

# Characterization of the virulence potential of *Staphylococcus condimenti* isolated from a patient with severe soft tissue infection

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## Abstract

The coagulase-negative bacterium *Staphylococcus condimenti* and closely related species are commonly isolated from or found in starter cultures of fermented sausage as well as fish and soy sauces, and have traditionally been considered nonpathogenic. Recently, however, a case of catheter-related bacteraemia caused by *S. condimenti* was reported. In the present study we identified and characterized a strain of *S. condimenti* isolated from a patient with a severe soft tissue infection, comparing it to *S. condimenti* and *S. carnosus* type strains in order to elucidate the virulence potential of the clinical strain. Genome comparison showed high degree of conservation between the clinical strain and the type strain used in food industry, as well as with *S. carnosus*. The genome of the clinical *S. condimenti* strain contains few horizontally transferred regions and 37 putative virulence genes, including genes with similarity to leucocidin and genes involved in immune evasion, proinflammatory and cytolytic activity. However, it remains to be tested whether these putative virulence genes are expressed and functional. Although uncommon, *S. condimenti* may cause severe infection in previously healthy persons.

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**Keywords:** Coagulase negative, genome, *S. condimenti*, soft tissue infection, staphylococci, virulence

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## Introduction

The genus *Staphylococcus* consists of more than 50 species, which have traditionally been grouped in coagulase-positive and coagulase-negative staphylococci (CoNS). The majority of CoNS are commonly found on human and animal skin and mucous membranes, and these have been regarded as nonpathogenic or less virulent compared to the coagulase-positive staphylococci, most notably *S. aureus*. However, some CoNS, especially *S. epidermidis* and *S. haemolyticus*, are

major nosocomial pathogens frequently causing biofilm-associated infections related to prosthetic and other indwelling devices [1]. *S. carnosus*, *S. piscifermentans* and *S. condimenti* make up a phylogenetic subgroup of CoNS that are commonly isolated from or are used in starter cultures of fermented sausage as well as fish and soy sauces [2,3]. These species have until recently not been associated with human disease. The first reported case of a human infection caused by *S. condimenti* was catheter-related bacteraemia in a 17-year old female patient with severe dilated cardiomyopathy [4]. Given their role in food production, investigating the potential pathogenicity of these species is of importance.

In this study, we identified and characterized a strain of *S. condimenti* isolated from a patient with a severe soft tissue infection, comparing it to the *S. condimenti* type strain DSM 11674 and the related *S. carnosus* TM300 in order to elucidate the virulence potential of the clinical strain.

## Case Presentation

While on holiday, a 7-year-old girl from a Central European country with no history of disease was admitted to St Olavs University Hospital in August 2014. The patient had a laceration after cutting her left knee on a fragment of glass in a plastic swimming pool the previous evening. Debridement of the wound and irrigation of the knee were performed, and the patient was discharged the following day without antibiotics.

At follow-up 4 days later, the patient presented with a temperature of 39°C, a painful knee and foul-smelling discharge from the wound. Blood samples showed C-reactive protein of 199 mg/L, erythrocyte sedimentation rate of 108 mm/h, and normal leukocyte count. The patient was readmitted, and revision surgery was performed promptly. A synovial fluid aspirate and deep biopsy samples from infected tissues were collected and submitted for microscopy and culture. After surgery, intravenous antibiotic treatment was initiated with dicloxacillin and gentamicin.

Synovial fluid and deep tissue samples from the left knee were cultured on aerobic and anaerobic media as well as media for the cultivation of mycobacteria. Microscopy of the samples showed presence of Gram-positive cocci. White catalase-positive and coagulase-negative morphologically similar colonies grew abundantly from synovial fluid and all tissue samples on 5% bovine blood agar and chocolate agar after an incubation period of 2 days. A representative isolate was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to the genus level, with the best hit being *S. condimenti* (log scores ranging from 1.27 to 1.99), while biotyping gave an unacceptable profile identification (API ID 32 Staph profile 375152000), the nearest significant taxa being *S. chromogenes*, *S. caprae*, *S. carnosus* and *S. simulans*. 16S rRNA gene sequencing analysis did not provide sufficient discrimination towards related species (99.8% sequence similarity with *S. carnosus* and *S. piscifermentans*). PCR analyses for *mecA*, *nuc*, *TSST-I*, *ETA* and *ETB* genes were negative. The isolate was found to be susceptible to all antibiotics tested (Table 1). After an incubation period of 10 weeks, growth of acid-fast bacteria was not detected. *S. condimenti* was not recovered in samples from repeat revision surgeries. Histologic analysis of tissue samples showed inflammatory changes and necrosis of the superficial layer of the muscle tissue.

Throughout the hospital stay, a total of 12 revision surgeries were performed due to severe infectious myositis with extensive involvement of the soft tissues of the right thigh up to the ischial tuberosity, and also involvement of the contralateral hip and thigh. Between revisions, the surgical wounds were left

**TABLE 1.** Antibacterial susceptibility of *Staphylococcus condimenti* StO 2014-01 and DSM 11674 strains

Antibiotic	<i>S. condimenti</i> StO 2014-01		<i>S. condimenti</i> DSM 11674	
	S-I-R	MIC (mg/L)	S-I-R	MIC (mg/L)
Ciprofloxacin		0.125		0.125
Clindamycin	S	0.125	S	0.125
Cloxacillin/dicloxacillin	S		S	
Daptomycin		0.125		0.016
Doxycycline	S		S	
Erythromycin	S		S	
Fusidic Acid	S		S	
Gentamicin	S	0.5	S	0.064
Linezolid	S	1.0	S	1.0
Penicillin G		0.064		
Rifampicin		0.016		0.008
Trimethoprim/sulfamethoxazole	S		S	
Vancomycin		1.0		0.5

I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

open. On the basis of results of antimicrobial susceptibility testing, antibiotic treatment was adjusted to intravenous clindamycin and dicloxacillin from day 7.

Two weeks after the first revision surgery, the surgical wounds were closed. The patient was discharged after 4 weeks' hospitalization with dicloxacillin tablets for 20 days' treatment. Clinical follow-up was to be scheduled in her home country.

## Materials and Methods

### Bacterial strains and typing

The bacterial strains used in this study were the clinical isolate of *Staphylococcus condimenti* from the described case, hereafter referred to as *S. condimenti* StO 2014-01, and the *S. condimenti* DSM 11674 type strain (CCUG 39902T) [3] isolated from soy sauce mash. Bacterial cultures were identified by MALDI-TOF MS (Bruker Daltonics) with MALDI Biotyper software. Identification to the species level was defined as log scores  $\geq 2.0$ , and identification to the genus level on log scores between 1.7 and 1.9, based on the manufacturer's guidelines. Antibacterial susceptibility testing was performed with agar disc diffusion and minimum inhibitory concentration testing by agar gradient diffusion. Interpretation of results was based on EUCAST/NordicAST breakpoints for staphylococci. 16S rDNA was sequenced with PCR primers covering VI–V9 and Sanger sequencing using BigDye Terminator v 3.1 and ABI Hitachi 3130 XL Genetic Analyser (Applied Biosystems).

### Whole genome sequencing and assembly

Bacterial cells were treated with proteinase K (2 mg/mL) and lysostaphin (0.1 mg/mL) for 15 minutes with shaking at 37°C, before heating for 15 minutes at 65°C. Genomic DNA was isolated using the Qiagen MagAttract DNA Mini M48 kit on

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