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Dissemination of *Staphylococcus epidermidis* ST22 With Stable, High-Level Resistance to Linezolid and Tedizolid in the Greek-Turkish Region (2008–2016)

To the Editor—Linezolid resistance is increasingly described among the 3 most prevalent clones of linezolid-resistant *Staphylococcus epidermidis* (LRSE) that are occasionally involved in large outbreaks: sequence type 2 (ST2), ST5, and ST22.[1] The resistant phenotype has been related to the occurrence of mutations in genes coding for the V domain of 23SrRNA and ribosomal proteins L3/L4 or *cfr* acquisition.[1] The LRSE-ST22 isolates have been associated with infection and colonization cases in Spain, France, Germany, and, particularly, Greece, and most of these LRSE-ST22 arise after treatment with linezolid.[1–7] In this study, we aimed to characterize the first methicillin- and linezolid-resistant *S. epidermidis* from a patient without previous linezolid exposure in Turkey and to assess its genetic similarity to the close geographical *S. epidermidis* from Greece.

In October 2016, a hypertensive 70-year-old male attended the emergency service at a hospital in Rize, Turkey, and was hospitalized with syncope and poor general condition (day 1). He was hospitalized in the neurology ward, where his symptoms deteriorated. These symptoms included fever, stiff neck, and confusion, and a diagnosis of clinical meningitis was established. A cerebrospinal fluid sample was collected, which was negative on cultural and microscopic analyses. Antibiotic therapy on day 2 included ceftriaxone, netilmicin, and vancomycin. The patient's condition deteriorated, and he was transferred to intensive care with room and contact isolation. Multidrug-resistant (MDR) *S. epidermidis* exhibiting resistance to oxacillin and linezolid was identified in 2 blood samples collected on days 14 and 15. The patient died on day 16 from multiple organ failure. Only a previous hospitalization for blood pressure control was registered 2 years before in the same hospital. Linezolid was never given to the patient, and additional linezolid-resistant gram-positive isolates were not detected before this case or until December 2017.

An LRSE isolate was sent to our laboratory for further characterization. The susceptibility to linezolid and vancomycin was confirmed by broth microdilution, to daptomycin and tedizolid by Etest, and to other 12 antibiotics by disk diffusion.[8] Using PCR and type sequencing, we searched *cfr*, *cfr(B)*, *optrA*, *mecA*, *mecC* genes, mutations in the 23S-rRNA-V-domain, and genes coding for L3/L4/L22 ribosomal proteins. Clonality was evaluated using pulsed-field gel electrophoresis (PFGE) and multilocus type sequencing (MLST; www.pubmlst.org). Antibiotic resistance stability (linezolid/tedizolid) was assessed after 100 daily passages in antibiotic-free Mueller-Hinton agar. Linezolid dependence was evaluated because it is a possible factor contributing to the emergence of ST22-*S. epidermidis* in Greece.[5] The Turkish LRSE and 2 LRSE-ST22 isolates from a high number of patients under linezolid therapy in 2 Greek regions (Patris and Athens) during 2008–2012[2,3] were compared by performing additional experiments not available in those studies: PFGE, ribosomal protein mutations, minimum inhibitory concentration (MIC)-tedizolid.

The Turkish LRSE-ST22 expressed resistance to linezolid (MIC ≥256 mg/L), tedizolid (MIC >32 mg/L), vancomycin (MIC = 4 mg/L), cefoxitin (*mecA*), and 8 other antibiotics. Linezolid resistance was related to T2504A and C2534T mutations in the 23S-rRNA-V domain and to the amino acid changes L94V, G152D, D159Y in L3 and N158S in L4 proteins (*S. epidermidis* RP62A numbering). The *cfr, cfr(B)* and *optrA* genes were not detected. The high linezolid and tedizolid MIC values were stably maintained after 100 serial passages, suggesting the absence of a biological burden linked to the identified mutations in non-selective contexts. Linezolid dependence was not observed in the conditions tested (Figure S1), suggesting a variable phenotype potentially dependent on previous linezolid exposure, as has been described for some strains.[5] The 3 Greek and Turkish isolates presented the same ribosomal mutations, MIC values for linezolid and tedizolid, and the same pulsotype A (Figure S2; Table 1).[4]

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TABLE 1. Characteristics of Linezolid-Resistant *Staphylococcus epidermidis* ST22 From the Greek-Turkish Region

Date	Location	No.	Source	I/C	PFGE	Linezolid Exposure	Linezolid MIC (mg/L)	MRSE	Resistance to Other Antibiotics	Antibiotic Susceptibility Pattern	Mutations (V domain of the[a] 23S rDNA)		Ribosomal Proteins[a,b] L3			L4	Ref
2016	Rize, Turkey	1	BL	I	A	No	>256	Yes	GEN, CLI, FUS, CIP, SXT, TOB, TED, NET, CLO	RIF, ERY, TET, TIG, DAP, VAN	T250-4A	C253-4T	L94V	G152D	D159-Y	N158S	This study
2010 to 2012	Patras, Greece	26	BL, CT	I, C	A	Yes[c]	>256	Yes	GEN, CLI, FUS, CIP, SXT, KAN, TED[d]	RIF, TET, VAN, DAP	T250-4A	C253-4T	L94V[d]	G152D[d]	D159-Y[d]	N158-S[d]	2; this study
2008 to 2009	Athens, Greece	12	BL, CT	U-K	A[e]	Yes[c]	>256	Yes	GEN, CLI, FUS, CIP, ERY, TOB, TED[d]	RIF, TET, Q/D, TIG, DAP, VAN, TEC	T250-4A	C253-4T	L94V[d]	G152D[d]	D159-Y[d]	N158-S[d]	3; this study

NOTE. BL, blood; CT, catheter; I, infection; C, colonization; MIC, minimum inhibitory concentration; MRSE, methicillin-resistant *Staphylococcus epidermidis*; Ref, reference. Antibiotics: CIP, ciprofloxacin; CLI, clindamycin; CLO, chloramphenicol; DAP, daptomycin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; KAN, kanamycin; LIN, linezolid; RIF, rifampicin; SXT, cotrimoxazol; TEC, teicoplanin; TED, tedizolid; TET, tetracycline; TIG, tigecycline; TOB, tobramycin; VAN, vancomycin; UK, unknown.

[a]Numeration according to *S. epidermidis* RP62A (GenBank no. CP000029.1).

[b]Mutations among ribosomal protein L22 were not detected.

[c]Previous linezolid exposure occurred in some patients.

[d]Results of published strains[2,3] obtained in this study.

[e]PFGE pulsotype "A" (E Petinaki, MD, PhD, written personnel communication, December 2017) was identified in all ST22 isolates (n = 12) from reference 3.

This study reports the dissemination of an LRSE-ST22 strain in the Greek-Turkish region at least since 2008. The Turkish LRSE-ST22 is one of the few LRSE infections described without previous linezolid exposure nor related to a hospital outbreak, which could have been facilitated by the stability of both oxazolidinones resistance, allowing its persistence in linezolid nonselective contexts. Linezolid was scarcely used in this Turkish hospital due to the low rate of vancomycin-resistant enterococci (6%) or the absence of vancomycin-resistant *Staphylococcus* spp. The origin of this strain (eg, transfer from this or other patient's microbiota, healthcare staff, or environment) remains to be elucidated, but the hypothesis of LRSE-ST22 transmission from the community cannot be ruled out, similarly to methicillin-resistant *Staphylococcus aureus*. Notably, the limited remaining last-resort therapeutic options (vancomycin and daptomycin) both require parenteral administration (in contrast to oxazolidinones) for the treatment of infections caused by these and other LRSE-ST22 strains.[1] Moreover, the high MIC value for vancomycin in the Turkish case (4 mg/L) and the coresistance to teicoplanin observed in other recent LRSE-ST22 strains[4] suggests the possibility of poor patient outcomes when glycopeptides are used.

This study demonstrates that identical MDR LRSE-ST22 strains expressing resistance to last-resort antibiotics, including linezolid and tedizolid, are spreading among hospitals in different countries. Additional studies including community sources are crucial to better understanding the factors driving the emergence and transmission routes of these strains and to timely optimization of antimicrobial stewardship.

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SUPPLEMENTARY MATERIAL

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