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Comparative In vitro antibacterial activity of nemonoxacin and other fluoroquinolones in correlation with resistant mechanisms in contemporary methicillin-resistant *Staphylococcus aureus* blood isolates in Taiwan

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Abstract

Background Nemonoxacin is a new quinolone with an antibacterial efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA). Certain sequence types (STs) have been emerging in Taiwan, including fluoroquinolone-resistant ST8/USA300. It's an urgent need to determine nemonoxacin susceptibility against ST8/USA300 and other emerging lineages, if any. Additionally, molecular characterization of nemonoxacin resistance among different lineages has yet to be defined.

Methods Non-duplicated MRSA blood isolates from five hospitals during 2019–2020 were collected and genotyped by pulsed-field gel electrophoresis, and further correlated to their STs. Antimicrobial susceptibility testing for all antibiotics was performing by using Sensititre standard panel, except nemonoxacin by using agar dilution method. Selected isolates with nemonoxacin MICs ≥ 0.5 mg/mL were sequenced for quinolone resistance-determining regions (QRDRs).

Results Overall, 915 MRSA isolates belonged to four major lineages, ST8 (34.2%), ST59 (23.5%), ST239 (13.9%), and clonal complex 45 (13.7%). Two-thirds of tested isolates were non-susceptible to moxifloxacin, especially ST8/USA300 and ST239. Of them, proportions of nemonoxacin non-susceptibility by a tentative clinical breakpoint (tCBP) of 1 μg/mL among four major lineages appeared to be different ($P = 0.06$) and highest in ST239 (22.2%), followed by ST8/USA300 (13.5%). Among 89 isolates sequenced, 44.1% of ST8 and all ST239 isolates had ≥ 3 amino acid substitutions (AAS) in *gyrA/parC* (group A) or 2 AAS in *gyrA/parC* with additional AAS in *gyrB/parE* (group B). Compared to other AAS patterns, isolates in group A had the greatest non-susceptible proportions to nemonoxacin (86.9%; overall/pair-wised comparisons, $P < 0.05$).

Conclusions Our study confirmed ST8/USA300 MRSA has disseminated in Taiwan. Using a tCBP defined by a higher parenteral daily dosage, nemonoxacin retained potency against moxifloxacin non-susceptible isolates. Patterns of AAS in QRDRs among different lineages may contribute to difference of nemonoxacin susceptibility.

Keywords Moxifloxacin, Levofloxacin, Ciprofloxacin, Quinolone resistance determination regions, Sequence type 8

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Backgrounds

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a large antimicrobial resistance burden worldwide and substantially threatens human health in both community and healthcare settings [1]. Fluoroquinolones have a specific role in managing diverse clinical MRSA disease entities. Fluoroquinolones also are of importance in treating community-acquired pneumonia, especially as empirical therapy due to their covering broader spectrums of respiratory pathogens, including community-acquired MRSA (CA-MRSA). In addition, because of high oral bioavailability, relatively low protein binding and acceptable safety profile, fluoroquinolones in oral formulations are usually assigned as the backbone agent in combination regimens of early oral step-down therapy for uncomplicated MRSA bloodstream infections (BSIs) or of prolonged therapy for deep-seated infections, including endocarditis and osteoarticular infections [2, 3].

Nemonoxacin, a nonfluorinated quinolone also targeting the quinolone resistance-determining regions (QRDRs) of DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*), has shown potent antibacterial activities against a broad spectrum of Gram-positive and Gram-negative pathogens and enhanced potency against MRSA by substituting a methoxy group at its C-8 position [4, 5]. The substituents of the methoxy group at the C-8 position of nemonoxacin was also contributed to reduced mutant selection among different Gram-positive bacteria compared to other fluoroquinolones, including ciprofloxacin, levofloxacin, and moxifloxacin [6]. Before 2010, studies showed that nemonoxacin had potent in vitro activity against MRSA, especially CA-MRSA lineages (low MIC_{50} ranging 0.03–0.25 μg/mL) [5, 7, 8]. Nevertheless, among ciprofloxacin-resistant MRSA isolates, the nemonoxacin MIC_{50} values ranged between 0.5–1 μg/mL in Asia [7, 8] and was of 4 μg/mL in Canada [5]. Meanwhile, it's also concerned about hospital-acquired MRSA (HA-MRSA) lineages, which might have a nemonoxacin MIC_{50} of 4 μg/mL [5].

In Taiwan, the molecular epidemiology of MRSA lineages has changed recently. Specifically, sequence type (ST)8/USA300 MRSA was increasingly isolated from variable clinical specimens in Northern Taiwan before 2018 [9–11]. A study demonstrated that ST8/USA300 has replaced ST59, the traditional CA-MRSA clone, to dominate as the leading clone for CA-MRSA BSIs in Taiwan [10]. Meanwhile, that study also found ST45 rapidly expanded in healthcare-associated community onset (HACO) setting and HA setting, albeit ST239, the traditional HA-MRSA clone in Taiwan, remained the most prevalent HA-MRSA clone. Despite being classified as a CA-MRSA clone, ST8/USA300 showed surprisingly low susceptibility rates to fluroquinolones similar to those of ST239 (all < 5%). Clonal shifting and/or expansion among these four major MRSA lineages in Taiwan, consisting of ST8/USA300, ST239, ST59, and ST45, might alter the overall susceptibility to fluroquinolone. In addition, it is important to ascertain whether nemonoxacin retains its in vitro activities against new emerging MRSA lineages, including ST8/USA300 and ST45, and the associated mutations in QRDRs among different lineages.

Herein, we collected a contemporary set of non-duplicated MRSA blood isolates from five hospitals in Taiwan to delineate the molecular epidemiology of MRSA by multilocus sequence type (MLST), to test in vitro susceptibility of nemonoxacin and other key antibiotics, to perform molecular characterizations of QRDRs and to compare mutation difference among four major lineages further.

Methods

Study design and MRSA isolates

All MRSA blood isolates were initially identified by MALDI-TOF MS (Bruker, except bioMérieux VITEK in hospital E) in the clinical microbiology laboratories and prospectively stored by using the glycerol-containing screw-cap vials in freezers at each of the participating hospitals. We retrospectively collected the first MRSA blood isolate of each patient from these five participating hospitals between January 1st, 2019 and December 31st, 2020. Participating hospital A was located in northern Taiwan, hospital B in middle Taiwan, and hospitals C, D, & E in two cities in southern Taiwan. All participating hospitals were medical centers and provided primary and tertiary medical service. Before performing the following microbiological experiments, we reidentified and confirmed all non-duplicated bacterial isolates as MRSA by microscopic and biochemical methods, and cefoxitin screening method as previously described [10]. We also collected the onset setting of MRSA BSIs caused by each of these non-duplicated isolates. We epidemiologically categorized these isolates as into three groups: hospital-acquired (HA; index culture obtained ≥ 48 h after admission); healthcare-associated, community onset (HACO; at least one healthcare-associated risk factor); and community-associated (CA) as previously described [10].

Molecular typing

After extracting bacterial genomic DNA using a Blood and Tissue Genomic DNA Miniprep system kit (VIOGENE), we primarily performed pulsed-field gel electrophoresis (PFGE) of all isolates, analyzed their banding patterns by using BioNumerics software (version 8.1, Applied Maths, Ghent, Belgium), and clustered isolates by a band pattern similarity threshold of ≥ 80% for the

same pulsotype as previously described [10]. We used the reference strain FPR3757, belonging to USA300 with multidrug resistance, as a standard for confirming whether a clinical MRSA isolate belonged to USA300 as previously described [10]. Then, we randomly selected representative isolates within each pulsotype and those without a specific pulsotype to determine their STs by a MLST scheme [10]. We further performed clustering analysis and assigned each ST to specific clonal complexes by goeBURST algorithm [12] if different STs were identified in the same pulsotype.

In vitro susceptibility testing

We randomly selected half of isolates belonging to four major lineages (ST8/USA300, ST239, ST59, and ST45) in each hospital, and half isolates of each ST (if $n \geq 10$) and all isolates of each ST (if $n < 10$) among other STs for the following testing. We utilized the commercialized broth microdilution method by the Sensititre standard panel GPALL1F, using lyophilized plates, to determine in vitro susceptibility to three fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) and other key antibiotics, including clindamycin, daptomycin, erythromycin, gentamicin, linezolid, oxacillin, rifampin, tetracycline, trimethoprim/sulfamethoxazole (SXT), and vancomycin according to manufacturer's instructions. We interpreted the susceptibility test results by using the clinical breakpoint (CBP) criteria provided by the Clinical Laboratory Standards Institute [13]. We performed agar dilution method with a dilution range of 0.008–32 μg/mL to determine in vitro susceptibility to nemonoxacin (TaiGen Biotechnology Co. Ltd.) [14]. Tentative epidemiological cutoff value (ECOFF) value of nemonoxacin was 0.125 μg/mL for *S. aureus*, and dose-specific tentative *S. aureus* CBPs of 0.5 & 1 μg/mL for intravenous (IV) 500 mg & IV 750 mg nemonoxacin [14]. We used *S. aureus* ATCC 29213 as a reference strain for quality control.

Sequencing of QRDRs and characterization of QRDRs mutation patterns

For QRDRs target sequencing, we randomly selected certain isolates in each of following 16 categories, which were categorized by nemonoxacin MICs of 0.5, 1, 2, and ≥ 4 μg/mL among the four major lineages. Ten isolates were randomly selected when the isolate number within the category was ≥ 10, and all isolates were selected when the isolate number within the category was < 10. We used the primers of target genes, consisting of *gyrA*, *gyrB*, *parC* and *parE*, as previously described [6]. Then, we performed sanger sequencing of QRDRs for the above isolates and compared complete sequence fragments of each target genes to the reference strain, wild-type *S. aureus* NCTC 8325. According to reports of nonsynonymous mutations of *gyrA* and *parC* to confer quinolone resistance [15], we further categorized the combined patterns of amino acid substitutions among four groups: ≥ 3 amino acid substitutions in *gyrA*/*parC* with or without additional amino acid substitutions in *gyrB* and *parE* (group A), 2 substitutions in *gyrA*/*parC* with additional substitutions in *gyrB* and *parE* (group B), only 2 substitutions in *gyrA*/*parC* (group C), and no substitutions in *gyrA*/*gyrB*/*parC*/*parE* (group D).

Statistics

Categorical variables were expressed by numbers (percentages) and compared by using the chi-square test with Bonferroni-adjusted α for pair-wise comparisons as post-hoc analysis. A two-sided P value < 0.05 was considered significant. We analyzed the correlation between the MICs of nemonoxacin and other three fluoroquinolones by using Pearson correlation coefficient (r) [16]. All statistical analyses were performed using Stata software (version 17; StataCorp, College Station, TX).

Results

Molecular typing of MRSA

A total of 915 MRSA blood isolates with a range of 148 to 266 isolates in each of 5 hospitals was collected. Overall, we found 780 (85.2%) isolates were clustered within the four major lineages, and the other 135 isolates belonged to 19 other STs. ST8 accounted for 34.2% (n = 313), followed by ST59 (23.5%), ST239 (13.9%), and clonal complex (CC)45 (13.7%) (Supplementary Table 1). All ST8 isolates belonged to USA300. Among CC45, we identified ST45 and ST508 in the same pulsotype, and both STs were clustered within the same group in goeBURST algorithm. ST8 (range, 28.3% [hospital A]–39.9% [hospital B]) and ST59 (range, 18.4% [hospital B]–33.1% [hospital C]) were the most and second common STs in each hospital, respectively, even though the distribution of STs proportions were significantly different among hospitals ($P = 0.02$) (Fig. 1A; Supplementary Table 1).

Among 498 blood isolates with available onset setting, we identified HACO-MRSAB as the most common onset setting (40.0%, n = 199), followed by CA-MRSAB (33.1%, n = 165), and HA-MRSAB (26.9%, n = 134). Of them, ST8 was the most common ST for CA-MRSAB (38.2%) and HACO-MRSAB (31.1%), and shared the leading STs with ST59 (26.1% and 29.1%, respectively) (Fig. 1B; Supplementary Table 1). Notably, the proportions of CC45 differed among three onset settings, from 7.9% as CA-MRSAB, 15.1% as HACO-MRSAB, to 19.4% as HA-MRASB.

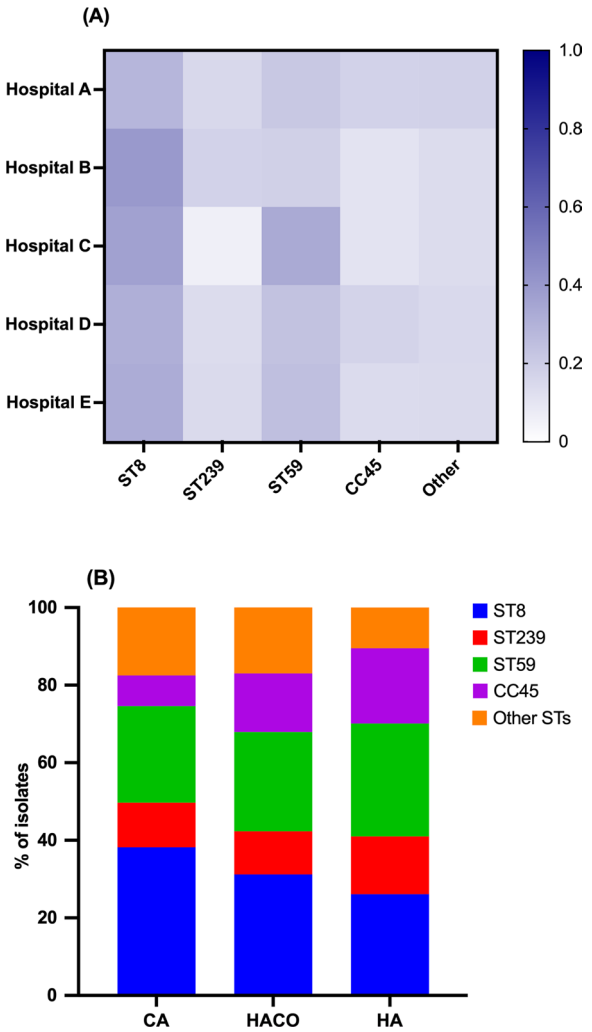

Fig. 1 Comparisons of sequence types by hospital (**A**) and by onset setting (**B**). CA, community acquired; HACO, healthcare-associated community onset; HA, hospital acquired

In vitro susceptibility testing of MRSA

For in vitro susceptibility testing, we selected 501 isolates, consisting of 157 ST8, 64 ST239, 108 ST59, 63 CC45, and 109 other STs isolates (Table 1). Compared to the susceptible proportions of other antibiotics tested by Sensititre, three tested fluroquinolones (moxifloxacin, levofloxacin, and ciprofloxacin) had the lowest susceptible rates, ranging from 35.3% to 37.5%, except that of erythromycin (19.6%). The susceptibility proportions to the three tested fluroquinolones were different among four major lineages. Specifically, few ST8 and ST239 isolates were susceptible to moxifloxacin, levofloxacin, and ciprofloxacin (≈1.5%), while the majority of ST59 were susceptible to three fluroquinolones tested (all ≥ 80.0%). For CC45, only one-third of isolates remained susceptible to three fluroquinolones tested. Other antibiotics also showed susceptible variations among the four major lineages, except all isolates were highly susceptible (≥ 90.0%) to vancomycin, daptomycin, linezolid, and rifampin, irrespective of STs.

To compare MIC distributions between nemonoxacin and other fluoroquinolones, Pearson correlation showed the highest coefficient (r) between MICs of nemonoxacin and moxifloxacin (0.711), followed by that (0.614) between those of nemonoxacin and levofloxacin, and that (0.582) between those of nemonoxacin and ciprofloxacin (all $P < 0.001$), indicating nemonoxacin MICs having the strongest correlation with moxifloxacin MICs. Thus, we depicted the distributions of nemonoxacin MICs by moxifloxacin susceptibility and STs (Table 2).

Nemonoxacin MIC_{50}/MIC_{90} for all isolates was 0.5/1 μg/mL with a range of 0.015 to 8 μg/mL. Overall, the distributions of nemonoxacin non-susceptibility proportions by a tentative clinical breakpoint (tCBP) of 1 μg/mL among four major lineages were numerically different although not statistically different ($P = 0.06$) and were highest in ST239 (22.2%), followed by ST8/USA300 (13.5%). Among 188 moxifloxacin susceptible isolates, most of them (183, 97.3%) had nemonoxacin MICs lower or equal to the tentative ECOFF of 0.12 μg/mL, and three-fifth of these 188 isolates belonged to ST59 and CC45. For 313 moxifloxacin non-susceptible isolates, 31.9% (n = 100) or 84.0% (n = 263) of them remained susceptible to nemonoxacin by dose-specific tentative CBPs of 0.5 μg/mL or 1 μg/mL, respectively, despite nemonoxacin MICs for most of isolates (n = 309; 97.8%) were higher than tentative ECOFF. Among moxifloxacin non-susceptible isolates, nemonoxacin MIC_{50}/MIC_{90} for ST59 and CC45 was 0.5/1 μg/mL, one dilution lower than that for the rest of STs, especially ST8 and ST239.

QRDRs mutations of MRSA

Eighty-nine isolates were sequenced for QRDRs, including 34 ST8, 23 ST239, 10 ST59, and 22 CC45 isolates. Among them, there were four different amino acid substitutions in *gyrA* (S84L, S85P, and E88A/K), and two in *gyrB* gene (D437E and P456S), respectively (Table 3). Whilst *parC* and *parE* gene had five (S80F/Y, S81P, and E84G/K) and three (D432N/V and R444C) different amino acid substitutions, respectively.

Each of four major lineages possessed specific combinations of amino acid substitutions at different genes in QRDRs. Fifteen of ST8 isolates (44.1%) had two or three substitutions in *gyrA* and *parC* with additional substitutions in *gyrB* and/or *parE* (group A or group B), while all ST239 isolates belonged to these two groups of amino acid substitutions in QRDRs (Table 3 and Fig. 2A). The majority of ST59 (7; 70.0%) and CC45 (17; 77.3%) only had S84L in *gyrA* and S80F in *parC*.

Table 1 In vitro susceptibility testing of key antibiotics against different sequence types of 501 methicillin–resistant *Staphylococcus aureus* blood isolates

	Total (n = 501)	ST8 (n = 157)	ST239 (n = 64)	ST59 (n = 108)	CC45[a] (n = 63)	other STs (n = 109)
Moxifloxacin						
Range[b] (μg/mL)	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4
MIC_{50}/MIC_{90}(μg/mL)	2/ > 4	2/ > 4	> 4/ > 4	≤ 0.25/2	2/4	≤ 0.25/4
S[c], n (%)	188 (37.5)	2 (1.3)	1 (1.6)	93 (86.1)	21 (33.3)	71 (65.1)
Levofloxacin						
Range[b] (μg/mL)	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4
MIC_{50}/MIC_{90}(μg/mL)	> 4/ > 4	> 4/ > 4	> 4/ > 4	0.25/ > 4	> 4/ > 4	0.5/ > 4
S[c], n (%)	188 (37.5)	2 (1.3)	1 (1.6)	93 (86.1)	21 (33.3)	71 (65.1)
Ciprofloxacin						
Range[b] (μg/mL)	1– > 2	1– > 2	1– > 2	1– > 2	1– > 2	1– > 2
MIC_{50}/MIC_{90}(μg/mL)	> 2/ > 2	> 2/ > 2	> 2/ > 2	1/ > 2	> 2/ > 2	1/ > 2
S[c], n (%)	177 (35.3)	2 (1.3)	1 (1.6)	87 (80.6)	21 (33.3)	66 (60.6)
Vancomycin						
Range[b] (μg/mL)	0.5–4	0.5–2	1–2	0.5–4	0.5–2	0.5–2
MIC_{50}/MIC_{90}(μg/mL)	1/1	1/1	1/2	1/1	1/1	1/1
S[c], n (%)	500 (99.8)	157 (100.0)	64 (100.0)	107 (99.1)	63 (100.0)	109(100.0)
Daptomycin						
Range[b] (μg/mL)	≤ 0.5–2	≤ 0.5–1	≤ 0.5–2	≤ 0.5–2	≤ 0.5–1	≤ 0.5–1
MIC_{50}/MIC_{90}(μg/mL)	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/1	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5
S[c], n (%)	499 (99.6)	157 (100.0)	63 (98.4)	107 (99.1)	63 (100.0)	109(100.0)
Linezolid						
Range[b] (μg/mL)	≤ 1–4	≤ 1–4	≤ 1–2	≤ 1–2	≤ 1–4	≤ 1–4
MIC_{50}/MIC_{90}(μg/mL)	2/2	2/2	2/2	2/2	2/2	2/2
S[c], n (%)	501 (100.0)	157 (100.0)	64 (100.0)	108 (100.0)	63 (100.0)	109(100.0)
Gentamicin						
Range[b] (μg/mL)	≤ 2– > 16	≤ 2– > 16	16– > 16	≤ 2– > 16	≤ 2– > 16	≤ 2– > 16
MIC_{50}/MIC_{90}(μg/mL)	8/ > 16	≤ 2/16	> 16/ > 16	16/ > 16	4/16	8/ > 16
S[c], n (%)	246 (49.1)	128 (81.5)	0 (0)	38 (35.2)	32 (50.8)	48 (44.0)
Rifampin						
Range[b] (μg/mL)	≤ 0.5– > 4	≤ 0.5– > 4	≤ 0.5– > 4	≤ 0.5–1	≤ 0.5– > 4	≤ 0.5– > 4
MIC_{50}/MIC_{90}(μg/mL)	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/2
S[c], n (%)	475 (94.8)	156 (99.4)	61 (95.3)	108 (100.0)	61 (96.8)	89 (85.6)
TMP/SMX						
Range[b] (μg/mL)	≤ 0.5– > 4	≤ 0.5– > 4	≤ 0.5– > 4	≤ 0.5– > 4	≤ 0.5–2	≤ 0.5– > 4
MIC_{50}/MIC_{90}(μg/mL)	≤ 0.5/ > 4	≤ 0.5/ ≤ 0.5	> 4/ > 4	≤ 0.5/ ≤ 0.5	≤ 0.5/ ≤ 0.5	≤ 0.5/1.2
S[c], n (%)	426 (85.0)	154 (98.1)	3 (4.7)	106 (98.1)	63 (100.0)	100 (91.7)
Tetracycline						
Range[b] (μg/mL)	≤ 2– > 16	≤ 2– > 16	≤ 2– > 16	≤ 2– > 16	≤ 2– > 16	≤ 2– > 16
MIC_{50}/MIC_{90}(μg/mL)	2/ > 16	2/16	> 16 > 16	2/16	> 16/ > 16	2/ > 16
S[c], n (%)	317 (63.3)	140 (89.2)	2 (3.1)	79 (73.1)	26 (41.3)	70 (64.2)
Clindamycin						
Range[b] (μg/mL)	≤ 0.5– > 2	≤ 0.5– > 2	≤ 0.5– > 2	≤ 0.5– > 2	≤ 0.5– > 2	≤ 0.5– > 2
MIC_{50}/MIC_{90}(μg/mL)	≤ 0.5/ > 2	≤ 0.5/ ≤ 0.5	> 2/ > 2	> 2/ > 2	≤ 0.5/ > 2	≤ 0.5/ > 2
S[c,d], n (%)	277 (55.3)	144 (91.7)	2 (3.1)	15 (13.9)	54 (85.7)	62 (56.9)
Erythromycin						
Range[b] (μg/mL)	≤ 0.25– > 4	≤ 0.25– > 4	0.5– > 4	≤ 0.25– > 4	≤ 0.25– > 4	≤ 0.25– > 4
MIC_{50}/MIC_{90}(μg/mL)	> 4/ > 4	> 4/ > 4	> 4/ > 4	> 4/ > 4	> 4/ > 4	> 4/ > 4
S[c,d], n (%)	98 (19.6)	20 (12.7)	1 (1.6)	10 (9.3)	27 (42.9)	40 (36.7)

Table 1 (continued)

ST sequence type, *CC* clonal complex, MIC_{50}/MIC_{90} the lowest concentration of the antibiotics at which 50% and 90% of the isolates were inhibited, respectively, *S* susceptible, *TMP/SMX* trimethoprim/sulfamethoxazole

[a] Among CC45, ST45 and ST508 were clustered within a band pattern similarity of ≥ 80% by PFGE

[b] Dilution range of each antibiotic tested are listed as follows: 0.25–4 μg/mL for moxifloxacin and levofloxacin, 1–2 μg/mL for ciprofloxacin, 0.25–32 μg/mL for vancomycin, 0.5–4 μg/mL for daptomycin, rifampin and erythromycin, 1–8 μg/mL for linezolid, 2–16 μg/mL for gentamicin and tetracycline, 0.5/9.5–4/76 for TMP/SMX, and 0.5–2 μg/mL for clindamycin

[c] The CLSI clinical breakpoints for each antibiotic are as follows: S ≤ 0.5 μg/mL for moxifloxacin, S ≤ 1 μg/mL for levofloxacin, S ≤ 1 μg/mL for ciprofloxacin, S ≤ 2 μg/mL for vancomycin, S ≤ 1 μg/mL for daptomycin, S ≤ 4 μg/mL for linezolid, S ≤ 4 μg/mL for gentamicin, S ≤ 1 μg/mL for rifampin, S ≤ 2/38 for TMP/SMX, S ≤ 4 μg/mL for tetracycline, S ≤ 0.5 μg/mL for clindamycin, and S ≤ 0.5 μg/mL for erythromycin

[d] For isolates that tested erythromycin resistant and clindamycin susceptible or intermediate, testing for inducible clindamycin resistance by broth microdilution was performed to determine susceptibility to clindamycin

Table 2 Comparisons of nemonoxacin minimum inhibitory concentration (MIC) distributions by moxifloxacin susceptibility and sequence types of 501 methicillin–resistant *Staphylococcus aureus* blood isolates

	MIC (μg/mL)														
	≤ 0.008	0.015	0.03	0.06	0.12	0.25	0.5[a]	1[a]	2	4	8	16	32	MIC_{50}/MIC_{90}	GM
Total (n = 501)	0	7	84	83	16	4	93	164	42	6	2	0	0	0.5/1	0.291
Moxifloxacin non-susceptible[b]															
Subtotal (n = 313)	0	0	3	3	1	1	92	163	42	6	2	0	0	1/2	0.867
ST8 (n = 155)	0	0	0	0	0	0	58	76	17	4	0	0	0	1/2	0.863
ST239 (n = 63)	0	0	0	0	0	0	0	49	11	2	1	0	0	1/2	1.219
ST59 (n = 15)	0	0	2	2	1	0	5	4	1	0	0	0	0	0.5/1	0.031
CC45 (n = 42)	0	0	1	1	0	1	22	15	1	0	1	0	0	0.5/1	0.618
Other STs (n = 38)	0	0	0	0	0	0	7	19	12	0	0	0	0	1/2	1.095
Moxifloxacin susceptible															
Subtotal (n = 188)	0	7	81	80	15	3	1	1	0	0	0	0	0	0.06/0.12	0.047
ST8 (n = 2)	0	0	1	1	0	0	0	0	0	0	0	0	0	–[c]/–[c]	–[c]
ST239 (n = 1)	0	0	1	0	0	0	0	0	0	0	0	0	0	–[c]/–[c]	–[c]
ST59 (n = 93)	0	5	49	29	9	1	0	0	0	0	0	0	0	0.03/0.12	0.042
CC45 (n = 21)	0	2	17	1	0	0	1	0	0	0	0	0	0	0.03/0.03	0.033
Other STs (n = 71)	0	0	13	49	6	2	0	1	0	0	0	0	0	0.06/0.12	0.061

MIC minimum inhibitory concentration, MIC_{50}/MIC_{90} the lowest concentration of the antibiotics at which 50% and 90% of the isolates were inhibited, respectively, *GM* geometric means, *ST* sequence type, *CC* clonal complex; –, not applicable

[a] Tentative *S. aureus* clinical breakpoints are 0.5 & 1 μg/mL for IV 500 mg & IV 750 mg nemonoxacin

[b] All 313 isolates had moxifloxacin MICs of ≥ 2 μg/mL, except one isolate with moxifloxacin MIC of 1 μg/mL, categorized as intermediate by CLSI clinical breakpoint criteria

[c] Only 2 ST8 isolates and 1 ST239 isolate were susceptible to moxifloxacin. Accordingly, we didn't calculate nemonoxacin MIC_{50}/MIC_{90} and MIC GM

Nemonoxacin GMs of MICs elevated as accumulation of the amino acid substitutions in each of major four lineages (Table 3). The proportions of non-susceptibility to nemonoxacin using a tentative CBP of 1 μg/mL were significantly different among four groups (overall and pairwise comparisons, $P < 0.05$, except that for group C vs. group D; supplementary Table 3), and isolates ≥ 3 amino acid substitutions in *gyrA*/*parC* with or without additional amino acid substitutions in *gyrB* and *parE* (group A) had the greatest non-susceptible proportions (86.9%) to nemonoxacin (Fig. 2B). Given nemonoxacin MICs for 89 isolates sequenced for QRDRs were ≥ 0.5 μg/mL, non-susceptibility to moxifloxacin, levofloxacin, and ciprofloxacin were nearly 100% among them without group differences, reflecting that three fluroquinolones might lose their in vitro potency against MRSA once amino acid substitutions in QRDRs develop.

Discussions

This multicenter study depicted the latest molecular epidemiology of MRSA in Taiwan, indicating clonal expansion of ST8/USA300 in both community and hospitals. Compared to ST59, CC45 and other STs, ST8/USA300 and ST239 expressed much higher resistance to three tested fluroquinolones (≈98.5%). For moxifloxacin non-susceptible isolates, which were mainly clustered within ST8 and ST239, 84% of the studied isolates remained susceptible to nemonoxacin if a tentative CBP of 1 μg/mL

Table 3 Comparisons of amino acid substitutions in the quinolone resistance-determining regions and corresponding minimum inhibitory concentrations of nemonoxacin and fluoroquinolone comparators among 89 MRSA blood isolates by sequence types

Genetic lineages[a]	No	*gyrA*			*gyrB*		*parC*			*parE*		Group[b]	MICs(μg/mL)				
		84	85	88	437	456	80	81	84	432	444		NEM		CIP	LEV	MOX
		S	S	E	D	P	S	S	E	D	R		Range	GM	Range	Range	Range
ST8	1	L	P				Y		K			A	2–4	3.5	> 2–> 2	> 4–> 4	> 4–> 4
	3	L	P				Y			V							
	1	L					Y	P									
	1	L					Y			V		B	1–2	1.9	> 2–> 2	> 4–> 4	≥ 4
	9	L					Y			N							
	19	L					Y					C	0.5–1	0.7	> 2–> 2	4–> 4	1–4
ST239	5	L	P				F			N		A	1–4[c]	2.2	> 2–> 2	> 4–> 4	> 4–> 4
	1	L		A			F			N							
	1	L		K			F			N							
	1	L			E		F			N		B	1–8[d]	1.5	> 2–> 2	> 4–> 4	4–> 4
	1	L				S	F			N							
	14	L					F			N							
ST59	7	L					F					C	0.5–2	0.7	> 2–> 2	> 4–> 4	2–4
	3											D	0.5–1	0.8	> 2–> 2	> 4–> 4	2–> 4
CC45	1	L	P				F		K			A	1–8[e]	2.5	> 2–> 2	> 4–> 4	> 4–> 4
	1	L					F		K								
	1	L					F		G								
	1	L					F			V		B	1	1.0	> 2–> 2	> 4–> 4	4–4
	1	L					F				C						
	17	L					F					C	0.5–1	0.7	> 2–> 2	> 4–> 4	2–4

MIC minimum inhibitory concentration, *No.* numbers of isolates, *ST* sequence type, *CC* clonal complex, *NEM* nemonoxacin, *CIP* ciprofloxacin, *LEV* levofloxacin, *MOX* moxifloxacin, *GM* geometric means

[a] Numbers of isolates in each genetic lineages were 34 isolates in ST8, 23 isolates in ST239, 10 isolates in ST59, and 22 isolates in CC45

[b] Groups consist of ≥ 3 amino acid substitutions in *gyrA*/*parC* with or without additional amino acid substitutions in *gyrB* and *parE* (group A), 2 substitutions in *gyrA*/*parC* with additional substitutions in *gyrB* and *parE* (group B), only 2 substitutions in *gyrA*/*parC* (group C), and no substitutions in *gyrA*/*gyrB*/*parC*/*parE* (group D)

[c] Among ST239 isolates in group A, 7 isolates had nemonoxacin MICs ≥ 2 μg/mL, except one having MIC = 1 μg/mL

[d] Among ST239 isolates in group B, 16 isolates had nemonoxacin MIC ≤ 2 μg/mL, except one having MIC = 8 μg/mL

[e] Among CC45 isolates in group A, 2 isolates had nemonoxacin MICs ≥ 2 μg/mL, and. One had MIC = 1 μg/mL

was applied. Among isolates with nemonoxacin MICs of ≥ 0.5 μg/mL and sequenced for QRDRs, we characterized 44.1% of ST8 and all ST239 isolates had ≥ 3 amino acid substitutions (AAS) in gyrA/parC (group A) or 2 AAS in gyrA/parC with additional AAS in gyrB/parE (group B); in contrast, over 70% of ST59 and CC45 isolates possessed only 2 amino acid substitutions in QRDRs (Fig. 2A). Further, we found both nemonoxacin MICs and proportions of nemonoxacin nonsusceptibility increased by accumulations of amino acid substitutions in QRDRs (Table 3 and Fig. 2B). These findings suggested that the difference of nemonoxacin susceptibility among four major lineages in Taiwan may be attributed to their underlying patterns of amino acid substitutions in QRDRs.

In Taiwan, the traditional CA-MRSA clone was ST59 carrying SCC*mec* type IV or V [17], while ST8/USA300 was an emerging CA-MRSA clone since 2010 [18]. Overall, this study demonstrated that ST8 dominated in various onset settings of BSIs, even in HA setting. ST59 was the second major MRSA clone across all participating hospitals. Despite the proportions of rest MRSA lineages may vary in each hospital, the overall proportion of ST239, the traditional HA-MRSA clone in Taiwan, declined to the third place after ST8 and ST59, highlighting that introduction and intermixing of different MRSA lineages (ST8 and ST45) from the community and long-term care facilities into hospitals results in clonal shift of HA-MRSA BSIs. Since previous studies illustrating the emergence of ST8/USA300 were conducted in Northern Taiwan before 2018 [10, 11], the current study extended the previous findings of ST8/USA300 epidemic has widely and substantially spread in different geographic regions of Taiwan. Given the case number of COVID-19 in Taiwan was extremely low in 2020, we couldn't further analyze the impact of COVID-19 on disease prevalence,

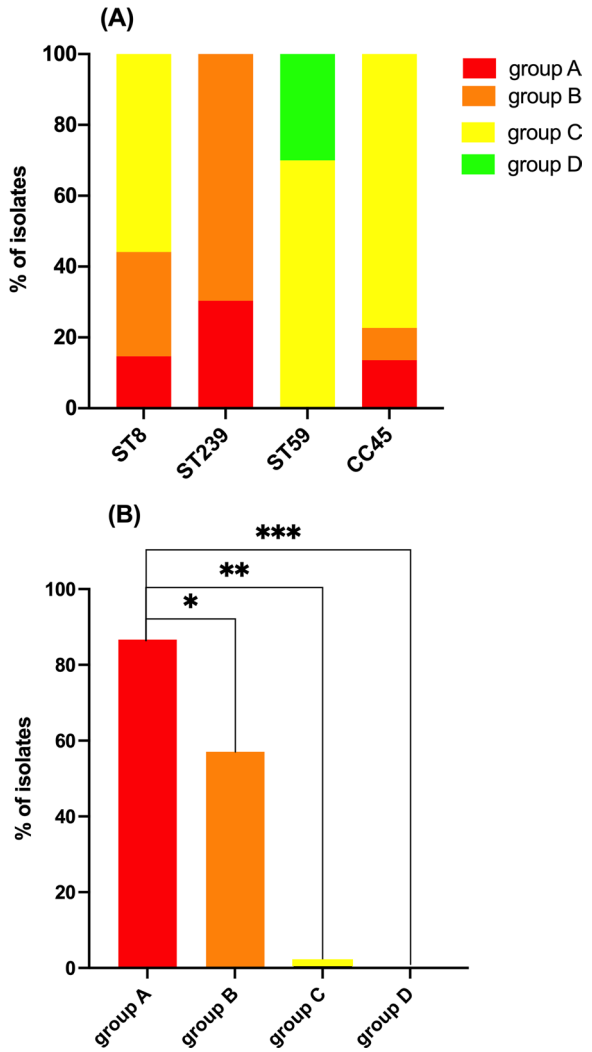

Fig. 2 Comparisons of proportions of amino acid substitutions groups of by sequence types (**A**), and those of nemonoxacin non-susceptibility by amino acid substitutions groups (**B**). Amino acid substitutions groups consisted of ≥ 3 amino acid substitutions in *gyrA*/*parC* with or without additional amino acid substitutions in *gyrB* and *parE* (group A), 2 substitutions in *gyrA*/*parC* with additional substitutions in *gyrB* and *parE* (group B), only 2 substitutions in *gyrA*/*parC* (group C), no substitutions in *gyrA*/*gyrB*/*parC*/*parE* (group D), and referred to supplementary Table 2 for the details. Panel B only showed *P* values of pairwise comparisons between group A and each of other 3 groups (*, < 0.05; **, < 0.01; ***, < 0.001), and referred to supplementary Table 3 for the details

antibiotic resistance patterns, and molecular epidemiology of MRSA.

In the current study, we found a bimodal distribution of nemonoxacin MICs among these contemporary MRSA blood isolates and was correlated to susceptibility to moxifloxacin. On the one hand, one group of wild type (WT) to nemonoxacin had modal MIC values between 0.03 and 0.06 μg/mL and most were susceptible to moxifloxacin. On the other hand, another group of non-WT to nemonoxacin had a model MIC of 1 μg/mL and most were non-susceptible to moxifloxacin. However, the distribution of nemonoxacin MICs were not significantly different between different epidemiological onset settings (supplementary Table 4). This might be due to intermixing of different MRSA lineages with varied nemonoxacin susceptibilities in the community and hospitals as mentioned above.

Nemonoxacin MIC_{50}/MIC_{90} for moxifloxacin non-susceptible isolates in this study were 1/2 μg/mL, both around the tentative CBP of 1 μg/mL. Generally, direct comparisons of MIC data generated from different institutions in different study periods may underestimate MIC differences due to site heterogeneity and lack of quinolone consumption data. Whereas MIC_{50}/MIC_{90} values of nemonoxacin determined in the present study were one dilution higher than those in previous studies from Taiwan before 2010. We speculated these findings might indicated MIC creep of nemonoxacin over time in Taiwan. However, considering MIC_{50}/MIC_{90} values were greater for specific MRSA lineages, this phenomenon might be in part attributed to clonal introduction and expansion of ST8/USA300 or intermixing with others, such as ST5 (nemonoxacin MICs of 1 and 2 μg/mL, n = 10 and 1, respectively), and ST188 (nemonoxacin MICs of 1 and 2 μg/mL, n = 7, and 2, respectively). Besides, we couldn't rule out the possibility that the selection pressure of quinolones in the community and hospitals in Taiwan would further contribute to nemonoxacin MIC creep. Simultaneously, the selection pressure of quinolones may also contribute to clonal expansion of ST8/USA300 in Taiwan due to its nature of highly resistant to fluoroquinolone [10].

By QRDRs target sequencing, we confirmed nemonoxacin remained its in vitro activity against quinolone non-susceptible MRSA isolates with 2 amino acid substitutions [19]. And it was also illustrated that nemonoxacin MICs increased as amino acid substitutions accumulated further. The above phenomenon was consistently observed among different sequence types in the present study, which was similar to others in different MRSA genetic backgrounds [20]. Taken together, these data provided molecular insights to explain difference of nemonoxacin susceptibilities among tested MRSA isolates.

To further explore the effects of different patterns of amino acid substitutions, we found isolates possessing ≥ 3 substitutions in *gyrA* and *parC* irrespective of substitutions in *gyrB*/*parE* (group A in Fig. 2B) had the highest non-susceptibility proportions, and half of them (n = 7) had nemonoxacin MICs ≥ 4 μg/mL. Comparatively, those possessing 2 substitutions

in *gyrA* and *parC* with other substations in *gyrB* and *parE* (n = 28, group B in Fig. 2B) only had one isolate (3.6%) with nemonoxacin MIC ≥ 4 μg/mL. These findings highlighted *gyrA* and *parC* are both the key genes to determine nemonoxacin non-susceptibility [15], while other substations in *gyrB* and *parE* might play a minor role in nemonoxacin susceptibilities. Further, no amino acid substitutions were identified in three isolates non-susceptible to moxifloxacin, levofloxacin, and ciprofloxacin, suggesting these isolates possessing other quinolone resistance mechanisms, such as overexpression of efflux pumps.

The current tentative nemonoxacin CBPs for *S. aureus* have different cut-off values by taking drug formulations and dosages into account [14]. It should be cautious that these CBPs were determined by the pharmacokinetics/pharmacodynamics data from healthy volunteers and clinical and microbiological response in clinical trials [14]. In line with MIC creep and clonal changes of MRSA, field studies are warranted to ascertain clinical and microbiological effectiveness of nemonoxacin in management of MRSA infections in the modern era and further to revise nemonoxacin CBPs by integrating population pharmacokinetics/pharmacodynamics analysis if indicated.

Some limitations may affect the interpretations of our findings. First, we only collected MRSA blood isolates for analysis, so generalization of our molecular epidemiology results is limited. However, other studies in Taiwan also demonstrated ST8/USA300 was the prevalent clone in skin and soft tissue infections, osteoarticular infections, and vascular infections [9, 21, 22]. We believed ST8/USA300 has successfully expanded in Taiwan. Second, we sequenced QRDRs among four major lineages to compare molecular mechanisms of quinolone resistance among them. However, we only selected some of isolates with nemonoxacin MICs ≥ 0.5 μg/mL, which was above a tentative ECOFFs of 0.125 μg/mL, for comparisons. This may limit our findings apply to the whole populations of a specific MRSA lineage. Third, this study didn't analyze other resistance mechanisms, such as overexpression of efflux pump systems [15]. Lastly, we didn't perform in vitro susceptibility testing of delafloxacin, so our results might not indicate resistant mechanisms of delafloxacin. Notably, some investigations showed multiple amino acid substitutions in QRDRs conferred delafloxacin resistance, especially those with concurrent overexpression of efflux pumps [23, 24].

Conclusions

Collectively, we found ST8/USA300 substantially disseminated in Taiwan. Nemonoxacin retained in vitro activity against contemporary MRSA blood isolates, while the nemonoxacin MIC creep might be present. The "creep" is in part due to the emergence of ST8/USA300 expansion and about half of them possessing ≥ 3 amino acid substitutions in QRDRs. Consequently, if nemonoxacin is indicated, we suggest using a higher intravenous dose of 750 mg/day to treat MRSA infections, especially for pneumonia. Future studies are needed to determine the appropriate doses of nemonoxacin for different MRSA clinical syndromes.

Abbreviations

MRSA	Methicillin-resistant *Staphylococcus aureus*
CA-MRSA	Community-acquired methicillin-resistant *Staphylococcus aureus*
HA-MRSA	Hospital-acquired methicillin-resistant *Staphylococcus aureus*
HACO	Healthcare-associated, community onset
QRDRs	Quinolone resistance-determining regions
ST	Sequence types
MLST	Multi-loci sequence type
CC	Clonal complex
PFGE	Pulsed-field gel electrophoresis
CBP	Clinical breakpoint
ECOFF	Epidemiological cut-off

Supplementary Information

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Supplementary material 1.

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Author contributions

Pao-Yu Chen and Jann-Tay Wang conceived and designed the study. Pao-Yu Chen and Jann-Tay Wang performed data analysis and interpretation. Pao-Yu Chen wrote the manuscript, which was reviewed and approved by Mao-Wang Ho, Po-Liang Lu, Hung-Jen Tang, and Cheng len SY. Jann-Tay Wang revised the article critically for important intellectual content and final approval of the version to be submitted. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board in each participating hospital (National Taiwan University Hospital, 202102038RIPA; China Medical University Hospital, CMUH110-REC3-103; Chi Mei Medical Center, 11010-002; Kaohsiung Medical University Hospital, KMUHIRB-E(I)-20210168; Kaohsiung Veteran General Hospital, KSVGH21-CT11-06).

Competing interests
The authors declare no competing interests.

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References

1. Antimicrobial RC. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399:629–55. https://doi.org/10.1016/S0140-6736(21)02724-0.
2. Buis DTP, Prins JM, Betica-Radic L, de Boer MGJ, Ekkelenkamp M, Kofteridis D, Peiffer-Smadja N, Schouten J, Spernovasilis N, Tattevin P, Ten Oever J, Sigaloff KCE. Current clinical practice in antibiotic treatment of *Staphylococcus aureus* bacteraemia: results from a survey in five European countries. J Antimicrob Chemother. 2022;77:2827–34. https://doi.org/10.1093/jac/dkac237.
3. de Kretser D, Mora J, Bloomfield M, Campbell A, Cheng MP, Guy S, Hensgens M, Kalimuddin S, Lee TC, Legg A, Mahar RK, Marks M, Marsh J, McGlothlin A, Morpeth SC, Sud A, Ten Oever J, Yahav D, Tong SY, Davis JS, Walls G, Goodman AL, Bonten M, Staphylococcus aureus Network Adaptive Platform Study Group m. Early oral antibiotic switch in *Staphylococcus aureus* bacteraemia: The *Staphylococcus aureus* Network Adaptive Platform (SNAP) trial early oral switch protocol. Clin Infect Dis. 2023. https://doi.org/10.1093/cid/ciad666.
4. Peterson LR. Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity. Clin Infect Dis. 2001;33(Suppl 3):S180–6. https://doi.org/10.1086/321846.
5. Adam HJ, Laing NM, King CR, Lulashnyk B, Hoban DJ, Zhanel GG. In vitro activity of nemonoxacin, a novel nonfluorinated quinolone, against 2,440 clinical isolates. Antimicrob Agents Chemother. 2009;53:4915–20. https://doi.org/10.1128/AAC.00078-09.
6. Yang JJ, Cheng A, Tai HM, Chang LW, Hsu MC, Sheng WH. Selected mutations by nemonoxacin and fluoroquinolone exposure among relevant gram-positive bacterial strains in Taiwan. Microb Drug Resist. 2020;26:110–7. https://doi.org/10.1089/mdr.2019.0048.
7. Chen YH, Liu CY, Lu JJ, King CH, Hsueh PR. In vitro activity of nemonoxacin (TG-873870), a novel non-fluorinated quinolone, against clinical isolates of Staphylococcus aureus, enterococci and *Streptococcus pneumoniae* with various resistance phenotypes in Taiwan. J Antimicrob Chemother. 2009;64:1226–9. https://doi.org/10.1093/jac/dkp370.
8. Lauderdale TL, Shiau YR, Lai JF, Chen HC, King CH. Comparative in vitro activities of nemonoxacin (TG-873870), a novel nonfluorinated quinolone, and other quinolones against clinical isolates. Antimicrob Agents Chemother. 2010;54:1338–42. https://doi.org/10.1128/AAC.01197-09.
9. Peng KT, Huang TY, Chiang YC, Hsu YY, Chuang FY, Lee CW, Chang PJ. Comparison of Methicillin-Resistant Staphylococcus aureus Isolates from Cellulitis and from Osteomyelitis in a Taiwan Hospital, 2016–2018. J Clin Med. 2019. https://doi.org/10.3390/jcm8060816.
10. Chen PY, Chuang YC, Wang JT, Sheng WH, Chen YC, Chang SC. Sequence type 8 as an emerging clone of methicillin-resistant *Staphylococcus aureus* causing bloodstream infections in Taiwan. Emerg Microbes Infect. 2021;10:1908–18. https://doi.org/10.1080/22221751.2021.1981158.
11. Huang YC, Chen CJ. USA300 (sequence type 8) has become a major clone of methicillin-resistant *Staphylococcus aureus* in northern Taiwan. Int J Antimicrob Agents. 2022;59:106534. https://doi.org/10.1016/j.ijantimicag.2022.106534.
12. Francisco AP, Bugalho M, Ramirez M, Carrico JA. Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. BMC Bioinformatics. 2009;10:152. https://doi.org/10.1186/1471-2105-10-152.
13 CLSI. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. 33rd ed. Malvern: Clinical and Laboratory Standards Institute; 2023.
14. Jean SS, Chang LW, Hsueh PR. Tentative clinical breakpoints and epidemiological cut-off values of nemonoxacin for *Streptococcus pneumoniae* and *Staphylococcus aureus* isolates associated with community-acquired pneumonia. J Glob Antimicrob Resist. 2020;23:388–93. https://doi.org/10.1016/j.jgar.2020.10.017.
15. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol. 2014;22:438–45. https://doi.org/10.1016/j.tim.2014.04.007.
16. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. Anesth Analg. 2018;126:1763–8. https://doi.org/10.1213/ANE.0000000000002864.
17. Wang JL, Wang JT, Chen SY, Chen YC, Chang SC. Distribution of Staphylococcal cassette chromosome mec types and correlation with comorbidity and infection type in patients with MRSA bacteremia. PLoS ONE. 2010;5:e9489. https://doi.org/10.1371/journal.pone.0009489.
18. Huang YC, Chen CJ, Kuo CC, Lu MC. Emergence, transmission and phylogeny of meticillin-resistant *Staphylococcus aureus* sequence type 8 (USA300) in Taiwan. J Hosp Infect. 2018;100:355–8. https://doi.org/10.1016/j.jhin.2018.02.014.
19. Qin X, Huang H. Review of nemonoxacin with special focus on clinical development. Drug Des Devel Ther. 2014;8:765–74. https://doi.org/10.2147/DDDT.S63581.
20. de Oliveira TLR, Cavalcante FS, Chamon RC, Ferreira RBR, Dos Santos KRN. Genetic mutations in the quinolone resistance-determining region are related to changes in the epidemiological profile of methicillin-resistant *Staphylococcus aureus* isolates. J Glob Antimicrob Resist. 2019;19:236–40. https://doi.org/10.1016/j.jgar.2019.05.026.
21. Lee CY, Fang YP, Wu TH, Chang YF, Sung CH. Sequence types 8, 59, and 45 methicillin resistant *Staphylococcus aureus* as the predominant strains causing skin and soft tissue infections in Taiwan's prisons and jails. J Microbiol Immunol Infect. 2022;55:1239–45. https://doi.org/10.1016/j.jmii.2021.08.013.
22 Kao CC, Lai CH, Wong MY, Huang TY, Tseng YH, Lu CH, Lin CC, Huang YK. Insight into the clonal lineage and antimicrobial resistance of *Staphylococcus aureus* from vascular access infections before and during the COVID-19 pandemic. Antibiotics. 2023. https://doi.org/10.3390/antibiotics12061070.
23. Iregui A, Khan Z, Malik S, Landman D, Quale J. Emergence of delafloxacin-resistant *Staphylococcus aureus* in Brooklyn, New York. Clin Infect Dis. 2020;70:1758–60. https://doi.org/10.1093/cid/ciz787.
24. de la Rosa JMO, Fernandez MA, Rodriguez-Villodres A, Casimiro-Soriguer CS, Cisneros JM, Lepe JA. High-level delafloxacin resistance through the combination of two different mechanisms in *Staphylococcus aureus*. Int J Antimicrob Agents. 2023;61:106795. https://doi.org/10.1016/j.ijantimicag.2023.106795.

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