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Vibrio parahaemolyticus-associated gastroenteritis in Italy: persistent occurrence of O3:K6 pandemic clone and emergence of O1:KUT serotype

Donatella Ottaviani[a,*], Francesca Leoni[a], Elena Rocchegiani[a], Cristina Canonico[a], Stefano Potenziani[a], Sabrina Santarelli[a], Laura Masini[a], Stefania Scuota[a], Antonio Carraturo[b]

[a]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Centro di Referenza Nazionale Controllo Microbiologico e Chimico Molluschi Bivalvi Vivi (CEREM), 60126 Ancona, Italy*

[b]*Laboratorio di Patologia Clinica, Ospedale Santa Maria Goretti, ASL Latina, 04100 Latina, Italy*

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Abstract

We report 2 cases of O3:K6 and O1:KUT *Vibrio parahaemolyticus* gastroenteritis associated with consumption of local mussels in Italy in 2008. Serotypic, antibiogram, toxigenic, and pulsed-field gel electrophoresis patterns of these strains were compared to those of other isolates collected from local clinical and seafood samples in 2007 to 2008. We underline the recurrent presence of O3:K6 pandemic clone and the emergence of *trh*-positive O1:KUT serotype in Italy.

Keywords: Pandemic *Vibrio parahaemolyticus*; Italy; Serotyping; Antimicrobials; Pulsed-field gel electrophoresis (PFGE); Gastroenteritis

Vibrio parahaemolyticus has been recognized as an important cause of foodborne illness, and its outbreaks have been associated with consumption of raw or undercooked shellfish (Potasman et al., 2002). *V. parahaemolyticus* O3:K6 serotype and its clonal derivatives with the O4:K68, O1:K25, or O1:KUT serotype have been linked to pandemic disease in Asia, Africa, and America since 1996 (Nair et al., 2007). The pandemic strains are characterized by the ability to rapidly spread, the presence of genes for the thermostable direct hemolysin (*tdh*) and the pandemic marker (*toxRS*), and by the lack of the TDH-related hemolysin gene (*trh*) (Matsumoto et al., 2000).

V. parahaemolyticus infections have been rarely reported in Europe in the past decades (Lemoine et al., 1999; Lozano-Leòn et al, 2003). Based on this, such microorganism is not included in the European Network for Epidemiologic Surveillance and Control of Communicable Diseases and in the Microbiological Surveillance System for Infectious Gastroenteritis. However, since 2001, pandemic *V. parahaemolyticus* O3:K6 has been detected from clinical samples in France, Russia, Spain (Nair et al., 2007), and Italy (Ottaviani et al., 2008).

Here, we report 2 new cases of acute gastroenteritis by *V. parahaemolyticus* that occurred in Central Italy in 2008, which are associated with consumption of local shellfish.

The first case involved a healthy 65-year-old woman in August 2008 (patient A), whereas the second case involved a healthy 55-year-old man in September 2008 (patient B). Both patients consumed local mussels purchased from different hawkers and cooked before consumption. However, mussels are often consumed by preference without sufficient cooking, and this habit may determine an insufficient thermal inactivation of *V. parahaemolyticus*. About 18 h later, patient A developed severe profuse diarrhea, abdominal cramps, nausea, and vomiting, whereas patient B experienced more severe symptoms, including hypotension, myalgias, fever, and diarrhea with mucus. Both were hospitalized because of dehydration and gastrointestinal pain. Epidemiologic information reported that both the patients did not travel recently to other countries. For the patient A, more details about the exact geographical origin of consumed mussels were not available. For the patient B, the

* Corresponding author. Tel.: +39-71-41760; fax: +39-71-42758.
E-mail address: d.ottaviani@izsum.it (D. Ottaviani).

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Table 1
Properties of *V. parahaemolyticus* isolates from clinical and seafood samples, Italy, 2007 to 2008

Isolate	Serotype	Source	Origin	*toxR*	*tlh*	*tdh*	*trh*	GS-PCR	Antimicrobial resistance
CEREM 38845	O3:K6	Stool	Diarrheal patient, Central Italy 2007	+	+	+	−	+	AMP, AMC, CEF, LEX
CEREM 40145	O3:K6	Stool	Diarrheal patient, Central Italy 2008	+	+	+	−	+	AMP, AMC, CEF, LEX
CEREM 36805	O1:KUT	Stool	Diarrheal patient, Central Italy 2008	+	+	−	+	−	AMP, AMC, CEF, LEX, COL
CEREM VS 50	O1:KUT	Mussels	Adriatic Sea, Central Italy 2008	+	+	−	+	−	AMP, AMC, CEF, LEX, COL

AMP = ampicillin; AMC = amoxicillin–clavulanic acid; CEF = cephalothin; LEX = cephalexin; COL = colistin.

eaten mussels came from the Adriatic Sea (Central Italy). Stool specimens were submitted for culture before patient treatment with intravenous hydration and appropriate antibiotics. Patients were treated with ciprofloxacin and recovered completely. They were discharged from the hospital after 48 h (patient A) and 96 h (patient B).

From the feces of both the patients were isolated Gram-negative, oxidase-positive, curved bacteria, in the absence of other enteric pathogens. The isolates, biochemically identified as *V. parahaemolyticus* (Ottaviani et al., 2003), were characterized by polymerase chain reaction (PCR) for the presence of the species-specific genes *toxR* and *tlh* (Kim et al., 1999; Wagley et al., 2008) and to detect the genes *tdh* and *trh* (Ottaviani et al., 2005). Group-specific (GS)-PCR method to detect the pandemic clone (Matsumoto et al., 2000) was performed. Lipopolysaccharide (O) and capsular (K) antigens were determined by the slide agglutination test using specific commercial antisera (Denka Seiken, Tokyo, Japan) according to the manufacturer's instruction. Pulsed-field gel electrophoresis (PFGE) (Wagley et al., 2008) was used as method of molecular subtyping. PFGE patterns were classified as identical, similar (differed by 1–3 bands), or distinct (differed by ≥4 bands), according to Tenover criteria (Tenover et al., 1995). Susceptibilities to 12 antimicrobial agents were examined using the disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). Antibiotics tested were as follows: amoxicillin–clavulanic acid, ampicillin, cephalothin, cephalexin, cefoperazone, ciprofloxacin, colistin, gentamicin, kanamycin, oxolinic acid, tetracycline, and trimethoprim/sulfamethoxazole. Interpretative criteria for each antibiotic tested were as published by CLSI (2006) guidelines or followed the recommendations of the antimicrobial agent suppliers.

The clinical isolate of August 2008 was identified as *V. parahaemolyticus* belonging to the pandemic clone of O3:K6 serotype. Its molecular and antimicrobial susceptibility profiles are summarized in Table 1. This strain was compared with the pandemic O3:K6 strain isolated in our laboratory in 2007 (Ottaviani et al., 2008), revealing that these were identical in antibiogram patterns and PFGE profiles (Table 1, Fig. 1). This report documents the second clinical isolation of pandemic *V. parahaemolyticus* O3:K6 in Italy, with local mussels as the most probable source of the infection. This evidence indicates that this clone is persistent in Italian coastal areas. Particularly, we underline the detection twice in Italy of pandemic *V. parahaemolyticus* O3:K6 with identical PFGE genomic fingerprinting in clinical samples. It remains to understand *V. parahaemolyticus* O3:K6 origin and how it has been introduced into Italian coastal waters. For this purpose, in future studies, we would like to compare the phenotypic and genotypic properties, including DNA fingerprints, of the Italian O3:K6 *V. parahaemolyticus* strains isolated from clinical sources with those from Italian environments (bivalve mollusks, seawater, etc.), including our previous isolates from mussels (Ottaviani et al., 2005).

The clinical isolate of September 2008 was identified as *V. parahaemolyticus* O1:KUT (untypeable) serotype. Its molecular and antimicrobial susceptibility profiles are

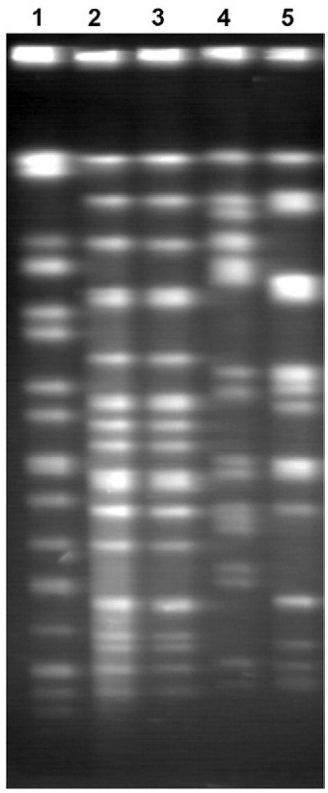

Fig. 1. PFGE patterns of *Not*I digests of O3:K6 and O1:KUT *V. parahaemolyticus* isolated from stools and mussels in Italy, 2007 to 2008. Lane 1, molecular mass marker of *Salmonella* serotype Braenderup H 9812; lane 2, clinical *V. parahaemolyticus* O3:K6 isolated in 2007 (CEREM 38845); lane 3, clinical *V. parahaemolyticus* O3:K6 isolated in 2008 (CEREM 40145); lane 4, clinical *V. parahaemolyticus* O1:KUT isolated in 2008 (CEREM 36805); lanes 5, O1:KUT *V. parahaemolyticus* isolated in 2008 from mussels (CEREM VS 50).