Microbial Pathogenesis 104 (2017) 12–16

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Distribution of resistance genetic determinants among *Vibrio cholerae* isolates of 2012 and 2013 outbreaks in IR Iran

Niloofar Rezaie, Bita Bakhshi*, Shahin Najar-Peerayeh

Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Article history:
Received 24 November 2016
Received in revised form
23 December 2016
Accepted 2 January 2017
Available online 3 January 2017

Keywords:
Vibrio cholerae
Antimicrobial resistance
SXT
Iran
Outbreak

ABSTRACT

The objective of this study was to characterize antimicrobial resistance determinants in relation to antimicrobial susceptibility and genotyping profile in 20 clinical isolates of *Vibrio cholerae*. All of the isolates were resistant to streptomycin. The second most prevalent resistance was observed to trimethoprim (75%), co-trimoxazole (60%), tetracycline (50%), and minocycline (45%). About 50% of the isolates fulfilled the criteria of Multi Drug Resistance (MDR) phenotype. None of the isolates carried *tet A, B, C,* and, *D* determinants. This finding shows that tetracycline resistance determinants recognized so far, does not satisfactorily describe the 50% tetracycline resistance phenotype in this study, suggesting the possible contribution of other not yet characterized resistance mechanisms involved. Class 1 integron, widely distributed among enteric bacteria, was not detected among *V. cholerae* strains under study. Conversely, 100% of the isolates harbored SXT constin$_{(int)}$, among which 70% were positive for *dfrA1*, *strA,* and *strB* genes. The *sul1*gene was present in 60% of the isolates while none of them contained *floR* gene. All the isolates uniformly appeared to be identical in fingerprinting profiles expected from outbreak strains. In conclusion, SXT element with its mosaic structure was the exclusive antimicrobial resistance determinant of clonal *V. cholerae* isolates taken from outbreaks of 2012 and 2013 in Iran.

1. Introduction

Vibrio cholerae is a Gram negative bacterium responsible for cholera outbreaks mainly in developing countries [1–3]. Cholera outbreaks are mainly caused by consumption of water and food contaminated with *V. cholerae* O1 or O139 serogroups. CTX bacterioghage which is integrated within the *V. cholerae* genome is responsible for cholera toxin expression [4].

Cholera treatment is rehydration with fluid replacement in combination with antimicrobial therapy [5]. Today, increase in antimicrobial resistance has become one of the most serious public health concerns worldwide. In this regard, some Asian countries are experiencing rapid increases in the prevalence of antimicrobial resistance in major bacterial pathogens [6]. Among several antimicrobial agents, tetracycline is one of the important antibiotics which are commonly used for bacterial infections, including cholera. Recently, resistance to this antibiotic is increasing due to its determinants carriage being on plasmids, transposons, and other mobile genetic elements [7].

Integrons particularly Class1 have fundamental role in spreading the resistance genes among Gram negative bacteria and in carrying several resistance gene cassettes under a functional promoter [8]. The SXT transmissible genetic element which can be located in *V. cholerae* genome, carries resistance genes including sulfomethoxazole, trimethoprim, chloramphenicol, and streptomycin as an integrative conjugative transposon (ICE) [9,10]. Moreover, some conjugative plasmids like R plasmid can be major carriers for antibiotic resistance including tetracycline with increasing frequency [11].

The contribution of different mobile genetic elements in cholera resistance emphasizes on the need for monitoring the molecular genotype of cholera outbreak isolates in order to assess their epidemiological correlation. Different molecular typing techniques have been used to characterize outbreaks or sporadic cases of cholera so far [12,13]. However, because of the speed in analysis, ease of use, and reproducibility of the process, PCR-based methods including RS-PCR (ribosomal gene spacer sequence-PCR) are frequently applied. This technique is based on the length and sequence polymorphisms in the spacers within the rrn loci, which

* Corresponding author. Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Jalal-Ale-Ahmad Ave., Tehran 14117-13116, Iran.
E-mail addresses: n.rezayi@modares.ac.ir (N. Rezaie), b.bakhshi@modares.ac.ir (B. Bakhshi), najarp_s@modares.ac.ir (S. Najar-Peerayeh).

http://dx.doi.org/10.1016/j.micpath.2017.01.005
0882-4010/

is repeatedly used to discriminate between different bacterial species and strains [14].

The objective of this study was to characterize antimicrobial resistance determinants in relation to antimicrobial susceptibility profile in clinical isolates of *V. cholerae*. Also, we aimed to determine the heterogeneity of the isolates taken from 2012 and 2013 outbreaks.

2. Materials and methods

2.1. Bacterial strains

A total of 20 suspected *V. cholerae* strains were randomly selected from cholera cases referred to major hospitals in Sistan-Baluchestan province of Iran during 2012–2013 outbreaks. The outbreaks were occurred during summer of each year (July to September). Conventional Biochemical assays including oxidase test, motility, growth in 0% NaCl, arginine dehydrolase, ornithine decarboxylase, methyl red, and indole test were done for the identification of strains [15]. A species-specific primer pair amplifying the conserve 16s-23s rRNA intergenic region was applied for molecular confirmation of *V. cholerae* isolates. *V. cholerae*ATCC14035 was used as a control in each biochemical and molecular assay.

2.2. Serogrouping and biotyping

Serogrouping of isolates was performed by O1 polyvalent and ogawa/Inaba monospecific antisera (Mast Diagnostics Ltd., Bootle, Merseyside, UK). Biotyping of the isolates was done by voges-proskauer (VP) test and hemolysis of sheep erythrocytes [15,16].

2.3. Antimicrobial resistance profile of isolates

Antimicrobial susceptibility testing was performed against 6 antimicrobial agents including tetracycline (30 μg), minocycline (30 μg), streptomycin (5 μg), chloramphenicol (30 μg), co-trimaxazole (25 μg), and trimethoprim (5 μg) according to CLSI guidelines. All antimicrobial agents were purchased from MAST company (Mast Companies, UK) and chosen to include representatives from antimicrobial classes related to genetic elements under study [17]. *Escherichia coli* ATCC 25922 was used as quality control for antimicrobial susceptibility assay.

2.4. Exploration of resistance determinants related to Class 1 integrons

Class 1 integron was explored among *V. cholerae* isolates under study using primers which specifically anneal to conserved integrase gene (intF/int-R) of Class 1 integrons. (Table 1). All of the conventional PCR reactions were performed in a 25 μL reaction mixture containing 1 × reaction buffer, 0.2 mm L^{-1}dNTPs, 0.5 mmL^{-1} MgCl2, 1 U of Taq polymerase, 25 pmL^{-1} of each primers, and 5 μL genomic DNA.

2.5. Conjugal self-transmissible integrating element (SXT constin) and related resistance genes and cassettes

The presence of SXT element was investigated by primers specifically chosen to amplify a conserve region of this element (sxt-F/sxt-R). The presence of resistance genes which may be located in SXT element was explored by primers specific for *sul1*, *strA*, *strB*, *floR*, and *dfrA* genes (Table 1).

2.6. Tetracycline resistance genes

Tetracycline resistance gene content of isolates was explored by *tetA, tetB, tetC*, and *tetD* specific primer pairs which are supposed to be more frequently associated with tetracycline resistance in *V. cholerae* (Table 1).

2.7. RS-PCR genotyping of V. cholerae isolates

To assess the clonal correlation of isolates taken from 2012 and 2013 outbreaks, RS-PCR was performed by a method described elsewhere [18]. Briefly, the process was carried out under an initial denaturation at 95 °C for 7 min, followed by 30 cycles of denaturation at 94 °C for 1 min, and annealing at 55 °C for 1 min with a final extension at 72 °C for 10 min. The amplification was carried out in a 25 μL reaction mixture containing 1 × reaction buffer, 0.3 mmol l^{-1}dNTPs, 1.2 mmol l^{-1} MgCl2, 1 U of Taq polymerase, 50 pmol l^{-1} of each primers, and 5 μL genomic DNA.

3. Results

3.1. Characterization of V. cholerae isolates under study

Conventional biochemical tests and molecular assay confirmed the identity of all suspected 20 *V. cholerae*, and an amplification band of 295 bp related to species-specific sequence of 16S–23S intergenic region was obtained for all of the isolates. Serogrouping and biotyping assays revealed that all of the isolates belonged to O1serogroup, Inaba serotype, and El Tor biotype.

3.2. Antimicrobial resistance profiles and related genetic elements

Antimicrobial susceptibility testing showed that 100% of the isolates were resistant to streptomycin. The second most prevalent resistance was observed to trimethoprim (75%). The least resistance was seen to co-trimoxazole (60%), tetracycline (50%), and minocycline (45%) while no resistance was revealed against chloramphenicol. Half of the isolates (50%) fulfilled the criteria of Multi Drug Resistance (MDR) phenotype with resistance to ≥3 antimicrobial classes (Table 2).

3.3. Distribution of integrons and tetracycline resistance genetic elements

Attempt to detect tetracycline resistance determinants revealed no *tetA*, *tetB*, *tetC*, and *tetD* genes among the isolates under study. Class 1 integron was also absent from all the isolates as no amplification was occurred by *int* primers, except for *Escherichia coli* and *Shigella sonnei* control strains producing an amplification band of 900 bp, which were further confirmed by sequencing.

3.4. SXT constin genetic element and related resistance cassettes

Conversely, 100% of the isolates produced an amplification band of 592 bp related to SXT $constin_{(int)}$, among which 70% were positive for *dfrA1*(O1 trimethoprim resistance), *strA*, and *strB* genes (streptomycin resistance) with amplification bands of 278 bp, 384 bp, 470 bp, respectively. The *sul1*gene (encoding sulfamethoxazole resistance) with an amplicon of 626 bp in size was present in 60% of the isolates while none of the isolates contained floR gene (resistance to chloramphenicol). SXT related genes correlating to antimicrobial susceptibility patterns are shown in Table 2.