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Original Article

Genetic diversity of toxigenic *Vibrio cholerae* O1 from Sabah, Malaysia 2015

Myo Thura Zaw [a], Nor Amalina Emran [a], Mohd Yusof Ibrahim [b], Maria Suleiman [c], Tajul Ariffin Awang Mohd [d], Aza Sherin Yusuff [b], Khin Saw Naing [b], Than Myint [b], Muhammad Jikal [e], Mohd Azmi Salleh [f], Zaw Lin [a,*]

[a] *Pathobiological and Medical Diagnostics Department, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
[b] *Department of Community Medicine, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
[c] *Sabah State Health Department, Jalan Tunku Abdul Rahman, Bandaran, 88000 Kota Kinabalu, Sabah, Malaysia*
[d] *Kota Kinabalu Public Health Laboratory, Bukit Padang, Jalan Kolam, 88850 Kota Kinabalu, Sabah, Malaysia*
[e] *Kudat Area Health Office, 89509 Kudat, Sabah, Malaysia*
[f] *Pathology Department, Hospital Duchess of Kent, KM3.2 Jalan Utara, 9000 Sandakan, Sabah, Malaysia*

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KEYWORDS
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Abstract *Background:* Cholera is an important health problem in Sabah, a Malaysian state in northern Borneo; however, *Vibrio cholerae* in Sabah have never been characterized. Since 2002, serogroup O1 strains having the traits of both classical and El Tor biotype, designated as atypical El Tor biotype, have been increasingly reported as the cause of cholera worldwide. These variants are believed to produce clinically more severe disease like classical strains.
Purpose: The purpose of this study is to investigate the genetic diversity of *V. cholerae* in Sabah and whether *V. cholerae* in Sabah belong to atypical El Tor biotype.
Methods: ERIC-PCR, a DNA fingerprinting method for bacterial pathogens based on the enterobacterial repetitive intergenic consensus sequence, was used to study the genetic diversity of 65 clinical *V. cholerae* O1 isolates from 3 districts (Kudat, Beluran, Sandakan) in Sabah and one environmental isolate from coastal sea water in Kudat district. In addition, we studied the biotype-specific genetic traits in these isolates to establish their biotype.
Results: Different fingerprint patterns were seen in isolates from these three districts but one of the patterns was seen in more than one district. Clinical isolates and environmental isolate have different patterns. In addition, Sabah isolates harbor genetic traits specific to both

* Corresponding author.
E-mail address: zawlin@ums.edu.my (Z. Lin).

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classical biotype (*ctxB-1*, *rstR*^{Cla}) and El Tor biotype (*rstR*^{ET}, *rstC*, *tcpA*^{ET}, *rtxC*, VC2346).
Conclusion: This study revealed that *V. cholerae* in Sabah were genetically diverse and were atypical El Tor strains. Fingerprint patterns of these isolates will be useful in tracing the origin of this pathogen in the future.

Introduction

Cholera is a life-threatening acute diarrheal disease caused by gram-negative bacterium *Vibrio cholerae*.[1] *V. cholerae* is a normal resident of coastal and estuarine water. Such aquatic environment represents the potential reservoir of this pathogen especially in coastal areas.[2] Based on somatic O antigens, *V. cholerae* can be divided into more than 200 O serogroups. However, only the toxigenic strains of serogroups O1 and O139 can produce cholera toxin (CT) and cause cholera. *V. cholerae* O1 strains can be further divided into three serotypes - Ogawa, Inaba and Hikojima, but Hikojima serotype is rare and not even recognized by some authorities.[3] In addition, among O1 strains, two biotypes, classical and El Tor, are recognized based on phenotypic differences. Classical strains were responsible for the first six of seven major cholera pandemics that occurred since the begining of 19th century; however El Tor strains were responsible for seventh pandemic that began from Sulawesi Island, Indonesia in 1961.[1] In 1992, serogroup O139 emerged in India subcontient and spread across Asia but later replaced by O1 strains that continue to cause cholera until now.[4] Classical strains are associated with higher case-to-infection ratio compared to El Tor strains.[1]

Traditonally, El Tor strains are differentiated from classical strains by phenotypic traits.[5] However, genetic studies have revealed the biotype-specific alleles or genotypes of three genes — the *ctxB* gene encoding subunit B of CT, the *rstR* gene encoding CTXϕ-prophage repressor protein and the *tcpA* gene encoding major structural unit of toxin-coregulated pilus.[6] In addition, there are two genes specifically associated with El Tor strains, *rstC* and *rtxC*. The *rstC* gene is part of RS1 satellite phage, which is found only in El Tor strains and lies adjacent to CTXϕ prophage. This RS1 satellite phage is similar to the RS2 region of CTXϕ prophage but contain an additional gene, *rstC* that encodes the antirepressor protein promoting the expression of CTXϕ gene.[6] The *rtxC* gene is part of the *rtxABCD* gene cluster coding for a cytotoxin and found only in El Tor strains because classical strains have an internal deletion in this gene cluster disrupting the *rtxC* gene.[6] Although phenotypic differences are traditionally the basis of biotyping O1 strains, genotyping biotype-specific genes and the detection of El Tor-specific genes can also differentiate the two biotypes.

In 2002, Nair et al. reported new variants of *V. cholerae* O1 strains from cholera cases in Matlab, Bangladesh that have phenotypic and genotypic traits of both classical and El Tor biotypes.[7] Since then, several studies have reported these variants as responsible for cholera outbreaks in many countries.[8–15] Most important characteristic of these variants, designated as hybrid or atypical El Tor biotype, is that they possess the classical allele of *ctxB* gene in CTXϕ prophage and the El Tor genetic backbone harboring the El Tor allele of *tcpA* and El Tor-specific *rtxC* gene.[6] CT production by these atypical El Tor strains was reported to be higher than prototype El Tor strains and similar to that of classical strains, suggesting these strains might produce more severe diarrhea like the classical strains.[16]

Sabah is one of the two Malaysian states on Borneo island. Cholera is an important health problem in rural areas of Sabah. A total of 134 cases of cholera were reported throughout Sabah in 2014 with overall incidence rate of 3.8. Even a larger number was observed in 2015: a total of 234 cases were reported with overall incidence rate of 6.7.[17] However, *V. cholerae* isolates from Sabah have never been characterized. In this study, we investigated the genetic diversity of 66 *V. cholerae* isolates from three districts in Sabah — Kudat, Beluran and Sandakan, (Fig. 1) including 65 clinical isolates and one environmental isolate from coastal sea water. For this purpose, we used a method of repetitive element PCR that utilize the primers targeting the enterobacterial repetitive intergenic consensus (ERIC) sequence, referred to as ERIC-PCR.[18] As the number and distribution of these ERIC sequences differ among the bacteria of different origin, PCR assay with ERIC primers generates amplicons of different sizes with different bacterial isolates and the fingerprint pattern thus obtained can be used to differentiate bacterial isolates. This method has been successfully utilized in subtyping *V. cholerae* isolates from environment and food.[19–21] We chose this method because it does not require expensive reagents and sophisticated equipment. In addition, we performed the genotyping of three biotype-specific genes in these isolates, *ctxB*, *rstR* and *tcpA*, and screened these isolates for two El Tor-specific genes, *rstC* and *rtxC*, and VC2346 gene, which is a marker of El Tor strains causing seventh cholera pandemic.[22]

Materials and methods

V. cholerae isolates

V. cholerae isolates analyzed in this study included 41 clinical isolates from Kudat district, 16 clinical isolates from Beluran district, 8 clinical isolates from Sandakan district and one environmental isolate from coastal sea water in Kudat. The geographical locations of these districts in Sabah are shown in Fig. 1. All Kudat isolates were collected in January 2015. Beluran isolates were collected from

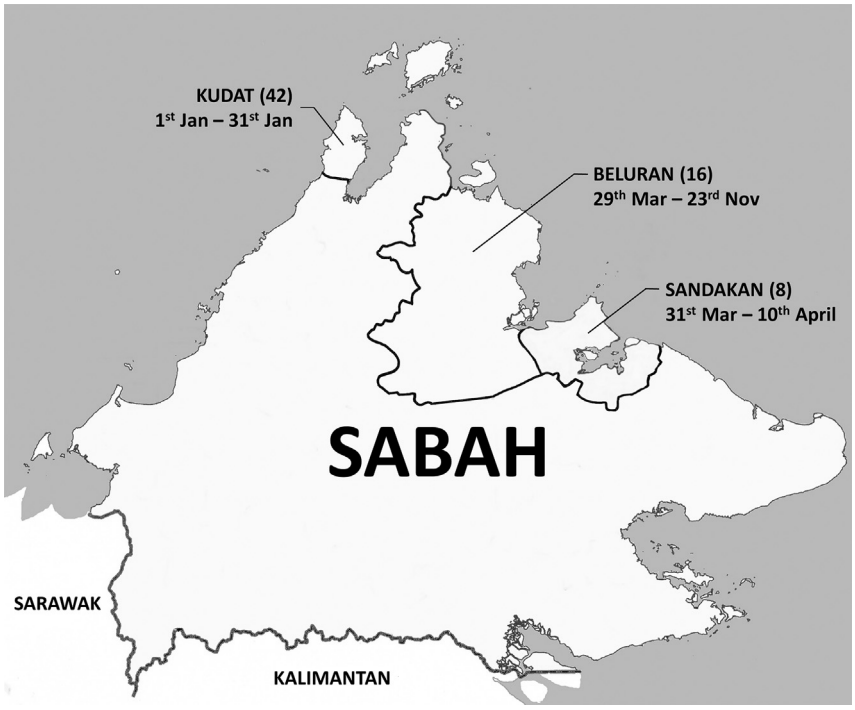

Figure 1. Map of Sabah showing Kudat, Beluran and Sandakan districts, which are the source of *V. cholerae* isolates analyzed in this study. Number in bracket indicates the number of isolates from each district analyzed in this study. Time of collection of isolates from each district is also mentioned. All of the isolates in this study are clinical isolates from rectal swab except for one isolate from coastal sea water in Kudat district.

March 29 to November 23, 2015, and Sandakan isolates were collected from March 31 to April 10, 2015. *V. cholerae* was isolated from rectal swab or sea water by basic bacteriological methods, confirmed as *V. cholerae* serogroup O1 by the slide agglutination test with polyvalent O1 antiserum (BD, New Jersey, USA), and subjected to serotyping with Ogawa and Inaba mono-specific antisera (BD, New Jersey, USA). Phenotypic tests for biotyping *V. cholerae* isolates were performed as described previously.[1]

Genomic DNA preparation

Bacteria were grown in Luria—Bertani broth (BD, New Jersey, USA) at 37 °C for 24 h, harvested by centrifugation at 6149 g for 2 min, and resuspended in distilled water. Bacterial suspension was boiled at 95 °C for 10 min to lyse the cells and release the DNA. Lysate was centrifuged at 6149 g for 10 min and supernatant was used as template DNA in PCR assays and fingerprinting analysis.

PCR assays

PCR amplification of *ompW*, O1 serogroup-specific *rfbN* sequence, *ctxA*, *ctxB*, *tcpA*, *rstR*, *rstC*, *rtxC*, VC2346 and *rfbT* genes was performed as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C, 57 °C, 58 °C, 60 °C, 62 °C or 66 °C (depending on primer sets as shown in the Table 1) for 1 min and extension at 72 °C for 1 min and final extension at 72 °C for 10 min in the total reaction volume of 30 μl containing 6 μl of template DNA, 1.5 μl of each primer (10 μM), 3 μl of 10x Taq buffer (Takara Bio Inc, Shiga, Japan), 2.5 μl of dNTP mix (2.5 mM each) (Takara Bio Inc, Shiga, Japan), 0.5 μl of recombinant Taq DNA polymerase (5 U/μl) (1st BASE Singapore Ltd, Singapore) and 15 μl of nuclease-free distilled water. PCR products were separated by electrophoresis in agarose gel and visualized by staining with ethidium bromide and UV illumination. The primers designed in this study were designed based on the sequences of whole genome-sequenced reference classical strain O395 (accession number CP000626.1 and CP000627.1) and reference El Tor strain N16961 (accession number AE003852.1 and AE003853.1) (Table 1).

Fingerprinting with ERIC primers

Fingerprint patterns were generated from each isolate by PCR amplification of genomic DNA with the primers ERIC1R (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′).[19] Amplification conditions were as follow: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 1 min, annealing at 45 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 20 min. The resulting amplicons were separated by electrophoresis in agarose gel and visualized as mentioned above. Fingerprint patterns obtained were analyzed using PyElph software[28] and dendrogram was deduced based on the unweighted pair group method with arithmetic means.

Sequencing analysis

The *ctxB, tcpA* and *rfbT* genes were amplified from genomic DNA with respective primers (Table 1) and the amplicons were sequenced with amplfication primers at First BASE

Table 1 Primers used in this study.

Target genes	Primers	Primer sequence (5'→3')	Amplicon size (bp)	Annealing temperature	Reference
ompW	ompW-F	CACCAAGAAGGTGACTTTATTGTG	588	58 °C	23
	ompW-R	GAACTTATAACCACCCGCG			
O1-specific *rfbN*	O1rfbN-F	GTTTCACTGAACAGATGGG	192	58 °C	24
	O1rfbN-R	GGTCATCTGTAAGTACAAC			
ctxA	ctxA-F	CTCAGACGGGATTTGTTAGGCACG	301	58 °C	23
	ctxA-R	TCTATCTCTGTAGCCCCTATTACG			
ctxB	ctxB-F	GGTTGCTTCTCATCATCGAACCAC	460	52 °C	25
	ctxB-R	GATACACATAATAGAATTAAGGATG			
tcpA	tcpA-F	CGACACCTTGTTGGTATTTT	878	60 °C	This study
	tcpA-R	TGACTTTGTGTGGTTAAATGT			
rstR	rstA-CF	CAAGAGTGAAAATCTGCTTTTTCAT	398 (Cla)	52 °C	This study
	rstR-1R (Cla)	GATTCAATCATTCGGATTTTCGATT	489 (ET)		
	rstR-2R (ET)	CTAAGCACCATGATTTAAGATGC			
rstC	rstC-F	AACAGCTACGGGCTTATTC	245	60 °C	26
	rstC-R	TGAGTTGCGGATTTAGGC			
rtxC	rtxC-F	CGACGAAGATCATTGACGAC	263	57 °C	27
	rtxC-R	CATCGTCGTTATGTGGTTGC			
VC2346	VC2346-F	ATGGATGGCTCACTTTTT	624	62 °C	This study
	VC2346-R	TCAGTCATTCAAAGATTGTTC			
rfbT	rfbT-F	TTATTGGTCAACAATGCCCTT	1066	66 °C	This study
	rfbT-R	AATTCACAGCACATCGCTAT			

Cla, classical; ET, El Tor.

Laboratories Sdn. Bhd. using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, USA). The *rfbT* amplicon from environmental isolate SA/1/886/K/2015 from coastal sea water was 2122 bp in size and required further sequencing analysis with two internal primers: rfbTintF (5′- AGCTTACTAAACCAAGAGTTCTCA -3′) and rfbTintR (5′-CACTCTGGGAAAACAATGCC -3′).

Results

It was revealed that all clinical *V. cholerae* isolates analyzed in this study belonged to serogroup O1 and Ogawa serotype except for the environmental isolate from sea water in Kudat, which was Inaba serotype. Before fingerprinting analysis, all isolates were further confirmed as toxigenic *V. cholerae* serogroup O1 by PCR detection of species-specific *ompW* gene, O1 serogroup-specific *rfbN* gene sequence and *ctxA* gene encoding the subunit A of CT using the respective primers (Table 1). All the isolates were positive for these genes. Fingerprinting analysis with ERIC primers showed that 8 fingerprint patterns (FPs) were observed among these isolates (Fig. 2). FP 1 was seen in all 41 clinical isolates from Kudat district (Table 2). Interestingly, the environmental isolate from coastal sea water in Kudat district showed a fingerprint pattern different from FP 1, which was designated as FP 2. Diverse fingerprint patterns (FP 3, FP 4, FP 5, FP 6, FP 7, FP 8) were seen among clinical isolates from Beluran. In contrast, a single fingerprint pattern (FP 3) was seen in Sandakan isolates. As mentioned in Table 2, FP 1 is the predominant pattern and it is seen in the majoritiy of isolates, more than 62%, analyzed in this study but seen only in clinical isolates from Kudat district. FP 3 is the second most dominant pattern accounting for nearly 23% of all isolates and seen in isolates from Beluran as well as Sandakan. FP 2, FP 4, FP 5, FP 6, FP 7 and FP 8 are seen in the remaining 15% of isolates. The only Inaba isolate, the environmental isolate from sea water in Kudat, was associated with FP 2, and this pattern was different from those of clinical isolates which are Ogawa serotype.

The sequencing analysis of *rfbT* gene amplicon and the database search using amplicon sequence as the query revealed that *rfbT* gene in clinical isolates was identical to the *rfbT* gene in whole genome-sequenced reference *V. cholerae* Ogawa strain EL-1786 (accession number CP003069.1 and CP003070.1) but the *rfbT* gene in environmental isolate was 2122 bp in length and thus required the primer working analysis with two internal primers, rfbTintF and rfbTintR to obtain the compete sequence. Upon database search, *rfbT* gene in this isolate was found to contain an 1261 bp insertion in 5' end relative to the reference *rfbT* gene in EL-1786 and be completely identical to the *rfbT* gene in Inaba strain GX06002 (accession number JX565687) from Guangxi Province, China, which was reported to contain the transposase *orfAB* gene at A^{41}ACC site.[3]

Biotyping of *V. cholerae* O1 strains traditionally involve the study of phenotypic traits so we did performed two phenotypic tests, polymixin B sensitivtiy and Voges-Proskauer test. Result revealed that Sabah isolates were resistant to polymixin B and gave positive reaction in Voges-Proskauer test, indicating that they are phenotypically similar to El Tor strains. Since genotypic traits are commonly studied for biotyping *V. cholerae* O1 strains these days, we performed the genotyping of biotype-specific genes, *ctxB* gene, *rstR* gene and *tcpA* gene, and PCR secreening of El Tor-specific genes, *rstC*, *rtxC* and VC2346 genes. PCR amplification and sequencing analysis of

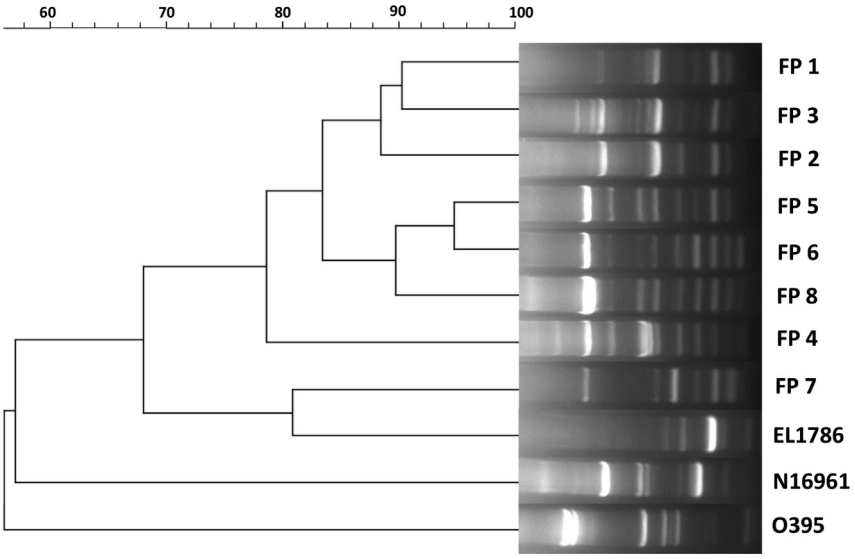

Figure 2. Eight fingerprint patterns observed among *V. cholerae* isolates from Sabah in 2015 and the dendrogram constructed using the unweighted pair-group method with arithmetic means based on these fingerprint patterns. EL1786, N16961, O395 are the reference strains for atypical El Tor, El Tor and classical strain respectively.

Table 2 ERIC-PCR fingerprint patterns among *V. cholerae* isolates from Sabah.

Fingerprint pattern	Number of isolates (%)								
	All	Kudat	Sandakan		Beluran				
		2015 January	2015 March	2015 April	2015 March	2015 April	2015 July	2015 October	2015 November
FP 1	41 (62.12)	41 (62.12)	—	—	—	—	—	—	—
FP 2	1 (1.52)	1 (1.52)	—	—	—	—	—	—	—
FP 3	15 (22.73)	0	2 (3.03)	6 (9.09)	3 (4.55)	—	1 (1.52)	2 (3.03)	1 (1.52)
FP 4	2 (3.03)	—	—	—	—	1 (1.52)	—	1 (1.52)	—
FP 5	2 (3.03)	—	—	—	—	2 (3.03)	—	—	—
FP 6	2 (3.03)	—	—	—	—	2 (3.03)	—	—	—
FP 7	1 (1.52)	—	—	—	—	1 (1.52)	—	—	—
FP 8	2 (3.03)	—	—	—	—	1 (1.52)	—	1 (1.52)	—

ctxB gene and *tcpA* gene revealed that Sabah isolates possessed *ctxB-1*, classical allele of *ctxB* gene, and *tcpA*^{ET}, El Tor allele of *tcpA* gene. PCR assay with specific primers for *RstR* gene showed that both classical and El Tor allele of *rstR* gene, *rstR*^{Cla} and *rstR*^{ET}, were present in our isolates. Furthermore, our isolates were also positive for El Tor-specific *rstC* and *rtxC* genes, and VC2346, which was considered the specific marker of El Tor strains causing the seventh cholera pandemic. In other word, Sabah isolates possessed genetic elements from both classical and El Tor strains and belonged to the recently described atypical El Tor or hybrid biotype.

Discussion

We choose the ERIC-PCR to study the genetic diversity of *V. cholerae* O1 isolates from Sabah because it has been shown to produce more bands and consequently be more discriminatory with *V. cholerae* than other PCR-based DNA fingerprinting methods targeting ribosomal gene spacer (RS) sequence and repetitive extragenic palindromic (REP) sequences.[20] In addtion, ERIC-PCR has been the proven method for subtyping *V. cholerae* O1 strains and utilized in a number of studies to investigate the genetic diversity of *V. cholerae*.[19–21] According to our fingerprinting analysis, the same fingerprint pattern (FP 1) is seen among the Kudat isolates suggesting that these isolates are genetically identical and clonal in origin, and indicating that cholera cases in Kudat district represent an outbreak resulting from the transmission from a common source. Diversity of fingerprint patterns among Beluran isolates indicates that they are genetically diverse and multiple clones of *V. cholerae* O1 were responsible for cholera cases in Beluran. One probable reason is that Beluran isolates were collected over a longer period of time from March 2015 to November 2015 compared to Kudat isolates which was collected within January 2015. The presence of a single fingerprint pattern (FP 3) among Sandakan isolates indicates that they are genetically identical and belong to a single clone arising from the common source. Actually FP 3

was also observed in Beluran isolates but this was not surprising because two districts were not too far away from each other.

V. cholerae is a resident of marine environment and such enviroment could be the source of this pathogen in coastal areas. Although the environmental isolate from Kudat district was isolated from coastal sea water near the place where the cholera cases were reported, it could not be the source of clinical cases given the difference in fingerprint pattern and serotype. This is further confirmed by the sequencing analysis of *rfbT* gene in this isolate and representative clinical isolates from Kudat district. The *rfbT* gene encodes an enzyme required for the expression of Ogawa-specific antigen and the changes in this gene are associated with serotype conversion to Inaba.[3] Accordingly *rfbT* gene is conserved in Ogawa strains but it harbors polymorphism in Inaba strains and is even suggested as the tracing marker in studying the clonality of *V. cholerae*.[3] Our results reveal that the clinical isolates in our study have the *rfbT* gene completely identical to that of reference *V. cholerae* Ogawa strain EL-1786 and the environmental isolate has the *rfbT* gene completely identical to that of a Chinese Inaba strain GX06002. It is interesting to see in one of our Sabah isolates the *rfbT* gene mutation causing Ogawa-Inaba serotype conversion from a Chinese strain. Anyway, this result confirmed that this toxigenic environmental isolate represented neither the source of *V. cholerae* causing cholera in Kudat district nor the result of fecal contamination of coastal sea water by cholera cases, and instead simply represented a distinct clone existing in the coastal sea water of Kudat. However, it contributed further evidence to the observation that toxigenic *V. cholerae* O1 strains were present in such aquatic environment.

As mentioned earlier, hybrid or atypical El Tor biotype of *V. cholerae* O1 strains having both classical and El Tor traits has emerged worldwide. So we studied the biotype-specific genes, *ctxB*, *rstR*, *rstC*, *tcpA* and *rtxC* in 18 representative Sabah isolates including the environmental isolate from sea water in order to establish their biotype. The *ctxB* gene coding for the subunit B of CT is the most important biotype-specific genetic element in *V. cholerae* O1 strains. It was even suggested that difference in *ctxB* gene and subunit B of CT between two biotypes was responsible for difference in severity of diseases caused by classical and El Tor strains.[6] Previous studies have revealed that nine different genotypes of *ctxB* gene exist and are associated with different serogroups; *ctxB-1*, *ctxB-2*, *ctxB-3* and *ctxB-7* in O1 strains; *ctxB-3*, *ctxB-4*, *ctxB-5* and *ctxB-6* in O139 strains; *ctxB-8* and *ctxB-9* in toxigenic non-O1 and non-O139 strains.[6] Among *ctxB* genotypes seen in O1 strains, classical strains are associated with *ctxB-1* while El Tor strains are associated with *ctxB-3* except for El Tor strains from Australia and US Gulf Coast, which possess *ctxB-2*.[25] *ctxB-7* allele is a relatively new genotype similar to *ctxB-1* and first detected in strains from Orissa, Eastern India in 2007.[29] Sequencing analysis revealed that *ctxB* gene in representative Sabah isolates was completely identical to that of reference classical strain O395, indicating that Sabah isolates possessed *ctxB-1* genotype associated with classical strains. As for the biotyping of *rstR* gene encoding CTXϕ phage repressor protein, we performed duplex PCR assay with allele-specific primers (Table 1). The result revealed that Sabah isolates were positive for both classical and El Tor alleles of *rstR* gene, $rstR^{Cla}$ and $rstR^{ET}$, indicating two different copies of *rstR* gene were present in the genomes of Sabah isolates. Actually, such strain harboring $rstR^{Cla}$ and $rstR^{ET}$ had already been detected in Thailand isolates collected between 1986-1992.[30] As mentioned earlier, *rstC* gene encodes a CTXϕ phage-related protein and is found only in El Tor strains that possessed the RS1 satellite phage together with CTXϕ phage. This *rstC* gene was also detected in Sabah isolates indicating the presence of El Tor-specific RS1 satellite phage in these isolates.

The *tcpA* gene in Sabah isolates was completely identical to that of reference El Tor strain N16961, indicating that El Tor allele of *tcpA* gene, $tcpA^{ET}$, was present in Sabah isolates. In fact, *tcpA* gene in classical and El Tor strains have only 78% identity at nucleotide level. Moreover, these isolates were positive for El Tor-specific *rtxC* gene. These two results together suggested that Sabah isolates had El Tor genetic backbone. Furthermore, we screened for VC2346 gene, which was considered the confirmatory marker for seventh pandemic El Tor clone and all isolates were positive for this gene further confirming the El Tor lineage of Sabah isolates. Table 3 compares the biotype-specific genetic traits in representative Sabah isolates and whole-genome sequenced reference strains. MJ-1236 (accession number CP001485.1 and CP001486.1) is one of the *V. cholerae* O1 strains from Bangladesh first described as atypical El Tor.[7] EL-1786 is an atypical El Tor strain from 2010 cholera outbreak in Haiti.[13] Our Sabah isolates possessed El Tor genetic backbone, as reflected by presence of $tcpA^{ET}$, *rtxC* and VC2346, together with CTXϕ prophage that harbored both classical biotype-specific elements (*ctxB-1*, $rstR^{Cla}$) and El Tor-specific elements ($rstR^{ET}$, *rstC*). These findings indicated that *V. cholerae* O1 isolates from Sabah did not belong to either classical or El Tor biotype, but were indeed atypical El Tor or hybrid strains.

During seventh cholera epidemic, classical strains were replaced by El Tor strains. Now again, atypical El Tor strains emerged and replaced prototype El Tor strains in many countries. It would be possible to investigate the time of emergence of these strains in Sabah if the collection of clinical *V. cholerae* isolates from the past two or three decades were available. In addition, biotype-specific genes, *ctxB* and *tcpA*, are useful markers of clonal dissemination. The $tcpA^{ET(CIRS)}$ is a variant of $tcpA^{ET}$ having a single nucleotide polymorphism (A→G) at nucleotide position 266 and first

Table 3 Biotype-specific genes in *V. cholerae* O1 isolates from Sabah in comparison with reference strains.

Strain (s)	*ctxB*	*tcpA*	*rstR*	*rstC*	*rtxC*	VC2346
Sabah isolates	*ctxB-1*	ET	Cla+ET	+	+	+
O395[a]	*ctxB-1*	Cla	Cla	−	−	−
N16961[b]	*ctxB-3*	ET	ET	+	+	+
MJ-1236[c]	*ctxB-1*	ET	Cla	−	+	+
EL-1786[c]	*ctxB-7*	ET(CIRS)	ET	+	+	+

[a] Classical.
[b] El Tor.
[c] Atypical El Tor.
Cla, classical; ET, El Tor.

described in Bangladesh strain CIRS101.[31] As mentioned earlier, *ctxB7* allele was first described in India.[29] *V. cholerae* strains positive for *ctxB-7* and/or *tcpA*$^{ET(CIRS)}$ alleles had globally disseminated from their origin in Indian subcontinent and are seen in the country as far as Haiti.[13] However, surprisingly, such strains were not observed among Sabah isolates.

Conclusion

This study revealed that *V. cholerae* isolates from Sabah in 2015 were genetically diverse and there were eight genetically different clones among these isolates. This study is an example of how fingerprinting with ERIC primers can be utilized to study the genetic diversity of this pathogen and trace the source of infection. Although variation in reagent and gel electrophoresis system may compromise reproducibility in repetitive element PCR methods including ERIC-PCR, this will not be an issue if the use of same reagent and equipment is ensured. This fingerprint pattern of isolates in this study will serve as the baseline in tracing the source of infection in future cholera outbreaks; however, this will require the continuous surveillance of *V. cholerae* strains circulating in the area. In addition, this study also documented the existence of *V. cholerae* O1 atypical El Tor strains in Sabah. Classical strains are more likely to produce severe diarrhea whereas asymptomatic infections are more frequently observed with El Tor strains.[6] However, concrete evidence is still lacking on the clinical spectrum of infection with atypical El Tor strains. Further studies should focus on this aspect of these variants and Sabah will be one of the places that is suitable for conducting such studies.

Conflicts of interest

Authors in this study do not have any conflict of interest to declare.

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