Complicated sea urchin-induced wound infection caused by *Vibrio alginolyticus* and *Staphylococcus lugdunensis* in a 14-year-old boy

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**Introduction:** Wound infections with *Vibrio alginolyticus*, a Gram-negative bacterium found in all temperate oceans, are rarely reported. However, a rising incidence of wound infections caused by *V. alginolyticus* requires better knowledge about this infectious agent.

**Case presentation:** We report the case of a 14-year-old boy suffering from a wound infection caused by *V. alginolyticus* and *Staphylococcus lugdunensis* after stepping on a sea urchin. Despite wound debridement and antibiotic therapy with cefaclor, the lesion did not heal over several weeks. After identification of the pathogens and antibiotic-susceptibility testing, antibiotic therapy was switched to ciprofloxacin, followed by trimethoprim/sulfamethoxazole. Two months after the accident the wound was re-epithelialized. Follow up after 6 months revealed a painful scar.

**Conclusion:** Non-cholera vibrios like *V. alginolyticus* should be considered as possible causative agents in seawater-contaminated wounds. *S. lugdunensis* is a relevant pathogen in mixed wound infections. Early microbiological diagnosis and antibiotic-susceptibility testing is crucial to prevent therapeutic failure.

**Keywords:** Vibrio; *Vibrio alginolyticus*; coagulase-negative staphylococci; *Staphylococcus lugdunensis*; wound; sea urchin.

**Abbreviations:** CLSI, Clinical and Laboratory Standards Institute; DDBJ, DNA Database of Japan.

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(Fig. 1). The wound was treated immediately by the beach warden using hot oil and lemon juice. This treatment led to a minor burn with blistering.

Immediately afterwards, the patient was taken to the local hospital. There a burn blister on top of the wound was opened and disinfected with an iodine-containing ointment. The wound was covered with sterile dressing, which was changed daily. During the next 2 weeks, until the boy’s departure from Egypt, the lesion became livid and kept oozing.

Back in Germany, the patient presented with the non-healing wound and an additional tonsillitis at a paediatric outpatient clinic. The wound was cleaned and empiric antibiotic therapy was started with cefaclor (500 mg three times daily). Seven days later, the patient returned to the clinic with continuing impaired wound healing, while the tonsillitis had resolved. A wound swab was submitted for microbiological analysis. The patient was then referred to the local hospital’s surgical outpatient clinic, where an extensive wound debridement was performed. Four days afterwards, the patient returned for a control examination and antibiotic therapy was changed to ciprofloxacin (200 mg twice daily).

Two bacterial isolates were obtained from the wound swab and identified as *V. alginolyticus* and *S. lugdunensis*. Numerous colonies were present up to the second (*V. alginolyticus*) and third (*S. lugdunensis*) streak area of the initial streak plates. After receiving antibiotic-susceptibility test results, therapy was switched to trimethoprim/sulfamethoxazole (160/800 mg twice daily) to which both isolates were susceptible. Antibiotic therapy with trimethoprim/sulfamethoxazole was continued for 20 days.

**Investigations**

Aerobic and anaerobic cultures were performed using Columbia blood agar, chocolate agar, Columbia CNA agar, MacConkey agar and Schaedler agar with/without kanamycin, using standard microbiological procedures. A Gram-negative rod and coagulase-negative staphylococci were grown and identified to the species level using appropriate VITEK 2 ID cards (VITEK 2 GN and GP-cartridge; bioMérieux) as *V. alginolyticus* and *S. lugdunensis*. Antibiotic-susceptibility testing was performed using appropriate VITEK 2 cards, AST N263 and AST P619, respectively, and interpreted according to current Clinical and Laboratory Standards Institute (CLSI) guidelines (Table 1).

For confirmation of the *V. alginolyticus* identification, the strain was sub-cultured on *Vibrio* selective agar (thiosulfate-citrate-bile-sucrose agar; Becton-Dickinson) at 37°C. After 24 h, colonies grew on the plates turning the colour of the agar to yellow as expected for *V. alginolyticus*. Additionally, matrix-associated laser desorption ionization-time of flight MS identification using a MALDI Biotyper (Bruker Daltonics) using software version 3.1 was performed, revealing *V. alginolyticus* with a score of 1.94. The next most closely related species was *Vibrio mytili* with a score of 1.84.

As the MALDI Biotyper revealed only an identification at the probable genus level, further confirmation was sought using 16S rRNA gene sequencing applying the method of Harmsen et al. (2003). The resulting 0.5 kbp amplicons were sequenced with a 3500XL Genetic Analyzer (Thermo Fisher Scientific). Using the curated database of EZbiocloud (Kim et al., 2012) and criteria for microbial identification using DNA target sequences (CLSI, 2008), similarities larger than 99% were found for numerous species of the genus *Vibrio*, including *V. alginolyticus*, without sufficient discrimination for identification at the species level. Similarly, the 16S rRNA gene sequence was analysed using BLASTN 2.2.26 and the DNA Database of Japan (DDBJ) due to its large number of well-documented *Vibrio* spp. genome sequences (http://ddbj.nig.ac.jp/blast/; Altschul et al., 1997). More than 200 strains of *Vibrio* spp. shared the best-reached similarity of 98% to our isolate, including 10 different species (*Vibrio fischeri*, *Vibrio para- haemolyticus*, *Vibrio harveyi*, *V. alginolyticus*, *Vibrio campbellii*, *Vibrio communis*, *Vibrio orientalis*, *Vibrio rotiferianus*, *Vibrio owensii* and *Vibrio antequarius*).

In the next step, additional multiplex PCR for the conserved transcriptional regulator genes VptoxR, VctoxR and VvtoxR (Osorio & Klose, 2000), according to Bauer & Rørvik (2007), was performed. There was a negative result for all toxR genes, leading to an exclusion of the species *V. parahaemolyticus, Vibrio cholerae* and *Vibrio vulnificus* from the identification.

Finally, rpoB sequencing applying the method and primers of Tarr et al. (2007) delivered two sequences of the rpoB gene (456 bp upstream, 528 bp downstream), which were analysed using DDBJ and BLASTN as described above.

**Fig. 1.** Lesion on the medial margin of the sole of the left foot 28 days after the accident. Diameter approximately 2 cm.
Our isolate showed identity of 100% to more than 50 strains of *V. alginolyticus* (upstream) and 99% to more than 200 strains of *V. alginolyticus* (downstream). There was one 99% match with *V. harveyi* (upstream and downstream) and one with *V. parahaemolyticus* (upstream). The sequence data were analysed with Bionumerics (Applied Maths, version 7.1; Applied Maths) and compared to previously published sequences of the most common pathogenic *Vibrio* spp. in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/nuccore). To visualize the phylogenetic relationship, the unweighted pair group method with arithmetic mean based on multiple alignments between the *rpoB* sequences was used (Fig. 2).

Table 1. MIC and antimicrobial-susceptibility test. Interpretive categories were according to the CLSI guidelines for *Vibrio* spp. (CLSI, 2015a) and *Staphylococcus* spp. with special regard to *S. lugdunensis* (CLSI, 2015b)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>V. alginolyticus</em></th>
<th><em>S. lugdunensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg l⁻¹)</td>
<td>Interpretation</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>NT*</td>
<td>NT</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≥32</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>NT*</td>
<td>NT</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>16</td>
<td>I</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.25</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤0.12</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤0.25</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤0.25</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>≤20</td>
<td>S</td>
</tr>
</tbody>
</table>

1, Intermediate susceptibility; NT, not tested; R, resistant; S, susceptible.  
*Testing not recommended for this species.

(http://ddbj.nig.ac.jp/blast/; Altschul et al., 1997). Our isolate showed identity of 100% to more than 50 strains of *V. alginolyticus* (upstream) and 99% to more than 200 strains of *V. alginolyticus* (downstream). There was one 99% match with *V. harveyi* (upstream and downstream) and one with *V. parahaemolyticus* (upstream). The sequence data were analysed with Bionumerics (Applied Maths, version 7.1; Applied Maths) and compared to previously published sequences of the most common pathogenic *Vibrio* spp. in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/nuccore). To visualize the phylogenetic relationship, the unweighted pair group method with arithmetic mean based on multiple alignments between the *rpoB* sequences was used (Fig. 2).

Because of the former biochemical identification results and the large number of perfect homologies to strains identified in various taxonomic studies (Ki et al., 2009; Oberbeckmann et al., 2011), we accepted *V. alginolyticus* as the final identification. For coagulase-negative staphylococci, biochemical identification is widely used and commonly accepted (Becker et al., 2014). Therefore, we accepted the Vitek 2-based identification of *S. lugdunensis* described above.

**Diagnosis**

*V. alginolyticus* and *S. lugdunensis* co-infection of a sea urchin-induced wound.

**Outcome and follow-up**

In the following months, wound healing continued slowly until the wound was epithelialized about 2 months later. When examined for follow-up 6 months after the initial accident, it was noticed that there remained an induration of the former wound with tenderness on palpation.

**Discussion**

In this case, we identified three major reasons for the prolonged, complicated wound infection. First of all, insufficient first aid and the resulting burn necrosis led to an environment where *V. alginolyticus* and *S. lugdunensis* could survive repeated debridement and disinfection. Lack of protection because of burned skin enables secondary bacterial infections.

Second, *V. alginolyticus* is well known for its numerous chromosomal and plasmid-mediated antibiotic-resistance
determinants (French et al., 1989; Li et al., 1999). Many of
the expressed β-lactamas lead to resistance to ampicillin
and second-generation cephalosporins, as seen in our iso-
late (Li et al., 1999). Resistance to trimethoprim/sulfameth-
oxazole is commonly reported (Li et al., 1999). Some isolates
also show resistance to third-generation cephalo-
sporins and fluoroquinolones, as reported by Ye et al.
(2016). According to this evolution of antibiotic resistance
and because of the typical mixed flora in chronic wound
infections (like S. lugdunensis in our case; Altoparlak et al.,
2004), early antibiotic-susceptibility testing is important to
prevent therapeutic failure.

Lastly, the presence of S. lugdunensis may have triggered
the progression of the disease. Compared to many other coagu-
lose-negative staphylococci, S. lugdunensis has higher patho-
genic potential. It can cause serious infections, i.e. soft
tissue and wound infections as well as infective endocardi-
itis, and has to be considered as a relevant pathogen (Becker
et al., 2014).

When treating the patient, the chosen therapy in
the hospital with ciprofloxacin was an appropriate choice
for the infection. However, fluoroquinolone use in children
is still off label for many indications (except, for example,
cystic fibrosis), especially if there is an alternative treatment
(Bradley et al., 2011). Therefore, therapy was changed suc-
cessfully to trimethoprim/sulfamethoxazole.

Altogether, this case and its course are an example of the
need to consider Vibrio-mediated infections in similar cir-
cumstances. Even if it is a rare disease at present, a rising
incidence has been observed, as indicated above. Warming
of the oceans will probably make this a global trend as the
first cases from northern shores suggest (Reilly et al., 2011;
Schets et al., 2006). Identification of V. alginolyticus is less
than straightforward and requires a combination of classical
biochemical identification methods, as well as appropriate
selective media and advanced molecular identification
methodology.

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