

Molecular Characterization of Epidemic Isolates of *Vibrio cholerae* O1 by Arbitrarily primed PCR (AP-PCR)

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Abstract

Background: Epidemic and endemic cholera is a major public health problem for many countries. Aim of this study was to evaluate AP-PCR for investigation of clonal relatedness among the strains of *Vibrio cholerae* recovered from an outbreak occurred in different parts of Iran in 2005.

Methods: The study was conducted during the cholera outbreak occurred in some of provinces in Iran in summer 2005. Bacterial isolation and identification was carried out according to the standard bacteriological methods. Arbitrarily primed PCR (AP-PCR) used to study the genetic relatedness between the *V.cholerae* isolates.

Results: Thirty-nine isolates of *V.cholerae* O1 were identified. All isolates belonged to serotype Inaba. AP-PCR could differentiate the isolates into five groups. AP-PCR cluster types 1 and 2 were the most prevalent groups, accounting for 36% and 41%, respectively, of *V.cholerae* isolates.

Conclusion: The most of epidemic strains of *V.cholerae* O1 isolated in the year 2005 could be attributed to two predominant clusters including AP-PCR cluster types 1 and 2 accounting for more than 77% of isolates. In conclusion, a few epidemic clones were responsible for the apparently epidemic occurrence of cholera in provinces studied.

Keywords: *Vibrio cholerae* O1, AP-PCR, Molecular epidemiology

Introduction

Vibrio cholerae is a gram-negative bacterium that causes cholera (1). Cholera is a widespread, severe diarrheic disease which continues to be a global threat (2). Strains of *V.cholerae* belonging to serogroup O1 biotype El Tor and serogroup O139 have been described as causative agents of diarrhea and localized outbreaks (3).

In many countries, epidemic and endemic cholera is a major public health problem and continues to be an important cause of morbidity and mortality in many parts of the Asia and Middle East (4). Molecular approach has provided powerful tools for diagnosis, epidemiological surveillance and tracking bacterial pathogens during outbreaks (5). Many traditional and molecular typing methods such as, biotyping, phage typing, serotyping,

ribotyping, restriction fragment length polymorphisms, arbitrarily primed PCR (AP-PCR) and random amplified polymorphic-DNA(RAPD) typing have been used for the epidemiological investigation of outbreaks caused by *Vibrio* spp (6,7).

AP-PCR uses oligonucleotide primers with arbitrary sequences that can serve for the study of several bacterial species (8). Under standardized reaction conditions, it gives a distinct pattern for each sample analyzed, which can be compared with others (9-12). AP-PCR is less laborious and time-consuming than other DNA-based typing techniques and has economic advantages over other DNA-based techniques. This technique has been widely used for the typing of bacteria in epidemiological and ecological studies, and its advantages and disadvantages are well known.

Even though this method does not require prior special knowledge of molecular biology of the investigated organisms and specific sequence of DNA target, it can potentially detect polymorphisms and genetic relatedness among strains. The study of genetic changes of *V.cholerae* is important in understanding the epidemiology and evaluation of the cholera bacteria as cause of diarrhea. In addition, there is a few regional information about the epidemiology of cholera in Iran (13- 21).

In this study, we employed AP-PCR to characterize and compare the strains of *V.cholerae* isolated from an outbreak occurred in different parts of Iran, in summer 2005.

Materials and Methods

Bacterial Samples A total of 39 *V.cholerae* O1 isolates collected from the cholera outbreaks which occurred in some part of Iran in summer 2005 were analyzed (Table 1). The isolates had been identified by bacteriology laboratory of Pasteur institute of Iran. Cultures were minimally passed and stored in 20% glycerol Luria-Bertani broth at -70° C for further molecular investigation.

Extraction of genomic DNA Bacterial strains were grown aerobically in tryptocasein soy broth at 37° C. Two ml of overnight culture was centrifuged at 4,000 rpm for 20 min. The pellet was resuspended in 620 ml of lysis buffer (10 mM Tris-HCl, 50 mM EDTA, 100 mM NaCl, pH 8) containing 1% SDS and 0.4 mg/ml of proteinase K. The mixture was incubated for 1 h at 56° C and then at 100° C for 1 h. An equal volume of phenol/chloroform/isoamylalcohol was then added to mixture and centrifuged at 10,000 rpm for 10 min. The supernatant was added to an equal volume of chloroform and after centrifuging at 10000 rpm, the top layer was collected and DNA was precipitated with two volumes of cold isopropanol at -20° C for 10 min. The pellet was obtained by centrifugation for 20 min and washed with 1.5 ml of 70% cold ethanol. Finally the pellet was resuspended in 100 ml of TE 1X buffer (10 mM Tris-Cl, 1 mM Na₂ EDTA, pH 8) and 1 µl was used for AP-PCR reaction (22).

AP- PCR The AP-PCR fingerprinting was carried out using a primer with sequence of 5'-GTTTCGCTCC-3' as previously described (23, 24). DNA templates were amplified in a total reaction volume of 50 µl containing 2.5 U of AmpliTaq Gold thermostable polymerase (Roche), 50 pmol of each primer, 200 µM of each deoxynucleotide, 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), and 50 mM KCl.

Amplification was carried out with denaturation at 94° C for 5 min, followed by 40 cycles according to the following program: 94° C for 30 sec, annealing at 40° C for 1 min, and extension at 72° C for 1 min, plus a final extension of 10 min at 72° C to complete partial polymerizations. The PCR product was run and visualized in 2% agarose gels stained with ethidium bromide.

Table 1: Province distribution and AP-PCR cluster types of *V. cholerae* O1 strains

AP-PCR clusters	Number of isolates	Provinces			
		Golestan	Ghom	Zahedan	Tehran
1	14	6	5	1	3
2	16	3	3	1	8
3	1				1
4	2			1	1
5	1				1

Results

All the strains belonged to serotype Inaba. The most strains (36%) were isolated from the patients of Tehran Province followed by those from Golestan Province (23%). AP-PCR produced 2 to 7 amplified bands ranging from 200 to 1700 bp. DNA band of 600 bp was detected in all strains. As shown in Fig. 1, AP-PCR patterns designations were 1-5. Frequency of the isolates was 14, 16, 1, 2 and 1 for the patterns 1 to 5, respectively. The strains belonging to cluster types 1 and 2 were prevalent and distributed among all provinces while single isolate in the cluster types 3 and 5 were restricted to Tehran Province.

In comparison with cluster type 2, cluster type 1 contained an additional slight DNA band. The lowest number (two) of DNA bands were seen in the cluster type 3 represented only by a single strain isolated from Tehran. Five strains could not be typed by the AP- PCR. Table 1 shows the provinces distribution and AP-PCR cluster types of *V.cholerae* O1 strains studied.

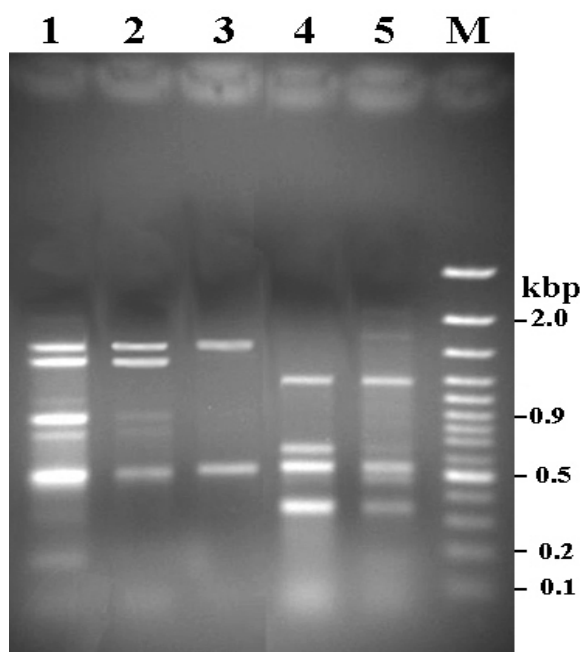


Fig. 1: Genomic typing by AP-PCR fingerprinting. Lanes 1-5 are representative patterns (1-5) of *V.cholerae* O1 strains. Lane M: molecular size marker

Discussion

V.cholerae is an important cause of diarrhoea in Iran, and restricted outbreaks of cholera have previously been reported in this country (19, 20).

In the past, the ability to differentiate individual strains of *V.cholerae* for epidemiological purposes has been hampered by the low discriminatory capability of available typing systems, such as biochemical and serologic identifications. The use of a discriminative molecular scheme for bacterial typing has allowed public health laboratories to follow the movement of *V.cholerae* strains and to identify their origins. The use of DNA-based typing methods would reveal epi-

demio relationships and the association of certain clones with potential sources of contamination (25- 27).

We used AP- PCR because of its many advantages such as its simplicity and shorter time consumption. The results showed that more than 77% of isolates belonged to two major AP-PCR clusters 1 and 2 that represented by 14 and 16 strains, respectively.

We concluded that the most of epidemic strains of *V.cholerae* isolated in the year 2005 could be attributed to these two closely clusters and a few epidemic clones are responsible for the apparently epidemic occurrence of cholera in the provinces studied. The results obtained from the current study will be useful to public health authorities in designing and implementing prevention and intervention strategies for cholera in Iran.

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