

Isolation and Characterization of Cholera Toxin Gene-Positive *Vibrio cholerae* Non-O1/Non-O139 Isolated from Urinary Tract Infection: A Case Report

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Background: Urinary tract infection (UTI) caused by *V. cholerae* is rare and less common. *V. cholerae* is a Gram-negative bacterium motile using single polar flagellum and, originally, is a waterborne microbe found in aquatic and estuarine environments. Toxigenic *V. cholerae* is well-known as a causative agent of acute and excessive watery diarrhea after ingesting food and water contaminated with this bacterium.

Case Presentation: A 27-year-old male patient presented to the emergency department on 17th July 2021 with burning micturition, normal vital signs, and no fever, vomiting, or diarrhea. In 2017, the patient complained of short stature and vitamin D deficiency. He was on human growth hormone from January 2018 till October 2019. The diagnosis was *V. cholerae* Non-O1/non-O139 urinary tract infection (UTI). Considering a urinary tract infection, empirical treatment with Lornoxicam and Ciprofloxacin was initiated, while the result of urine culture was still pending. The patient was discharged on the same day and without any complications.

Conclusion: *V. cholerae* non-O1/non-O139 is primarily a marine inhabitant and is associated with sporadic cases resulting in cholera-like diarrhea after consumption of contaminated seafood and exposure to seawater. Extraintestinal infection associated with this bacterium should no longer be ignored as this change in the behavior of cholera bacteria mechanism of pathogenicity might be related to some associated virulence genes.

Keywords: non-O1/non-O139 *V. cholerae*, virulence genes, extraintestinal infection, ERIC-PCR

Background

Vibrio cholerae is Gram negative-bacteria that habituate marine and estuarine environments. Cholera infection remains a potential public health problem, and it is not restricted to underdeveloped countries but has been seen in developing countries.^{1,2} Toxigenic strains of *V. cholerae* are responsible for causing acute dehydrating diarrhea that occurs in an epidemic form in many developing countries. *V. cholerae* is currently classified into more than 200 serogroups, and the toxigenic strains producing cholera toxin, that are responsible for epidemics and pandemics, belong to serogroups O1 and O139.³ Non-O1/non-O139 *V. cholerae* (NOVC), which does not agglutinate with O1 and O139 antisera, could also cause sporadic cases of gastroenteritis and other extraintestinal infections such as bacteremia, keratitis and wound infections.^{2,4-7}

The cholera toxin (CT) is the major secreted toxin by *V. cholerae* serogroup O1 and O139 and is responsible for severe watery diarrhea, which could lead to dehydration and death if left untreated.⁸ The other virulence factor is toxin-coregulated pilus (TCP), which is required for small intestine colonization.⁸ It has been documented that NOVC isolates are lacking CT and TCP, therefore, they establish infections through other virulence factors such as zonula occludens toxin (zot), accessory cholera enterotoxin (ace), and hemolysin (hlyA).^{9,10} Mechanism of extraintestinal infections caused by NOVC is not yet fully

understood, but it has been accepted that more than 85% of NOVCs have a capsule that may be involved in combination with other virulence factors in causing extraintestinal infections.¹¹ We here present a case of non-O1/non-O139 *V. cholerae* (NOVC) causing a urinary tract infection in a 27-year-old male patient. To our knowledge, this is the first case report in Saudi Arabia reporting the occurrence of urinary tract infection caused by *V. cholerae* non-O1/non-O139.

Case Presentation

A 27-year-old Saudi male from Al-Khobar City in the Eastern Province of Saudi Arabia presented to the emergency department at King Fahd Hospital of the University (KFHU) on 17th July 2021 with burning micturition. The patient had a history of swimming the day before in Halfmoon beach area situated on the coast of the Arabian Gulf. His urine analysis revealed negative nitrite, 3+ leukocytes, numerous white blood cells (30–50), red blood cells more than 200, and 2+ bacteria. Urine culture was done on blood agar plate and revealed β -hemolytic colonies. Bacterial colonies were identified as *V. cholerae* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), Biomérieux, France. The identification by MALDI-TOF MS was verified on thiosulfate citrate bile salts sucrose (TCBS) agar. Colonies on TCBS revealed sucrose fermenting yellow colonies of typical characteristic of *V. cholerae*, which was further confirmed using Vitek2 (Biomérieux, France). The serological of isolate was determined by slide agglutination test using *V. cholerae* O1 and *V. cholerae* O139 polyvalent antisera (MAST ASSURE Antiserum *Vibrio cholerae* polyvalent, UK). The isolate gave a negative result with both polyvalent antiserum and reported as non-O1/non-O139 *V. cholerae* strain according to the manufacturer's instruction. Antimicrobial susceptibility testing was carried out using Vitek2 (Biomérieux, France), and the isolate was resistant to ampicillin and susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole. The isolate was further confirmed as *V. cholerae* by PCR targeting the *V. cholerae* species specific *toxR* (Figure 1A) and *ompU* genes (Figure 1B) and strain tested positive for both genes. Furthermore, the strain was tested positive for major cholera toxin (*ctx*) gene and lack the TCP gene which is required for colonization (Figure 2). Multiplex-PCR analysis revealed *V. cholerae* strain was harboring gene encoding hemolysin (*hlyA*) which play significant role in disease mechanism and invasion, while it was lacking the zonular occludens toxin (*zot*) and accessory colonization enterotoxin (*ace*) genes (Figure 3). ERIC-PCR was used as genomic fingerprinting for determination of clonality and relatedness of isolated *V. cholerae* strain from urine with three *V. cholerae* control strains (Figure 4).

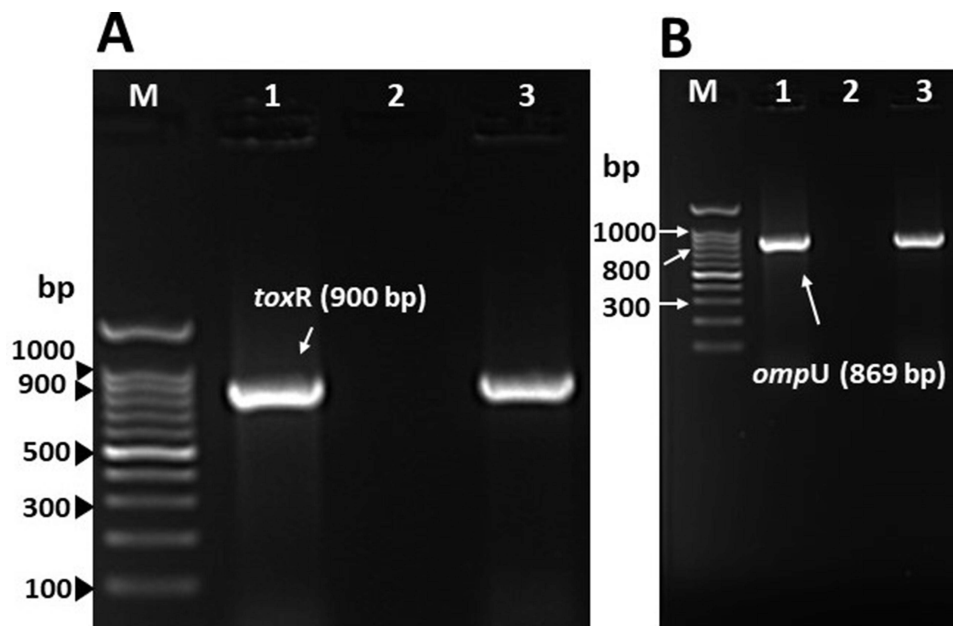


Figure 1 (A) PCR analysis for *toxR* gene of genomic DNA of *V. cholerae* non-O1/O139 strain isolated from urine specimen. Lane M, 100 base pair (bp) DNA ladder; lane 1, *V. cholerae* O1 Ogawa-Classical NIH41 (positive control for *toxR* gene); lane 2, negative control; lane 3, *V. cholerae* non-O1/O139 strain isolated from urine specimen. (B) PCR analysis for *ompU* gene of genomic DNA of *V. cholerae* non-O1/O139 strain isolated from urine specimen. Lane M, 100 base pair (bp) DNA ladder; lane 1, *V. cholerae* O1 Ogawa-Classical NIH41 (positive control for *ompU* gene); lane 2, negative control; lane 3, *V. cholerae* non-O1/O139 strain isolated from urine specimen.

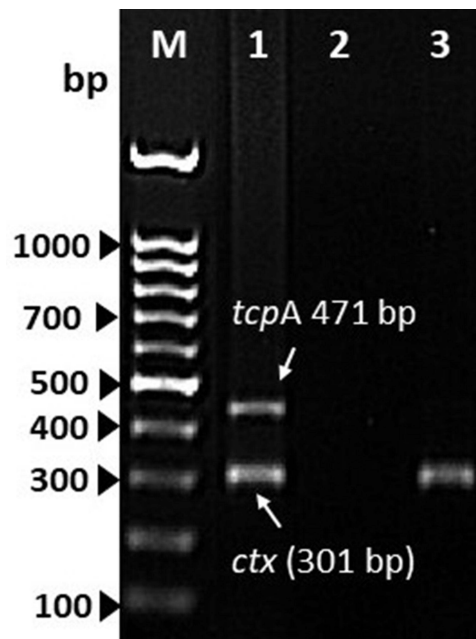


Figure 2 Multiplex-PCR analysis for *ctxA* and *tcpA* genes. Lane M, 100 bp DNA ladder; lane 1, *V. cholerae* O1 Ogawa-Classical NIH41 (positive control for *ctxA* and *tcpA* gene); lane 2, negative control; lane 3, *V. cholerae* non-O1/O139 strain isolated from urine specimen.

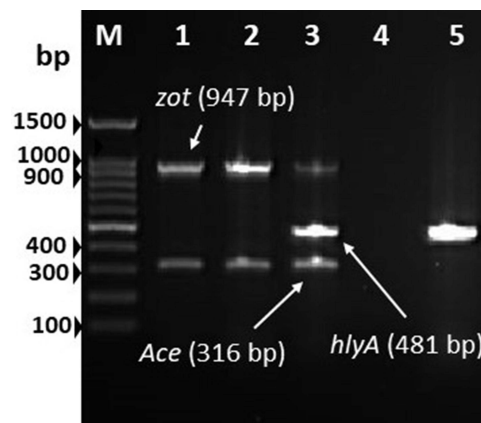


Figure 3 Multiplex-PCR analysis for *zot*, *ace* and *hlyA*. Lane M, 100 bp DNA ladder; lane 1, *V. cholerae* O1 Ogawa-Classical NIH41 (positive control for *ctxA* and *tcpA* gene); lane 2, negative control; lane 3, *V. cholerae* non-O1/O139 strain isolated from urine specimen.

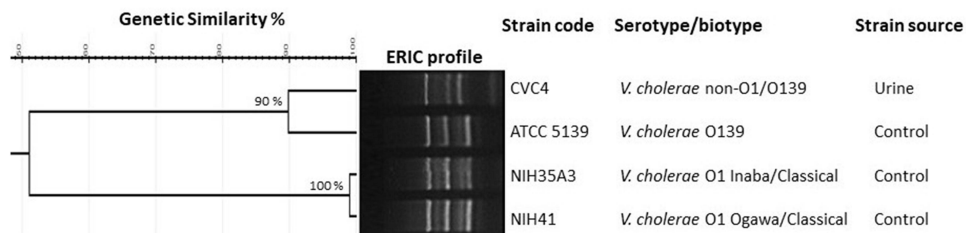


Figure 4 Dendrogram is based on the unweighted-pair group method with arithmetic means (UPGMA) and Pearson correlation coefficient, showing genetic similarity among toxigenic *V. cholerae* non-O1/O139 isolated from urine and reference control strains of *V. cholerae* O1 Ogawa-Classical NIH41, *V. cholerae* O1 Inaba-Classical NIH35A3, and *V. cholerae* O139 ATCC 5139. All strains were typed by ERIC-PCR DNA fingerprinting and electrophoretic patterns analyzed (UPGMA and Pearson correlation coefficient) with the Gelj software. Two control strains of *V. cholerae* O1 Ogawa-Classical NIH41 and *V. cholerae* O1 Inaba-Classical NIH35A3 with 100% genetic similarity, whereas toxigenic strain of *V. cholerae* non-O1/O139 isolated from urine showed 90% genetic similarity homogenous with control strain of *V. cholerae* O139 ATCC 5139.

Based on analyzed result of ERIC fingerprint patterns using UPGMA and Pearson correlation coefficient, isolated *V. cholerae* strains share 90% genetic similarity with the control strain of *V. cholerae* O139 ATCC 5139 (Figure 4).

Discussion

V. cholerae is a Gram-negative bacterium and highly motile by the means of single polar flagellum, whose natural habitat is usually costal water and marine environment. Among more than 200 serogroups that have been identified in *V. cholerae*, only O1 and O139 are related to cholera pandemic outbreak. *V. cholerae*, particularly serogroups O1 and O139, is well-known for causing severe watery diarrhea, a potentially life-threatening illness if left untreated. Transmission typically occurs through contaminated water and food. Additionally, non-O1/non-O139 *V. cholerae* and other non-cholera *Vibrio* species, such as *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, and *V. fluvialis*, can cause infections, usually acquired through consumption of contaminated seafood or exposure to seawater.^{12,13}

Both O1 and O139 serogroup produce cholera toxin (ctx) and toxin co-regulated pilus (TCP), which are the major virulence factors associated with *V. cholerae* infection. However, *V. cholerae* non-O1/non-O139 strains are associated with sporadic cases of diarrhea and extra-intestinal infections, and some of these strains have been reported to possess virulence factors encoding *zot*, *ace* and *hlyA*.^{9,10,14} In recent years, cases of extra-intestinal infections caused by *V. cholerae* non-O1/non-O139 are on the rise and have been reported from different parts of the world causing infections such as septicemia, meningitis, cellulitis, and keratitis.^{4,5,15,16} It is well-known that *V. cholerae* is the causative agent of cholera, a gastrointestinal disease, after ingestion of contaminated food or water, where the infection leads to voluminous watery diarrhea causing extreme dehydration. Non-O1/non-O139 may cause a cholera-like illness similar to pandemic *V. cholerae* serogroup O1 and O139, however, distinct pathways of pathogenesis mechanisms may exist.¹⁴ Some non-O1/non-O139 strains may harbor cholera toxin gene or produce similar cholera toxin that have been seen in most diarrheal cases. Both serogroups of pandemic *V. cholerae* O1 and O139 are a non-invasive intestinal bacterial pathogens and have never been isolated from body fluids other than fecal stool specimen, because these serogroups do not breach the mucosal surface of the intestine. In contrast, *V. cholerae* non-O1/non-O139 strains are potentially invasive and implicated in invasive infections leading to septicemia and other extra-intestinal infections.^{4,17}

In this case study, reporting the isolated strain harboring the *ctxA* gene encoding the A subunit of CT is considered important. Usually, strains of *V. cholerae* non-O1/non-O139 are lacking the pathogenicity island of CT and TCP but harbor other virulence factors such as heat stable enterotoxin, hemolysin, and zonula occludens toxin. The association of these virulence gene factors with extra-intestinal infections has been documented by several studies.^{18,19}

In Saudi Arabia, cases that are unrelated to international travel of extra-intestinal *V. cholerae* non-O1/non-O139 infections has been reported by several studies.^{6,20–23} *V. cholerae* strain in the current case was investigated and compared with control strains of *V. cholerae* O1 Ogawa (Classical biotype), *V. cholerae* O1 Inaba (Classical biotype) and *V. cholerae* O139 (Bengal) using ERIC-PCR to find the possibility of epidemiological link. Therefore, urine strain of *V. cholerae* was grouped with control strain of *V. cholerae* O139 and shared 90% genetic similarity. Further molecular epidemiology investigation of this strain and other strains of *V. cholerae* non-O1/non-O139 isolated from other intestinal and extra-intestinal infections including coastal water and sewerage on future studies is essential and should be conducted to identify the potential sources and route of infections. In addition, the possible effects of global warming on infectious diseases may broaden the endemic area of cholera infection since *V. cholerae* growth is abundance in warm waters of tropical and subtropical climate.

Conclusion

This study suggests that more attention should be directed towards the potential for future endemicity of *V. cholerae* in areas where few cases are currently reported. The current report emphasizes that *V. cholerae* non-O1/non-O139 should be suspected in patients who present symptoms of urinary tract infection. Moreover, similar infections may increase in the future in an area where *V. cholerae* is not endemic such as Saudi Arabia due to the climate change effects on infectious diseases. From reviewed case reports in the literature, several studies reported rare and unusual cases of extra-intestinal infections associated with *V. cholerae* non-O1/non-O139; therefore, more intensive studies are needed to understand this change in the behavior of *V. cholerae* non-O1/non-O139 from sporadic intestinal infections to broad extra-intestinal infections. However, this higher likelihood of a potential increase in *V. cholerae* non-O1/non-O139 extra-intestinal infection cases is reported globally, and

there is a possibility that this increase could be linked to the increased number of vulnerable patients who are immunocompromised and to the climate change. Clinical awareness in hospitals and laboratories is required to ensure optimal patient outcomes by considering *V. cholerae* non-O1/non-O139 might cause urinary tract infections after swimming exposure to seawater. To our knowledge, this is the first report of *V. cholerae* non-O1/non-O139 (*ctx*⁺) isolated from urine in Saudi Arabia.

Data Sharing Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics Approval

The patient had given its written informed consent for the use of his personal and medical information for the publication of this study. This study was approved by the Institutional Review Board (IRB) of Imam Abdulrahman Bin Faisal University (IRB approval number: IRB-2022-03-220).

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Disclosure

The authors report no conflicts of interest in this work.

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