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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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Title:

Molecular characterization of nasal MRSA isolates showing increasing prevalence of mupirocin resistance and associated multi-drug resistance following attempted decolonization.

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Running title: Mupirocin resistance during MRSA decolonization

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Abstract

Sequential methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients following attempted mupirocin nasal decolonisation 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.

Eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage minimizes MRSA transmission.^{1,2,3} Mupirocin is used for nasal decolonization despite increasing mupirocin-resistance (MR), i.e. low-level MR (LLMR) and high-level MR (HLMR) rates of 1-81%.^{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 enrolment. 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 four 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 acquisition 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 enrolment in the study 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, to 10% (3/30) amongst isolates recovered at recruitment. Among the 19 final isolates available, six were MR. Assuming that those successfully decolonised (n=11) did not harbour 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 ($p=0.47$, Fisher's exact test), however a two 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 was 9.1%, when a 'screen and treat' policy (similar to our hospital) was implemented, but 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 characterisation of MRSA isolates from the 19 patients with persistent carriage after mupirocin nasal decolonisation 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 14-28 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 MS 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 harboured *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., 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 multi-drug resistance (MDR), 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), was found in 68.4% (13/19) of MRSA isolates from patients with persistent carriage. 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, guide systemic prophylaxis and/or

antimicrobial therapy. While MDR/mupirocin resistance association has been reported in isolates causing infection (including bloodstream infection),^{12,13,14} our investigation revealed several MDR genes in addition to *ileS2* and/or *qacA* among MR-MRSA colonizing isolates. Furthermore, co-carriage 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 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 centre 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 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 de-colonization agent.

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Conflict of interest

HH is in receipt of research support from Pfizer and Astellas and has provided professional advice or education to Pfizer and Cepheid. Other authors have no conflict of interest to declare.

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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 decolonisation.

ID	Duration of MRSA carriage Years	Recorded number of mupirocin courses		Mupirocin susceptibility		<i>spa</i> type		Presence of antibiotic resistance gene in MRSA from persistent carrier at study end							
		Prior to RCT	During RCT	Recruit -ment	At RCT end	Recruit -ment	RCT end, 14 -28 days later	<i>mecA</i>	<i>blaZ</i>	<i>ileS2</i>	<i>erm(C)</i>	<i>aphA3</i>	<i>sat</i>	<i>tet(K)</i>	<i>qacA</i>
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	+	+	-	+	-	-	-	-

RCT- randomized controlled trial, *mecA* - alternate penicillin binding protein 2, *blaZ* - beta-lactamase gene, *ileS2* – high-level mupirocin

resistance gene, *erm(C)* – encodes resistance to macrolides, lincosamides and streptogramin B (MLS_B) compounds, *aphA3* – encodes

resistance to aminoglycosides, *sat* - streptothricin, *tet(K)*- tetracycline, *qacA* - quaternary ammonium compound. #S – mupirocin susceptible

^a patient isolate developed high level mupirocin resistance (HLMR) (>1024 mg/L) following 2 courses of mupirocin, ^b patient isolate was HLMR

at start and end of study, ^c patient isolate low level mupirocin resistance (LLMR) (8-256 mg/L) at study start but HLMR following 2 courses of

mupirocin, ^d patient isolate developed LLMR following 2 courses of mupirocin.