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Emergence of *ileS2*-Carrying, Multidrug-Resistant Plasmids in *Staphylococcus lugdunensis*

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Of 137 *Staphylococcus lugdunensis* isolates collected from two nephrology centers in Hong Kong, 10 (7.3%) and 3 (2.2%) isolates had high-level and low-level mupirocin resistance, respectively. Isolates with high-level resistance contained the plasmid-mediated *ileS2* gene, while isolates with low-level resistance contained the mutation V588F within the chromosomal *ileS* gene. All but one of the *ileS2*-positive isolates belong to the predominating clone HKU1. Plasmids carrying the *ileS2* gene were mosaic and also cocarry multiple other resistance determinants.

Staphylococcus lugdunensis resembles *Staphylococcus aureus* in its ability to cause catheter-related bacteremia, endocarditis, and exit site infections in renal dialysis patients (1, 2). While antibiotic resistance in *S. lugdunensis* is not regarded as a major clinical problem, resistance to aminoglycosides, macrolides, oxacillin, and tetracycline have been increasingly reported (3–5). In dialysis settings, topical mupirocin has been shown to substantially reduce the risk for *S. aureus* infections (6). However, long-term application may result in the development of mupirocin resistance in *S. aureus* and other colonizing staphylococci (7). In dialysis patients, a high prevalence of *S. lugdunensis* carriage has been reported, but data on mupirocin resistance in *S. lugdunensis* are lacking (1, 3, 8). In staphylococci, low-level mupirocin resistance (LLMR) occurs as the result of a point mutation in the chromosomal *ileS* gene that reduces the binding of mupirocin. Conversely, high-level mupirocin resistance (HLMR) is conferred by the plasmid-borne *ileS2* gene that encodes an alternative isoleucyl-tRNA synthetase that is not bound by mupirocin. The goal of this study was to determine the prevalence and mechanisms of mupirocin resistance in *S. lugdunensis*.

We previously screened 252 adult patients from two nephrology centers (A and B) from November 2013 to February 2014 in Hong Kong and obtained 137 unique *S. lugdunensis* isolates, including 116 methicillin-sensitive *S. lugdunensis* (MSSL) isolates and 21 methicillin-resistant *S. lugdunensis* (MRSL) isolates (3). Pulsed-field gel electrophoresis (PFGE) divided 129 isolates into 10 clones (designated HKU1 to HKU10) and eight singletons (3). The predominant clone, HKU1 (sequence type 38), accounted for 79 isolates (3). This study was approved by the Institutional Review Boards of the University of Hong Kong and the Hospital Authority (reference numbers UW13-351 and KW/EX-13-138-69-15). Informed consent was obtained from the patients.

In the present study, a disk diffusion (5-μg disk) method was used to screen for mupirocin resistance, and resistant isolates (inhibition zone, ≤13 mm) were further examined by Etest (9). PCR assays were used to detect *ileS2* and to map the IS*257*-*ileS2* spacer regions (see Table S1 in the supplemental material) (10). Six isolates were sequenced by an Illumina sequencing platform (5 by MiSeq and 1 by HiSeq) at >200-fold coverage (11, 12). The plasmids were assembled *de novo* and annotated as previously described, and gaps were closed by additional PCRs and Sanger sequencing (see Table S1) (11–14). The six assembled *ileS2*-carrying plasmids were correlated with the results from S1 nuclease-PFGE and Southern blotting using an *ileS2* probe. In four strains (8G, 20G, 63N, and 93G), the *ileS2*-carrying plasmid is the only plasmid detected (see Table S2 in the supplemental material). In two strains (15G and 33G), an extra small plasmid (~4 kb) was found in addition to the large *ileS2*-carrying plasmid. One of the isolates (93G) was further sequenced by PacBio, resulting in a single-con-

TABLE 1 Univariate analysis of potential risk factors for carriage of *S. lugdunensis* with HLMR

Factor	Carriage of *S. lugdunensis* with HLMR		
	No (n = 242)	Yes (n = 10)	P value[b]
Male (%)	54.5	90.0	0.027
Age, ≥65 yr (%)	45.5	70.0	0.127
Hemodialysis (%)	49.6	30.0	0.225
Duration of dialysis (mean ± SD [yr])	4.1 ± 5.1	4.9 ± 2.6	0.594
Topical gentamicin use (%)[a]	25.2	10.0	0.274
Topical mupirocin use (%)[a]	49.2	100.0	0.002
Old age home resident (%)	14.9	10.0	0.669
Diabetes mellitus (%)	47.5	20.0	0.087
Chronic skin disease (%)	8.3	0.0	0.343
Medical care in nephrology center A (%)	38.4	90.0	0.001

[a] Topical gentamicin and mupirocin use indicate use in the past 4 weeks.
[b] These values were obtained by Student's t (continuous variable) or chi-square (categorical variable) tests. A P value of less than 0.05 was considered to be statistically significant.

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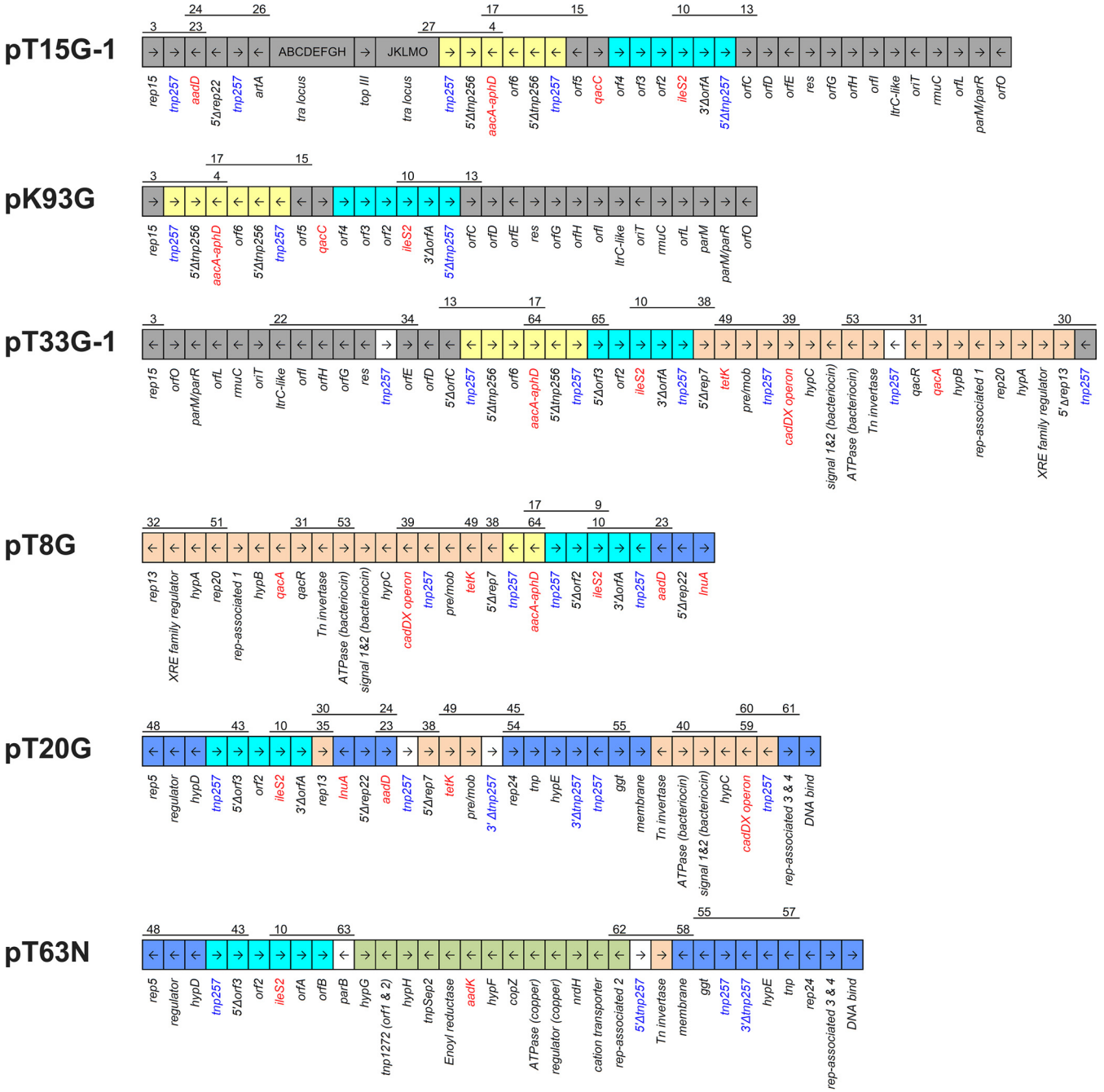

FIG 1 Comparative analysis of linear plasmid maps for six plasmids from mupirocin-resistant *Staphylococcus lugdunensis* carriage isolates from Hong Kong. Open reading frames and genes are represented by boxes and are colored to show shared regions: pSK41 region (gray; GenBank accession no. AF051917), Tn*4001*-IS*257* hybrid (yellow), IS*257*-*ileS2* region (light blue), pT33G region (light orange), pT20G region (deep blue), and pSP01 region (light green; GenBank accession no. KR230047). The numbers and horizontal lines above the linear maps showed the locations of the primers, PCRs, and the Sanger sequencing performed to confirm the assemblies. All genes in the plasmids are shown, but the genes and regions in the maps are not drawn exactly in proportion to the length of the sequences.

tig *ileS2*-carrying plasmid (pK93G), which is concordant with the assembled plasmid using Illumina data.

Overall, 3 (2.2%) and 10 (7.3%) isolates were found to have LLMR (MIC, 32 μg/ml) and HLMR (MIC, ≥1,024 μg/ml), respectively (see Table S2 in the supplemental material). All isolates with LLMR were MSSL, and all of them were from nephrology center B. The 10 isolates with HLMR included two MRSL and eight MSSL isolates. All except one MSSL isolate with HLMR were from nephrology center A. There is no significant difference in the prevalences of HLMR among MRSL and MSSL isolates (9.5% [2/21] and 6.9% [8/116], respectively; $P = 0.65$). The clones of the three isolates with LLMR were diverse (3 different pulsotypes). In contrast, 9 of the 10 isolates with HLMR were members of the HKU1 clone. The remaining isolate (from nephrology center A)

belongs to HKU3. Prevalence of HLMR among HKU1 isolates was significantly higher than that among non-HKU1 isolates (12.7% [9/71] and 1.5% [1/66], respectively; $P = 0.018$).

The proportions of patients in nephrology centers A and B with recent exposure to topical mupirocin were 73.5% (75/102) and 36.0% (54/150), respectively ($P < 0.001$); those for topical gentamicin were 6.9% (7/102) and 36.7% (55/150), respectively ($P < 0.001$). In univariate analysis, the carriage of *S. lugdunensis* with HLMR was significantly and positively associated with the male sex, the recent use of topical mupirocin, and medical care in nephrology center A (Table 1). In multivariate analysis, medical care in nephrology center A was the only variable that was independently associated with the carriage of *S. lugdunensis* with HLMR (odds ratio, 14.4; 95% confidence interval, 1.8 to 115.7; $P = 0.012$).

The three isolates with LLMR were *ileS2* negative but had a V588F mutation in the chromosomal *ileS* gene (see Table S2 in the supplemental material). All 10 isolates with HLMR were *ileS2* positive, and seven had either an A839V ($n = 6$) or G591R ($n = 1$) mutation in the chromosomal *ileS* gene. V588F and G591R are mutations within the mupirocin-binding pocket and have been correlated with low-level resistance (15). A839V is not a significant mutation. The following five amplification patterns were obtained for the IS*257*-*ileS2* spacer regions (see Fig. S1 in the supplemental material): types I ($n = 2$), II ($n = 5$), III ($n = 1$), IV ($n = 1$), and V ($n = 1$). The complete sequences of six plasmids carrying *ileS2* were obtained (Fig. 1). The number of plasmid replication initiation (*rep*) genes in each plasmid ranges from one to five. The families of the *rep* genes include *rep5* ($n = 2$), *rep7* ($n = 3$), *rep13* ($n = 3$), *rep15* ($n = 3$), *rep20* ($n = 2$), *rep22* ($n = 3$), and *rep24* ($n = 2$) (14). In the plasmids, one to six other genes encoding resistance to aminoglycosides (*aacA-aphD*, *aadD*, and *aadK*), lincosamide (*lnuA*), tetracyclines (*tetK*), biocides (*qacA* and *qacC*), and heavy metals (*cadDX*) were detected. Two plasmids (pT15G-1 and pK93G) shared extensive homology with pSK41, including a Tn*4001*-IS*257* hybrid inserted downstream of *orf5* (16). In the two plasmids, the IS*257*-*ileS2* region was inserted downstream of the *qac* gene. The other four plasmids (pT33G-1, pT8G, pT20G, and pT63N) were mosaic, with modules having high homologies to regions in pSK41 and pSP01 or were shared among each other (Fig. 1). pSK41 and pSP01 are two completely sequenced *ileS2*-negative plasmids that were previously described in staphylococci (14, 17).

This study revealed that mupirocin resistance is emerging among *S. lugdunensis* isolates in dialysis settings. Notably, this involves the expansion of a predominating HKU1 clone and mosaic multidrug-resistant plasmids cocarrying IS*257*-associated *ileS2* and other resistance determinants. In staphylococcal plasmids, it is known that IS*257* elements can integrate small plasmids into larger, multireplicon plasmids (13, 18). During the study period, the routine prescriptions for Tenckhoff catheter exit site infection prophylaxis in nephrology centers A and B were topical mupirocin and gentamicin, respectively. Mupirocin was seldom used in center B except for in special situations, such as gentamicin intolerance. In center A, all new peritoneal dialysis patients were screened for *S. aureus* carriage, and topical mupirocin was used for the elimination of carriage. These differences in mupirocin use policy may explain why HLMR prevalence is higher in center A than in center B. It has previously been described that coagulase-negative staphylococci may acquire HLMR following nasal decolonization of *S. aureus* with mupirocin (19). It is worrying that 6 of the 10 isolates with HLMR had coresistance to gentamicin (*aacA-aphD* positive) because this may undermine efforts to prevent resistance emergence through rotational use of mupirocin and gentamicin (20). In conclusion, our findings highlight the potential for the dissemination of mupirocin resistance through successful *S. lugdunensis* clones and multidrug-resistant mosaic plasmids. Infection control practices should be enhanced to reduce the spread of the resistant clones.

Accession number(s). The sequences of the plasmids depicted in Fig. 1 have been deposited in GenBank under accession numbers KU882681 (pT15G-1, 42,253 bp), KU882682 (pK93G, 25,674 bp), KU882683 (pT33G-1, 46,415 bp), KU882684 (pT8G, 31,767 bp), KU882685 (pT20G, 37,435 bp), and KU882686 (pT63N, 40,583 bp).

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