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Case Report Open Access

Vibrio alginolyticus Infections: Report of Two Cases from Spain with Literature Review

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Abstract

Vibrio alginolyticus is rarely reported as a human pathogen. However, we report two cases from Spain. The first case involved a 69-year-old male with a rectum adenocarcinoma that developed enterocolitis and septic shock. The second case was associated with a previously healthy 37-year-old male patient with a pretibial ulcer exposed to the Mediterranean Sea. The isolates were identified as *V. alginolyticus* by MALDI-TOF MS and *rpoD* gene sequencing. The strain from the first patient was incorrectly attributed to *Aeromonas* sp. in the preliminary identification by API 20E and API 20NE. The antibiotic susceptibility against 13 antibiotics was tested and both strains were resistant to penicillin and ampicillin. This study is intended to raise awareness about the increasing incidence of *V. alginolyticus* and to that aim a review of the previous cases is also provided. Additionally, this study alerts that *V. alginolyticus* can be confused with *Aeromonas* based on the used identification method.

Keywords: *Vibrio alginolyticus*, *Aeromonas*, MALDI-TOF, rpoD gene, API 20E.

Introduction

Vibrio alginolyticus is a Gram-negative halophilic bacterium found in the aquatic environment, especially in temperate oceans but also able to grow in extremely high salty environments [1-5]. The virulence of this bacterium is directly related to its capacity to produce hemolysis, hemagglutination and proteases [2,5,6]. This microorganism is considered a rare human pathogen causing mainly diarrhea. However, it has been etiologically associated with otitis and wound infections, producing occasionally life-threatening infections in immunocompromised individuals [2,5,7,8]. The majority of isolates are resistant to penicillin, ampicillin and second-generation cephalosporins by the acquisition of resistance genes that are present in mobile elements [3,9,10]. Recent studies in USA, indicate that in 2011 the incidence of *V. alginolyticus* infections was only 0.048 cases per million [11]. However, the description of several new cases confirms that a rising incidence has occurred in the last years [1-3,8]. Therefore, a better knowledge about this infectious agent and its resistance to antibiotics is needed. This study is intended to contribute describing in detail two cases of infection produced by *V. alginolyticus* from Spain, providing a review of the literature and alerting of the potential confusion with *Aeromonas* depending on the employed identification method.

Case Presentation

Case presentation 1

A 69-year-old male patient visited his doctor complaining of watery diarrhea with a duration of 24 hours, abdominal pain, nausea and vomiting, with no fever. He had a previous history of hyperuricemia, osteoporosis, Widal syndrome, a right femur prosthesis and a proximal rectum adenocarcinoma that was treated with chemotherapy and surgical exeresis. The patient was referred to the Emergency Department of University Hospital Sant Joan de Reus (Catalonia, Spain). Upon arrival the patient exhibited hypoxemia (pO_2 31.7 mmHg, oxygen saturation 43.4%) and hypotension (blood pressure 85/65 mmHg). Laboratory data showed a white blood cell count of 16.8×10^9/L with a relative reduction in lymphocytes (5.3%) and an increase in neutrophils (91.1%). A computer axial tomography showed dilatation of the gut with internal fluid accumulation compatible with acute enterocolitis. The patient was admitted to the intensive care unit where he developed a septic shock with acute renal insufficiency, metabolic acidosis and high levels of lactate (8.75%) and then an intravenous treatment with imipenem (500 mg/6 h) was empirically initiated.

A stool sample was obtained and examined for intestinal parasites and for several bacteria i.e., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica, Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp. and *Campylobacter* spp. Investigation of intestinal parasites was negative. However, the stool yielded a positive culture in Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) medium (Becton Dickinson Diagnostics, USA) after 24 h of incubation in aerobiosis at 37°C. The recovered isolate, labeled 1182C, was identified based on the biochemical test API 20E (BioMérieux, Marcy l'Etoile, France), and the 7-digit code obtained after three repetitions were: 0046126, identifying this strain as: 50% *Aeromonas* spp./43% *V. fluvialis*/5% *V. alginolyticus*; 1046126: 70% *Aeromonas* spp./29% *V. fluvialis* and 1045126: 99% *Aeromonas* spp. However, with the API 20NE (BioMérieux, Marcy l'Etoile, France) the result of the 7-digit code without repetition was 6030444, identifying the strain: 71% *V. vulnificus*/27% *V. alginolyticus*. Also, based on the MALDI-TOF MS Biotyper v. 3.1, the isolate was identified as *V.*

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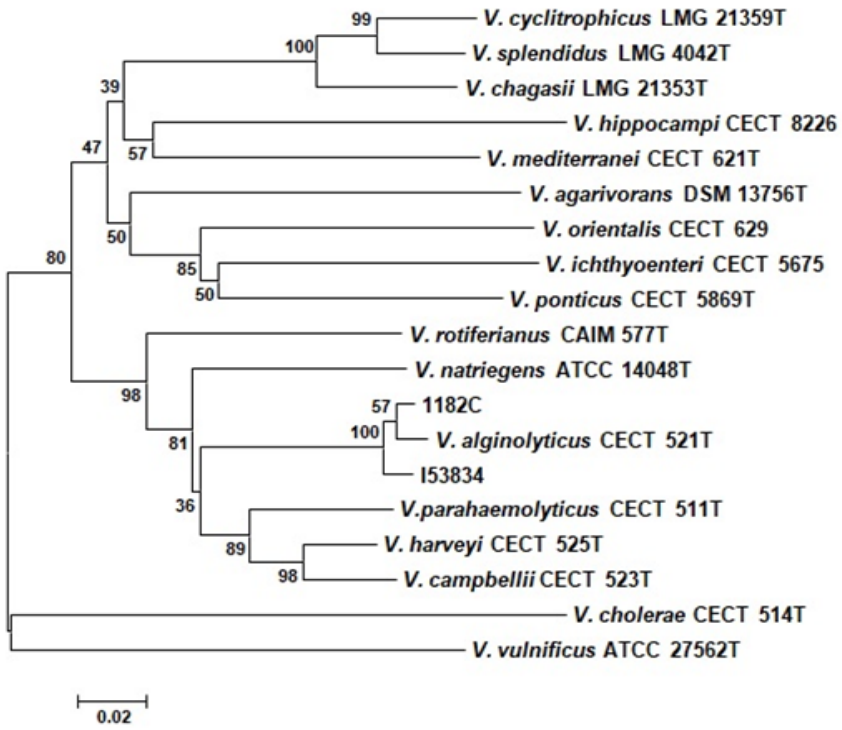
alginolyticus with a score >2.0. The antibiotic susceptibility of the strain 1182C was tested with MicroScan Walkaway and the results were analyzed according to the CLSI guidelines [12]. The results showed that the strain was resistant to ampicillin and susceptible to amoxicillin/clavulanic acid, ciprofloxacin and imipenem (Table 1). Considering these results and the good clinical status of the patient, the oral diet was restarted progressively. Also, on day 7 imipenem was changed to oral ciprofloxacin 500 mg/12 h until discharge from the hospital 9 days after admission, but treatment was continued at home for an additional period of 5 days. The strain 1182C was sent to the Unit of Microbiology at the University Rovira i Virgili for the re-identification using the sequences of the *rpoD* gene. The DNA extraction, amplification and sequencing were performed using the primers and the conditions previously described [13]. Nucleotide Blast (Blastn) analysis with the obtained *rpoD* sequence revealed a 99% similarity with *V. alginolyticus* (GenBank accession number JF930400). Considering these results, a Neighbour-joining phylogenetic tree was constructed adding also the *rpoD* sequences (479 bp) of the type strains using the MEGA 6 software. The phylogenetic tree showed that the isolate 1182C clustered with the sequence of the type strain of *V. alginolyticus* (Figure 1). In addition the antibiotic-susceptibility against 13 antibiotics was tested again using the disk diffusion method and interpretation was again done following the CLSI guidelines [12]. The strain 1182C was susceptible to all antibiotics tested except for penicillin and ampicillin (Table 2).

Case presentation 2

A 37-year-old male patient with no previous history of disease was attended at the University Hospital Miguel Servet (Zaragoza, Spain) in September 2016, because he had an apparently infected left pretibial ulcer, after swimming in the Mediterranean Sea in August. The wound occurred in January after the patient fell down the stairs. However, the wound was still not completely cured, when he noticed that the aspect of the wound had worsened. A sample of the wound exudate was collected and cultured in Blood Agar (BA). The culture was positive after 24 h of incubation in aerobiosis at 37°C and the strain recovered was named I53834 and was identified as *V. alginolyticus* / *V. parahaemolitycus* with MALDI-TOF MS Biotyper v 3.1 with a final score of 1.967 and 1.949, respectively for each microbe (Table 3). With Etest (BioMérieux, Marcy l'Etoile, France) the strain was susceptible to cefotaxime, ceftazidime, ciprofloxacin and tetracycline. An empiric antibiotic treatment was initiated with vibriamycin (doxycycline) 100 mg/12 h.

The isolate I53834 was sent to the Unit of Microbiology at the University Rovira i Virgili for re-identification using the sequences of *rpoD* gene that was performed as described above for the other case. The Blastn analysis of the obtained sequence showed a 99% similarity with a *V. alginolyticus* sequence (GenBank accession number JQ015344). In the phylogenetic tree, constructed as indicated above, the sequence of the strain I53834 clustered with the sequence of the type strain of *V. alginolyticus* and with the other sequence of this species (strain 1182C) from the other case (Figure 1). The antibiotic susceptibility against 13 antibiotics was additionally tested using the disk diffusion method and results were interpreted following the CLSI guidelines [12]. The strain I53834 showed to be susceptible to all tested antibiotics except to



*Numbers at nodes indicate bootstrap values (percentage of 1000 replicates). Bar 0.02 estimated nucleotide substitutions per site

Figure 1: Neighbour-joining phylogenetic tree obtained with *rpoD* sequences (479 bp) showing the position of the strains 1182C and I53834.

Antimicrobial agent	MIC (µg/ml)	Pattern
Amikacin	≤8	S
Ampicillin	≤8	R
Amox/Clavulat K	≤8/4	S
Aztreonam	≤1	S
Ceftazidime	≤1	S
Cefalotine	≤8	I
Cefotaxime	≤1	S
Cefoxitin	≤8	S
Cefazolin	≤8	S
Ciprofloxacin	≤0.5	S
Cefepime	≤1	S
Cefuroxime	8	S
Ertapenem	≤0.5	I
Gentamicin	≤2	S
Imipenem	≤1	S
Nalidixic acid	≤16	I
Piperacillin tazobactam	≤8	S
Trimet/Sulfa	≤2/38	S
Tigecycline	≤1	S
Tobramycin	≤2	I

Table 1: Response of strain 1182C to several antimicrobial agents obtained with the MicroScan Walkaway at the University Hospital Sant Joan de Reus. Susceptible (S), resistant (R) or intermediate (I).

Antimicrobial agents	Zone Diameter (mm)		Breakpoints		
	1182C	I53834	Sensitive	Intermediate	Resistance
Amikacin	S (19)	S (18)	≥ 17	15-16	≤ 14
Ampicillin	R (12.5)	R (13)	≥ 17	14-16	≤ 13
Amoxicillin-clavulanate	S (21)	S (22)	≥ 18	14-17	≤ 13
Cefotaxime	S (29)	S (29.5)	≥ 26	23-25	≤ 22
Ciprofloxacin	S (22)	S (23)	≥ 21	16-20	≤ 15
Cefepime	S (27)	S (26)	≥ 25	19-24	≤ 18
Penicillin*	R (17)	R (17)	≥ 21	19-20	≤ 18
Gentamicin	S (17)	S (17.5)	≥ 15	13-14	≤ 12
Imipenem	S (25)	S (26)	≥ 23	20-22	≤ 19
Piperacillin	S (23)	S (23.5)	≥ 21	18-20	≤ 17
Piperacillin-tazobactam	S (23.5)	S (≤ 22)	≥ 21	18-20	≤ 17
Tetracycline	S (16)	S (17)	≥ 15	12-14	≤ 14
Trimetoprim-sulfamethoxazole	S (17)	S (17)	≥ 16	11-15	≤ 10
*This antibiotic does not appear in the CLSI guidelines					

Table 2: Susceptibility profile of strains 1182C and I53834 to 13 antibiotics evaluated with the disk diffusion method at the University Rovira i Virgili (results expressed in mm).

S No	First option		Second option	
	Score	Specie	Score	Specie
1	1.998	*V. alginolyticus*	1.992	*V. parahaemolyticus*
2	1.713	*V. parahaemolyticus*	1.708	*V. alginolyticus*
3	1.985	*V. alginolyticus*	1.896	*V. parahaemolyticus*
4	1.967	*V. alginolyticus*	1.949	*V. parahaemolyticus*

Table 3: Results of the preliminary identification of the strain I53834 with MALDI-TOF MS Biotype. The method was performed four times and the two higher scores are presented.

Patient age (years)	Country	Presentation	Condition	References
M/22	United States	Leg ulcer	Anemia and chronic leg ulcers	[14]
N/A	United States	Wound infection in the right calf	None	[14]
M/42	United States	Trauma (ulcer) in the right leg	Chronic venous insufficiency in the leg	[7]
F/55	Japan	Abdominal pain and diarrhea	Therapy of submandibular cyst	[22]
M/20	United States	Headache and fever (intracranial infection)	None	[24]
M/26	United States	Respiratory distress and dehydration (bacteremia)	Osteogenic sarcoma	[26]
M/65	United States	Cellulitis in the right leg	None	[15]
F/40	United Kingdom	Laceration in the left leg	None	[16]
M/17	Portugal	Nausea, fever and frontal headache (sphenoiditis)	None	[25]
M/31	China	Necrotizing Fasciitis in the leg	Cirrhosis and hepatitis B	[27]
M/38	United States	Watery non-bloody stool	Odynophagia and dysphagia	[23]
F/23	South Korea	Abdominal pain and diarrhea	Hepatitis B	[21]
F/52	South Korea	Abdominal pain, vomit and diarrhea	Treatment for pulmonary tuberculosis 30 years ago	[21]
F/48	Colombia	Necrotizing Fasciitis in the leg	Exacerbation of her Asthma (steroids)	[28]
M/57	Turkey	Otitis	None	[17]
M/37	Japan	Wound infection in replanted fingers	Amputation and replantation of two fingers	[18]
M/59	Korea	Shock septic with pain in the legs	Hepatitis B	[5]
M/50	Italy	Painful cutaneous ulceration	None	[19]
M/70	Italy	Wound in the right leg	None	[20]
F/70	United Kingdom	Infected wound on her lower leg	None	[1]
M/66	Spain	Painful ulcer in the left foot	Chronic radiation-induced dermatitis	[8]
F/47	Turkey	Meningitis	None	[2]
M/14	Egypt	Wound on the left foot	None	[3]

Table 4: Different cases associated to *Vibrio alginolyticus* reported at the literature. Male (M), female (F) and not available (N/A).

Characteristics	Number	Percentage
Patient		**%**
Gender	**n= 1307**	
Male	913	70
Female	392	30
Age	**n=1276**	
<1-9	213	17
10-19	250	20
20-29	135	10
30-39	149	12
40-49	156	12
50-59	130	10
>60	243	19
Clinical features		**%**
Infection	**n=1331**	
Gastrointestinal	62	5
Blood	56	4
Skin	1162	87
Unknown	51	4
Outcomes	**n=variable**	
Hospitalization	235/1202	20
Death	12/1170	1
Antibiotics	1047/1156	91
Transmission		**%**
Foodborne	**101/1331**	8
Non-foodborne	**1141/1331**	86
Water	829/998	83
Sea water	706/802	88
Other	**89/1331**	6

Table 5: Characteristics of *Vibrio alginolyticus* infections in USA between 1988-2012 (adapted from Jacobs Slikfa et al.[29]).

penicillin and ampicillin (Table 2).

Discussion

A review of the literature of infections produced by *V. alginolyticus* showed that the most common presentations were superficial wound and ear infections [1-3,7,8,14-20]. These were followed by cases of diarrhea [21-23]. Sphenoiditis, intracranial infections, necrotizing fasciitis, bacteremia and even septic shock were the other infections [5,24-29]. The case reports found at the literature are summarized in Table 4. In addition, an epidemiological study on *V. alginolyticus* infections that occurred in USA between 1988-2012 and that included 1331 cases was performed by Jacobs Slikfa et al. [29]. The main data obtained in the Jacobs Slikfa et al. study are listed in Table 5. As shown in the Tables 4 and 5, the majority of the cases occurred in patients that showed no undelaying diseases and were associated with wound infections or ulcers that have occurred in contact with seawater [5,8,19,24,28,29]. The latter infections occurred in coastal waters of temperate and tropical regions [2,3,29,30]. In addition, in the summer months, the incidence of these infections increased significantly [2,28,31]. The impact of climate change in water temperature observed in recent years had led to an increased incidence of *V. alginolyticus* both in tropical waters and in the otherwise colder waters of northern Europe [20,29].

One of the cases reported in this study involved a patient that was immunocompromised as occurred in other cases described in the literature [5,26-29]. Therefore, it is important to consider this condition, because this agent is an emerging pathogen that in immunocompromised individuals can cause important infections. Interestingly, the patient of our second case had a wound that was exposed to seawater of the Mediterranean Sea, considered, as commented, an important risk factor in this type of infections [2,8,15,17,28].

The *V. alginolyticus* infections usually respond well to appropriate antibiotics and only occasionally causes life-threatening infections as shown in Table 5 [2,4,29]. Recent literature reported that *V. alginolyticus* is generally resistant to penicillin, ampicillin and vancomycin but susceptible to ciprofloxacin, chloramphenicol, aminoglycosides and some beta-lactams [32]. The antimicrobial profiles in this study were analyzed with an automatized system, MicroScan Walkaway and with the disk diffusion method. The results of the antimicrobial profile obtained in this study are in agreement with the resistance to ampicillin and penicillin obtained in other studies and the susceptibility to trimethoprim-sulfamethoxazole, tetracycline and gentamicin [32-35]. Regarding the identification of *Vibrio*, many clinical microbiology laboratories still routinely rely on the use of phenotypic methods, such as API 20E system that is a biochemical panel for identification and differentiation of members of the *Enterobacteriaceae* family. The results of twenty mini-test chambers provide a 7-digit code, that is introduced in the API catalog or apiweb to get an identification of the bacteria strain with a determined probability (%). In our first case the strain was incorrectly identified by API 20E as *Aeromonas* sp. This result confirms that an accurate phenotypic identification of *Vibrio* species is problematic, largely because of the great variability in biochemical characteristics [36-38]. O'Hara et al. evaluated six commercial systems for the ability to identify the 12 species of *Vibrio* found in clinical samples and the results showed that one strain of *V. cholerae* was also identified as *Aeromonas hydrophila* with API 20E [36-38]. Confusion between *Aeromonas* and *Vibrio* spp. occur approximately in 6% of the biochemical identifications, therefore a genus probe to avoid this problem have been developed at our laboratory [38-42].

MALDI-TOF MS is a mass spectrometry technique, that provides the determination of molecular weights of biomolecules, mostly of the proteins associated with the 16S rRNA gene from a bacteria isolate in a few minutes [43,44]. The profiles or protein spectra are specific to each bacteria genus and species [43,44]. The identification is obtained comparing the profiles or spectra with the ones available at the database of the system and providing a score value that describes the degree of accuracy i.e., >2.0, indicates accuracy at species level [45]. If the reference database doesn't contain sufficient spectra from all the *Vibrio* spp., the accuracy of the identification is poor (score <2.0). Furthermore, considering that *V. alginolyticus* and *V. parahaemolyticus* are very closely related species that show a 16S rRNA similarity of 99.4%, the probability that they can be correctly identified on the basis of the MALDI-TOF MS is low, because as indicated above the system relay on the proteins associated to the 16S rRNA gene [46]. These are the reasons that explains the ambiguous results obtained with MALDI-TOF MS in our second case where the strain could not be assigned either to *V. alginolyticus* or to *V. parahaemolyticus* giving for both a score <2.0.

Conclusion

Our two reports on human infections by *V. alginolyticus* is intended to raise awareness about the infections caused by this bacterium. It is essential to consider this emerging pathogen in patients with cancer or with other immunosuppressed conditions and in healthy patients with skin or soft tissue lesions that have been in contact with seawater. Furthermore, there is a need to report new cases to determine if the incidence in our country and in other regions is also increasing.

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