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Methicillin-Resistant *Staphylococcus aureus* Harboring *mecC* Still Eludes Us in East London, United Kingdom

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ABSTRACT Reports of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the *mecC* gene have increased in the UK since first being described. To our diagnostic *S. aureus* multiplex PCR, a *mecC* primer set was designed and implemented, and then the prevalence in our patient population was investigated. Fewer than 1% of the clinical isolates possessed the *mecC* gene, confirming that *mecA* remains the dominant genetic determinant of MRSA in East London.

KEYWORDS MRSA, PCR, *mecC*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is such a significant health care issue that routine screening of all hospital admissions in the United Kingdom has been in place since 2009 (1). Screening for MRSA typically involves subculture of the patient sample to a selective chromogenic agar, followed by cefoxitin susceptibility testing and, if available, penicillin-binding protein 2′ (PBP2′) detection; PBP2′ is the protein encoded by *mecA*, which confers methicillin resistance (2–4). However, 2011 saw the emergence of a *mecA* homologue, now known as *mecC*, which defied these traditional screening methods and has since been reported in farm animals, animal products, and humans (5–8). Despite these reports, there have been few cases of *mecC* detection described in the UK patient population (9, 10).

Therefore, we sought to determine the *mecC* prevalence in our patient population in East London by modifying our existing *S. aureus* multiplex PCR and incorporating a *mecC* target.

MATERIALS AND METHODS

We are a district general hospital responsible for a patient population in East London of approximately 2.5 million, with specialist centers in trauma, cancer, cardiac, and emergency care.

***S. aureus* screening.** Any *S. aureus* isolates recovered in the laboratory undergo cefoxitin (30 μg) susceptibility testing, by disk diffusion, according to EUCAST guidelines (11). Isolates that are cefoxitin resistant are submitted for extended antibiotic susceptibility testing using the MicroScan (Beckman Coulter, UK). Where disk diffusion and automated susceptibility results disagree, latex agglutination for PBP2′ (Oxoid, UK) is performed. On the rare occasion that the latex agglutination and susceptibility results also disagree, and for suspect Panton-Valentine leucocidin (PVL) toxin producers, *S. aureus* isolates are investigated using the in-house *S. aureus* PCR.

***S. aureus* multiplex PCR.** The diagnostic *S. aureus* multiplex PCR includes primers for *femB*, the PVL toxin, and *mecA* (Table 1). PCR was performed using HotStarTaq Plus mastermix (Qiagen, Germany), with primers at a final concentration of 0.2 μM (PVL) and 0.48 μM (*femB* and *mecA*), under the following conditions: initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 45 s, 50°C for 45 s, and 72°C for 1 min; and a final elongation at 72°C for 2 min. PCR products were resolved and sized by gel electrophoresis.

Addition of *mecC* to the *S. aureus* PCR. As we already had an established *S. aureus* PCR as part of our diagnostic service, it was important to source *mecC* primers which were compatible with this assay. Therefore, the literature was reviewed for a *mecC* primer set that would anneal to target DNA at 50°C and produce a product that was either >651 bp or <310 bp. To maximize the primer choice, alternative *mecC* primers were also designed using Primer3 and the *S. aureus* M10/0061 *mecC* gene sequence (GenBank accession no. NG_047955).

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TABLE 1 *S. aureus* PCR primers used in this study

Primer	Sequence	Size (bp)	Reference
FemB1	TTACAGAGTTAACTGTTACC	651	21
FemB2	ATACAAATCCAGCACGCTCT		
PVL1	ATCATTAGGTAAAAGTCGGAC	433	22
PVL2	GCATCAAGTGTATTGGATAGCAA		
MecA1	GTAGAAATGACTGAACGTCCGAT	310	
MecA2	CCAATTCCACATTGTTTCGGTCT		
MecC Design_1 (F)	TGAACGAAGCAACAGTACACC	238	This study
MecC Design_1 (R)	AGATCTTTTCCGTTTTCAGCCT		
MecC Design_2 (F)	CCCGAATTATTGGTAAATCTGGC	163	
MecC Design_2 (R)	GCATTATAGCTGGCCATCCC		

***mecC in silico* analysis.** The NCBI database (https://www.ncbi.nlm.nih.gov/) was interrogated for all *S. aureus* strains with identified complete coding sequences for the *mecC* gene. The chosen *mecC* primer set was then aligned to each sequence in turn to determine the likely accuracy of detection, using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). In addition, the *mecC* primers were aligned to *S. aureus* strains harboring *mecA* to determine if there would be any cross-amplification.

Study isolates. The following were used as control strains in this study: *S. aureus* M10/0061 (*mecC* positive), *S. aureus* LGA_{251} (*mecC* positive), *S. aureus* NCTC 12493 (*mecA* positive), and *S. aureus* NCTC 6571 (*mecA* and *mecC* negative). All *mecA*- and *mecC*-harboring control strains were methicillin resistant, as determined by cefoxitin disk susceptibility testing, with the exception of M10/0061, which was cefoxitin susceptible.

The number of isolates needed to determine the prevalence of *mecC* in our *S. aureus* population was calculated using the equation described by Jones et al., using a 95% confidence level, a 5% precision level, and an expected proportion of 6.2% based on the recently reported *mecA* prevalence (10, 12). Based on this equation, at least 89 isolates would need to be tested.

Therefore, 78 MRSA and 64 methicillin-sensitive *S. aureus* (MSSA) nonduplicate isolates, collected sequentially in the laboratory between July 2018 and February 2019, were used to evaluate the modified PCR. These isolates included 127 recovered from patient samples (Table 2), 7 external quality assessment (EQA) isolates, and 8 internal quality assessment (IQA) isolates. These isolates represented approximately 6% of the total annual *S. aureus* isolates recovered in the laboratory from MRSA and MSSA patient screens.

RESULTS

Primer selection. Based on our *S. aureus* PCR cycling conditions, the *mecC* primer sets from two studies were compatible with our assay (5, 13) and evaluated alongside the two primer sets designed as part of this study (Table 1).

TABLE 2 Patient demographics for the 127 clinical *S. aureus* isolates recovered from patient samples

Demographic	No. (%)
Sex	
Male	76 (60)
Female	51 (40)
Age (yr)	
0–18	39 (31)
19–65	64 (50)
>65	24 (19)
Health care setting	
Inpatient	41 (32)
Outpatient	80 (63)
Unknown	6 (5)
Sample/isolate origin	
Surveillance screen	34 (27)
Skin and soft tissue infection	65 (51)
Protected site	5 (4)
Sepsis	12 (9)
Respiratory infection/cystic fibrosis	8 (6)
Unknown	3 (3)

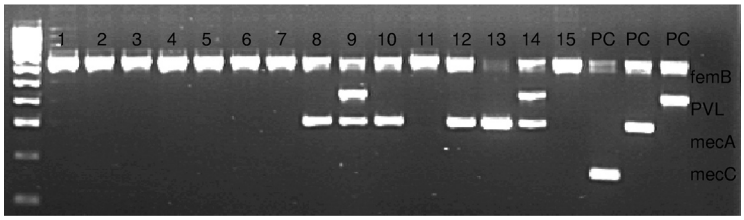

FIG 1 Gel electrophoresis results for *S. aureus* isolates screened using our diagnostic assay. The ladder is composed of 100-bp increments. Lanes 1 to 15, clinical *S. aureus* isolates. PC, positive control. There are 3 PCs, one for *mecC* (163 bp), one for *mecA* (310 bp), and one for PVL (433 bp). All 3 controls are also positive for *femB* (651 bp).

All four potential *mecC* primer sets were run within the *S. aureus* multiplex PCR using the PCR control strains. The two published primer sets failed to amplify the *mecC* target, whereas both designed primer sets successfully amplified the gene in the M10/0061 and LGA251 control strains (data not shown). For optimal gel resolution, the Design_2 primer set (here referred to as the *mecC* primer set) was selected for further testing, as it amplified a smaller DNA fragment compatible with the sizing of the other *S. aureus* targets (Fig. 1).

***In silico* analysis.** A comparison of the chosen *mecC* primer set to complete *mecC* coding sequences in the GenBank database revealed the primer sequences and gene sequences to be identical for all *mecC*-harboring strains. The *mecC* primer set was also compared to complete MRSA *mecA* coding sequences to determine if there would be any cross-amplification. *In silico* analysis revealed the *mecA* sequence to differ in 9/23 positions in the *mecC* forward primer and 3/20 positions of the *mecC* reverse primer.

***mecC* PCR results.** Following the addition of the *mecC* primers to the *S. aureus* PCR, 142 *S. aureus* isolates were analyzed using this assay. Of these, *mecC* was detected in just one MRSA isolate, which was recovered from an EQA sample. All other MRSA isolates were positive for the *mecA* gene. The MSSA isolates were negative for both the *mecC* and *mecA* genes.

DISCUSSION

Despite the limited reports of *mecC* in human isolates, a number of commercial *S. aureus* PCR assays, available in the UK, now include a *mecC* target (9, 14, 15). Similarly, various research groups have designed *mecC* primers for their *S. aureus* PCR assays, which we also found to be the most appropriate method when selecting a *mecC* target for our established *S. aureus* PCR (16–18).

When designing primers for an assay, the concern for nonspecific amplification or cross-amplification of closely related genes is high. Given that *mecC* is a homologue of *mecA*, we not only confirmed that our *mecC* primers would specifically anneal to *mecC* genes only, but we also ensured there would be no cross-amplification of *mecA* genes. Lefever et al. reported that >5 mismatches between primer sequences and target sequences will result in PCR inhibition, and as the number of mismatches increases, the annealing temperature becomes affected (19). Our *mecC* primer sequences differed from the *mecA* primer binding regions in 9 and 3 positions, respectively, preventing cross-amplification of *mecA* by our *mecC* primers. This was clearly demonstrated by our study, with all *S. aureus* targets correctly amplified in their respective PCR control and the MRSA isolates possessing *mecA* or *mecC*, but not both (Fig. 1).

Though the specificity of our *mecC* primers was high, screening of our clinical *S. aureus* isolates identified just one *mecC*-harboring MRSA strain, which mirrors the low prevalence (0.45%) reported elsewhere in the UK (9, 10). However, this is also likely to be an artifact of our testing criteria, which predominantly focus on suspected PVL producers in recurrent skin infections, regardless of methicillin resistance, with fewer MRSA isolates analyzed for *mecA* and *mecC* detection and confirmation. In addition, as a hospital-based laboratory, we only test human isolates, which are reported to have a lower *mecC* prevalence than that of animal isolates, with livestock being the purported

mecC reservoir (7, 9, 20). With such a lower number of *mecC*-positive isolates included in our study, it is important that more *mecC* strains are tested in the future to confirm the validity of our modified assay.

Our study demonstrates that *mecA* remains the dominant genetic determinant in human MRSA isolates in East London, and the multiplex PCR described here provides an additional appealing assay for the investigation of recurrent *S. aureus* skin infections.

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