

# Wound infection caused by *Photobacterium damsela* in a 32-year-old woman: case report and review of the literature

## Abstract

The case of a 32-year-old woman is reported, who was affected by a persisting wound infection caused by *Photobacterium damsela* after an accident in the Mediterranean Sea. Besides the clinical case, microbiological characteristics based on the phenotypic and genotypic description of the isolate (including whole genome data) are presented and discussed.

**Keywords:** *Photobacterium damsela*, wound infection, whole genome data, MALDI TOF MS, antimicrobial profile

## Zusammenfassung

Wir berichten über den Fall einer 32-jährigen Frau, die nach einem Badeunfall im Mittelmeer von einer persistierenden Wundinfektion, welche durch *Photobacterium damsela* verursacht wurde, betroffen war. Neben der Beschreibung des klinischen Falls werden mikrobiologische Charakteristika des Isolats vorgestellt und diskutiert. Diese beinhalten phänotypische und genotypische Beschreibungen (auch unter Bezugnahme des gesamten bakteriellen Genoms).

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## Introduction

*Photobacterium damsela* belongs to the family of the Vibrionaceae. The species was first described by Love et al. as *Vibrio damsela* in 1981 [1]. The first reclassification followed in 1985 and the species was included into the genus *Listonella* [2]. In 1991, Smith et al. undertook a reevaluation of the genus *Listonella* and *P. damsela* was finally introduced into bacterial taxonomy [3].

*P. damsela* has been detected in sea water and is a well-known fish pathogen [4], [5], [6]. The two haemolysins damselysin (Dly) and phobalysin (PhlyP) have been identified as the main virulence factors for fish. The corresponding genes (the *dly* respectively *hlyA* gene) are encoded by the virulence plasmid pPHDD1 [5].

Besides this, infections in humans, occasionally with a fatal outcome, have also been described. In most of these cases, a previous contact with sea water or fish was reported and infections often originated from minor injuries, which many patients could not remember. Fatal courses

of the disease were usually caused by a rapidly progressing necrotizing fasciitis, sepsis or are mediated by bacterial toxins. However, there are also localized infections of the skin, which mostly resulted in a complete healing. Here we report on a persisting wound infection of a 32-year-old woman, which was caused by *P. damsela* after an accident in the Mediterranean. Furthermore, we also describe the bacterial isolate both phenotypically and genotypically.

## Case description

In August 2019, a 32-year-old female presented to our emergency department and reported that she had injured herself at a rotor leaf of a boat engine after falling off a dinghy into the salt water of the Mediterranean Sea on a trip to Spain 10 days before. Initially, the patient had received medical treatment by wound cleaning, disinfection and surgical stapling. Unfortunately, there is no in-

formation available on an antimicrobial therapy that has already been given in Spain.

Clinically we saw four laceration wounds (each approximately 4–5 cm) to the lateral thigh and calf of the left leg. While the two proximal wounds (thigh) were inconspicuous, the distal wounds on the calf displayed a local hyperemia, swelling and pressure pain. Additionally, there was slight bleeding and purulent secretion. The peripheral sensitivity, strength and mobility were unaffected. Moreover, the patient showed no systemic signs of infection. Except for a marginal elevation of the inflammation parameter CRP (14.7 mg/L) all further laboratory findings were normal. Radiographic imaging was not altered either. Apart from a nicotine (20 packyears) and alcohol abuse (2–3 drinks per day) the medical history of the patient was empty.

The patient was admitted to the hospital and treated surgically. Intraoperatively, the swelling revealed to be an infected hematoma. After collection of microbiological samples, the hematoma was removed and the wound cavity was lavaged thoroughly. A drainage was inserted and the wound was then closed layer by layer. After the surgical treatment the patient received an immediate, empirical, intravenous antibiotic treatment with a cephalosporin (cefuroxime 4x 1.5 g per day). After confirmation of infection by *P. damsela*, the antibiotic treatment was adjusted to a combination of ampicillin (1 g) and sulbactam (2 g). The antibiotic was administered three times a day. After seven days of intravenous treatment, the patient was discharged from the hospital. We recommended an additional oral antibiotic treatment with amoxicillin (875 mg) and clavulanic acid (125 mg) for seven further days. The antibiotic was administered three times a day. A scheduled appointment for clinical reevaluation was not met by the patient.

## Microbiological methods and results

### Cultivation

In total, three specimens were sent to the Institute for Medical Microbiology and Hygiene of the Technical University Dresden: the first sample was collected from a wound swab (collected one day prior to the operation, isolate DSM 110633). The second (intraoperative wound swab, isolate DSM 110632) and the third sample (biopsy, isolate DSM 110634) were obtained in the course of the operation. The samples were cultured on Columbia agar with 5% sheep blood (Oxoid, Wesel, Germany), bile chrysoidin glycerol agar (Oxoid, Wesel, Germany), brain heart infusion (Becton Dickinson, Heidelberg, Germany) and Schaedler broth (bioMérieux, Nürtingen, Germany). The agar plates were incubated for 18 hours at 37°C (without CO<sub>2</sub> infusion). After incubation, smooth, glossy and slightly transparent bacterial colonies showing a strong beta haemolysis were detected on all Columbia blood agars (Figure 1). Bacterial growth was also detected

in brain heart infusion (indicated by turbidity) and bile chrysoidin glycerol agar.



**Figure 1:** *Photobacterium damsela* DSM 110634 growing on Columbia blood agar containing 5% sheep blood (Oxoid, Wesel, Germany). The bacteria were incubated for 18 hours.

### Identification

MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) was used for primary species identification. All isolates were identified as *P. damsela* with score values >2.0, which indicates a high confidence identification (DSM 110632: 2.131; DSM 110633: 2.334; DSM 110634: 2.395). The results were additionally confirmed by sequencing of the 16S rRNA gene using 27F (agagtttgatcmtggctcag) as forward and 1498R (cggttacctgtttacgactt) as reverse primer. The data were analysed using the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The species *P. damsela* was confirmed in all isolates (99% identity). The PCR product covers a length of 1,378 bases. A total of four mismatches were found.

### Antimicrobial susceptibility testing

Bacterial colonies originating from the third specimen (DSM 110634, biopsy) were inoculated in physiological sodium chloride solution (Fresenius, Bad Homburg, Germany) and a McFarland standard of 0.5 was created. The bacterial suspension was plated on Mueller-Hinton agar (bioMérieux, Nürtingen, Germany) using a plate rotator (bestbion dx, Köln, Germany). Gradient diffusion test strips (bestbion dx, Köln, Germany) were then placed on the agar plates and incubated at 37°C for 18 hours. The interpretation of the MIC values was performed according to the EUCAST guidelines (PK/PD breakpoints) published in 2020 [7]. The isolate was susceptible towards ampicillin (0.25 mg/L), ampicillin sulbactam (0.25 mg/L), amoxicillin-clavulanate (0.5 mg/L), piperacillin (0.25 mg/L), piperacillin-tazobactam (0.064 mg/L),

cefotaxime ( $\leq 0.016$  mg/L), ceftazidime (0.125 mg/L), imipenem (0.5 mg/L), meropenem (0.032 mg/L), ciprofloxacin (0.008 mg/L), levofloxacin (0.004 mg/L) and moxifloxacin (0.016 mg/L). However, it showed resistance towards the aminoglycosides gentamicin (1.0 mg/L) and amikacin (4.0 mg/L). There are no breakpoints available for fosfomycin since there is insufficient evidence for its usefulness in the clinical setting.

## Next generation sequencing and whole genome data analysis

It could be assumed that all the isolates were identical since *P. damsela* is only extremely rarely detected as a pathogen in humans and beyond that, only pure cultures were found. For this reason, only one isolate (DSM 110634) was chosen for whole genome sequencing. Sequencing of a Nextera XT DNA Library (Nextera XT DNA Library Prep Kit; Illumina, San Diego, CA, USA) was performed on an Illumina NextSeq 550 instrument (Illumina, San Diego, CA, USA) followed by genome assembly using SPAdes 3.14 and an automated annotation applying NCBI Prokaryotic Genome Annotation Pipeline [8], [9]. Screening for resistance genes was performed using ResFinder 2.1 as recently described and CARD5 [10], [11]. Pylogenomic analyses were carried out performing digital DNA-DNA hybridization (dDDH). Type (Strain) Genome Server (TYGS) was used as database [12]. The results showed an identity of 88.2% to *P. damsela* CIP 102761, thus confirming the species [13]. Resistance gene analysis using both ResFinder 2.1 and CARD 5 did not lead to an explanation for the observed resistance against aminoglycosides. The genome sequence was submitted to NCBI GenBank under accession number JAATTX000000000. In total three genes encoding for haemolysis were detected: *dly*, *hlyA* and (chromosomally encoded) *hlyA*. Interestingly, the complete virulence plasmid pPHDD1 was not found.

## Discussion

To the best of our knowledge, a total of 29 case descriptions of *P. damsela* infections in humans (including the present report) are described so far (Table 1). All reports (except one) have in common that an infection has always been preceded by contact with sea water (the natural reservoir of *P. damsela*), fish or other sea animals (see Table 1) [14]. Additionally, a closer look at the available literature reveals that fatal cases tend to affect (but are not exclusive to) older patients ( $n=10$ ; average age=63.1 years). In contrast, there are young patients who have recovered from the infection ( $n=19$ ; average age=38.9 years) (Table 1). The patient in the present case was 32 years old. Based on our current knowledge, the course of the disease was also positive, which is congruent with the observations mentioned before.

Basically, there are two possible explanations for a worse course of the infection. On the one hand, the deteriorating function of the immune system with age (immunosenescence) should be mentioned [15]. On the other hand, the different virulence properties of the strains must be taken into account. So far, the two haemolysins damselysin (Dly) and phobalysin (PhlyP) have been described as the most important virulence factors [5]. Our isolates also showed strong  $\beta$ -haemolysis. Genome analysis of the entire genome of the *P. damsela* strain DSM 110634 showed both haemolysins being present with 100% amino acid identity (Swissprot IDs D1J6Q4 and D1J6Q5). Both genes were found in one cluster similar to their assembly in pPHDD1. Moreover, the corresponding assembled contig, namely NODE\_21, carried the *parA* replication gene showing clearly both haemolysin genes are encoded on a plasmid. However, the complete plasmid pPHDD1 (RefSeq ID NC\_014653) was not found as such. Obviously, the virulence plasmid structure differs within our isolate. Surprisingly, a second chromosomally encoded haemolysin gene was found with 92% amino acid identity to *hlyA* (NODE\_13:122725.120917).

We were also able to reliably identify our isolate using the MALDI TOF MS since it was in accordance to results obtained from sequencing of the 16S rRNA gene and the dDDH. However, a general statement about the suitability of MALDI TOF MS for the identification of *P. damsela* can only be made using a larger collection of well-characterized strains derived from clinical specimens.

The data currently available are insufficient to make general statements about the antimicrobial resistance of *P. damsela*. However, previous reports suggest that the species is sensitive to most antibiotics (including most of the  $\beta$ -lactams, cotrimoxazole, aminoglycosides, fluoroquinolones) [16]. However, resistance to aminoglycosides (as in the present case report) has also been described [17]. Unfortunately, a search for genes providing aminoglycoside resistance using both ResFinder 2.1 and CARD 5 did not obtain any results. There are basically three possible explanations for the development of resistance to aminoglycosides:

1. reduction of the concentration of aminoglycosides within the bacterial cell (e.g. efflux pump),
2. changes in the target structure for aminoglycosides (e.g. 16S methylation or ribosomal mutations) and
3. enzymatic inactivation (e.g. aminoglycoside acetyltransferases, aminoglycoside nucleotidyltransferases, aminoglycoside phosphotransferases) [18].

It is important to note that amikacin is not inactivated by enzymes, which act on gentamycin or tobramycin [18]. However, both gentamycin and amikacin are resistant in our case. For this reason, a resistance mechanism is suggested in our isolate, which affects the entire class of aminoglycosides (e.g. an efflux pump).

Table 1: Overview of reports on human infections caused by *Photobacterium damsela*

Publi- cation No.	Age	Gender	Region where the infection was probably acquired	Affected tissue	Underlying disease	Species identification by	Out- come	Additional information	Refer- ence
1	64	m	USA (Texas)	right hand (up to elbow)	atherosclerotic heart disease, ventricular arrhythmias	n.g.	fatal	death probably caused by bacterial toxins	[18]
2	58	m	Japan (Okinawa)	left hand (and arm)	diabetes mellitus	API20E, sequencing of the 16S rRNA gene, DNA- DNA hybridisation	fatal	rapid disease progression and death due to organ failure (kidney)	[17], [19]
3	58	m	Japan (Okinawa)	left hand (and arm)	diabetes mellitus	API20E, sequencing of the 16S rRNA gene, DNA- DNA hybridisation	fatal	rapid disease progression and death due to organ failure (kidney)	[17], [19]
	76	m	Japan (Okayama)	right thumb, hand to shoulder	none	API20E, sequencing of the 16S rRNA gene, DNA- DNA hybridisation	fatal	rapid disease progression and death due to multiple organ failure	[17]
4	64	m	Australia (estuary of the Murchison River)	right lower leg	no previous illnesses reported	GN-card (VITEK 2 compact), MALDI TOF MS, sequencing of the 16S rRNA gene	cured	<i>Vibrio harveyi</i> additionally detected (not reliably identified, could also be <i>Vibrio rotiferanus</i> or <i>Vibrio communis</i> )	[20]
5	22	f	USA (Puerto Rico)	urinary tract infection	no previous illnesses reported	n.g.	cured	pregnancy (23 <sup>rd</sup> week of gestation), contact with sea water	[21]
6	69	m	USA (Boston, Massachusetts)	left fifth digit (up to shoulder, neck, back, chest, flank)	no previous illnesses reported	n.g.	fatal	rapid disease progression based on a soft tissue infection, several successive operations, multiple organ failure, intensive care withdrawn due to poor prognosis	[22]
7	2	m	Jamaica (Kingston)	right buttock wound/ blood	sickle-cell disease	VITEK 2	cured	<i>E. coli</i> in urine specimen, contact with fish	[23]
8	68	m	Japan (Yamaguchi)	right lower leg	diabetes mellitus	n.g.	cured		[24]

(Continued)

Table 1: Overview of reports on human infections caused by *Photobacterium damsela*

Publication No.	Age	Gender	Region where the infection was probably acquired	Affected tissue	Underlying disease	Species identification by	Outcome	Additional information	Reference
9	43	m	USA (Florida/Tampa Bay)	right lower leg	n.g.	n.g.	cured		[25]
10	46	m	Korea (Seoul)	blood	liver cirrhosis (Child-Pugh Class B)	GN-card (VITEK 2), API20E	fatal	food (raw fish meal) associated	[26]
11	41	m	n.g.	wound	no previous illnesses reported	n.g.	cured	<i>Peptostreptococcus</i> spp. additionally detected	[14]
	26	m	n.g.	wound	no previous illnesses reported	n.g.	cured	<i>S. aureus</i> additionally detected	
	55	m	n.g.	wound (foot)	no previous illnesses reported	n.g.	cured	Clostridial species additionally detected	
	32	m	n.g.	wound (leg)	no previous illnesses reported	n.g.	cured		
	40	f	Bahamas	wound (foot)	reported as healthy	n.g.	cured		
	47	m	n.g.	wound	no previous illnesses reported	n.g.	cured	<i>Enterococcus</i> spp., <i>Acinetobacter</i> spp., <i>P. putrefaciens</i> additionally detected, no obvious contact with water	
12	61	m	USA (Texas)	left hand (up to shoulder)	alcoholism, pancreatitis, diabetes mellitus	API 20 E, DMS-rapid NFT system, cultural standard methods	fatal	rapid disease progression, disseminated intravascular coagulation, acute kidney failure and death in cardiac arrest, contact with fish, two variants/strains of <i>V. damsela</i> (different hemolytic properties)	[27]
13	38	m	USA (Texas)	right middle finger (up to axilla)	diabetes mellitus	n.g.	cured		[28]
14	62	m	China (Hong Kong)	left palm (up to shoulder including M. pectoralis major and M. latissimus dorsi)	no previous illnesses reported	cultural standard methods, AMS GNI card (Vitek® Systems)	fatal	rapid disease progression, septic shock, disseminated intravascular coagulation, acute kidney failure, necrotizing fasciitis	[16]

(Continued)

Table 1: Overview of reports on human infections caused by *Photobacterium damsela*

Publication No.	Age	Gender	Region where the infection was probably acquired	Affected tissue	Underlying disease	Species identification by	Outcome	Additional information	Reference
15	63	m	Korea	left arm and hand	alcoholic liver disease, diabetes mellitus	n.g.	fatal	rapid disease progression, septicemia	[29]
16	75	m	Australia (Sydney)	right hand and forearm	hypertention, hypercholesterolemia, penicillin allergy	MALDI TOF MS	cured	<i>Vibrio harveyi</i> additionally detected	[30]
17	70	m	New Jersey (USA)	third finger of the left hand (up to entire arm and left chest wall)	mitral valve replacement, coronary artery bypass	n.g.	fatal	rapid disease progression, phlegmon, septicemia, contact with fish	[31]
18	62	m	China (Hong Kong)	left hand and forearm (up to entire arm and chest wall)	no previous illnesses reported	n.g.	fatal	necrotizing fasciitis	[32]
19	14	m	USA (Florida, Jacksonville)	right ankle	no previous illnesses reported	n.g.	cured		[33]
20	20	m	Australia (Sydney)	right leg	n.g.	n.g.	cured	<i>Vibrio alginolyticus</i> additionally detected	[34]
	35	m	Australia (near Sydney)	right ankle	n.g.	n.g.	cured	<i>Vibrio parahaemolyticus</i> additionally detected	
	11	m	Australia (near Sydney)	right and left thigh	n.g.	n.g.	cured		
21	74	f	USA (Worcester, Massachusetts)	right foot	(controlled) hypertension	n.g.	cured		[35]
22	32	f	Spain	left lower leg	alcoholism nicotine abuse	MALDI TOF MS, sequencing of the 16S rRNA gene	Probably cured		current report

n.g. = not given

## Conclusion

We describe the 29<sup>th</sup> case of a human infection caused by the marine bacterium *P. damsela*. Infections affecting younger patients (like the patient described here) seem to show a more favorable course of healing than those affecting older patients. Since the patient in this report did not keep a follow-up visit at the hospital after the therapy, we assume the healing process has been uncomplicated. Using whole genome sequencing, we could detect three haemolysins serving as virulence factors. However, the issue whether those proteins really contribute to human infections as well needs to be elucidated in more detail in further studies. Moreover, a larger strain collection is needed to be able to create a reliable resistance profile.

## Notes

### Competing interests

The authors declare that they have no competing interests.

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