The first case of *Staphylococcus aureus* ST398 causing bacteremia in an immunocompromised patient in Greece

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Abstract

We describe a case of catheter-related bloodstream infection, in a patient with colon cancer, caused by a methicillin-sensitive *Staphylococcus aureus* strain, nontypeable by pulsed field gel electrophoresis of *Sma*I macrorestriction fragment analysis, belonging to ST398. The patient recovered after daptomycin therapy. This is the first report that documents the emergence of ST398 in Greece.

Key words: *Clones, ST398, Staphylococcus aureus*

Introduction

It has recently become apparent that livestock constitutes a new methicillin-resistant *Staphylococcus aureus* (MRSA) reservoir and can be a source of a novel and rapidly emerging type of MRSA. These livestock-associated clones are nontypeable by pulsed- field electrophoresis with *Sma*I and belong to sequence type (ST) 398.[1] MRSA ST398 account for 20% of all MRSA in the Netherlands and it has been described worldwide.[1,2] Although ST398 transmission has been reported primarily between animals, persons with occupational exposure to livestock are at higher risk for *S. aureus* carriage than the general population, with an interest of whether MRSA may be transmitted between animals and humans.[1,2] ST398 usually cause colonization; however, several cases of infections with variable clinical relevance have been described over the past few years.[1,2] We report a case of catheter-related bloodstream infection (CRBSI) in a 68-year-old patient with cancer under

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treatment, in Greece, caused by *S. aureus* of clonal lineage ST398.

Case Report

The patient had been diagnosed 3 years ago with colon cancer with metastatic disease in the liver. He was treated initially with chemotherapy (oxaliplatin, 5-fluorouracil, leucovorin), followed by surgical resection of the primary tumor, but the liver metastases were unresectable. Postsurgery, he received 6 months of chemotherapy (irinotecan, 5-fluorouracil, leucovorin) in combination with bevacizumab (a monoclonal antibody targeting vascular endothelial growth factor) administered intravenously every 14 days through a surgically implanted central venous catheter (port-a cath). Subsequently, bevacizumab was continued as maintenance therapy.

Patient was treated for upper respiratory tract infection with amoxicillin/clavulanic acid a month before admission. Therapy was discontinued 3 days later because of side effects from the gastrointestinal system.

Patient felt well till 5 days before admission. He experienced headache, nausea and anorexia, without other symptoms, and begun to have fever as high as 39°C. There was no history of exposure to animals, residence in a rural area, recent travel or risk factors for human immunodeficiency virus infection. He did not use tobacco or alcohol.

On admission, his physical examination was unrevealing. The blood pressure was 110/70 mmHg, the pulse was 90 and respirations 16. No rash, petechiae, septic cutaneous lesions or lymphadenopathy was found. Inspiratory crackles were heard at both lung bases. The heart rhythm was regular without any murmur. The abdomen was soft and nontender. There was no peripheral edema or cyanosis and a neurological examination disclosed no focal abnormalities

The hematocrit was 37.3% and the white blood cell count 19,280/mm[3] with 90.5% neutrophils. The platelet count was 402,000/mm^3 (Sysmex XT-1800i, Roche Diagnostics, Basel, Switzerland). Prothrombin time was 15.8 seconds with a control of 13 seconds; partial thromboplastin time was 43.5 sec (STA Compact, Stago, Parsippany, NJ, USA). Sodium was 130.6 mmol/L, potassium 4.5 mmol/L, calcium 8.8 mg/dL, protein 6.1 gr/dL (albumin 2.8 gr/dL), (ACHITECT C8000, Abbott Laboratories, Abbott Park, IL, USA) and C-reactive protein 16.2 mg/dL (BECKMAN Coulter IMMAGE 800, Immunochemistry System, Beckman Coulter International SA, 1260 Nyon, Switzerland).

Specimens of blood and urine were obtained for culture and empirical treatment with teicoplanin and piperacillin/tazobactam intravenously was initiated. Blood cultures were performed routinely by the BacT/Alert 3D System (bioMerieux S.A. 69280, Marcy l'Etoile, France) at the Department of Microbiology, University Hospital of Patras (UHP), in Greece.

The central catheter was removed and the catheter tip was sent to the Microbiology Laboratory for culture. *S. aureus* was isolated from two different sets of blood samples as well as the catheter tip. Isolates were identified by Gram stain (Gram stain kit, bioMerieux), catalase and coagulase production (slidex Staph plus test, bioMerieux) and verified by molecular methods (PCR for 16S rDNA, *nuc* and *mecA* genes) [Table 1].[3] Antibiotic susceptibility testing was performed by the disk diffusion method against antistaphylococcal agents: Cefoxitin, tetracycline, rifampicin, gentamicin, kanamycin, erythromycin, clindamycin, fusidic acid, ciprofloxacin and trimethoprim-sulphamethoxazole (SXT) (SirScan, i2a, Parc de la Mediterranée 34470, Pérols, France).[4] The MICs of oxacillin, vancomycin, teicoplanin, linezolid and daptomycin were determined by the Etest (bioMerieux).[4] Isolates were forwarded to the National Reference Laboratory for Staphylococcal infections for typing by means of *Sma*I (Roche Diagnostics GmbH, Mannheim, Germany) macrorestriction pattern and pulsed-field gel electrophoresis (PFGE), as previously described.[5] The mechanism of tetracycline resistance was investigated by PCR [Table 2].[6] The genes encoding Panton-Valentine leukocidin (PVL) (*luk*F/*luk*S-PV), toxic shock syndrome toxin-1 (*tst*), exfoliative toxins A and B (*eta, etb*), *egc* operon (*sem, seg* genes), and *agr* groups were investigated by PCRs [Table 1].[7,8] Multi-locus Sequencing Typing (MLST) was performed as described (genes encoding: Carbamate kinase *arcC,* shikimate dehydrogenase *aroE,* glycerol kinase *glpF,* guanylate kinase *gmk,* phosphate acetyltransferase *pta,* triosephosphate isomerase *tpi*, acetyl coenzyme A acetyltransferase *yqiL*) [Table 1].[9] All *S. aureus* isolates were of the same phenotype and genotype. The strain was susceptible to vancomycin (MIC: 1.5 mg/L), teicoplanin (0.5 mg/L), linezolid (1 mg/L), daptomycin (0.25 mg/L), and intermediate resistant to oxacillin (3 mg/L). It showed resistance to tetracycline and SXT. It was negative for the presence of *mecA* (MSSA) and the toxin genes tested, while it carried *tet(M)*. PCR for the *agr*-specific types indicated that the isolate belonged to *agr* group 1. By PFGE of *Sma*I macrorestriction fragments it was nontypeable, while by MLST belonged to ST398.

Antibiotic therapy was switched to daptomycin, 6 mg/kg/day intravenously, for 14 days combined with gentamicin, 80 mg every 8 hours daily, for 5 days. The patient became afebrile 48 hours later, while, additional blood cultures demonstrated no bacterial growth.

After treatment, patient and household contacts were tested and found to be negative for MRSA/*S. aureus* nasal and pharyngeal carriage by conventional and molecular methods (MRSA/*S.aureus* nasal, Gene Xpert, Cepheid, Bromma, Sweden).

Table 1: Primers used for *S. aureus* typing

Genes	Primers	Sequences 5'-3'	Reference
16SrDNA	Staph756F	AACTCTGTTATTAGGGAAGAACA	[3]
	Staph750R	CCACCTTCCTCCGGTTTGTCACC	
nuc	Nuc1	GCGATTGATGGTGATACGGTT	[3]
	Nuc2	AGCCAAGCCTTGACGAACTAAAGC	
mecA	MecA1	GTAGAAATGACTGAACGTCCGATAA	[3]
	MecA2	CCAATTCCACATTGTTTCGGTCTAA	
lukF/lukS	PVL1	ATCATTAGGTAAAATGTCTGGACATGATCCA	[7]
	PVL2	GCATCAASTGTATTGGATAGCAAAAGC	
tst	tst-1	TTCACTATTTGTAAAAGTGTCAGACCCACT	[7]
	tst-2	TACTAATGAATTTTTTATCGTAAGCCCTT	
eta	etA1	ACTGTAGGAGCTAGTGCATTTGT	[7]
	etA3	TGGATACTTTTGTCTATCTTTTTCATCAAC	
etb	etB1	CAGATAAAGAGCTTTATACACACATTAC	[7]
	etB2	AGTGAACTTATCTTTCTATTGAAAAACACTC	
sem	sem-1	CTATTAATCTTTGGGTTAATGGAGAAC	[7]
	invsei-1	CCTACACCAATATCACCTTGAG	
seg	seg-1	TAAGGGAACTATGGGTAATGTAATG	[7]
	seg-2	GAACAAAAGGTACTAGTTCTTTTTTAGG	
agr1	agr I	GTCACAAGTACTATAAGCTGCGAT	[8]
	pan	ATGCACATGGTGCACATGC	
agr2	agr II	TATTACTAATTGAAAAGTGGCCATAGC	[8]
	pan	ATGCACATGGTGCACATGC	
agr3	agr III	GTAATGTAATAGCTTGTATAATAATACCCAG	[8]
	pan	ATGCACATGGTGCACATGC	
agr4	agr IV	CGATAATGCCGTAATACCCG	[8]
	pan	ATGCACATGGTGCACATGC	
arcC	arcC1	TTGATTCACCAGCGCGTATTGTC	[9]
	arcC2	AGGTATCTGCTTCAATCAGCG	
aroE	aroE1	ATCGGAAATCCTATTTCACATTC	[9]
	aroE2	GGTGTTGTATTAATAACGATATC	
glpF	glpF1	CTAGGAACTGCAATCTTAATCC	[9]
	glpF2	TGGTAAAATCGCATGTCCAATTC	
gmk	gmk1	ATCGTTTTATCGGGACCATC	[9]
	gmk2	TCATTAACTACAACGTAATCGTA	
pta	pta1	GTTAAAATCGTATTACCTGAAGG	[9]
	pta2	GACCCTTTTGTTGAAAAGCTTAA	
tpi	tpi1	TGGTTCATTCTGAACGTCGTGAA	[9]
	tpi2	TTTGCACCTTCTAACAATTGTAC	
yqiL	yqiL1	CAGCATACAGGACACCTATTGGC	[9]
	yqiL2	CGTTGAGGAATCGATACTGGAAC	

Discussion

A peculiarity of *S. aureus* of clonal lineage ST398 is the indigestibility of whole cellular DNA by restriction enzyme *Sma*I, because of protection by a novel DNA methylation enzyme.[1] Therefore, *Sma*I macrorestriction patterns generate only one large fragment, as it happened with our isolates.

The patient had no underlying cardiac predisposing conditions or clinical signs of endocarditis and a transthoracic echocardiography did not detect any vegetations. Identification of rare ST398 human outbreaks[2] supports the hypothesis that the scarce number of infections reported so far may be due to the still-limited spread of ST398 among critically ill patients, while emergence among pigs is thought to be recent.

In Greece, ST80 is the predominant clone among MRSA infections and is spread in the community and the hospitals.[10] Among bloodstream infections caused by MRSA, the most frequent characterized clone is ST80, followed by the hospital-associated clone ST239 and sporadic cases of ST30 and ST97 clones (personal data). MRSA of clonal lineage ST398 was not characterized in Greece till now.[2] In the present case, even though the isolates from all clinical specimens were methicillin-sensitive, as proved by molecular methods, they were further analyzed because of the severity

Table 2: Primers used for *S. aureus* tetracycline resistance investigation

Genes	Primers	Sequences 5'-3'	Reference
tet(A)	tet(A)F	GCTACATCCTGCTTGCCTTC	[6]
	tet(A)R	CATAGATCGCCGTGAAGAGG	
tet(B)	tet(B)F	TTGGTTAGGGGCAAGTTTTG	[6]
	tet(B)R	GTAATGGGCCAATAACACCG	
tet(C)	tet(C)F	CTTGAGAGCCTTCAACCCAG	[6]
	tet(C)R	ATGGTCGTCATCTACCTGCC	
tet(D)	tet(D)F	AAACCATTACGGCATTCTGC	[6]
	tet(D)R	GACCGGATACACCATCCATC	
tet(E)	tet(E)F	AAACCACATCCTCCATACGC	[6]
	tet(E)R	AAATAGGCCACAACCGTCAG	
tet(G)	tet(G)F	GCTCGGTGGTATCTCTGCTC	[6]
	tet(G)R	AGCAACAGAATCGGGAACAC	
tet(G)	tet(G)F	CAGCTTTCGGATTCTTACGG	[6]
	tet(G)R	GATTGGTGAGGCTCGTTAGC	
tet(K)	tet(K)F	TCGATAGGAACAGCAGTA	[6]
	tet(K)R	CAGCAGATCCTACTCCTT	
tet(L)	tet(L)F	TCGTTAGCGTGCTGTCATTC	[6]
	tet(L)R	GTATCCCACCAATGTAGCCG	
tet(M)	tet(M)F	GTGGACAAAGGTACAACGAG	[6]
	tet(M)R	CGGTAAAGTTCGTCACACAC	[6]
tet(O)	tet(O)F	AACTTAGGCATTCTGGCTCAC	[6]
	tet(O)R	TCCCACTGTTCCATATCGTCA	
tet(S)	tet(S)F	CATAGACAAGCCGTTGACC	[6]
	tet(S)R	ATGTTTTTGGAACGCCAGAG	
tetA(P)	tetA(P)F	CTTGGATTGCGGAAGAAGAG	[6]
	tetA(P)R	ATATGCCCATTTAACCACGC	
tet(Q)	tet(Q)F	TTATACTTCCTCCGGCATCG	[6]
	tet(Q)R	ATCGGTTCGAGAATGTCCAC	
tet(X)	tet(X)F	CAATAATTGGTGGTGGACCC	[6]
	tet(X)R	TTCTTACCTTGGACATCCCG	

of patients' disease. This is the first case of *S. aureus* ST398 emergence in Greece. Patient and household contacts had no animal contact and were found negative for *S. aureus* carriage. Therefore, we were unable to locate the source of patient's infection. Three MSSA belonging to ST398 already characterized from humans and animals in other countries are deposited in MLST data base (www.mlst.net).

In conclusion, epidemiological investigation and attention should be given to systematic and deep-seated *S. aureus* infections, despite methicillin resistance. Even though ST398 strains are associated with intensive animal farming, continuous surveillance in staphylococcal severe human infections should monitor the extent of disease from ST398 strains.

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