

Fast-Track Communication

First Clinical Report of Pandemic *Vibrio parahaemolyticus* O3:K6 Infection in Italy[▽]

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Vibrio parahaemolyticus is a gram-negative, halophilic bacterium widely distributed in coastal waters worldwide that is associated with gastroenteritis due to the consumption of raw or improperly cooked seafood. The thermostable direct hemolysin (TDH) and TDH-related hemolysin are the major virulence factors. In the past decades, diverse serotypes, including O3:K6, have been related to human gastrointestinal diseases. However, since 1996 the emergence of a new clone of serotype O3:K6 with pandemic potential, which has accounted for an increased incidence of cases in Asia, Africa, and America, has been reported (5). Previous studies have demonstrated that *V. parahaemolyticus* clinical isolates belonging to both the old and new O3:K6 clones have distinct molecular patterns but do not differ in biological characteristics such as TDH production and susceptibilities to antibiotics and environmental stresses (4, 10). In Europe, pandemic *V. parahaemolyticus* O3:K6 strains have been detected in clinical samples in France (8), Russia (5), and Spain (9).

Here, we report the characterization of a pandemic *V. parahaemolyticus* O3:K6 strain isolated from a stool sample of a diarrheal patient hospitalized in central Italy in the summer of 2007. The reported epidemiological information indicated that the patient had eaten fresh shellfish, bought from a local seller, 24 h before hospitalization. The microbiology laboratory of the hospital isolated a gram-negative, oxidase-positive curved bacterium in the absence of other enteric pathogens. The isolate, presumptively identified as belonging to the *Vibrio* genus, was sent to the Italian Reference Laboratory for Bacteriological Contamination of Shellfish (CEREM) for definitive identification. Suspect colonies grown on thiosulfate citrate bile salt agar (Oxoid Ltd., Basingstoke, England) were subcultured on Trypticase soy agar (Oxoid) with 1% NaCl and biochemically identified at the genus and species levels (6). Moreover, all sucrose-negative colonies were tested by PCR for the presence of *V. parahaemolyticus* species-specific genes (*toxR* and *tlh*) and the *tdh* and *trh* genes (3, 7). A group-specific PCR (GS-PCR) method, based on the sequence variation in the *toxRS* gene, was also performed to detect the pandemic clone (4). O (lipopolysaccharide) and K (capsular) serotypes were determined by using specific commercial antisera (Denka; Seiken Corp., Tokyo, Japan). The susceptibilities of the isolate to 11 antimicrobial agents were determined by the disk diffusion method on Mueller-Hinton agar plates according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (2). The antibiotics tested were amoxicillin-clavulanic acid, ampicillin, cefoperazone, cephalexin, cephalothin, colistin, gentamicin, kanamycin, oxolinic acid, tetracycline, and trimethoprim-sulfamethoxazole. The interpretative criteria to determine the susceptibilities to the tested antimicrobials were as published by the CLSI (2) or followed the recommendations of the antimicrobial agent suppliers.

The strain, identified by the biochemical protocol as *V. parahaemolyticus*, had the *toxR* and *tlh* genes and was serotyped as

O3:K6. This isolate was also characterized as *tdh* positive, *trh* negative, and GS-PCR positive (Fig. 1) and was resistant to amoxicillin-clavulanic acid, ampicillin, cephalexin, and cephalothin. The pattern of antimicrobial susceptibilities was similar to those of other O3:K6 clinical strains previously isolated (10). GS-PCR analysis indicated that the isolate was unequivocally linked to the pandemic O3:K6 clone of *V. parahaemolyticus* (4) (Fig. 1). In further investigations, this *V. parahaemolyticus* O3:K6 strain will be characterized by molecular typing methods in order to compare it with other pandemic strains of environmental and clinical origins circulating worldwide.

To our knowledge, this report documents the first clinical isolation of a pandemic *V. parahaemolyticus* O3:K6 strain in Italy, with local shellfish as the most probable source of the infection. This evidence, in association with the previous reports of the presence of the pandemic O3:K6 clone in other European countries, demonstrates the spread of this clone to Italian coastal areas. Recently, we isolated toxigenic *V. parahaemolyticus* strains, serotyped as O1:K untypeable (data not shown), from local shellfish in central Italy. Previous studies have shown the pandemic potential of this serotype and its close phylogenetic relationship to the new O3:K6 clone (4). Moreover, in May 2007, a *V. parahaemolyticus* strain with pandemic potential was detected in a seawater sample from the northern Adriatic Sea (Italy) (1). All this evidence should prompt official veterinary and health authorities to pay more attention to the epidemiological roles of environmental microorganisms in local food-borne diseases and to increase the microbial

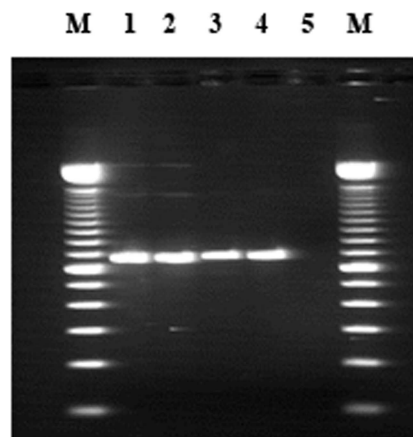


FIG. 1. Results of the GS-PCR assay. Lanes: M, molecular size markers (100-bp DNA ladder; Invitrogen); 1 and 2, *V. parahaemolyticus* O3:K6 strain isolated in this study; 3 and 4, *V. parahaemolyticus* ATCC BAA-238 (positive control); 5, PCR water (negative control).

surveillance of pathogenic *V. parahaemolyticus* strains isolated from environmental, seafood, and clinical sources.

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Donatella Ottaviani*

Francesca Leoni

Elena Rocchegiani

Sabrina Santarelli

Cristina Canonico

Laura Masini

Vittoria DiTrani

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche

Centro di Referenza Nazionale Controllo Microbiologico e Chimico

Molluschi Bivalvi Vivi (CEREM)

Via Cupa di Posatora 3

60126 Ancona, Italy

Antonio Carraturo

Laboratorio di Patologia Clinica

Ospedale Santa Maria Goretti

Azienda Unità Sanitaria Locale di Latina

Via Canova

04100 Latina, Italy

*Phone: 39-71-41760

Fax: 39-71-42758

E-mail: d.ottaviani@izsum.it

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