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Original Article

Molecular characteristics and clinical features of *Staphylococcus epidermidis* healthcare-associated late-onset bacteremia among infants hospitalized in neonatal intensive care units

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Abstract *Background:* Though *Staphylococcus epidermidis* was the most common pathogen of late-onset sepsis (LOS) in neonatal intensive care units (NICUs), there haves been scanty reports on molecular epidemiology of *S. epidermidis* isolates from infants stayed in NICU and on correlation of molecular characteristics with clinical features in these infants.
Methods: We collected and characterized *S. epidermidis* bloodstream isolates from infants hospitalized in NICU of a medical center in Taiwan between 2018 and 2020. Medical records of these infants were retrospectively reviewed.
Results: A total of 107 isolates identified from 78 episodes of bacteremia in 75 infants were included for analysis. Of the 78 isolates (episodes), 24 pulsotypes, 11 sequence types (STs), and 5 types of staphylococcal chromosomal cassette (type I–V) were identified. ST59 and its single locus variant ST1124 (37.2%) comprised the most common strain, followed by ST35 (14.1%), ST2 (11.5%), and ST89 (10.3%). All but 5 isolates (73/78, 93.6%) belonged to clonal complex (CC) 2. Comparing infants infected with genetically different strains, the patients with underlying immune disease were significantly associated with ST2 infection ($P = 0.021$), while no statistically significant differences were found in terms of clinical and laboratory characteristics. Only 3.8% of the isolates were susceptible to oxacillin.

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Conclusions: More than 90% of *S. epidermidis* bloodstream isolates from infants in NICU in Taiwan were resistant to oxacillin. Though diverse, more than 90% of the isolates (episodes) belonged to CC2. No statistically significant differences were found in terms of clinical characteristics among the infants infected with genetically different strains.
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Introduction

Coagulase-negative staphylococci (CoNS) were considered to be apathogenic constituents of the healthy human skin and mucosa microbiota.[1] However, CoNS emerged as common causes of healthcare infections. Factors contributing to the growing clinical impact of CoNS include an immature or compromised immune system, breaking of the natural skin and mucosa barrier and presence of indwelling medical devices.[1—5] More than 50 different CoNS species have been described but only a few species are believed to be associated with a higher clinical significance and include *Staphylococcus epidermidis*.[1—5]

With the technical and medical advances in neonatal intensive care units (NICUs), the overall survival and prognosis of the preterm babies have been improved markedly. Then, preterm birth-related complications would occur and become the major cause of neonatal death.[6] Among these serious complications, healthcare-associated late-onset sepsis (LOS) is frequent in NICUs and between 20 and 30% of very low-birthweight preterm babies develop LOS at least once during their stay in the NICU. Undoubtedly, skin commensal CoNS are the most frequently involved pathogens and *S. epidermidis* is regarded as the main CoNS pathogen of LOS in this population. Most clonal lineages of *S. epidermidis* are commensals.[7—13] However, some globally spreading healthcare-associated methicillin-resistant *S. epidermidis* (HA-MRSE) clones, namely sequence type (ST) 2 and ST5, are major causes of the difficult-to-treat invasive infections.[7—13] There has been scanty reports on molecular epidemiology of *S. epidermidis* causing LOS in infants stayed in NICU and on correlation of molecular characteristics with clinical features in these infants.

In Taiwan, CoNS are also a leading cause of LOS in NICU in most hospitals and CoNS accounted for approximately 40% of LOS etiologies in several large-scale studies.[14—18] However, most studies did not identify the CoNS isolates to the species level, not mentioning molecular epidemiology of any designated CoNS species. With the introduction of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to clinical microbiologic laboratory of our hospital in 2013, most clinical CoNS isolates can be identified to the species level, which made the research on molecular epidemiology of CoNS isolates possible. In this study, we first molecularly characterized *S. epidermidis* bloodstream isolates from infants hospitalized at NICUs of our hospital between 2018 and 2020 and then we collected, analyzed and compared demographic data, predisposing factors, clinical manifestations, laboratory data, management and outcome of these infants, stratified by the genetic background of the isolates.

Methods

Subjects and setting

This study was conducted in Chang Gung Pediatric Medical Center, which is a part of Chang Gung Memorial Hospital (CGMH) at Linkou and is a university-affiliated teaching hospital in northern Taiwan. There are five neonatal units, including three neonatal intensive care units (NICUs) and two special care nurseries (SCNs), distributed on 2 floors, in this Pediatric Medical Center. Currently, there are 16, 24 and 10 beds in NICU-1 (3L), NICU-2 (5L), and NICU-3 (3L) respectively. For SCNs, there are 24 beds in SCN-1 (3L) and 30 beds in SCN-2 (5L).

According to the microbiologic database of CGMH, we retrieved *S. epidermidis* bloodstream isolates from infants hospitalized at NICUs between January 2018 and December 2020, which were systematically collected, preserved and stored in our hospital. Infants with bloodstream isolates, which were identified from blood cultures obtained at least 72 h after admission to NICUs and were also available for further analysis, were enrolled in this study. Because the significance of community-onset *S. epidermidis* bacteremia was doubted, we only focused on healthcare-associated infection of *S. epidermidis* in this study and thus isolates from blood cultures obtained within 72 h after admission to NICUs were excluded due to the high probability of community-onset. This study was approved by the institutional review board of Chang Gung Memorial Hospital.

Data collection

We retrospectively reviewed the medical records of enrolled patients and collected the following data: demographic data, comorbidities of prematurity, clinical manifestations, laboratory data, presence of a central venous catheter, usage of mechanical ventilation and antimicrobial therapy within the 30 days preceding infection, treatment courses for bacteremia, and clinical outcomes. All recorded data describing the episodes of late-onset sepsis caused by *S. epidermidis* were reviewed by two investigators (Y.-H.H. and Y.-R.Y.) for face validity.

Microbiologic methods

In our NICUs, blood cultures were drawn from patients with suspected sepsis at the discretion of the attending

physicians or residents on duty. Blood specimens were collected after aseptic preparation, and cultured in trypticase soy broth (Becton Dickinson, MD, USA). Positive culture results were detected using an automated detection system (BD BACTEC™ FX; Becton Dickinson, MD, USA). Blood was drawn from positive blood culture bottles and spread onto blood plate agar for subculture (Becton Dickinson, MD, USA). The agar and broth were incubated in a 37 °C CO_2 incubator for 18—24 h. Single colonies grown on agar plates were selected for further analysis. *S. epidermidis* was identified based on colony morphology, a coagulase test and Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics GmbH, Bremen, Germany).

Definitions

The diagnosis of *S. epidermidis* bacteremia was based on the criteria of the Vermont Oxford Network Database,[19—22] requiring clinical septic signs and symptoms and intravenous antibacterial therapy for at least 5 days after obtaining blood culture, or until death. Clinical signs and symptoms of sepsis included fever or hypothermia, hyper- or hypoglycemia, apnea or tachypnea, bradycardia or cyanosis, frequent desaturation with increased requirement of ventilator support, feeding intolerance, jaundice, abdominal distension, decreased activity, skin mottling and hypotension etc. Only symptoms that occurred during the episode were recorded.

Empirical antibiotics for coverage of both Gram-positive and Gram-negative organisms were usually prescribed at the onset of clinical sepsis. Modification of antimicrobial regimens would be decided by the attending physicians in charge according to the results and antibiotic susceptibility patterns of blood cultures. All therapeutic antimicrobial regimens were administrated through intravenous routes, and the optimal dosages, according to the standard guidelines, were confirmed by both the attending physicians and pharmacists.

Separated episodes in an individual patient were considered when *S. epidermidis* was identified with an interval greater than 14 days and a full course of appropriate antimicrobial agents as judged by the in-vitro susceptibility testing were administered with at least one negative blood culture. Persistent bacteremia was defined as at least one consecutive blood cultures positive for genetically identical *S. epidermidis* isolate, at least 48 h apart, after an in-vitro susceptible antibiotic therapy for at least three days during a single sepsis episode[23]; otherwise, recovery was defined as at least one negative blood culture after a 7-14-day course of in-vitro susceptible antibiotic therapy.

All patients were followed up until death or discharge. The definitions of underlying diseases and all comorbidities of prematurity, including bronchopulmonary dysplasia and necrotizing enterocolitis, were based on the latest updated diagnostic criteria in the standard textbook of neonatology.[24]

Antibiotic susceptibility study

The antimicrobial susceptibility of all *S. epidermidis* isolates to nine antibiotics, including oxacillin, trimethoprim/sulfamethoxazole (TMP—SMX), penicillin, linezolid, rifampicin, teicoplanin, clindamycin, vancomycin, and erythromycin, was tested in accordance with the guideline of Clinical and Laboratory Standard Institutes[25] by using the disk-diffusion method.

Molecular characterizations

All the *S. epidermidis* isolates were molecularly characterized, and the molecular methods used included pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion, *Staphylococcal* chromosomal cassette *mec* (SCC*mec*) typing, and multilocus sequence type (MLST). Presence of Panton-Valentine leukocidin (PVL) genes[27] was also examined. The details of the procedures were described elsewhere previously.[26—30] The pulsotypes of PFGE were designated in Arabic numbers. PFGE patterns with 4- or more band differences were classified as a different type and designated consecutively. PFGE patterns with $<$ 4-band differences from an existing genotype were defined as subtypes. MLST was examined for selective isolates of representative PFGE patterns.[30]

Statistics

For analyzing and comparing the demographic and clinical characteristics of LOS due to *S. epidermidis*, we would classify the patients to several subgroups according to their genotypes if any major clones were identified. Pearson's chi-squared test was used to analyze categorical variables and One-Way ANOVA was used to compare means of numerical variables.

The definition of statistical significance was $p < 0.05$. Post hoc test was analyzed when there was $p < 0.05$ in One-Way ANOVA results. All statistical analyses were performed using IBM SPSS version 19.0.

Results

Identification of late-onset sepsis due to *S. epidermidis*

During the study period, a total of 148 potential *S. epidermidis* bloodstream isolates from 118 infants hospitalized at our NICUs were identified. 41 patients were excluded from this study (Fig. 1) because blood cultures were obtained within 72 h of admission (n = 27), multiple pathogens were identified from blood cultures (n = 9), no or incomplete antimicrobial therapy were administered (n = 4), and no any isolate was available for analysis (n = 1). Of the 77 patients, 5 (6.5%) patients and 1 (1.3%) patient experienced 2 and 3 episodes of *S. epidermidis* LOS, respectively. Among the 5 patients with 2 episodes, three patients had no isolates available in their second episodes. The patient with 3 episodes had isolates available in only two episodes. Hence, three patients with two episodes were included for analysis. As a result, a total of 77 patients with 80 episodes of *S. epidermidis* bacteremia treated during the study period were included for further analysis in this study.

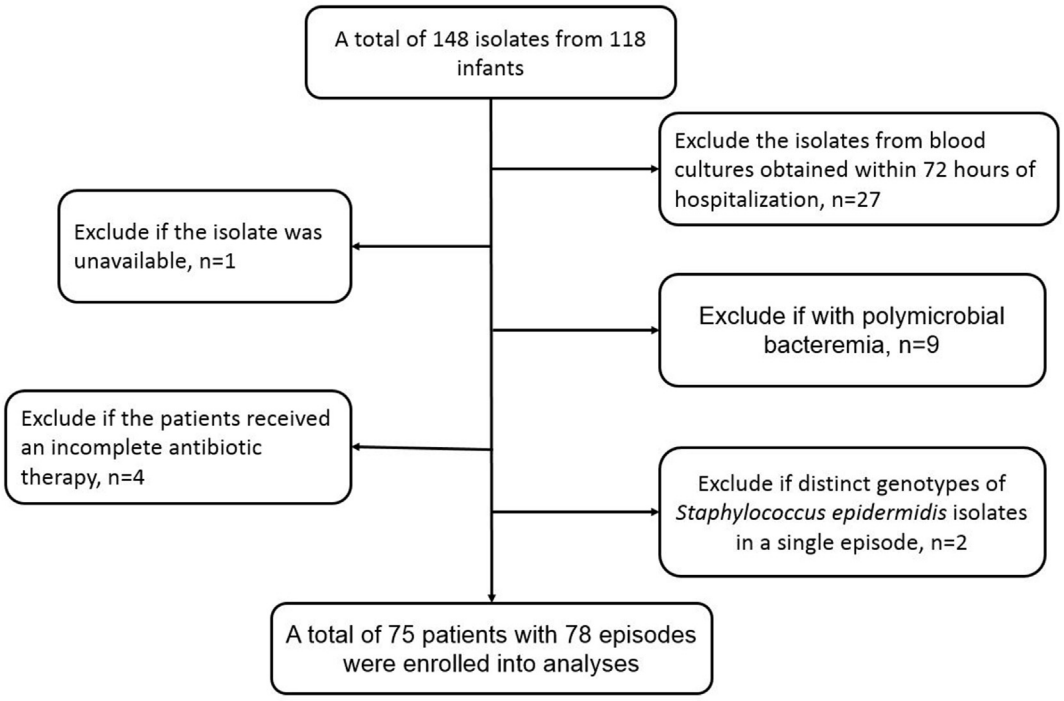


Figure 1. Flow chart of the patients who were enrolled in this study.

Molecular characterization

Of the 107 *S. epidermidis* isolates from 77 patients with 80 episodes available for molecular characterization, a total of 24 pulsotypes were identified by PFGE (Fig. 2), named as pulsotypes 100—3500. Five SCC*mec* types were identified and included SCC*mec* type I, II, III, IV, and V. 18 isolates from 14 episodes had untypeable SCC. 43 isolates of 24 PFGE patterns were selected for MLST analysis and 11 sequence types (STs) were identified. None of them possessed Panton-Valentine leukocidin genes.

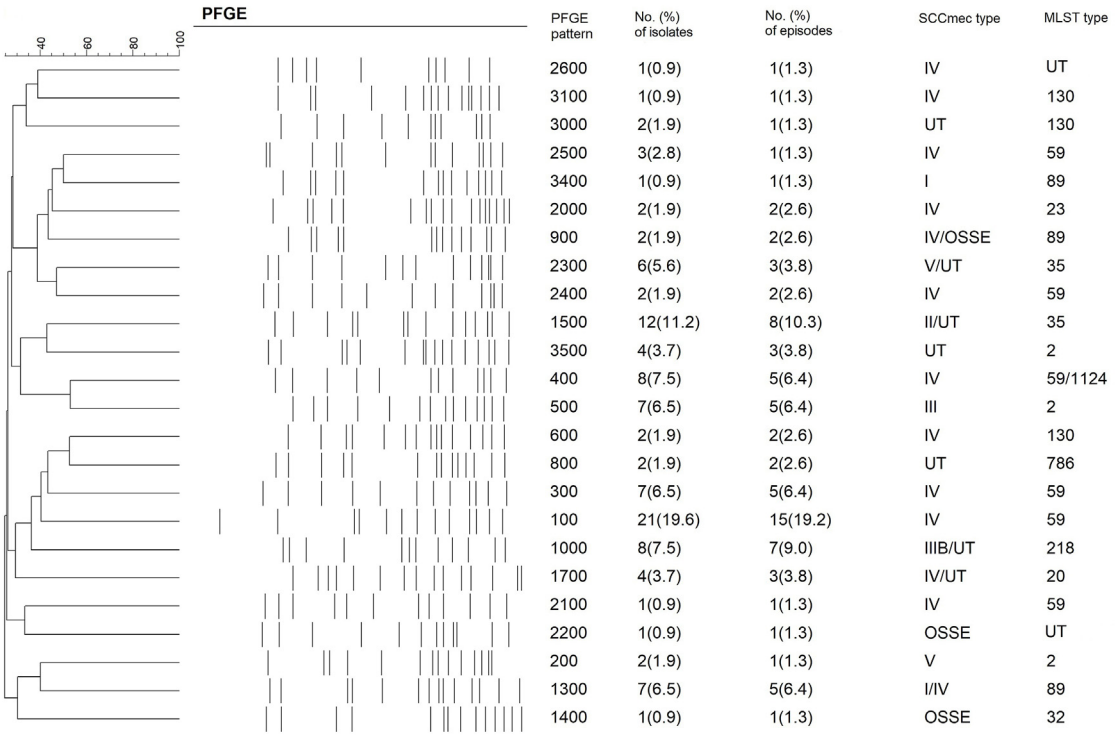
Of the 41 episodes excluded for analysis, 37 isolates were available for characterization and revealed 20 pulsotypes and 13 STs, respectively (9 pulsotypes and 6 STs, respectively, not identified in above 107 isolates) (Supplementary Table 1). Of the 144 isolates characterized, four novel sequence types (ST1124, 1125, 1126, 1131) were identified.

Of the 80 episodes in 77 patients, 19 episodes had multiple isolates from a single episode available for analysis, and all the *S. epidermidis* isolates from a single episode were genetically indistinguishable in 16 episodes, among which five episodes had 3 isolates and 11 episodes had two isolates. Of the remaining 3 episodes, both *S. epidermidis* isolates from a single episode were genetically distinct in two episodes; one episode had pulsotype 100 & 400 isolates identified, and the other episode had pulsotype 400 &1000 identified, in which we could not differentiate either pulsotype (isolate) to be the true pathogen. Hence, these two episodes were excluded for further clinical analysis. Whereas, the third episode had 4 isolates from a single episode; the first isolate identified at onset was characterized as pulsotype 100 (ST59) while the three sequential isolates from the blood cultures obtained on day 6, 9, and 10, respectively, were characterized as pulsotype 500 (ST2). We classified this case in the subgroup of pulsotype 500/ST2 for further analysis. Finally, a total of 75 infants with 78 episodes of *S. epidermidis* bacteremia were included for clinical analysis by sub-genogroups (Fig. 1). One isolate (genotype) for each episode was used to be classified into sub-genogroups.

Of the 78 isolates (episodes), pulsotype 100/sequence type (ST) 59/SCC*mec* IV (19.2%) and pulsotype 1500/ST35/SCC*mec* II (10.3%) were the two most common strains. One isolate belonged to ST1124, which is a single locus variant of ST59 and thus was classified together. Of the 11 STs identified, all STs but ST32 and ST786 belonged to clonal complex (CC) 2 (Table 1, Fig. 3), and ST59/1124 (37.2%), ST35 (14.1%), ST2 (11.5%), and ST89 (10.3%) were the four most common sequence types. ST59 dominated in each year during the study period, and accounted for 41.7%, 40.0%, and 32.0% of the isolates in 2018, 2019, and 2020, respectively. ST35 was identified since 2019, and accounted for 24.1% and 16% in 2019 and 2020, respectively. Based on the results of molecular characterization, we classified the patients into five sub-genogroups (ST59, ST89, ST35, ST2, and others) and we analyzed and compared the demographics and clinical features of these infants among the five groups.

Basic demographics

Demographic characteristics of the 75 patients with 78 episodes of LOS caused by *S. epidermidis* are shown in Table 2. Male accounted for 57.7% (n = 45/78). The mean gestational age was 31 weeks (range, 23—40 weeks). The average of birth body weight was 1705 g (range, 500—3410 g). Age of onset ranged from 4 to 150 days with a mean of 32.88 days.



PFGE pattern	No. (%) of isolates	No. (%) of episodes	SCCmec type	MLST type
2600	1(0.9)	1(1.3)	IV	UT
3100	1(0.9)	1(1.3)	IV	130
3000	2(1.9)	1(1.3)	UT	130
2500	3(2.8)	1(1.3)	IV	59
3400	1(0.9)	1(1.3)	I	89
2000	2(1.9)	2(2.6)	IV	23
900	2(1.9)	2(2.6)	IV/OSSE	89
2300	6(5.6)	3(3.8)	V/UT	35
2400	2(1.9)	2(2.6)	IV	59
1500	12(11.2)	8(10.3)	II/UT	35
3500	4(3.7)	3(3.8)	UT	2
400	8(7.5)	5(6.4)	IV	59/1124
500	7(6.5)	5(6.4)	III	2
600	2(1.9)	2(2.6)	IV	130
800	2(1.9)	2(2.6)	UT	786
300	7(6.5)	5(6.4)	IV	59
100	21(19.6)	15(19.2)	IV	59
1000	8(7.5)	7(9.0)	IIIB/UT	218
1700	4(3.7)	3(3.8)	IV/UT	20
2100	1(0.9)	1(1.3)	IV	59
2200	1(0.9)	1(1.3)	OSSE	UT
200	2(1.9)	1(1.3)	V	2
1300	7(6.5)	5(6.4)	I/IV	89
1400	1(0.9)	1(1.3)	OSSE	32

Figure 2. Distribution of pulsed-field gel electrophoresis patterns and other molecular characterizations of 107 *Staphylococcus epidermidis* isolates. PFGE, pulsed-field gel electrophoresis; SCC*mec*, staphylococcal chromosomal cassette *mec*; UT, untypable. OSSE, oxacillin sensitive *Staphylococcus epidermidis*; MLST, multilocus sequence type.

Table 1 Distribution of multilocus sequence types (MLST), clonal complex and genetic cluster by Bayesian analysis of population structure (BAPS) of 78 *Staphylococcus epidermidis* bloodstream isolates.

MLST type	Clonal complex	Genetic cluster by BAPS	No. of episodes	Percentage (%)
59[a]	2	6	29	37.2
35	2	1	11	14.1
89	2	1	8	10.3
2	2	5	9	11.5
218	2	1	7	9.0
20	2	1	3	3.8
786	—	—	2	2.6
23	2	5	2	2.6
130	2	6	4	5.2
UT	—	—	2	2.6
32	32	6	1	1.3
total			78	100.2[b]

[a] included one isolate of ST1124, which is a single locus variant of ST59.

[b] Owing to rounding to the nearest first decimal places in the percentage of each MLST type, there may be a slight discrepancy between the sum of individual items and the total as shown in the table, which made the total added up to 100.2%. UT, untypable.

Peripherally-inserted central venous catheters were used at the onset of bacteremia in all the 78 episodes. Catheters were removed/replaced after the onset of bacteremia and sent for bacterial culture in 58 episodes (74.4%). Catheter culture positive for *S. epidermidis* was found in 20 episodes. More than one-third of the patients had underlying diseases, such as cardiovascular disease (34.6%), gastrointestinal sequelae (11.5%), congenital anomalies (10.6%), and immune disease (2.6%). No statistically significant differences

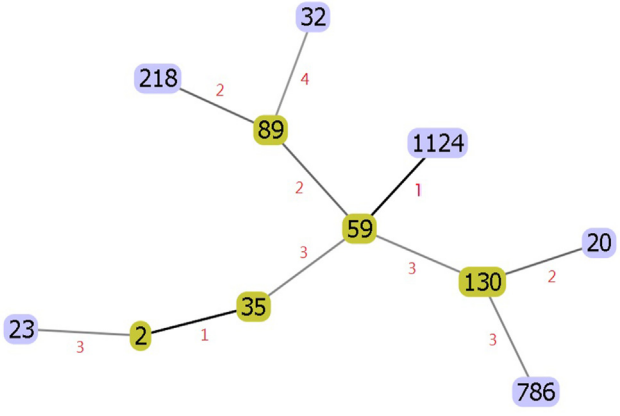


Figure 3. eBURST analysis of 78 *Staphylococcus epidermidis* isolates.

Table 2 Comparison of demographics, underlying conditions of 78 episodes of late-onset sepsis caused by *Staphylococcus epidermidis* in infants stayed in neonatal intensive care units, stratified by sequence types.

Characteristic	No. (%) of subjects						p-value[a]
	Total (n = 78)	ST 59 (n = 29)	ST 35 (n = 11)	ST 89 (n = 8)	ST 2 (n = 9)	Others (n = 21)	
Male gender	45 (57.7%)	16 (55.2%)	9 (81.8%)	3 (37.5%)	5 (55.6%)	12 (57.1%)	0.405
Birth weight							
Mean + −SD, gm	1705.3+-853.2 (500–3410)	1937.9+-912.9 (570–3410)	1445.5+-739.3 (600–3000)	1683.8+-876.5 (810–3380)	1963.3+-832.2 (720–3210)	1418.0+-764.5 (500–2900)	0.171
<1500 gm	38 (48.7%)	11 (37.9%)	7 (63.6%)	5 (62.5%)	3 (33.3%)	12 (57.1%)	0.546
1500～2000 gm	13 (16.7%)	6 (20.7%)	2 (18.2%)	0 (0%)	1 (11.1%)	4 (19%)	
Gestational age							
Mean, weeks	31.4+-4.8 (23–40)	32.3+-5.2 (23–40)	30.5+-4.4 (23–38)	31.0+-3.6 (27–36)	33.9+-4.9 (25–40)	29.8+-4.3 (24–38)	0.175
<30 weeks	29 (37.2%)	10 (34.5%)	4 (36.4%)	3 (37.5%)	1 (11.1%)	11 (52.4%)	0.337
30～37 weeks	38 (48.7%)	13 (44.8%)	6 (54.5%)	5 (62.5%)	5 (55.6%)	9 (42.9%)	
Underlying diseases[a]							
Congenital anomalies	8 (10.6%)	4 (13.8%)	1 (9.1%)	1 (12.5%)	0 (0%)	2 (9.5%)	0.933
Cardiovascular disease	27 (34.6%)	8 (27.6%)	4 (36.4%)	3 (37.5%)	5 (55.6%)	7 (33.3%)	0.643
Gastrointestinal sequelae	9 (11.5%)	4 (13.8%)	2 (18.2%)	1 (12.5%)	1 (11.1%)	1 (4.8%)	0.771
Immune disease	2 (2.6%)	0 (0%)	0 (0%)	0 (0%)	2 (22.2%)	0 (0%)	0.021
Retinopathy of prematurity	6 (7.7%)	2 (6.9%)	0 (0%)	0 (0%)	1 (11.1%)	3 (14.3%)	0.663
Surgical history (within one month)	27 (34.6%)	8 (27.6%)	3 (27.3%)	2 (25%)	6 (66.7%)	8 (38.1%)	0.283
Surgery during the episode	9 (11.5%)	3 (10.3%)	2 (18.2%)	1 (12.5%)	2 (22.2%)	1 (4.8%)	0.529
Age at onset, mean + −SD (days)	32.88+-29.48 (4–150)	28.83+-26.92 (4–120)	36.55+-36.13 (10–135)	25.88+-12.05 (10–45)	40.67+-27.40 (12–105)	35.90+-35.20 (6–150)	0.742
Use of NG tube during the episode	75 (96.2%)	29 (100%)	10 (90.9%)	8 (100%)	8 (88.9%)	20 (95.2%)	0.257
Use of CPAP during the episode	62 (79.4%)	23 (79.3%)	10 (90.9%)	6 (75%)	6 (66.7%)	17 (81%)	0.908
Use of endotracheal tube during the episode	51 (65.4%)	17 (58.6%)	7 (63.6%)	6 (75%)	6 (66.7%)	15 (71.4%)	0.883
Removal of CVC during the episode	58 (74.4%)	20 (69%)	9 (81.8%)	6 (75%)	7 (77.8%)	16 (76.2%)	0.94
CVC culture positive for *S. epidermidis*	20 (25.6%)	3 (10.3%)	3 (27.3%)	2 (25%)	4 (44.4%)	8 (38.1%)	0.099

[a] Pearson's chi-squared test was used to analyze categorical variables and One-Way ANOVA was used to compare means of numerical variables with post hoc test.
SD, standard deviation; NG, nasogastric; CPAP, continuous positive airway pressure; CVC, central venous catheters.

were noted among the 5 groups in terms of demographics and most underlying conditions except underlying immune disease which was significantly associated with patients with ST2 infection (P = 0.021).

Clinical and laboratory characteristics

Clinical symptoms at the onset of bacteremia are shown in Supplementary Table 2. The common symptoms recorded

during the LOS episode included fever (21.5%), tachycardia (17.9%)/bradycardia (34.6%), low O_2 saturation (50.0%), abdominal distention (32.1%), poor activity (15.4%), poor feeding (4.1%), hypotension (3.8%), cyanosis (1.3%), and jaundice (1.3%). There were no significant statistical differences among the five groups.

Laboratory characteristics are summarized in Table 3. The mean leukocytes count at onset was 10,400/μL, and leukocytosis, defined as WBC count ≧15,000/μL in patients older than 1-month-old and ≧30,000/μL in patients younger than 1-month-old, was noted in 14 episodes (17.9%). The average platelet count at onset was 218,500/μL and thrombocytopenia (platelet count <150,000/μL) was noted in 38 (48.7%) episodes. The mean C-reactive protein level at onset was 22.8 mg/L (normal, <5 mg/L), ranging from 0.23 to 138.4 mg/L. No statistically significant differences were found among the 5 groups.

Antibiotics treatment

The summary of antibiotics susceptibility of these 78 episodes are shown in Table 4. All the isolates were susceptible to linezolid, teicoplanin and vancomycin while only three (3.8%) and one isolates were susceptible to oxacillin and penicillin, respectively. Only three isolates were resistant to rifampin while 58 (74.4%), 46 (59%), and 22 (28.2%) of the isolates were susceptible to trimethoprim/sulfamethoxazole, clindamycin, and erythromycin, respectively. The isolates of ST59 were significantly associated with a higher susceptibility to clindamycin while lower susceptibility to trimethoprim/sulfamethoxazole than other sub-genogroups.

Among the 78 episodes, empirical combined antibiotics therapy was used in 68 (87.1%) episodes; and single empirical antibiotic (either first-generation cephalosporin, aminoglycoside, or glycopeptide) was used in the remaining 10 episodes (12.9%). Inadequate empirical antibiotic therapy, defined as not including in vitro susceptible antibiotics, was used in 18 episodes (23%) (Supplementary Table 3). Some patients also received antifungal agents and anti-anaerobic agents simultaneously, but no statistically significant differences were found among the 5 groups in term of the simultaneous usage of antifungal agents and anti-anaerobic agents.

Definite antibiotic therapy with vancomycin was used in 64 (82.1%) episodes; teicoplanin and oxacillin was used in one (1.3%) and two (2.6%) episodes, respectively; and vancomycin-containing regimen as combination therapy was used in the remaining 11 (14.2%) episodes (Supplementary Table 4). Persistent bacteremia was detected in three (3.8%) patients. Two of them were infected with ST2 strain, and the other one was infected with ST35 strain. All of them were prescribed with vancomycin. No statistically significant differences were found among the 5 sub-genogroups in terms of the antibiotics used and outcomes.

Discussion

To our knowledge, this is the first study to investigate molecular characteristics of *S. epidermidis* bloodstream isolates in neonates from Taiwan. Results from this study showed that more than 90% of *S. epidermidis* bloodstream isolates from infants stayed in NICUs in northern Taiwan were oxacillin-resistant. Of the 78 *S. epidermidis* bloodstream isolates, though diverse, four major sequence types, namely ST59, ST35, ST2 and ST89, were identified and each ST accounted for at least 10% of the 78 episodes. ST59/SCC*mec* IV was the most frequently identified strain, and accounted for more than one-third of the *S. epidermidis* isolates. All but 5 isolates (73/78, 93.6%) belonged to clonal complex 2 (CC2). The results of molecular characterization in the present study were similar to those reported worldwide previously, particularly from China. Also, this is the first study to correlate molecular genotypes of *S. epidermidis* with clinical characteristics of the NICU-hospitalized infants with LOS due to *S. epidermidis*; however, generally, no statistically significant differences were found among the 5 genogroups in terms of the demographics, underlying diseases, clinical symptoms/signs and laboratory data, antibiotics used and clinical outcomes.

Table 5 summarizes the results of molecular typing for *S. epidermidis* from five selected reports[11,31–34] worldwide. Among the five reports, a total of 485 clinical, infecting isolates and 60 colonizing isolates collected between 2002 and 2018 from four countries were characterized, and the results showed that the isolates were genetically heterogeneous in each study and the prevailing strains (STs) were also diverse in different studies, but most of the isolates, particularly those causing complicated infections, shared only a limited number of STs and belonged to clonal complex (CC) 2. Using genomics to analyzing isolates from 96 institutions in 24 countries, Lee et al. reveal that three multidrug-resistant, hospital-adapted lineages of *S. epidermidis* (two ST2 and one ST23) have emerged in recent decades and spread globally.[15,35] Altogether, the prevalent strains (STs) of *S. epidermidis* may differ in different regions, countries, and continents; however, the majorities of these strains (STs) belong to CC2.

Although we detected a diversity of SCC*mec* types in this study, the number size of SCC*mec* type IV was larger than those of SCC*mec* types I, II, III and V. All the ST59 isolates carried SCC*mec* type IV, which maybe can confer that SCC*mec* IV increased the dissemination ability.[36] According to the eBURST analysis,[37] *S. epidermidis* STs could be cluster into some clonal complex (CC). ST2, which was the global dominant *S. epidermidis* strain, belonged to CC2–I; while the major clone in our NICU, ST59, was classified in CC2-II. CC2–I had higher methicillin resistant rate (95.2%) than CC2-II (70.7%),[13] while in this study more than 90% of *S. epidermidis* isolates were oxacillin-resistant. Bayesian analysis of population structure (BAPS) analysis[11,38] classified *S. epidermidis* STs into 6 genetic clusters (GCs). ST2 belonged to GC5, while ST59 was brought into GC6. GC5 could form a hard biofilm and bring the virulence markers, such as *ica*A, making it have significant resistance to certain antibiotics, including oxacillin, clindamycin, gentamicin, and TMP–SMX.[39] Therefore, those properties made the clone well adapted to the nosocomial environment and became the most prevalent clone around the world. However, GC6 was also common in clinical specimens, but it possessed less virulence factors than GC5.[40]

Similar to previous studies, increasing incidence rate of LOS in infants with a lower birth weight and gestational age

Table 3 Comparison of laboratory characteristics of 78 episodes of late-onset sepsis caused by *Staphylococcus epidermidis* in infants stayed in neonatal intensive care units, stratified by sequence types (STs).

Laboratory data	Total (n = 78)	ST 59/1124 (n = 29)	ST35 (n = 11)	ST89 (n = 8)	ST2 (n = 9)	Others (n = 21	p-value[c]
WBC ([c]1000/μL) at onset, mean + −SD	10.4+-4.8 (2.2 −24.3)	10.9+-5.5 (2.2 −24.3)	9.8+-4.7 (5.3 −19.8)	12.6+-4.1 (5.6 −17.2)	9.5+-5.1 (4.6 −20.2)	9.6+-3.8 (4.6−18.1)	0.56
Peak WBC ([c]1000/μL) count, mean + −SD	14.2+-5.3 (6.5 −38.7)	14.8+-4.7 (7.4 −28)	13.0+-3.7 (6.8 −19.8)	14.2+-2.0 (11.8 −17.2)	14.5+-7.0 (6.9 −27.4)	13.8+-7.0 (6.5−38.7)	0.89
Nadir WBC ([c]1000/μL) count, mean + −SD	8.7+-3.8 (1.2 −22.1)	9.4+-4.4 (2.2 −22.1)	7.5+-3.7 (1.2−13)	10.3+-3.7 (5.6 −15.2)	8.0+-4.0 (4.6 −17.6)	8.1+-3.0 (3−13.5)	0.377
Leukocytosis (peak)[a]	14 (17.9%)	5 (17.2%)	2 (18.2%)	2 (25%)	3 (33.3%)	2 (9.5%)	0.557
Leukopenia (nadir)[b]	6 (7.7%)	2 (6.9%)	2 (18.2%)	0 (0%)	0 (0%)	2 (9.5%)	0.606
Hb (g/dL) at onset, mean + −SD	12.1+-1.9 (7.5 −19.4)	12.3+-2.3 (9.1 −19.4)	11.9+-1.7 (9 −14.3)	12.1+-1.3 (10.3 −13.7)	11.4+-2.1 (7.5 −13.8)	12.1+-1.5 (9.9−15.3)	0.816
Nadir Hb level (g/dL), mean + −SD	10.6+-1.7 (7.5 −17.2)	11.0+-1.8 (8.4 −17.2)	10.8+-1.5 (9 −13.7)	10.1+-0.7 (9.4 −11.2)	10.3+-1.8 (7.5 −12.9)	10.3+-1.8 (8−14.4)	0.516
Hb < 10 g/dL (nadir)	30 (38.5%)	7 (24.1%)	3 (27.3%)	4 (50%)	4 (44.4%)	12 (57.1%)	0.145
Platelet ([c]1000/μL) at onset, mean + −SD	218.5+-132.4 (28 −603)	254.2+-139.4 (37 −603)	202.2+-132.5 (61 −431)	194.9+-104.6 (58 −365)	242+-122.1 (67 −472)	176.8+-131.9 (28−492)	0.301
Nadir platelet count ([c]1000/μL), mean + −SD	174.3+-124.5 (11 −603)	207.2+-138.2 (30 −603)	153.1+-134.5 (12 −369)	174.4+-96.8 (11 −310)	181.8+-123.1 (25 −371)	136.6+-105.8 (14−325)	0.373
Platelet<150,000/μL (nadir)	38 (48.7%)	11 (37.9%)	7 (63.6%)	3 (37.5%)	5 (55.6%)	12 (57.1%)	0.49
CRP (mg/L) at onset, mean + −SD	22.8+-24.8 (0.23 −138.4)	19.3+-29.1 (0.23 −138.4)	30.3+-26.8 (2.4 −69.8)	26.2+-20.8 (0.4 −54.8)	17.8+-13.4 (1.03 −33.97)	25.2+-23.0 (0.28−68.23)	0.485
Highest C-reactive protein (mg/dL), mean + −SD	34.1+-30.5 (0.5 −158.35)	25.3+-28.4 (0.5 −138.4)	51.2+-41.3 (13.56 −158.35)	40.8+-37.2 (1.46 −98.2)	35.8+-17.3 (1.35 −56.87)	34.0+-26.8 (1.55−104.84)	0.18
CRP>40 mg/L (peak)	25 (32.1%)	5 (17.2%)	6 (54.5%)	3 (37.5%)	4 (44.4%)	7 (33.3%)	0.158

[a] Leukocytosis was defined as WBC count ≧15,000/μL in patients older than 1-month-old and ≧30,000/μL in patients younger than 1-month-old.
[b] Leukopenia was defined as WBC count <4500/μL.
[c] Pearson's chi-squared test was used to analyze categorical variables and One-Way ANOVA was used to compare means of numerical variables with post hoc test.
SD, standard deviation; WBC, white blood cell; Hb, hemoglobin; CRP, serum C-reactive protein.

Table 4 Comparison of antibiotic susceptibility of 78 *Staphylococcus epidermidis* isolates stratified by sequence types (STs).

Antibiotics	Total (n = 78) No. (%)	ST59# (n = 29) No. (%)	ST35 (n = 11) No. (%)	ST89 (n = 8) No. (%)	ST2 (n = 9) No. (%)	Others (n = 21) No. (%)	p-value[b]
Clindamycin	46 (59.0)	23 (79.3)	4 (36.4)	4 (50)	3 (33.3)	12 (57.1)	0.034
Erythromycin	22 (28.2)	6 (20.7)	1 (9.1)	1 (12.5)	2 (22.2)	12 (57.1)	0.018
Oxacillin	3 (3.8)	0	0	1 (12.5)	0	2 (9.5)	0.181
Penicillin	1 (1.3)	0	0	0	0	1 (4.8)	0.628
SXT	58 (74.4)	13 (44.8)	11 (100)	8 (100)	8 (88.9)	18 (85.7)	<0.001
Rifampin	75 (96.2)	28 (96.6)	11 (100)	8 (100)	9 (100)	19[a](90.5)	0.800
Linezolid	78 (100)	29 (100)	11 (100)	8 (100)	9 (100)	21 (100)	—
Vancomycin	78 (100)	29 (100)	11 (100)	8 (100)	9 (100)	21 (100)	—
Teicoplanin	78 (100)	29 (100)	11 (100)	8 (100)	9 (100)	21 (100)	—

[a] Both isolates resistant to rifampin belonged to ST23/pulsotype2000.
[b] Pearson's chi-squared test was used to analyze categorical variables and One-Way ANOVA was used to compare means of numerical variables with post hoc test.
ST, sequence type; SXT, trimethoprim/sulfamethoxazole.
#This group included one isolate of ST1124, which is a single locus variant of ST59.

Table 5 Summary of the results of molecular typing for *Staphylococcus epidermidis* from five selected reports worldwide.

References, study period	City, country	No. isolates	Sites isolation	Remarks
Guo et al., 2002—2014[31]	Wenzhou, China	223	Sterile, infecting	28 STs were identified and most of the clinical isolates belonged to CC2
Xu et al., 2018[32]	Tianjin, China	60	Hands, colonizing	ST59 (31.7%) and ST35 (6.7%) were the two most common clones detected
Bispo et al., 2009[33]	Brazil	30	Ocular, infecting	12 different STs were detected with a predominance of ST59 (30%), ST5 and ST6 (13.3% each). 93.3% of the isolates belonged to CC2 and grouped mainly within subcluster CC2-II (92.9%).
Mendes et al., 2010[11]	United States	71	Any, infecting	27 STs were detected, and ST5 (21.1%) and ST2 (16.9%) predominated. 87.3% of STs belonged to CC2.
Shelburne et al., 2011—2017[34]	United States and Spain	161	Bloodstream, infecting	49 sequence types (STs) were identified. All strains causing complicated infections were derived from five STs, namely ST2, ST5, ST7, ST16, and ST32.

ST, sequence type; CC, clonal complex.

were noted due to the frequent usage of invasive catheters and longer time of hospitalization.[41—43] In this study, nearly half of the patients born with a very low birth weight (<1500g), and more than one-third of the patients were born with a <30-week gestation. Also, more than one-third of the patients had underlying diseases. However, no statistically significant differences were noted among the 5 genogroups in terms of demographics and most underlying conditions except underlying immune disease which was significantly associated with patients with ST2 infection (P = 0.021). Age of onset ranged from 4 to 150 days with a mean of 32.88 days. Central venous catheters were used at the onset of bacteremia in all the 78 episodes and of the removed catheters sent for culture, more than one-third revealed a positive result for *S. epidermidis*, suggesting peripherally inserted central catheter-related bacteremia

likely. A scenario, in which a very-low-birth-weight premature infant with a peripherally-inserted central venous catheter for nutritional support developed LOS due to *S. epidermidis* weeks later, was frequently seen in modern NICUs.

As expected, clinical symptoms at the onset of *S. epidermidis* bacteremia are non-specific and included fever, tachycardia, bradycardia, desaturation, and abdominal distention etc. As for laboratory data, leukocytosis was noted in less than one-fifth of the episodes at the onset, while 7.7% patients showed leukopenia and thrombocytopenia (platelet count $<$150,000/μL) was noted in nearly half of the episodes. The mean C-reactive protein level at onset was 22.8 mg/L (normal, $<$5 mg/L), ranging from 0.23 to 138.4 mg/L, and a value of $>$40 mg/L was noted in nearly one-third of the episodes. These findings again suggest that LOS caused by *S. epidermidis* is clinically significant and cannot be ignored. No statistically significant differences were found among the 5 genogroups in terms of clinical symptoms/signs and laboratory data.

Recently, the increase of antibiotic resistance among *S. epidermidis* is an emerging issue worldwide.[35] The pathogenic mechanism of *S. epidermidis* is mostly because of its ability to colonize on indwelling medical devices and form an adherent multi-layered biofilm within 24 h of invasion,[4] which is a big problem in treating *S. epidermidis* infection because bacterial biofilm provides significant resistance to antibiotics.[4] In the current study, though oxacillin resistant rate was quite high ($>$95%), all the isolates were susceptible to linezolid, teicoplanin and vancomycin and only three isolates were resistant to rifampin. It is intriguing that the isolates of ST59/1124 were significantly associated with a higher susceptibility to clindamycin while lower susceptibility to trimethoprim/sulfamethoxazole than other subgenogroups. However, the three multidrug-resistant, globally-spread lineages of *S. epidermidis* revealed by Lee et al.[15] are resistant to rifampicin through acquisition of specific rpoB mutations and these rpoB mutations not only confer rifampicin resistance, but also reduce susceptibility to the last-line glycopeptide antibiotics, vancomycin and teicoplanin. This previously unrecognized international spread of a near pandrug-resistant opportunistic pathogen deserved further surveillance and observation for clinical impact.

There are several limitations in this study. First, this study was conducted in a single medical center and the epidemiologic features shown here may not represent the whole perspective in Taiwan. However, our hospital is the largest hospital in Taiwan and the case number in this study was not small, so it still can partly reflect the current status of *S. epidermidis* in NICU in Taiwan. Second, though the isolates were systemically collected, medical records of the patients were retrospectively reviewed, so some clinical features recorded and laboratory data examined in the patients were not consistent and the comparison may be misleading. However, the items we chose for comparison were consistent. Third, we included the infants with only one blood culture positive for *S. epidermidis*, which are considered to be not true infection, in this study. However, all the infants had clinical symptoms/signs, had a peripherally-inserted central catheter at the onset of LOS and received at least 5-day course of antimicrobial therapy. In addition, all the *S. epidermidis* isolates from a single episode were genetically indistinguishable in 16 of 19 episodes with multiple isolates from a single episode and 3 of 4 *S. epidermidis* isolates from a single episode were genetically indistinguishable in an additional case, suggesting that the true infection rate is high.

Conclusions

More than 90% of *S. epidermidis* bloodstream isolates from infants in neonatal units in northern Taiwan were resistant to oxacillin. Though diverse, more than 90% of the isolates (episodes) belonged to clonal complex 2, and four major strains of *S. epidermidis*, namely ST59, ST35, ST2 and ST89, accounted for more than 70% of the isolates. No statistically significant difference was found among the NICU-hospitalized infants infected with these 4 genotypes and other genotypes in terms of demographics, clinical symptoms/signs and laboratory data. In addition to oxacillin, resistance to clindamycin, and trimethoprim/sulfamethoxazole were identified in a substantial proportion of the isolates and was significantly associated with genotypes. No reduced susceptibility to glycopeptides or linezolid was noted among these isolates. Further surveillance is needed.

Ethics approval

The study was approved by the institutional review board of Chang Gung Memorial Hospital (Reference No: 202100078B0) and this was a retrospective study so the consent to participate was waived.

Consent to publish

Not applicable.

Availability of data and materials

All the data are presented in the paper.

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Authors' contribution

YHH & YRY: laboratory performance, acquisition of data, analysis and interpretation of data, drafting the manuscript.

RIL & MCC: acquisition of data, analysis and interpretation of data,

YCH: conception and design, analysis and interpretation of data, modifying and revising the manuscript.

All authors read and approved the final manuscript.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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References

1. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev* 2014;**27**:870—926.
2. Becker K, Both A, Weißelberg S, Heilmann C, Rohde H. Emergence of coagulase-negative staphylococci. *Expert Rev Anti Infect Ther* 2020;**18**:349—66.
3. Heilmann C, Ziebuhr W, Becker K. Are coagulase-negative staphylococci virulent? *Clin Microbiol Infect* 2019;**25**:1071—80.
4. Dong Y, Speer CP, Glaser K. Beyond sepsis: *Staphylococcus epidermidis* is an underestimated but significant contributor to neonatal morbidity. *Virulence* 2018;**9**:621—33.
5. Michels R, Last K, Becker SL, Papan C. Update on coagulase-negative staphylococci—what the clinician should know. *Microorganisms* 2021;**9**:830.
6. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016;**388**:3027—35.
7. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics* 2010;**126**:443—56.
8. Cortese F, Scicchitano P, Gesualdo M, Filaninno A, de Giorgi E, Schettini F, et al. Early and late infections in newborns: where do we stand? A review. *Pediatr Neonatol* 2016;**57**:265—73.
9. Thomas JC, Vargas MR, Miragaia M, Peacock SJ, Archer GL, Enright MC. Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. *J Clin Microbiol* 2007;**45**:616—9.
10. Harris LG, Murray S, Pascoe B, Bray J, Meric G, Mageiros L, et al. Biofilm morphotypes and population structure among *Staphylococcus epidermidis* from commensal and clinical samples. *PLoS One* 2016;**11**:e0151240.
11. Mendes RE, Deshpande LM, Costello AJ, Farrell DJ. Molecular epidemiology of *Staphylococcus epidermidis* clinical isolates from U.S. hospitals. *Antimicrob Agents Chemother* 2012;**56**:4656—61.
12. Li X, Arias CA, Aitken SL, Galloway Pena J, Panesso D, Chang M, et al. Clonal emergence of invasive multidrug-resistant *Staphylococcus epidermidis* deconvoluted via a combination of whole-genome sequencing and microbiome analyses. *Clin Infect Dis* 2018;**67**:398—406.
13. Lee JYH, Monk IR, Gonçalves da Silva A, Seemann T, Chua KYL, Kearns A, et al. Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. *Nat Microbiol* 2018;**3**:1175—85.
14. Datta MS, Yelin I, Hochwald O, Kassis I, Borenstein-Levin L, Kugelman A, et al. Rapid methicillin resistance diversification in *Staphylococcus epidermidis* colonizing human neonates. *Nat Commun* 2021;**12**:6062.
15. Du X, Larsen J, Li M, Walter A, Slavetinsky S, Both A, et al. *Staphylococcus epidermidis* clones express *Staphylococcus aureus*-type wall teichoic acid to shift from a commensal to pathogen lifestyle. *Nat Microbiol* 2021;**6**:757—68.
16. Ho TS, Wang SM, Wu YH, Shen CF, Lin YJ, Lin CH, et al. Long-term characteristics of healthcare-associated infections in a neonatal intensive care unit. *J Microbiol Immunol Infect* 2010;**43**:407—15.
17. Wu JH, Chen CY, Tsao PN, Hsieh WS, Chou HC. Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. *Ped Neonatol* 2009;**50**:88—95.
18. Tsai MH, Hsu JF, Chu SM, Lien R, Huang HR, Chiang MC, et al. Incidence, clinical characteristics and risk factors for adverse outcome in neonates with late-onset sepsis. *Pediatr Infect Dis J* 2014;**33**:e7—13.
19. Makhoul IR, Sujov P, Smolkin T, Lusky A, Reichman B. Epidemiological, clinical, and microbiological characteristics of late-onset sepsis among very low birth weight infants in Israel: a national survey. *Pediatrics* 2002;**109**:34—9.
20. Horbar JD, Soll RF, Edwards WH. *Vermont Oxford Network database manual of operations*, release 2.0. Burlington [VT]: Vermont Oxford Network; 1993.
21. Makhoul IR, Sujov P, Smolkin T, Lusky A, Reichman B. Israel Neonatal Network. Pathogen-specific early mortality in very low birth weight infants with late-onset sepsis: a national survey. *Clin Infect Dis* 2005;**40**:218—24.
22. Bizzarro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy-five years of neonatal sepsis at Yale: 1928-2003. *Pediatrics* 2005;**116**:595—602.
23. Khashu M, Osiovich H, Henry D, Khotani AA, Solimano A, Speert DP. Persistent bacteremia and severe thrombocytopenia caused by coagulase-negative Staphylococcus in a neonatal intensive care unit. *Pediatrics* 2006;**117**:340—8.
24. Taeusch HW, Ballard RA, Gleason CA. *Avery's diseases of the newborn*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2006.
25. Wikler MA. *Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement*. 16th ed. M100-S16. Clinical and Laboratory Standards Institute; 2006.
26. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob Agents Chemother* 2007;**51**:264—74.
27. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. *Clin Infect Dis* 1999;**29**:1128—32.
28. Lu SY, Chang FY, Cheng CC, Lee KD, Huang YC. Methicillin-resistant Staphylococcus aureus nasal colonization among adult patients visiting emergency department in a medical center in Taiwan. *PLoS One* 2011;**6**:e18620.
29. Huang YC, Ho CF, Chen CJ, Su LH, Lin TY. Comparative molecular analysis of community-associated and healthcare-associated methicillin-resistant Staphylococcus aureus isolates from children in northern Taiwan. *Clin Microbiol Infect* 2008;**14**:1167—72.
30. Sharma P, Satorius AE, Raff MR, Rivera A, Newton DW, Younger JG. Multilocus sequence typing for interpreting blood isolates of Staphylococcus epidermidis. *Interdiscip Perspect Infect Dis* 2014;**2014**:787458.
31. Guo Y, Ding Y, Liu L, Shen X, Hao Z, Duan J, et al. Antimicrobial susceptibility, virulence determinants profiles and molecular characteristics of Staphylococcus epidermidis isolates in Wenzhou, eastern China. *BMC Microbiol* 2019;**19**(1):1—11.
32. Xu Z, Cave R, Chen L, Yangkyi T, Liu Y, Li K, et al. Antibiotic resistance and molecular characteristics of methicillin-resistant Staphylococcus epidermidis recovered from hospital personnel in China. *J Glob Antimicrob Resist* 2020;**22**:195—201. https://doi.org/10.1016/j.jgar.2020.02.013.
33. Bispo PJM, Hofling-Lima AL, Pignatari ACC. Characterization of Ocular Methicillin-Resistant Staphylococcus epidermidis isolates belonging predominantly to clonal complex 2 subcluster II. *J Clin Microbiol* 2014;**52**:1412—7.

34. Shelburne SA, Dib RW, Endres BT, Reitzel R, Li X, Kalia A, et al. Whole-genome sequencing of Staphylococcus epidermidis bloodstream isolates from a prospective clinical trial reveals that complicated bacteraemia is caused by a limited number of closely related sequence types. *Clin Microbiol Infect* 2020;**26**: 646.e1—8.
35. Kosecka-Strojek M, Sadowy E, Gawryszewska I, Klepacka J, Tomasik T, Michalik M, et al. Emergence of linezolid-resistant *Staphylococcus epidermidis* in the tertiary children's hospital in Cracow, Poland. *Eur J Clin Microbiol Infect Dis* 2020;**39**: 1717—25. https://doi.org/10.1007/s10096-020-03893-w.
36. Trindade PA, McCulloch JA, Oliveira GA, Mamizuka EM. Molecular techniques for MRSA typing: current issues and perspectives. *Braz J Infect Dis* 2003;**7**:32—43.
37. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. EBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 2004;**186**:1518—30.
38. Corander J, Marttinen P, Siren J, Tang J. Enhanced Bayesian modeling in BAPS software for learning genetic structures of populations. *BMC Bioinf* 2008;**9**:539.
39. Thomas JC, Zhang L, Robinson DA. Differing lifestyles of Staphylococcus epidermidis as revealed through Bayesian clustering of multilocus sequence types. *Infect Genet Evol* 2014;**22**:257—64.
40. Tolo I, Thomas JC, Fischer RS, Brown EL, Gray BM, Robinson DA. Do Staphylococcus epidermidis genetic clusters predict isolation sources? *J Clin Microbiol* 2016;**54**(7):1711—9.
41. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 2002;**110**:285—91.
42. Odabasi IO, Bulbul A. Neonatal sepsis. *The Medical Bulletin of Sisli Etfal Hospital* 2020;**54**:142.
43. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. *Arch Dis Child Fetal Neonatal Ed* 2015;**100**:F257—63.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.08.005.