

Brief report

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First clinical isolation report of *Shewanella algae* from the stools of a patient with acute enteritis in Spain

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ABSTRACT

We report a case of acute enteritis caused by *Shewanella algae* in a cirrhotic patient. Biochemical identification systems revealed to be insufficient to identify the *Shewanella* isolate at the species level, thus requiring *16S rRNA* and *gyrB* partial gene sequencing. Even if co-infection by *Clostridium difficile* could not be ruled out, this is, to our knowledge, the first report of acute enteritis caused by *Shewanella algae* in Europe.

Key words: Enteritis, Molecular characterization, *Shewanella algae*, stools.

Primer aislamiento clínico en España de *Shewanella algae* en heces de un paciente con enteritis aguda

RESUMEN

Presentamos un caso de enteritis aguda causada por *Shewanella algae* en un paciente cirrótico. Los sistemas de identificación mediante pruebas bioquímicas no fueron adecuados para la identificación de *S. algae* a nivel especie, por lo que se requirió la secuenciación parcial de los genes *16S rRNA* y *gyrB*. Aunque la enfermedad debida a la co-infección por *Clostridium difficile* no podría ser descartado, este es, a nuestro entender, el primer caso de enteritis aguda causada por *Shewanella algae* en Europa.

Palabras clave: Enteritis, caracterización molecular, *Shewanella algae*, heces.

INTRODUCTION

More than 50 species conform the *Shewanella* genus. Among them, *Shewanella algae*, *Shewanella putrefaciens*, *Shewanella haliotis* and *Shewanella xiamenensis* can cause disease in humans with the former causing it more often [1]. The reason that explains the prominence of these species to cause disease, and *S. algae* being most abundant, are unknown. *S. algae* are a halophilic Gram-negative rod with a distinctive biochemistry: non-glucose fermenting, cytochrome oxidase positive, nitrate reduction test positive, catalase negative and H₂S producer. It can be found all over the world especially in seawater, aquatic animals and sediments. This microbe can cause disease in human beings. Case reports from the literature include soft tissue infections as preponderant, but also bacteremia, otitis and arthritis, among others [2]. Most of the cases come from warm coastal areas. Acute enteritis cases are scarce and have been reported in association with *Escherichia coli* as another pathogen [3] and as a chronic diarrhea case [4]. We present a case of acute enteritis that included *S. algae*.

CASE REPORT

We report the case of a 69 years old patient with liver cirrhosis, ischemic heart disease, high blood pressure and two admissions in the last two months. He lives in an urban area in southern Spain, 67 km away from the coast with his wife, retired for several years. Contact with wild animals, recent trips to the coast, consumption of unprocessed food, or contact with water or algae had not been reported.

The patient arrived at the emergency room with an acute onset, non-bloody diarrhea that had begun 7 days ago. In the next few hours, his condition deteriorated as he had developed shock, acute kidney injury, metabolic acidosis and hyperkalemia. He was then admitted to the ICU. After receiving adequate life support and empiric antibiotic therapy consisting of ciprofloxacin plus metronidazole, he recovered and was

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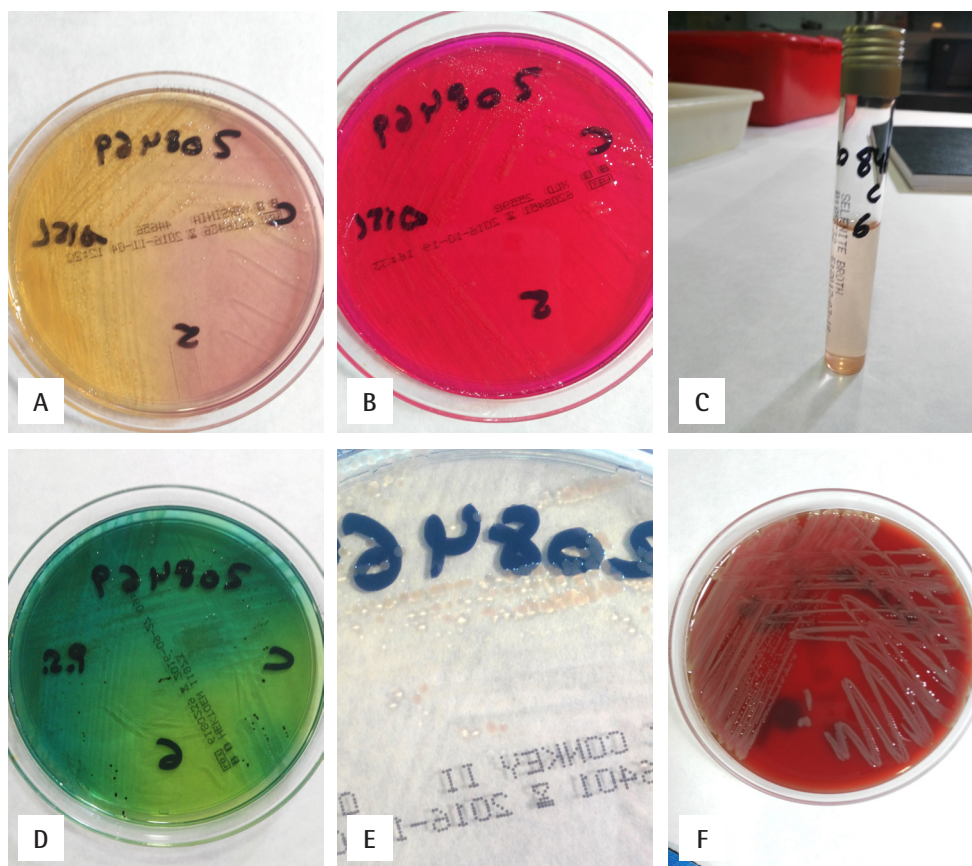


Figure 1 Growth of *Shewanella algae* on culture media (A = CIN, B = XLD, C = selenite broth, D = Hecktoen, E = MacConkey, and F = blood agar) for enteropathogenic bacteria.

transferred to the general Gastroenterology ward from where he was discharged. Stools samples obtained in the emergency room came back positive for *S. algae* and Toxin (A+B) *Clostridium difficile*. The *S. algae* isolate grew on all growth culture media that are commonly used for the isolation of enteropathogenic bacteria as well as on non-selective media like blood agar and in chromogenic medium specific for the isolation of uropathogens (Uriselet 4, BioRad, Madrid, Spain) (figure 1). The isolate was also found to be ornithine decarboxylase positive, indole negative, unable to produce acid from sucrose, and resistant to colistin. It was able to grow in 6.5% NaCl, to cause hemolysis and mucoid colonies in blood agar. *S. algae* grew at 42°C and were identified by mass spectrometry MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization, Bruker Daltonics, Madrid, Spain) (score 2.16). It was not identified by API 20NE (bioMérieux®, Marcy-l'Etoile, France) or MicroScan Walkaway (Beckman Coulter, L'Hospitalet de Llobregat, Spain).

As *S. algae* and *S. halotis* cannot be clearly differentiated on the basis of their biochemical characteristics or by MALDI-TOF [5], a molecular characterization of the isolate was conducted. Partial sequences of the *16S rRNA* and *gyrB* genes were obtained after PCR amplification with the primer pairs 8F

(AGAGTTTGATCCTGGCTCAG) / 1492R (GGTACCTTGTTACGACTT) [6], and SW-GYRB-F (GAAGTGGCKATGCAGTGGAA)/SW-GYRB-R (CGRCRAATACCACAGCCRAG) [7], respectively. Both sequences were deposited in the GenBank sequence database (NCBI) under the accession numbers KY774312 (*16S rRNA*) and KY774313 (*gyrB*), and the phylogenetic relationships of the isolate, hereafter designated as G1, were reconstructed (figure 2). Of note, *16S rRNA* sequencing revealed to be insufficient to provide identification at the species level (figure 2A). As shown in figure 2B, the taxonomical position of G1 falls univocally within the *S. algae* clade for the *gyrB* reconstruction.

Antibiogram performed by E-Test in Mueller-Hilton medium and aerobiosis using an inoculum matching a 0.5 McFarland turbidity standard revealed the following MICs (mg/L): ampicillin (0.125), ciprofloxacin (0.094), trimethoprim-sulfamethoxazole (0.19), cefotaxime (0.125), gentamicin (0.138) and colistin (3). The patient had another hospital admission due to acute diarrhea secondary to *C. difficile* infection one month after the reported incidence. A stool sample obtained one month later without the patient having signs of infection came back negative for *S. algae*.

Ethical statement. The study protocol was carried out

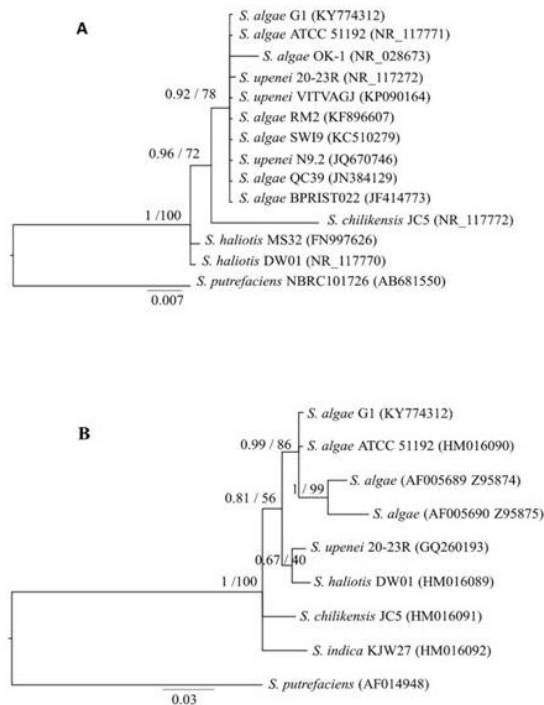


Figure 2 Bayesian phylogenetic trees of isolate G1 and reference strains based on partial *16S rRNA* sequences (1406 nt, Hasegawa-Kishino-Yano distance model, invariant sites) (A) and *gyrB* (597 nt, Tamura and Nei model with equal base frequencies, gamma distribution) (B). Accession numbers are given in parentheses. Maximum likelihood trees showed the same topology. Numbers at the nodes are the Bayesian posterior probability after 5×10^6 generations and bootstrap after 1000 replicates. The scale bar indicates the number of substitutions per site.

in accordance with the Declaration of Helsinki. This was a non-interventional study based solely on routine procedures using biological material only for standard gastrointestinal tract infection diagnostics as prescribed by attending physicians. There was no additional sampling or modification of the routine sampling protocol, and data analyses were carried out using an anonymous database. Therefore, ethical approval was considered unnecessary according to national guidelines. The Clinical Management Unit of Infectious Diseases and Clinical Microbiology of the University Hospital Virgen de las Nieves, Spain granted permission to access and use the data.

DISCUSSION

Even though most *Shewanella* infections are caused by *S. algae* and to a lesser extent *S. putrefaciens*, over the last few

years, two other species with a pathogenic potential to humans have been reported, namely *S. haliotis* [8] and *S. xiamenensis* [9]. A pathogenic potential of *S. algae* is increasingly recognized worldwide [1]. Since the *Shewanella* genus is abundant in marine environments, infection often occurs via contact with seawater or consumption of raw seafood. Strikingly, in some other cases like the one presented here, no apparent relationship to such risk factors exist. This pathogen has been identified in opportunistic infections in patients on peritoneal dialysis, with neoplastic disease, tuberculosis and hepatobiliary disease like the case we have presented [5].

While we cannot rule out a simultaneous infection with *C. difficile*, it is worth noting that the patient had already tested positive for *C. difficile* infection in a recent admission. At any rate, we were unable to find other *S. algae* plus *C. difficile* acute diarrhea cases in the literature. Sometimes, automated systems used in microbiology laboratories cannot yield a reliable identification of *Shewanella* spp. Thereby, it is necessary to resort to molecular analyses involving the sequencing of more than one gene, as in the case reported herein, since *16S rRNA* sequencing and MALDI-TOF mass spectrometry may not be enough to discriminate at the species level. There are no previous *S. algae* infection cases reported in our area. However, it is important to know its potential to cause severe gastrointestinal disease in immunocompromised patients in order to identify it readily. It is also worth highlighting the need for conducting accurate species identification given the limitations even of modern, up-to-date identification techniques.

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None to declare.

CONFLICTS OF INTEREST

The author declare that they have no conflicts of interest.

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