflora that plays a significant role in controlling many of the enteric pathogens [1,2]. The *Bacteroides* group is generally associated with the host's nutritional status as well as mucosal and systemic immunity [3]. However, certain strains of *Bacteriodes fragilis* termed enterotoxigenic *B. fragilis* (ETBF) are responsible for acute diarrhea and other inflammatory bowel diseases in children and travelers [4–6].

The problem of emerging antimicrobial resistance in ETBF has been reported in several countries [7–11]. Determination of antimicrobial resistance patterns of the pathogen(s) is important for clinical management of the infection and in the epidemiological surveillance. In our previous finding, we have demonstrated dissemination of different antimicrobial resistance genes among enteric pathogens due to extensive use of several antibiotics [12–14]. Susceptibility testing of pathogenic bacterial strains from diarrheal specimen is not routinely performed in India and the treatment is often relies on the decision of clinicians. This is partially because of acute nature of the infection that needs immediate treatment. It is in these contexts, the antimicrobial resistance pattern and the prevalence of resistance encoding genes in ETBF strains isolated from the children with diarrhea and urban communities in Kolkata, India has been undertaken. In addition, ETBF strains were also examined for the presence of integrons, which play important role in the acquisition of antibiotic resistance markers, in bacteria.

2. Materials and methods

2.1. Culture methods and bacterial strains

Twenty seven ETBF strains isolated from diarrheal children

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Table 1
List of target genes, primer sequences and PCR conditions used in this study.

Primer	Locus	Primer sequence (5′–3′)	Amplicon size (bp)	Reference	PCR condition	
					T^b	T^c
Int1-F	*intl1*	GTTCGGTCAAGGTTCTG	920	[14]	50	30
Int1-R		GCCAACTTTCAGCACATG				
Int2-F	*intl2*	ATGTCTAACAGTCCATTTTT	420	[14]	50	30
Int2-R		AAATCTTTAACCCGCAAAC				
5′CS (In-F)	Class 1 integron	GGCATCCAAGCAGCAAG	Variable	[14]	52	30
3′CS (In-B)		AAGCAGACTTGACCTGA				
Hep 51	Class 2 integron	GATGCCATCGCAAGTACGAG	Variable	[41]	60	60
Hep 74		CGGGATCCCGGACGGCATGCACGATTTGTA				
NIM-3	*nim*	ATGTTCAGAGAAATGCGGCGTAAGCG	458	[38]	72	60
NIM-5		GCTTCCTTGCCTGTCATGTGCTC				
strA-F	*strA*	CCA ATCGCAGATAGAAGGCAAG	580	[14]	65	30
strA-R		ATCAACTGGCAGGAGGAACAGG				
tet(A)-F	*tet(A)*	GGTCTTGCTCGTCTCGCTGG	690	[14]	62	30
tet(A)-R		AACGCCATCCATCCCCGTG				
tet(B)-F	*tet(B)*	CCTTATCATGCCAGTCTTGC	774		50	30
tet(B)-R		ACTGCCGTTTTTTCGCC				
tet(D)-F	*tet(D)*	TGGGCAGATGGTCAGATAAG	827		50	30
tet(D)-R		CAGCACACCCTGTAGTTTTC				
cat F1-F	*catA1*	AAGTTGGCAGCATTCACCCG	573	[14]	61	30
cat B2-R		TCGTGGTATTCACTCCAGAGCG				
sul1-F	*sul1*	TGGTGACGGTGTTCGGCATTC	790	[42]	62	30
sul1-R		GCGAAGGTTTCCGAGAAGGTG				
sul2-F	*sul2*	TTCGGCATTCTGAATCTCAC	822		50	30
sul2-R		ATGATCTAACCCTCGGTCTC				
faa-F	*aac(6′)Ib-cr*	GCAACGCAAAAACAAAGTTAGG	561	[14]	55	30
faa-R		GTGTTTGAACCATGTACA				
bla_{OxA-1}F	bla_{OxA-1}	GCAGCGCCAGTGCATCAAC	198	[42]	50	30
bla_{OxA-1}R		CCGCATCAAATGCCATAAGTG				
bla_{TEM}-F	bla_{TEM}	CATTTCCGTGTCGCCCTTATTCC	828	[14]	59	30
bla_{TEM}-R		GGCACCTATCTCAGCGATCTGTCTA				
qnrAF	*qnrA*	ATTTCTCACGCCAGGATTTG	516	[14]	64	60
qnrAR		GATCGGCAAAGGTTAGGTCA				
qnrBF	*qnrB*	GATCGTGAAAGCCAGAAAGG	476	[43]	64	60
qnrBR2		ATGAGCAACGATGCCTGGTA				
qnrCF	*qnrC*	GGGTTGTACATTTATTGAATCG	307		55	60
qnrCR		CACCTACCCATTTATTTTCA				
qnrDF	*qnrD*	CGAGATCAATTTACGGGGAATA	582	[44]	60	60
qnrDR		AACAAGCTGAAGCGCCTG				
qnrSmF	*qnrS*	GCAAGTTCATTGAACAGGGT	428	[14]	64	60
qnrSmR		TCTAAACCGTCGAGTTGCGCG				
tet(Q)-F	*tet(Q)*	TTGGCAAGACATACGAATCC	869	This study (AY515263[a])	55	30
tet(Q)-R		CACCGGCTGATTATTGG				
cepA-F	*cep(A)*	TATGTCCTGCCCTGGTAG	856	This study (L13472[a])	52	30
cepA-R		AATCTATCTGTTGCGTTAC				
cfxA-F	*cfx(A)*	TCATCTTGGTATTTTCATTGTTCC	809	This study (U38243[a])	52	30
cfxA-R		TAACAAATACCGCTAAGG				
bla_{CMY-F}	bla_{CMY}	ATAACCACCCAGTCACGC	631	[45]	55	30
bla_{CMY-R}		CAGTAGCGAGACTGCGCA				

Abbreviations: T^b, annealing temperature in °C; T^c, annealing time in sec.
[a] GenBank accession number.

admitted in the Infectious Diseases Hospital or B. C. Roy Memorial Hospital for children and from urban communities in Kolkata, India were included in this study. ETBF strains were isolated from the stool specimens using bile-esculin agar (BBE, Becton, Dickinson and Company, Sparks, MD) followed by incubation at 37 °C for 48 h in an anaerobic jar (BD GasPak EZ anaerobic systems). *B. fragilis* strains were identified by conventional methods [15] and ATB-32A system (bioMérieux, Marcy l'Etoile, France), followed by screening the strains for the presence of *bft* gene that encodes *B. fragilis* toxin [16]. The strains *B. fragilis* ATCC 25285 and a *bft* gene positive 520630 from our collection were used as the controls in all assays. Enterotoxigenic *Escherichia coli* strain E9 that harbored both *int1* and *int2* was used as a positive control strain in the Southern hybridization assay. This work was approved by the Ethical Committee of the National Institute of Cholera and Enteric Diseases, Kolkata, India.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the ETBF strains was tested using the E-test strips (bioMérieux-AB, Solna, Sweden) as previously reported [17–19]. The ETBF strains were first grown anaerobically on Colombia blood agar (CBA, bioMérieux) for 48 h at 37 °C. Inocula were prepared by suspending the ETBF cultures in sterile normal saline to a turbidity equivalent of 1 MacFarland standard (~3×10^8 CFU/ml), followed by spreading onto *Brucella* agar (Oxoid, Basingstoke, UK) supplemented with 5% lacked sheep blood, 5 mg/L hemin, and 1 mg/L vitamin K. E-strips were placed and the MIC values were recorded after anaerobic incubation at 37 °C for 48 h according to the manufacturers' instructions. E-strips of different antibiotics, namely amoxicillin (AMX), ampicillin (AMP), ampicillin/sulbactam (SAM), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), chloramphenicol (CHL), ciprofloxacin (CIP),