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Molecular Characterization of Nasal Methicillin-Resistant *Staphylococcus aureus* Isolates Showing Increasing Prevalence of Mupirocin Resistance and Associated Multidrug Resistance following Attempted Decolonization

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ABSTRACT Sequential methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients following attempted mupirocin nasal decolonization showed an increase in mupirocin resistance (MR) from 6.6% to 20%. MR isolates from patients who failed decolonization yielded indistinguishable *spa* types and carried multiple antimicrobial and antiseptic resistance genes, which may guide infection control and prevention.

KEYWORDS MRSA, *Staphylococcus aureus*, multidrug resistance, mupirocin

Eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage minimizes MRSA transmission (1–3). Mupirocin is used for nasal decolonization despite increasing mupirocin resistance (MR), i.e., low-level MR (LLMR) and high-level MR (HLMR) rates of 1 to 81% (4, 5). Among persistent carriers, knowledge of circulating colonizing MRSA clones, resistance genes, and antimicrobial susceptibility profiles might better inform antimicrobial choices for decolonization and treatment.

We recently described a randomized controlled trial (RCT) (CT number 2010-023408-28) in which 50 patients receiving 2% mupirocin for nasal decolonization were compared with 50 patients receiving medical-grade honey (MGH) (6). Triclosan (1% body wash) was used for concurrent skin decolonization. Here, we describe the development of MR in the mupirocin-treated group as a secondary outcome. In addition, we present the genotypic and phenotypic analyses of isolates obtained longitudinally during the RCT, correlated with MRSA nasal persistence following attempted decolonization with mupirocin.

All 50 patients in the mupirocin group were known MRSA carriers and had received at least two courses of mupirocin prior to study enrollment. Forty-four patients (44/50) completed the protocol. Of these, 20 received one additional course and 24 received two additional courses of mupirocin during the study. A single course comprised the application of mupirocin three times a day for five consecutive days. Isolates were obtained from patients when recruited and from persistent carriers within 4 weeks of completing mupirocin decolonization treatment.

Nineteen patients, 43% (19/44), failed decolonization. Excluding two of these, who were known HLMR cases, 23.5% (4/17) were new acquisitions of MR-MRSA, giving an overall incidence rate of 9.5% (4/42).

A historic isolate was available for 30/44 patients (taken previously between 2 months and 12 years prior to study enrollment as part of routine screening), and a final isolate was available for 19 of these 30 (the remaining 11 were successfully decolonized during the RCT). This facilitated a longitudinal analysis of MR-MRSA carriage among these 30 patients only. MR increased from 6.6% (2/30) among historic isolates to 10% (3/30) among isolates recovered at recruitment. Among the 19 final isolates available, six were MR. Assuming that those successfully decolonized ($n = 11$) did not harbor an MR isolate, the overall rate of MR was 20% (6/30) among these patients at study end. The difference in MR prevalence between recruitment (baseline) isolates and final isolates was not significant (P value of 0.47 by Fisher's exact test); however, a 2-fold increase in MR from recruitment to study end following mupirocin exposure was observed.

The increase in MR-MRSA among nasally colonized patients treated with mupirocin from 10% to 20% supports previous findings that mupirocin use strongly correlates with acquisition of MR (7, 8). Our findings in this longitudinal study confirm those in a simulation model in two London hospitals, where MR among MRSA isolates was 9.1% when a screen-and-treat policy (similar to that of our hospital) was implemented, but it was increased to 21.3% with subsequent universal mupirocin use (9). The findings reaffirm the importance of active surveillance and routine mupirocin susceptibility testing regardless of suppression therapy, as well as targeted or universal decolonization.

Further characterization of MRSA isolates from the 19 patients with persistent carriage after mupirocin nasal decolonization was undertaken using *spa* typing and DNA microarray analysis. The protocols and primers described by SeqNet (http://www.seqnet.org) were used for *spa* typing. Sanger sequencing was performed by GATC-Biotech, Germany. Comparing the final isolates from patients who failed to decolonize to their baseline isolates, taken 14 to 28 days previously, 89.4% (17/19) yielded an indistinguishable *spa* type (Table 1). Therefore, persistence of an indistinguishable *spa* type may be a useful predictor of future decolonization failure. This may inform risk assessment and targeted decolonization. For example, where the isolate is methicillin susceptible and suppression therapy is indicated, such as before surgical implant placement, *spa* type may be included in the decision regarding decolonization.

The antimicrobial resistance gene carriage of isolates taken from patients who failed nasal decolonization was investigated using the *S. aureus* genotyping kit 2.0 (Alere Technologies, Germany). All isolates harbored *mecA* and *blaZ*, encoding genotypic resistance to methicillin and beta-lactams, respectively (Table 1).

In total, 6/19 exhibited phenotypic MR, but only three of the six (50%) were *ileS2* positive. Two cases were *de novo* HLMR, i.e., the same *spa* type at recruitment and following two courses of mupirocin treatment. The occurrence of MR among isolates was observed by *de novo* acquisition as well as *spa* type replacement. Genotypic multidrug resistance (MDR) was found in 68.4% (13/19) of MRSA isolates from patients with persistent carriage. Genotypic MDR is defined as the carriage of three or more of the following antibiotic/antiseptic resistance genes: MRSA (*mecA*), beta-lactamase (*blaZ*), mupirocin (*ileS2*), macrolides, lincosamides and streptogramin B (MLS$_B$) compounds [*erm*(C)], tetracycline [*tet*(K), *tet*(M)], streptothricin (*sat*), aminoglycosides (*aacA-aphD*, *aadD*, and *aphA3*), and *qacA* (resistance to quaternary ammonium compounds). As *S. aureus* infection is often endogenous (10, 11), our study suggests that antimicrobial and antiseptic resistance gene profiles of the original colonizing isolate may inform stewardship and guide systemic prophylaxis and/or antimicrobial therapy. While MDR/mupirocin resistance association has been reported in isolates causing infection (including bloodstream infection) (12–14), our investigation revealed several MDR genes in addition to *ileS2* and/or *qacA* among MR-MRSA colonizing isolates. Furthermore, cocarriage of antimicrobial/antiseptic resistance genes was more frequent among isolates from patients with persistent colonization (data not presented here). As *qac* genes are plasmid associated and highly transmissible, infection with MDR MRSA strains and antiseptic-

TABLE 1 Mupirocin susceptibility and *spa* type changes of sequential isolates and genotypic resistance profile of 19 patients with persistent MRSA carriage after mupirocin nasal decolonization[e]

ID	Duration of MRSA carriage (yr)	Recorded no. of mupirocin courses		Mupirocin susceptibility at:		*spa* type at:		Presence of antibiotic resistance gene in MRSA from persistent carrier at study end							
		Prior to RCT	During RCT	Recruitment	RCT end	Recruitment	RCT end, 14 to 28 days later	*mecA*	*blaZ*	*ileS2*	*erm*(C)	*aphA3*	*sat*	*tet*(K)	*qacA*
1114	<1	2	2	S	S	t7636	t7636	+	+	−	+	−	−	−	−
1122[a]	<1	2	2	S	HLMR	t4559	t127	+	+	+	+	+	+	+	+
1126	3	>2	2	S	S	t4559	t4559	+	+	−	−	+	−	−	−
1131	<1	>2	2	S	S	t515	t515	+	+	−	+	+	−	−	−
1136[b]	9	>2	2	HLMR	HLMR	t032	t032	+	+	+	+	−	−	−	−
1138	3	>2	2	S	S	t032	t032	+	+	−	−	−	−	−	−
1141	4	>2	2	S	S	t032	t032	+	+	−	+	−	−	−	−
1152	5	2	2	S	S	t032	t032	+	+	−	+	−	−	−	−
1153	12	>2	2	S	S	t515	t515	+	+	−	−	−	−	−	−
1159	7	>2	2	S	S	t515	t515	+	+	−	−	−	−	−	−
1163	4	>2	2	S	S	t032	t032	+	+	−	−	−	−	−	−
1165	<1	>2	2	S	S	t032	t032	+	+	−	−	−	−	−	−
1180	6	>2	2	S	S	t032	t032	+	+	−	−	−	−	−	−
1181	<1	2	2	S	S	t022	t032	+	+	−	+	−	−	−	−
1184[c]	<1	2	2	LLMR	HLMR	t1612	t1612	+	+	−	+	−	−	−	+
1195[a]	<1	2	2	S	HLMR	t127	t127	+	+	+	+	+	+	+	+
1197[b]	2	2	2	HLMR	HLMR	t515	t515	+	+	−	+	−	−	−	−
1208[d]	<1	2	2	S	LLMR	t1612	t1612	+	+	−	+	−	−	−	−
1210	<1	2	2	S	S	t032	t032	+	+	−	+	−	−	−	−

[a]Patient isolate developed HLMR (>1,024 mg/liter) following 2 courses of mupirocin.
[b]Patient isolate was HLMR at start and end of study.
[c]Patient isolate LLMR (8 to 256 mg/liter) at study start but HLMR following 2 courses of mupirocin.
[d]Patient isolate developed LLMR following 2 courses of mupirocin.
[e]RCT, randomized controlled trial; S, mupirocin susceptible; *mecA*, alternate penicillin binding protein 2; *blaZ*, beta-lactamase gene; *ileS2*, high-level mupirocin resistance gene; *erm*(C), gene encoding resistance to macrolides, lincosamides, and streptogramin B (MLS$_B$) compounds; *aphA3*, gene encoding resistance to aminoglycosides; *sat*, streptothricin; *tet*(K), tetracycline; *qacA*, quaternary ammonium compound.

resistant characteristics present an additional challenge for topical decolonization and systemic treatment. MR phenotype should alert clinicians to potential MDR carriage and warrants additional investigation.

This study had some limitations. This was a single-center study, and a retrospective isolate was only available in 60% of patients (30/50) in the mupirocin group. Apart from MR, we report only antimicrobial and antiseptic resistance gene carriage, which does not always correlate with phenotypic resistance. Nonetheless, the longitudinal, sequential nature of this study revealed changes in susceptibility and *spa* type, and an association between MR phenotype and potential resistance to antibiotics and disinfectants, that may better inform decolonization and therapeutic strategies. While *spa*-type persistence alerts us to potential future decolonization failure, more discriminatory isolate typing methods (e.g., whole-genome sequencing) may better inform decolonization choice. Better controlled, evidence-based use of mupirocin may enable conservation of this valuable decolonization agent.

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