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 leucoidin 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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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 biofilmassociated 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.

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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 catalasepositive 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-1, 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	I. Antibacterial	susceptibility	of	Staphylococcus
condimen	nti StO 2014-01 and	d DSM 11674 st	rain	IS

		dimenti 014-01	S. condimenti DSM 11674			
Antibiotic	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		S			
Daptomycin		0.125		0.016		
Doxycycline	S		S			
Erythromycin			S			
Fusidic Acid	S S 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. I6S 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

Qiagen BioRobot M48. Illumina sequencing libraries were prepared using the Nextera XT sample prep kit, and were sequenced on the MiSeq platform with 300 bp paired-end reads (MiSeq Reagent Kit v3). MinION libraries were prepared from genomic DNA sheared by G-tube (Covaris) using the SQMAP-004 kit (Oxford Nanopore Technologies). The MinION library was sequenced on a R7.3 flow cell using the MinION sequencer (Oxford Nanopore Technologies). Raw data were basecalled using Metrichor software (r7.X 2D Basecalling rev 1.12), and extracted using Poretools [5]. Data from two Nextera XT libraries and one MinION library (2D filtered reads) were hybrid assembled using the SPAdes Genome Assembler (v3.5.0) [6]. Contigs were assembled and circularized using the Geneious Assembler (Biomatters). The complete genome was annotated using the Rapid Annotation using Subsystem Technology (RAST) [7]. The S. condimenti StO 2014-01 chromosome and plasmid sequences were deposited in DDBJ/ENA/GenBank with accession numbers NZ_CP018776 and NZ_CP018777 respectively.

Comparative genomics and identification of virulence factors

Accessions of the reference genome sequences used in this study are provided in Table 2. BRIG [8] was used for whole genome comparison. Proteins were considered to be homologs having at least 90% sequence identity over at least 60% alignment length. ClustalX2 [9] and FigTree v1.4.2 were used for sequence alignments and phylogeny. Putative virulence factors were identified by protein sequence blast against the Virulence Factors Database using BLAST+ and PfamScan against the Pfam-A database. Putative horizontally transferred pathogenicity islands were identified using Alien Hunter [10].

Results

The genome of S. condimenti StO 2014-01 was sequenced and assembled into a complete 2 665 650 bp chromosome and a

35 235 bp plasmid. The genome encodes a total of 2535 protein-encoding genes, of which 1866 (73.6%) were assigned to a function and 669 (26.4%) were defined as hypothetical, based on RAST. The plasmid (named pStO 2014-01) identified in *S. condimenti* StO 2014-01 displays very little sequence similarity (maximum 25% alignment length) to known sequences in National Center for Biotechnology Information's nucleotide collection, and thus appears to be a new plasmid.

Comparative genomics of S. condimenti and closely related species

We selected genomes of the most closely related species to S. *condimenti* for comparisons. These are strains originally isolated from food sources and are thus not known to be pathogenic. Furthermore, we selected genomes of more distantly related species such as S. *simulans*, S. *massiliensis*, S. *epidermidis* and S. *aureus* to include in whole genome comparisons (Table 2).

Whereas ribosomal gene (16S and 23S rDNA) alignments could not discriminate sufficiently between these staphylococcal species (results not shown), an alignment of the *sodA* gene encoding superoxide dismutase, which is commonly used for species-level identification of coagulase-negative staphylococci [11], provided discrimination between the closely related *S. condimenti* and *S. carnosus* strains (Fig. 1).

Alignments of the chromosomes of *S. condimenti* StO 2014-01 and *S. carnosus* TM300 (Fig. 2) indicate a high degree of synteny and sequence identity between these two species. Between the two *S. condimenti* strains StO 2014-01 and DSM 11674, there is an even higher degree of synteny and sequence identity, indicating that apart from a few horizontally transferred regions, the genomes are highly conserved in both structure and function.

Horizontally transferred genomic regions in S. condimenti StO 2014-01

Identification of putative horizontal gene transfer (HGT) regions in S. condimenti StO 2014-01 (Table 3, Fig. 2) revealed that

TABLE 2. Characteristics of staphylococcal strains and genomes used in this study.

Strain	Source	Туре	RefSeq accession no.	Size (Mb)	GC%	Protein	rRNA	tRNA
S. condimenti StO 2014-01	Human soft tissue infection	Complete	NZ CP018776-7	2.70	34.6	2535	15	58
S. condimenti DSM 11674	Soy sauce mash	Complete	NZ CP015114	2.66	34.7	2427	18	58
S. carnosus subsp. carnosus TM300	Meat starter culture	Complete	NC_012121	2.57	34.6	2357	15	58
S. carnosus subsp. utilis LTH7013	South Tyrolean Ham	Contigs	NZ LAIU0000000	2.63	34.4	2399	12	57
S. carnosus 336	Beef trim	Contigs	NZ_LISV0000000	2.67	34.4	2467	12	57
S. simulans ACS-120-V-Sch1	Human reproductive tract	Scaffolds	NZ_AGZX0000000	2.67	36.0	2399	14	55
S. simulans FDAARGOS 124	Human blood	Complete	NZ CP014016	2.65	36.0	2390	18	59
S. massiliensis CCUG 55927	Human brain abscess	Scaffolds	NZ_AKGE0000000	2.37	36.5	2106	3	55
S. massiliensis S46	Human healthy skin	Contigs	NZ AMSQ0000000	2.45	36.3	2251	7	53
S. epidermidis ATCC 12228	Reference strain	Complete	GCF_000007645	2.56	32.1	2482	16	60
S. aureus NCTC 8325	Conjunctiva, corneal ulcer	Complete	GCF_000013425	2.82	32.9	2767	16	61

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FIG. I. Midpoint rooted neighbourjoining phylogenetic tree based on sodA alignment (606 bp). Bootstrapping values (1000 resamplings) are displayed for each node. The sodA gene of Staphylococcus condimenti StO 2014-01 displays 100% pairwise sequence identity to sodA of S. condimenti DSM 11674, 96.54% identity to S. carnosus LTH7013 and S. carnosus 336, and 96.38% identity to sodA of S. carnosus TM300. Identity to S. simulans strains is 90.05%, to S. massiliensis strains 81.05% and to more distantly related S. aureus and S. epidermidis strains from 78 to 83%.

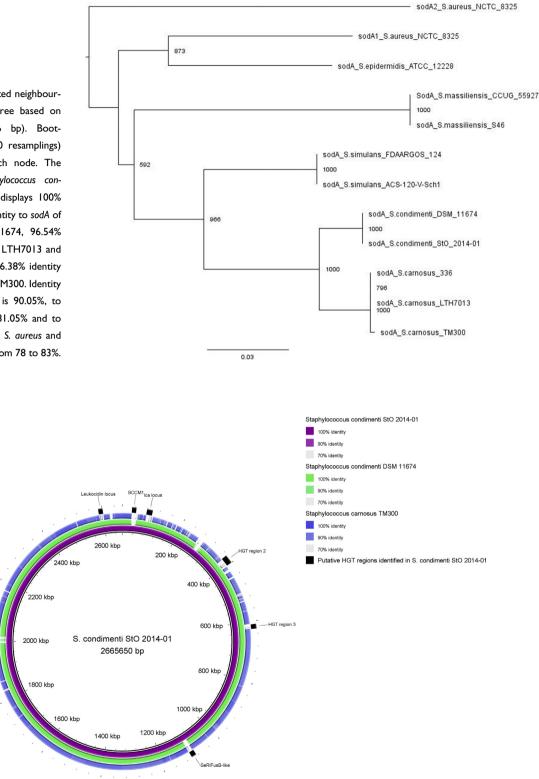


FIG. 2. Whole genome comparison of the chromosome of *Staphylococcus condimenti* StO 2014-01 to the chromosomes of *S. carnosus* TM300 and *S. condimenti* DSM 11674 created using BRIG [8]. Sequence identity is indicated by coloured key as specified. Putative horizontally transferred genomic regions identified in *S. condimenti* StO 2014-01 are marked on the outer circle in black.

Name	Туре	Locus	Putative virulence genes	BLAST (nt/nr) best hit (% identity and % alignment length)
pStO 2014-01	Plasmid	I-35 235 (plasmid)	Cadmium resistance operon, MSCRAMM	S. saphrophyticus strain FDAARGOS 168 plasmid unnamed 1 (94%, 25%)
SeRIFusB	Genomic island	087 33- 02 997	_ ·	S. epidermidis NTUH-5907 antibiotic resistance island SeRIFusB-9507 (91%, 56%)
SCCMI	Staphylococcal chromosomal cassette	30 654-44 604	_	S. aureus MI composite island SCCMI (99%, 100%)
Ica locus	Genomic island	78 554–97 039	IcaADBC	_
Leukocidin locus	_	2 588 090-2 602 243	Leukocidin genes	_
Prophage	Prophage	1 972 956-12 014 682	_ 5	S. aureus phage 37 (95%, 29%)
HGT region 2	Genomic island/ prophage remnant	372 500-400 000	MSCRAMMs	_
HGT region 3	Genomic island	610 000-625 000	Adhesins	_

TABLE 3. Horizontally	r transferred re	egions identified in	n Staphylococcus	condimenti StO 2014-01
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HGT, horizontal gene transfer; MSCRAMM, microbial surface component recognizing adhesive matrix molecules.

this strain contains a GroEL-integrated genomic island (SeR-IFusB-like) that appears to be related to several phage-related resistance islands previously described in *S. epidermidis* [12]. The genomic island however lacks the region encoding the *FusB* gene providing resistance to fusidic acid. The clinical strain also contains a staphylococcal chromosomal cassette (SCC) that is 99% identical to SCC_{M1} of *S. aureus* M1 [13]. The strain furthermore contains a 41.7 kb prophage similar to *S. aureus* phage 37 [14] as well as the well-characterized intercellular adhesion (ica) operon which is involved in biofilm formation [15]. Two other genomic regions (HGT 2 and HGT 3) identified as potentially horizontally transferred appear to be involved in adherence.

Putative virulence factors identified in S. condimenti StO 2014-01

We identified 37 putative virulence factors in S. condimenti StO 2014-01, most of which were also found in either S. condimenti DSM 11674 and to a lesser extent in S. carnosus TM300 (Table 4). Notably, S. condimenti StO 2014-01 encodes two proteins containing leukocidin domains that may form a putative bicomponent β -barrel toxin and have close homologs in S. condimenti DSM 11674. The S. condimenti clinical strain furthermore contains a number of genes which appear to encode functions related to immune evasion and suppression (Table 3). These include proteins encoding domains conserved in staphylococcal superantigens and toxins involved in modulation of T-cell responses [16], immunoglobulin-binding domain B of staphylococcal protein A (SpA), as well as a putative staphopain, extracellular fibrinogen-binding protein (Efb), staphylococcal complement inhibitor (SCIN) and secreted von Willebrand factor-binding protein (VWbp). As is common in staphylococci, S. condimenti StO 2014-01 encodes a number of cell wall-anchored proteins, including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [17]. The strain furthermore encodes two putative phenolsoluble modulins (PSMs), which have been implicated in proinflammatory and cytolytic activity, as well as in biofilm-associated infections [18].

Discussion

In this study we have identified, sequenced the genome of and identified putative virulence traits of a strain of *S. condimenti* which caused severe soft tissue infection with extensive and prolonged inflammation of the leg and thighs in a patient with no known immune defects or underlying disease. This is the first reported genome of a *S. condimenti* strain involved in human disease. We compared the genome of the clinical strain with those of closely related staphylococci, i.e. the *S. condimenti* type strain DSM 11674 and three strains of *S. carnosus*, all of which have been isolated from food sources and have been considered to be nonpathogenic, in an attempt to elucidate which virulence traits contributed to the severity of disease observed in this case.

S. condimenti was isolated in pure culture from both synovial fluid and several soft tissue samples collected via biopsy during the first revision surgery 4 days after the first treatment for knee injury. S. condimenti must therefore be considered as the definite cause of infection in this case. The infection must also be classified as a severe, life-threatening condition because it progressed, necessitating multiple surgical revisions despite adequate intravenous antibiotic treatment. The 7-year-old patient did not have any known underlying disease or history of infections indicating primary immunodeficiency which could explain her susceptibility to a low-virulent bacterium, in contrast to the recently reported case of catheter-related bacteraemia in a patient with severe dilated cardiomyopathy [4].

Except the single case of infection mentioned earlier, S. condimenti has not previously been reported as a cause of infection in humans. This could reflect the low virulence of the

TABLE 4. Putative virulence factors and associated protein domains identified in Staphylococcus condimenti StO 2014-01	and their
presence or absence in S. condimenti DSM 11674, S. carnosus TM300, S. carnosus subsp. utilis LTH7013 and S. carnosus 3.	36

Protein		Function and location			Presence in strain				
Locus	Domains	Putative function	Location	Group	StO 2014-01	DSM 11674	TM300	LTH 7013	336
87310-86081	Glyco_tranf_2_3(D)	Biofilm	Ica region	PIA	+	+	-	-	-
93502-90869	SdrG_C_C(D), Fn_bind(R), Gram_pos_anchor(F)	Adhesion (Fn binding)	Ū	MSCRAMMs	+	+	-	-	-
124775-125395	Gram_pos_anchor(F)				+	+	-	-	-
2804 - 29885	YSIRK_signal(M)				+	+	+	-	+
236818-240156	Gram_pos_anchor(F), SdrG_C_C(D)	Adhesion (Fn binding)		MSCRAMMs	+	-	+	+	+
356867-363328	Gram_pos_anchor(F)				+	-	-	-	-
381063-374938	Gram_pos_anchor(F)		HGT 2	MSCRAMMs	+	+	-	-	-
386132-381174	Gram_pos_anchor(F)		HGT 2		+	+	-	-	-
387035-386607	YSIRK_signal(M)		HGT 2		+	+	-	-	-
387441-396902	YSIRK_signal(M), Collagen(R)	Adhesion (Collagen binding)	HGT2		+	+	-	-	-
401777-397014	YSIRK_signal(M), Gram_pos_anchor(F)	Adhesion	HGT 2	MSCRAMMs	+	-	-	-	-
440878-445341	Cna_B(F), Gram_pos_anchor(F)	Adhesion (Collagen binding)		MSCRAMMs	+	+	-	-	-
533545-532070	Gram pos anchor(F)	Adhesion		MSCRAMMs	+	+	+	+	-
581719-582465	Wzz(F)				+	+	+	+	+
607537-608196	Wzz(F)				+	+	-	-	-
610182-615884	Gram_pos_anchor(F)	Adhesion	HGT 3		+	-	-	-	-
616174-616500	YSIRK_signal(M)		HGT 3		+	+	-	-	-
1108237-1106822	B(D)	Immune evasion		Spa	+	+	+	-	-
1164228-1163152	Virul_fac_BrkB(F)			•	+	+	+	+	+
1558049-1559167	Virulence_fact(F)				+	+	+	+	+
1730473-1729709	SSL_OB(D), Stap_Strp_tox_C(F)	Exotoxin/superantigen-like		Toxins	+	+	+	+	+
1732147-1730576		Coagulase			+	+	-	-	-
1774244-1769928	Gram_pos_anchor(F)	Adhesion		MSCRAMMs	+	+	-	-	+
1859898-1859704	Staph_haemo(F)	Haemolysis		PSMs	+	+	-	+	-
1860088-1859954	Staph_haemo(F)	Haemolysis		PSMs	+	+	+	+	+
1864696-1864199	efb-c(D)	Adhesion/immune evasion			+	+	-	-	-
2081226-2082389	Staphopain_pro(D), Peptidase_C47(F)	Immune evasion		Staphopains	+	+	-	-	-
2082420-2082749	Staphostatin_B(D)				+	+	+	+	+
2195344-2196078	Stap_Strp_tox_C(F)	Exotoxin/superantigen-like		Toxins	+	+	+	+	+
2513714-2511339	YSIRK_signal(M)				+	+	-	+	+
2589085-2588090	Leukocidin(D)	Leukocidin		Toxins	+	+	-	-	-
2590079-2589087	Leukocidin(D)	Leukocidin		Toxins	+	+	-	-	-
2597244-2596648	CompInhib_SCIN(F)	Immune evasion		SCIN	+	+	+	+	-
2631010-2628557	YSIRK_signal(M)				+	+	-	-	-
2644454-2640147					+	+	+	+	-
8288-6513	Strep_67kDa_ant(F)		Plasmid		+	-	-	+	-
31798-25526	Gram pos anchor(F)		Plasmid		+	-	-	-	-

HGT, horizontal gene transfer; MSCRAMM, microbial surface component recognizing adhesive matrix molecules; PSM, phenol-soluble modulin; SCIN, staphylococcal complement inhibitor; PIA, polysaccharide intercellular adhesin; D, domain; R, repeat; F, family; M, motif.

species, but it may also be a consequence of the difficulty in identifying the species by phenotypic methods available in routine laboratories before matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) was recently introduced [19]. Regardless, *S. condimenti* seems to be a rare cause of infections in humans. In a study including 8388 CoNS identified by MALDI-TOF MS from clinical samples in a hospital in France, only three isolates were identified as *S. condimenti* [19]. Similarly, this bacterial species was identified in only one other clinical sample (one of four blood cultures) in addition to this case in the period since MALDI-TOF MS was introduced in our university hospital laboratory in 2011.

Both the clinical isolate and the *S. condimenti* type strain were susceptible to all antibiotics tested, which is in accordance with previous findings reporting low prevalence of antibiotic resistance in this phylogenetic lineage of *Staphylococcus* [20]. The protracted course of infection in this patient was therefore not due to lack of susceptibility to the antibiotics used but rather to the aggressive nature of the infection. Comparative genomic analysis of strain StO 2014-01 with genomes of related species shows high degree of conservation between the two S. *condimenti* genomes with few horizontally transferred genomic regions, although the low number of available genomes for comparison makes interpretation of results uncertain. Two of the pathogenicity islands, SCC_{M1} and seRiFusB-like, had a high degree of similarity to pathogenicity islands in S. *aureus* and S. *epidermidis* respectively.

In this study we have identified a number of putative virulence factors in *S. condimenti* StO 2014-01 which may have contributed to the observed severity of disease. Among these were proteins with similarity to leukocidin, involved in immune evasion, and with proinflammatory and cytolytic activity. However, to what extent these putative virulence factors are expressed and functional was not tested. In a previous study of CoNS isolated from food and starter cultures, no toxin production was found in the two *S. condimenti* strains tested; however, one strain displayed moderate haemolytic activity on human blood agar [2]. Since most of the putative virulence genes identified in the strain StO 2014-01 were also present in

© 2017 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, NMNI, 18, 8–14 This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). the type strain S. *condimenti* DSM 11674, it seems likely that the virulence potential of our clinical strain may also apply to strains associated with food and food production.

In conclusion, we present a case of severe soft tissue infection caused by the CoNS S. *condimenti*, which has usually been considered nonpathogenic. Whole genome sequencing showed that the genome is highly conserved between the clinical strain and the type strain isolated from food. The genome contains several putative virulence factors, including leucocidin toxin–like proteins. Although uncommon, S. *condimenti* may cause severe infections in previously healthy persons.

Conflict of Interest

None declared.

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