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# Analysis of the Molecular Epidemiology of *Staphylococcus epidermidis* Clinical Isolates from USA Hospitals

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SENTRY ANTIMICROBIAL SURVEILLANCE

## AMENDED ABSTRACT

**Background**: Linezolid-resistant *Staphylococcus epidermidis* clinical isolates remain rare, and information related to the epidemiology of these strains is limited. This study addresses the genetic background of linezolid-resistant and -susceptible *S. epidermidis* responsible for infections in USA hospitals during the 2010 SENTRY Antimicrobial Surveillance Program.

**Methods**: A total of 69 *S. epidermidis* were selected. Representatives of methicillin-susceptible and -resistant strains were selected from each USA site contributing *S. epidermidis* (27 centers in 25 cities; 19 states; seven census regions). Susceptibility testing was performed by CLSI methods. Linezolid-resistant strains were screened for *cfr* and mutations in the 23S rRNA-, L3- and L4. All selected isolates were subjected to multilocus sequence typing (MLST) and results were analyzed using eBURST to infer the evolutionary relatedness of STs. Strains were also typed by PFGE and SCC*mec*.

**Results**: Overall, MLST analysis revealed 27 STs among which, five had not been previously recorded in the MLST database. The majority (60/69; 87.0%) of STs belonged to clonal complex (CC) 2, comprised of CC2-II (39/60; 65%) and 2-I (21/60; 35%), while the other STs clustered within CC23, 32 or singletons. Among methicillin-susceptible *S. epidermidis* (19/69; 27.5%), 13 (68.4%) isolates were CC2-II (one CC2-I), while 50 (72.5%) were methicillin-resistant and mostly associated with CC2-II (25/50; 54.0%) or CC2-I (20/50; 40.0%). Linezolid-susceptible strains (56/69; 81.2%) belonged mostly to CC2-II (36/56; 64.3%), and 11 (14.3%) strains were CC2-I. Linezolid-resistant strains (13; 18.8%) originated from 11 USA sites. Cfr-mediated resistance was associated with CC2-I (3/4; 75.0%), as were isolates possessing other resistance mechanisms (7/9; 77.8%).

**Conclusions:** The majority of *S. epidermidis* responsible for infections in USA hospitals were CC2. eBURST analysis demonstrated that methicillin- and linezolid-susceptible strains were predominantly associated with CC2-II, the most prevalent clone. In contrast, linezolid-resistant isolates appeared to be associated with CC2-I, regardless of the resistance mechanism.

## INTRODUCTION

*Staphylococcus epidermidis* is ubiquitous of the skin and mucosal human microflora. Infections caused by this species in immunocompetent patients occur when the integrity of the skin barrier is disturbed. The vast majority of infections have been associated with indwelling medical devices, such as intravascular and intrathecal catheter systems, pacemaker electrodes, urinary tract catheter and other polymer and metal implants, which are used as vehicles to enter the host.

Overall, the rates of methicillin (oxacillin) resistance among *S. epidermidis* exceed 70% in many institutions worldwide. It has been demonstrated that this organism possesses great capability for genetic recombination and gene acquisition, including resistant determinants. However, although antimicrobial resistance can compromise therapy, treatment failure has been greatly associated to this species ability to form biofilms on medical devices, which represents a common feature of many nosocomial pathogens.

Several reports have described the occurrence of nosocomial outbreaks caused by *S. epidermidis*, including linezolid-resistant isolates. This study addresses the genetic background of linezolid-resistant and -susceptible *S. epidermidis* responsible for infections in United States monitored hospitals during the 2010 SENTRY Antimicrobial Surveillance Program.

## MATERIALS AND METHODS

**Bacterial strain collection.** A total of 69 *S. epidermidis* clinical isolates collected during the 2010 sampling year for the SENTRY Program were included in this study. At least one methicillin-susceptible and one -resistant representative strains were selected, when available, from each USA medical institution contributing *S. epidermidis*. Additional strains were selected for each methicillin susceptibility group (up to 10% of total) from those medical sites contributing more than twenty strains in the study period. When multiple strains from a single site were included, isolates exhibiting different antimicrobial susceptibility profiles were chosen.

**Antimicrobial susceptibility testing.** Susceptibility testing was performed by broth microdilution methods, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A8, 2009). Minimum inhibitory concentration (MIC) interpretations were applied as described in M100-S21 (CLSI, 2011). *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were concurrently tested for quality assurance purposes. In addition, the inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event.

**Screening for linezolid resistance mechanisms.** All linezolid-resistant strains were screened for the presence of *cfr*, and mutations in the 23S rRNA and ribosomal proteins (L3 and L4) by PCR and sequencing. Amplicons were sequenced on both strands. Ribosomal proteins obtained were compared to those from wildtype *S. epidermidis* ATCC 12228 using the Lasergene® software package (DNAStar; Madison, Wisconsin).

**Molecular typing.** Multilocus sequence typing (MLST) was performed according to the methods published for *S. epidermidis* (Thomas et al., 2007). STs were determined using the *S. epidermidis* MLST databases (http:/www.mlst.net/) and the eBURST method to infer the evolutionary relatedness of STs. All selected strains were subjected to pulsed-field gel electrophoresis (PFGE). Smal-digested genomic DNA was resolved in CHEF-DR II (BioRad, Richmond, California) and PFGE profiles obtained were analyzed using the GelCompar II software (Applied Math, Kortrijk, Belgium). Percent similarities were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.0% and 0.8%, respectively. Isolates showing similarity coefficient ≥95% were considered as genetically identical (subtype), while those with similarity coefficient between 80.0 and 94.9% were classified as genetically related (type).

## RESULTS

- *S. epidermidis* originated from 27 centers in 25 cities (19 states; and seven Census regions) and the majority (47/69; 68.1%) of strains were recovered from blood, followed by other less prevalent (<13% each) specimen types (Table 1).
- Among the *S. epidermidis* isolates, 27 STs were observed. ST5 (21.7%) and ST2 (17.4%) were most commonly detected, followed by ST83 (8.7%), ST7 (5.8%) and ST59 (5.8%). Other STs had occurrences lower than 4.5% (Table 1).
- The eBURST algorithm clustered these STs into one major clonal complex (CC) (CC2; 60/69; 87%), two minor CCs (23 and 32 [2/69; 2.9% each]) and five singletons (Table 1). CC2 was comprised of clusters CC2-II (39/60; 65%) and CC2-I (21/60; 35%).

**Table 1.** Epidemiology, antimicrobial susceptibility interpretations, and demographic information related to the *S. epidermidis* included in this study.

| | | | Molecular typing[a] | | Antimicrobial susceptibility[b] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | State | City | CC | ST | OXA | LZD | CIP | CLI | TET | ERY | GEN | TEC | SXT | MDR[c] | Specimen[d] | Age[e] | Sex |
| 38307 | TX | Houston | 23 | ST23 | R | S | R | R | R | R | I | S | R | Y | WD | 86 | F |
| 7241 | KY | Louisville | 23 | ST23 | R | S | R | R | S | R | I | S | S | Y | BC | 35 | M |
| 11279 | MO | Kansas City | 32 | ST32 | S | S | S | S | S | R | S | S | S | N | UC | 6 | M |
| 18201 | MI | Detroit | 32 | ST358 | S | S | S | S | S | S | S | S | S | N | WD | 11 | F |
| 2758 | AR | Little Rock | 193 | ST193 | S | S | S | S | S | R | S | S | S | N | PF | 16 | M |
| 35920 | PA | Hershey | 249 | ST249 | S | S | S | S | S | R | S | S | S | N | BC | 68 | M |
| 18001 | LA | New Orleans | 347 | ST347 | S | S | S | S | S | S | S | S | S | N | BC | 90 | M |
| 22541 | MI | Lansing | 348 | ST348 | R | S | S | R | S | R | S | S | R | Y | SK | 22 | M |
| 18461 | MI | Detroit | 360 | ST360 | S | S | S | S | S | R | S | S | R | N | BC | 80 | F |
| 18676 | TX | Galveston | 2-I | ST16 | R | S | R | I | S | S | S | S | S | N | BC | 91 | F |
| 49671 | WI | Milwaukee | 2-I | ST16 | R | S | S | S | S | R | S | S | S | N | BC | 76 | M |
| 16644 | TN | Memphis | 2-I | ST16 | R | S | S | S | S | R | S | S | S | N | BC | 49 | F |
| 38449 | AZ | Tempe | 2-I | ST186 | R | R | R | R | S | I | I | S | S | Y | BC | 73 | M |
| 25438 | MA | Boston | 2-I | ST2 | R | R | R | I | S | I | R | S | R | Y | BC | 61 | M |
| 37506 | TX | Houston | 2-I | ST2 | R | R | R | I | S | I | R | R | R | Y | BC | 29 | F |
| 25437 | MA | Boston | 2-I | ST2 | R | R | R | I | S | R | R | S | R | Y | BC | 26 | M |
| 34444 | TX | Houston | 2-I | ST2 | R | R | R | I | S | R | R | S | R | Y | BC | 66 | M |
| 39867 | TX | Houston | 2-I | ST2 | R | R | R | I | S | R | I | S | R | Y | SN | 67 | M |
| 18006 | LA | New Orleans | 2-I | ST2 | R | R | R | S | S | S | R | S | R | Y | BC | 50 | M |
| 38531 | PA | Hershey | 2-I | ST2 | R | R | R | S | S | S | S | S | R | Y | SK | 42 | M |
| 13036 | KY | Lexington | 2-I | ST2 | R | S | R | R | S | R | S | S | R | Y | BC | 60 | M |
| 29257 | CT | Farmington | 2-I | ST2 | R | S | R | R | S | R | R | S | R | Y | BC | 50 | M |
| 35921 | PA | Hershey | 2-I | ST2 | R | S | R | R | S | R | R | S | R | Y | SK | 2 | M |
| 16669 | NY | New York | 2-I | ST2 | R | S | R | R | S | R | R | S | R | Y | WD | 84 | M |
| 5849 | KY | Lexington | 2-I | ST2 | S | S | R | R | S | R | R | S | R | Y | BC | 56 | F |
| 12676 | OH | Akron | 2-I | ST22 | R | R | R | R | S | I | I | S | R | Y | BC | 61 | F |
| 2907 | KY | Lexington | 2-I | ST22 | R | R | R | R | S | I | R | S | R | Y | BC | 55 | F |
| 9611 | NJ | New Brunswick | 2-I | ST22 | R | S | R | S | S | S | R | S | R | Y | BJ | 77 | M |
| 10560 | NY | New York | 2-I | ST346 | R | S | S | S | S | R | S | S | S | N | BC | 52 | M |
| 30077 | MN | St. Paul | 2-I | ST361 | R | S | R | S | S | S | R | S | R | Y | SK | 63 | M |
| 2763 | AR | Little Rock | 2-II-5 | ST130 | R | S | S | R | S | R | R | S | S | N | BC | 21 | M |
| 28062 | MO | Kansas City | 2-II-5 | ST136 | R | S | S | S | S | R | S | S | S | N | BC | 6* | F |
| 36018 | MA | Burlington | 2-II-5 | ST359 | R | S | R | S | S | S | S | S | S | N | BC | 55 | M |
| 4042 | MO | Kansas City | 2-II-5 | ST5 | R | R | R | R | S | R | S | S | S | Y | BC | 49 | F |
| 34374 | MI | Detroit | 2-II-5 | ST5 | R | R | R | S | S | I | R | S | R | Y | BC | 50 | M |
| 22291 | TN | Memphis | 2-II-5 | ST5 | R | R | R | S | S | I | R | S | R | Y | PL | 53 | M |
| 2969 | IA | Des Moines | 2-II-5 | ST5 | R | S | R | R | R | R | S | S | R | Y | BC | 50 | M |
| 30328 | KY | Lexington | 2-II-5 | ST5 | R | S | R | S | R | R | S | S | R | Y | PF | 39 | F |
| 17996 | LA | New Orleans | 2-II-5 | ST5 | R | S | R | S | S | R | S | S | S | N | BC | 40 | M |
| 34420 | MI | Detroit | 2-II-5 | ST5 | R | S | R | S | S | R | S | S | S | N | BC | 87 | M |
| 17994 | LA | New Orleans | 2-II-5 | ST5 | R | S | R | S | S | R | I | S | R | Y | UT | 80 | F |
| 30079 | MN | St. Paul | 2-II-5 | ST5 | R | S | R | S | S | S | R | S | S | N | BC | 69 | M |
| 42022 | PA | Hershey | 2-II-5 | ST5 | R | S | R | S | S | S | S | S | S | N | BC | 62 | M |
| 497 | NE | Omaha | 2-II-5 | ST5 | R | S | R | S | S | S | S | S | S | N | CT | 70 | M |
| 30078 | MN | St. Paul | 2-II-5 | ST5 | S | S | R | R | S | R | S | S | S | N | U | 72 | M |
| 16733 | OH | Akron | 2-II-5 | ST5 | S | S | R | S | R | R | S | S | S | N | BC | 56 | M |
| 13638 | NY | New York | 2-II-5 | ST5 | S | S | R | S | S | R | S | S | S | N | BC | 71 | F |
| 29249 | CT | Farmington | 2-II-5 | ST5 | S | S | R | S | S | S | S | S | S | N | BC | 50 | F |
| 25442 | MA | Boston | 2-II-5 | ST59 | R | S | R | I | S | R | I | S | S | N | UT | 80 | M |
| 35037 | NE | Omaha | 2-II-5 | ST59 | R | S | R | S | R | S | R | S | S | Y | BC | 71 | F |
| 34419 | MI | Detroit | 2-II-5 | ST59 | R | S | R | S | R | S | R | S | R | Y | BC | 77 | M |
| 69 | NJ | New Brunswick | 2-II-5 | ST59 | R | S | R | S | S | S | S | S | S | N | BC | 79 | F |
| 29784 | OH | Akron | 2-II-5 | ST69 | R | S | R | S | S | S | R | S | S | N | BC | 28 | M |
| 28061 | MO | Kansas City | 2-II-5 | ST7 | S | S | R | R | S | R | S | S | S | Y | BC | 3 | M |
| 8749 | WI | Milwaukee | 2-II-5 | ST7 | S | S | R | R | S | R | S | S | R | Y | BC | 52 | F |
| 22310 | TN | Memphis | 2-II-5 | ST7 | S | S | R | S | S | R | S | S | R | N | BC | 52 | M |
| 17995 | LA | New Orleans | 2-II-5 | ST7 | S | S | S | S | S | R | S | S | R | N | UT | 30 | F |
| 6711 | AZ | Tucson | 2-II-5 | ST83 | R | S | R | I | S | S | S | S | S | N | BC | 55 | F |
| 2730 | AR | Little Rock | 2-II-5 | ST83 | R | S | R | R | S | R | R | S | R | Y | WD | 2* | M |
| 13857 | OH | Cincinnati | 2-II-5 | ST83 | R | S | R | R | S | R | R | S | R | Y | BC | 59 | M |
| 48458 | MI | Detroit | 2-II-5 | ST83 | R | S | R | R | S | R | R | S | S | Y | BC | 79 | F |
| 2674 | NE | Omaha | 2-II-5 | ST83 | R | S | R | S | S | S | S | S | R | N | BJ | 48 | M |
| 22300 | TN | Memphis | 2-II-5 | ST83 | S | S | S | S | S | S | R | S | S | N | BC | 37 | M |
| 2820 | NJ | New Brunswick | 2-II-6 | ST345 | S | S | S | R | S | R | S | S | S | N | BC | 54 | F |
| 16675 | NY | New York | 2-II-6 | ST73 | S | S | S | I | R | S | S | S | S | N | WD | 60 | F |
| 11175 | UT | Salt Lake City | 2-II-85 | ST20 | R | S | S | R | S | R | R | S | S | Y | BC | 2* | M |
| 5687 | MO | Kansas City | 2-II-85 | ST20 | R | S | S | S | S | R | I | S | S | N | UC | 1* | M |
| 30259 | MI | Detroit | 2-II-89 | ST327 | S | S | S | S | S | S | S | S | S | N | BC | 80 | M |
| 1374 | NJ | New Brunswick | 2-II-89 | ST88 | R | S | S | S | S | S | S | S | S | N | BC | 80 | F |

a. Clonal complexes (CC) determined according to the eBURST analysis.
b. MIC interpretive criteria as published by CLSI M100-S21. S, susceptible; I, intermediate; and R, resistant. OXA, oxacillin; LZD, linezolid; CIP, ciprofloxacin; CLI, clindamycin; TET, tetracycline; ERY, erythromycin; GEN, gentamicin; TEC, teicoplanin; and SXT, trimethoprim/sulfamethoxazole.
c. MDR, multidrug resistance defined when isolate exhibited a resistance phenotype to at least four classes of antimicrobial agents.
d. WD, wound; UT, urinary tract; UC, urethral catheter; SK, skin; SN, sinus; BJ, joint/bone; CT, catheter; PF peritoneal fluid and; PL, pleural fluid.
e. * represents months.

- It has been proposed that cluster 2-II can be further subdivided into four groups, represented in this study by CC2-II-5 (33/39; 84.6%), CC2-II-6 (2/39; 5.1%), CC2-II-85 (2/39; 5.1%) and CC2-II-89 (2/39; 5.1%; Table 1).
- All *S. epidermidis* belonging to CC2 cluster I were methicillin (oxacillin)-resistant (20/21; 95.2%), except for one strain. Lower methicillin resistance rates were observed among CC2-II (27/39; 69.2%) and other CCs (6/15; 40.0%; Tables 1 and 2).
- Linezolid resistance was only observed among CC2-I (10/21; 47.6%) and CC2-II (3/39; 7.7%) *S. epidermidis* isolates. Among these strains, Cfr-mediated resistance was most often detected among CC2-I strains (3/4; 75.0%; Tables 1, 2 and 3).
- All linezolid-resistant isolates demonstrated at least one amino acid alteration in L3 (Table 3). Modifications in 23S rRNA were also observed and these were only detected among CC2-I isolates (8/10; 80.0%). In addition, a glycine insertion at position 71 of L4 was detected in some strains (9/13; 69.2%).
- The vast majority of CC2-I strains (17/21; 80.9%) were multidrug-resistant (resistant to at least four classes of drugs), while 35.9% (14/39) and 26.7% (4/15) of CC2-II and other CCs demonstrated this phenotype, respectively (Tables 1 and 2).
- Analysis of Smal PFGE patterns clustered the *S. epidermidis* isolates included in this study into 41 major types and 62 subtypes. Two PFGE types showed the highest number of strains (8/69; 11.6%) and were associated with CC2-I or CC2-II (data not shown).

**Table 2.** Occurrences of antimicrobial resistance phenotypes according to clonal complexes among *S. epidermidis* clinical strains.

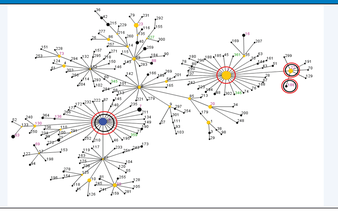
| Resistance phenotype | Clonal complexes[a] | Occurrence | Percentage (%) |
|---|---|---|---|
| Methicillin resistance | 2-I | 20/21 | 95.2 |
| | 2-II | 27/39 | 69.2 |
| | Other CCs | 6/15 | 40.0 |
| Linezolid resistance | 2-I | 10/21 | 47.6 |
| | 2-II | 3/39 | 7.7 |
| | Other CCs | 0/15 | 0.0 |
| Multidrug resistance[b] | 2-I | 17/21 | 80.9 |
| | 2-II | 14/39 | 35.9 |
| | Other CCs | 4/15 | 26.7 |

a. CC2-II includes subclusters 2-II-5 (33 strains), 2-II-6 (two strains), 2-II-85 (two strains) and 2-II-89 (two strains). Other CCs include CC23 (two strains), CC32 (two strains), CC193 (one strain), CC249 (one strain), CC347 (one strain), CC348 (one strain) and CC360 (one strain).
b. Multidrug resistance represents strains exhibiting a resistance phenotype to at least four different classes of antimicrobial agents.

**Table 3.** Linezolid resistance mechanisms observed among *S. epidermidis* strains recovered from USA hospitals and selected for this study.

| | | | | Linezolid | Molecular typing | | Linezolid resistance mechanisms | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | City | State | Site | MIC (µg/mL) | CC | MLST | cfr | 23S rRNA | L3 | L4 |
| 4042 | Kansas City | Missouri | 449 | 16 | 2-II-5 | ST5 | + | WT | A157R | WT |
| 34374 | Detroit | Michigan | 003 | 16 | 2-II-5 | ST5 | - | WT | H146Q/V154L/A157R | $_{71}G_{72}$ |
| 22291 | Memphis | Tennessee | 412 | 16 | 2-II-5 | ST5 | - | WT | H146Q/V154L/A157R | $_{71}G_{72}$ |
| 18006 | New Orleans | Louisiana | 448 | 32 | 2-I | ST2 | - | G2576T | H146P/M156T | WT |
| 38531 | Hershey | Pennsylvania | 453 | 32 | 2-I | ST2 | - | G2576T | V154L/M156T | WT |
| 39867 | Houston | Texas | 024 | 64 | 2-I | ST2 | - | G2576T | H146P/M156T | $_{71}G_{72}$ |
| 37506 | Houston | Texas | 116 | 64 | 2-I | ST2 | - | G2576T | H146R/M156T | $_{71}G_{72}$ |
| 34444 | Houston | Texas | 116 | 128 | 2-I | ST2 | - | G2576T | H146R/M156R | $_{71}G_{72}$ |
| 2907 | Lexington | Kentucky | 107 | 128 | 2-I | ST22 | + | WT | V154L/A157R | WT |
| 25438 | Boston | Massachusetts | 441 | 128 | 2-I | ST2 | - | G2576T | G137S/H146P/M156R | G69R/$_{71}G_{72}$ |
| 25437 | Boston | Massachusetts | 441 | 128 | 2-I | ST2 | - | G2576T | G137D/H146R/V154L/M156T | $_{71}G_{72}$ |
| 12676 | Akron | Ohio | 004 | >128 | 2-I | ST22 | + | WT | H146Q/V154L/A157R | $_{71}G_{72}$ |
| 38449 | Tempe | Arizona | 426 | >128 | 2-I | ST186 | + | C2534T | H146Q/V154L/A157R | $_{71}G_{72}$ |

**Figure 1.** eBURST analysis of *S. epidermidis* CC2 using all 312 STs available in the MLST database as of September 2011. Each ST is represented by a dot; lines connect single-locus variant. The blue dot ST5 represent the putative founder of CC2. Yellow dots represent putative subgroup founders. Pink STs represent those observed here and previously recorded in the MLST database, while green numbers represent new STs detected in this study. Red circles indicate STs associated with linezolid-resistant isolates and those inner black circles indicate STs associated with Cfr-mediated resistance.



## CONCLUSIONS

- Based on the PFGE analysis, a great degree of genetic diversity (41 PFGE types) was observed within this contemporary collection of *S. epidermidis*. However, this was expected due to the applied strain selection criteria.
- ST2 has usually been the most prevalent sequence type observed in previous epidemiology studies among nosocomial *S. epidermidis* worldwide. However, a greater proportion of ST5 strains (21.7%) were detected in this study.
- The vast majority of *S. epidermidis* responsible for infections in USA hospitals belonged to the major CC2. This CC was most represented (65%) by *S. epidermidis* clustering within CC2-II.
- Although less frequent, isolates belonging to CC2-I were more likely to exhibit antimicrobial resistance. Methicillin (95.2%) and linezolid (47.6%) resistance, and a MDR phenotype (80.9%) were more common among CC2-I strains compared to CC2-II *S. epidermidis* (69.2, 7.7 and 35.9%, respectively).
- This data suggests that CC2-I isolates may possess an enhanced capacity for acquisition of foreign DNA. In addition, the presence of mutations in the 23S rRNA, may confer a lower fitness cost for CC2-I strains, which may be more adapted to survive in the hospital and/or adverse environment than other CC2 clusters.

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