Multiple-locus VNTR Analyses of Methicillin-resistant 
Staphylococcus aureus from Jamaica

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ABSTRACT

BACKGROUND: This study assessed the antimicrobial susceptibilities and the presence of inducible macrolide–lincosamide–streptogramin B (iMLSB) resistance in methicillin-resistant Staphylococcus aureus (MRSA) of Jamaica as well as the relatedness using polymerase chain reaction-based staphylococcal cassette chromosome mec (SCCmec) and multiple-locus variable numbers of tandem repeat analyses (MLVAs).

MATERIALS AND METHODS: Antimicrobial susceptibility, the presence of MLS$_B$ resistance, and SCCmec and MLVA patterns were assessed for 61 nonduplicate isolates of MRSA from hospitalized patients.

RESULTS: While no isolate was resistant to vancomycin, 53 (86.9%) isolates were resistant to ciprofloxacin, 52 (85.3%) to erythromycin, 49 (80%) to lincomycin, and 45 (74%) to clindamycin. Of the 52 erythromycin-resistant isolates, 48% exhibited constitutive resistance and 8% showed inducible MLS$_B$ resistance. Most (85%) of typable isolates were SCCmec type IV, and among these, 16 MLVA patterns were identified.

CONCLUSION: Multidrug resistance continues to characterize MRSA. Among the erythromycin-resistant isolates, constitutive resistance and iMLS$_B$ resistance are common. These facts will complicate the treatment of MRSA infections and warrant continued surveillance and judicial use of antimicrobial agents.

KEYWORDS: MRSA, SCCmec, MLS$_B$ resistance, multiple-locus VNTR analysis

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of hospital-acquired (HA) infections and has established itself as a significant community-acquired pathogen. In most if not all cases, invasive infections because of MRSA are associated with significant morbidity and mortality and high costs to the healthcare system. Community-associated MRSA (CA-MRSA) is usually caused by emerging strains unlike those responsible for HA infections and can cause infections in otherwise healthy persons with no links to healthcare systems or no known risk factors for MRSA colonization. CA-MRSA is occurring with increasing frequency and tends to occur in conditions where people are in close physical contact, such as athletes involved in football and wrestling, soldiers kept in close quarters, inmates, childcare workers, and residents of long-term care facilities. CA-MRSA differs from HA-MRSA in that it does not generally belong to the major clonal groups of epidemic MRSA, it is susceptible to most non-$\beta$-lactam antibiotics, it contains the type IV, V, or VI staphylococcal cassette chromosome mec (SCCmec) element, and it frequently contains genes for Panton–Valentine leukocidin. In the main English-speaking Caribbean islands (Jamaica and Trinidad and Tobago) and Puerto Rico, it has been shown that the prevalence of methicillin resistance in $S$. aureus is increasing. Further, Chroboczek et al noted that the distribution of the major MRSA clones in these islands was different, and clones most closely resemble those circulating within the home countries of frequent tourist travelers.

Macrolide antibiotic resistance in $S$. aureus may be because of three factors: (1) cells harboring the linA gene that inactivates both lincomycin and clindamycin but resists high levels of lincomycin alone (L resistance), (2) an active efflux mechanism encoded by msrA that confers resistance to macrolides and type B streptogramins only, ie, macrolide–streptogramin (MS) resistance, and (3) ribosomal target modification that affects macrolide–lincosamide–streptogramin B (MLS$_B$) resistance. Strains with inducible MLS$_B$ (iMLSB$_B$) resistance demonstrate in vitro resistance to 14- and 15-member macrolides (eg, erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and type B streptogramins. On the other hand, strains with constitutive MLS$_B$ resistance show in vitro resistance to all of these agents.

Advances in molecular typing have been achieved by analysis of variable numbers of tandem repeat (VNTR) loci...
identified in the genomes of eukaryotic and prokaryotic species during genome sequencing projects. The number of repeat units at the same locus varies from strain to strain and can be detected by polymerase chain reaction (PCR) with flanking primers. The sequencing of the *S. aureus* genome indicated the presence of several VNTR loci, including *sdr*, *clfA*, *clfB*, *sspA*, and *spa*. Given that there is so much genetic variability in MRSA regionally and so little information available in Jamaica, the aims of this study were to: (1) determine the antimicrobial susceptibility patterns of isolates of MRSA submitted by patients admitted to public hospitals in the Kingston and St. Andrew metropolitan in Jamaica, (2) determine if *iMLSB* resistance is present in MRSA resistant to erythromycin, (3) group isolates using PCR-restriction fragment length polymorphism analysis of the SCCmec element, and (4) discriminate among isolates based on the multiple-locus VNTR analysis (MLVA) of five (*sdr*, *clfA*, *clfB*, *ssp*, and *spa*) tandem repeat loci.

**Materials and Methods**

**Isolates and antibiotic susceptibility testing.** Sixty-one nonduplicate isolates of MRSA were submitted by patients admitted to public hospitals in Kingston and St. Andrew, Jamaica (excluding the University Hospital of the West Indies (UHWI)). These public hospitals included two adult hospitals (over 750 beds) and a children’s hospital (283 beds), and they are served mainly by the government-run public health laboratory system. More than half of the patients admitted to the adult hospitals are from the surrounding inner city communities. The UHWI was excluded as several studies have been reported from that institution, and there is a robust infection control program in place. Patients were seen on the pediatric, medical, and surgical wards, and isolates were obtained between September 2011 and August 2012. It is unclear whether patients were treated with antibiotics prior to sample isolation. Isolates were recovered from wounds, sputum, midstream urine and catheter tip, ear and nasal swabs, and a knee aspirate, identified as *S. aureus* by standard biochemical techniques, and subsequently confirmed as MRSA by disc susceptibility results with 1 g oxacillin or by growth on plates containing oxacillin. Antimicrobial susceptibility tests were carried out on Mueller-Hinton agar using the disc diffusion technique as per the Clinical and Laboratory Standards Institute guidelines and using gentamicin (10 μg), rifampicin (30 μg), trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 μg), ciprofloxacin (5 μg), teicoplanin (30 μg), vancomycin (30 μg), lincomycin (2 μg), clindamycin (2 μg), erythromycin (15 μg), mupirocin (5 and 200 μg), linezolid (30 μg), chloramphenicol (30 μg), quinupristin–dalfopristin (15 μg), and tetracycline (30 μg). All isolates were first tested with the 5 μg mupirocin disc to determine low-level resistance (LLR). High-level resistance (HLR) was confirmed in isolates resistant to 5 μg mupirocin using 200 μg mupirocin discs.

**Disc induction testing.** The determination of the presence of *iMLSB* resistance in MRSA to erythromycin was carried out according to Novotna et al. Essentially, lincomycin, clindamycin, and quinupristin–dalfopristin discs were placed at the sides of an erythromycin disc, about 15 mm apart, and then the plates were incubated for 16–18 hours.

**SCCmec and MLVA of MRSA strains.** SCCmec analysis was carried out on chromosomal DNA extracted from the strains according to the scheme proposed by Yang et al., using the *ccrB* forward and reverse primers (Table 1) and appropriate controls. Subsequently, PCR products were digested with *Hinfl* and *BsmI* (New England Biolabs) at 37°C for three hours, and then the digested products were analyzed by 1% agarose gel electrophoresis. MLVA analysis was determined based on the scheme proposed by Sabat et al., using a set of PCR primers to simultaneously amplify the hypervariable VNTR regions of the *spa*, *sspA*, *clfA*, *clfB*, and *sdrCDE* genes (Table 1). PCR products were analyzed by 2% agarose gel electrophoresis, and a 100 bp DNA ladder was included in each run as a DNA size marker. Any two MLVA patterns differing by one or more bands were considered distinct types.

### Table 1. Primers and reaction conditions used in amplification reactions in this study.

| LOCUS TARGTED | PRIMER NAME | PRIMER SEQUENCE | ANNEALING TEMPERATURE (°C) |
|--------------|-------------|----------------|--------------------------|
| ccrB         | ccrB-F      | 5’-GGCTATTATCAAGGCAATTTCACC 5’-ACTTTATACGTTTGACTATTTCG | 50 |
|              | ccrB-R      | 5’-GATTCTGACCCAGTTCAGA 5’-CTGTAATCTGGTAAATGCTTTT | 55 |
| clfA         | clfA-F      | 5’-ATGGGATTTACAGGTAATTCC 5’-CATTATTGGGGTGAACCTTTT | 55 |
|              | clfA-R      | 5’-GGCTATTATCAAGGCAATTTCACC 5’-ACTTTATACGTTTGACTATTTCG | 50 |
| clfB         | clfB-R      | 5’-GGCTATTATCAAGGCAATTTCACC 5’-ACTTTATACGTTTGACTATTTCG | 50 |
| sdr          | sdrCDE-R    | 5’-GTAACAATTTACGATCGATG 5’-TACCTGTTCTGGTAATGCTTTT | 55 |
| spa          | spa-F       | 5’-AGGACAAAAAAGGGAAGAACAA 5’-GTTTAACGACATGTACCCGT | 55 |
|              | spa-R       | 5’-AGGACAAAAAAGGGAAGAACAA 5’-GTTTAACGACATGTACCCGT | 55 |
| ssp          | sspA-F      | 5’-ATCMATTTGCAAGYCATGACCA 5’-TTGTTCTGAATTATTTGCTGCC | 55 |
|              | sspA-R      | 5’-ATCMATTTGCAAGYCATGACCA 5’-TTGTTCTGAATTATTTGCTGCC | 55 |
Results

Antimicrobial susceptibility testing. The number and percentages of MRSA isolates resistant and susceptible to antimicrobial agents are presented in Table 2. While no isolate was resistant to vancomycin, 53 (86.9%) isolates were resistant to ciprofloxacin, 52 (85.3%) to erythromycin, 49 (80%) to lincomycin, 45 (74%) to clindamycin, and 39 (64%) to gentamicin. Furthermore, five (8%) isolates were resistant to rifampicin. Thirteen (23%) isolates showed LLR to mupirocin; 11 (85%) of these 13 isolates (18% of the 61 isolates) showed high-level mupirocin resistance. High-level mupirocin resistance was observed in two isolates recovered from nose swabs.

Eleven isolates showed multiple resistance to seven or more antibiotics. These isolates showed complete resistance to lincomycin, and 91% and 82% of isolates were resistant to erythromycin and clindamycin, respectively. Almost all (28 of 30) isolates that showed multiple resistance to five antibiotics were resistant to the same antibiotics (lincomycin, gentamicin, ciprofloxacin, erythromycin, and clindamycin). The other two isolates showed resistance to mupirocin, tetracycline, and trimethoprim/SXT.

Disc induction testing. Of the 52 erythromycin-resistant isolates, full cross-resistance occurred in only 3 (6%) isolates. Even in the presence of erythromycin (an inducer), 50 (96%) of these isolates were susceptible to at least one MLSB antibiotic (Fig. 1); in all cases, the isolates were susceptible to quinupristin–dalfopristin. The most common type was the ELC phenotype (ie, resistance to erythromycin, lincomycin, and clindamycin). The constitutive macrolide–lincosamide–streptogramin (MLS) resistance mechanism was evident in 25 (48%) isolates, MS resistance mechanism in 24 (46%) isolates, and inducible MLS resistance mechanism in 4 (8%) isolates.

SCCmec analysis. SCCmec typing confirmed a type in only 34 isolates. Analysis yielded 29 (85%) isolates as SCCmec type IV, 3 (9%) as type II, and 1 each (3%) as type I and type III, respectively (Table 3).

### Table 2. Antimicrobial susceptibility profiles of MRSA isolates in this study.

| ANTIMICROBIAL AGENT | RESISTANT | INTERMEDIATE | SUSCEPTIBLE |
|----------------------|-----------|---------------|-------------|
| Aminoglycosides       |           |               |             |
| Gentamicin           | 39 (64%)  | 3 (5%)        | 19 (40%)    |
| Ansamycins           |           |               |             |
| Rifampicin           | 5 (8%)    | 0             | 56 (92%)    |
| Antifolates          |           |               |             |
| Trimethoprim/sulfamethoxazole | 10 (16%) | 3 (5%) | 48 (79%) |
| Fluoroquinolones     |           |               |             |
| Ciprofloxacin        | 52 (85%)  | 1 (2%)        | 8 (13%)     |
| Glycopeptide         |           |               |             |
| Teicoplanin          | 0         | 1 (2%)        | 60 (98%)    |
| Vancomycin           | 0         | 0             | 61 (100%)   |
| Lincosamides         |           |               |             |
| Lincomycin           | 49 (80%)  | 3 (5%)        | 9 (15%)     |
| Clindamycin          | 45 (74%)  | 2 (3%)        | 14 (23%)    |
| Macrolides           |           |               |             |
| Erythromycin         | 52 (85%)  | 1 (2%)        | 8 (13%)     |
| Other antibiotics    |           |               |             |
| Mupirocin (5 µg)     | 13 (23%)  | 0             | 48 (79%)    |
| Mupirocin (200 µg)   | 11 (18%)  | –             | –           |
| Oxazolidinones       |           |               |             |
| Linezolid            | 0         | 0             | 61 (100%)   |
| Phenicols            |           |               |             |
| Chloramphenicol      | 4 (6%)    | 1 (2%)        | 56 (92%)    |
| Streptogramins       |           |               |             |
| Quinupristin–dalfopristin | 0     | 3 (5%) | 58 (95%) |
| Tetracyclines        |           |               |             |
| Tetracycline         | 16 (26%)  | 3 (5%)        | 42 (69%)    |

Note: Percentages may not add up to 100% because of rounding.
Figure 1. Phenotypes identified by a triple-disc induction test in erythromycin-resistant strains in this study.

Abbreviations: E, erythromycin resistant; L, lincomycin resistant; C, clindamycin resistant; Q, reduced susceptibility to quinupristin–dalfopristin; Li, lincomycin resistant after induction by erythromycin; Ci, clindamycin resistant after induction by erythromycin; cMLS, constitutive MLS resistance; iMLS, inducible MLS.

Table 3. Results from MLVA and SCCmec typing of MRSA isolates in this study.

| ISOLATE NUMBER | ERY | RESISTANCE PHENOTYPE | RESISTANCE MECHANISM | MLVA TYPE | SCCmec TYPE |
|----------------|-----|----------------------|----------------------|-----------|-------------|
| AHSW           | R   | ELCQ                 | cMLS                 | –         | –           |
| A001           | R   | E                    | MS                   | –         | –           |
| A42            | R   | ELC                  | MS & L               | –         | –           |
| A079           | R   | ELC                  | MS & L               | 2         | IV          |
| A086           | S   | –                    | –                    | –         | –           |
| A089           | R   | ELCQ                 | cMLS                 | –         | –           |
| A099           | R   | ELC                  | cMLS                 | 2         | IV          |
| A101           | R   | ELC                  | MS & L               | –         | –           |
| A122           | S   | –                    | –                    | 1         | IV          |
| A127           | R   | ELCi                 | iMLS & L             | 3         | IV          |
| A131           | R   | ELC                  | MS & L               | 2         | IV          |
| A182           | R   | ELC                  | cMLS                 | 5         | IV          |
| A244           | R   | ELC                  | MS & L               | 5         | II          |
| A253           | R   | ELC                  | MS & L               | 6         | IV          |
| A259           | R   | ELC                  | MS & L               | 7         | IV          |
| A278           | R   | ELC                  | cMLS                 | 6         | IV          |
| A283           | R   | ELC                  | cMLS                 | –         | –           |
| A287           | R   | E                    | MS                   | –         | –           |
| A294           | R   | ELC                  | cMLS                 | 5         | II          |
| A303           | R   | ELiCi                | iMLS                 | –         | –           |
| A304           | R   | ELC                  | cMLS                 | 6         | IV          |
| A305           | R   | ELC                  | MS & L               | 6         | IV          |
| A343           | R   | ELC                  | cMLS                 | 8         | IV          |
| A365           | R   | E                    | MS                   | –         | –           |
| A374           | R   | ELC                  | cMLS                 | –         | –           |
| B345           | R   | E                    | MS                   | 9         | II          |
| B018           | R   | ELC                  | cMLS                 | 10        | IV          |
| B029           | R   | –                    | –                    | –         | –           |
| B039           | R   | ELC                  | cMLS                 | 10        | IV          |
| B040           | R   | ELC                  | cMLS                 | 10        | IV          |
| B046           | R   | ELC                  | MS & L               | –         | –           |
| B048           | R   | ELC                  | cMLS                 | 10        | IV          |
| B057           | R   | ELC                  | cMLS                 | 10        | IV          |
VNTR analyses of methicillin-resistant *S. aureus* from Jamaica

Multigene analysis (genetic typing) of MRSA. Results were obtained for the 34 isolates that yielded an SCC\textit{mec} type. Sixteen MLVA patterns were identified based on the scheme proposed by Sabat et al.\textsuperscript{19} MLVA pattern 10 (with 12 isolates) was the most common, followed by patterns 6 (4 isolates) and 2 and 5 (3 isolates each). Six isolates were unrelated based on MLVA patterns (Table 3). Of the isolates that showed identical MLVA patterns, all isolates with patterns 2, 6, 10, and 16 had the same SCC\textit{mec} type (type IV).

### Discussion

*S. aureus*, and in particular MRSA, has long been one of the more serious and problematic nosocomial pathogens, repeatedly responding to the challenge of staphylococcal antibiotics by acquiring new resistance. In fact, its prevalence has increased globally, and it is clear that there are major differences in prevalence between countries and regions. In Jamaica, prevalence studies have largely been centered around the UHWI, the main teaching hospital in Kingston. However, this study sought to assess the characteristics of isolates outside of this controlled zone, where less stringent infection control is practiced.

In light of this, the highest overall susceptibility rates were observed for the glycopeptides and oxazolidinones (100% susceptible), followed by the susceptibility to ansamycins, phenicols, and streptogramins (93–95%). While parenteral glycopeptides remain the forefront treatment for systemic MRSA infections, not all of these infections have poor prognosis, and oral agents might be indicated, particularly when long-term therapy is required.\textsuperscript{22} For example, rifampicin and trimethoprim–SXT, with demonstrable better tissue penetration than the glycopeptides, might be better suited oral agents.\textsuperscript{22}

| ISOLATE NUMBER | ERY | RESISTANCE PHENOTYPE | RESISTANCE MECHANISM | MLVA TYPE | SCC\textit{mec} TYPE |
|----------------|-----|----------------------|----------------------|-----------|---------------------|
| B070           | R   | ELC                  | cMLs                 | 10        | IV                  |
| B086           | R   | ELC                  | MS & L               | 10        | IV                  |
| B091           | S   |                      |                      |           |                     |
| B101           | R   | ELC                  | cMLs                 |           |                     |
| B103           | R   | ELC                  | cMLs                 |           |                     |
| B117           | R   | E                    | MS                   |           |                     |
| B128           | S   |                      |                      |           |                     |
| C042           | R   | ELiCi                | iMLs                 |           |                     |
| C162           | S   |                      |                      |           |                     |
| C194           | S   |                      |                      |           |                     |
| D003           | S   |                      |                      |           |                     |
| D031           | R   | ELC                  | MS & L               | 10        | IV                  |
| D032           | R   | ELCi                 | iMLs & L             | 11        | IV                  |
| D034           | R   | ELC                  | MS & L               | 10        | IV                  |
| D050           | R   | ELC                  | cMLs                 |           |                     |
| D054           | R   | ELC                  | MS & L               |           |                     |
| D061           | I   |                      |                      | 10        | IV                  |
| D108           | R   | E                    | MS                   |           |                     |
| D132           | R   | ELC                  | cMLs                 | 12        | IV                  |
| D155           | R   | ELC                  | cMLs                 | 13        | I                   |
| D160           | R   | ELC                  | cMLs                 |           |                     |
| D224           | R   | ELC                  | MS & L               |           |                     |
| U020           | R   | ELC                  | cMLs                 | 15        | IV                  |
| U195           | R   | E                    | MS                   | 13        | III                 |
| U268           | R   | ELC                  | MS & L               | 16        | IV                  |
| U465           | R   | ELC                  | cMLs                 | 16        | IV                  |
| U636           | R   | ELCQ                 | cMLs                 |           |                     |
| U733           | R   | ELC                  | MS & L               |           |                     |

Note: –, no type determined. Details of antimicrobial resistance profiles, disc induction phenotypes and resistance mechanisms, and MLVA and SCC\textit{mec} types of MRSA isolates in this study are available in Supplementary Table 1.

Abbreviations: E, erythromycin resistant; L, lincomycin resistant; C, clindamycin resistant; Q, reduced susceptibility to quinupristin–dalfopristin; Li, lincomycin resistant after induction by erythromycin; Ci, clindamycin resistant after induction by erythromycin; cMLS, constitutive MLS; iMLS, inducible MLS.
and, as shown in this study, had good coverage rates against MRSA. On the other hand, the highest resistance rates were observed against fluoroquinolones and macrolides, as reported elsewhere. It is worth noting that fluoroquinolones select for methicillin resistance in staphylococci and with a 75% resistance rate, as seen in this study, suggest that these drugs can no longer be used in empirical therapy against MRSA infections.

As expected, no resistance was observed against either teicoplanin or linezolid; however, the one intermediate (reduced susceptibility) phenotype to teicoplanin and the three intermediate phenotypes to quinupristin–dalfopristin are of some concern as none of these drugs are used locally in Jamaica. However, the findings for linezolid are in concern with the results from other studies reviewed by Beibei et al., which indicated that linezolid has excellent success rates against MRSA in randomized control studies. While the authors noted that there was no difference in total adverse effects related to the use of linezolid versus vancomycin and vancomycin tended to result in more nephrotoxicity, there was superior clinical and microbiological outcomes with linezolid in S. aureus infections.

With a low resistance rate of 6%, chloramphenicol shows similar results to other studies: 10%, 13%, 5%, 10.7%, and 4.9% resistance.28 Result for rifampicin, with a resistance rate of 7%, is at the lower end when compared to other reports (3%, 29 18%, 22 and 53%)30, although higher resistance rates of rifampicin resistance in MRSA might be attributable to the treatment of tuberculosis.31,32 Trimethoprim/SXT and tetracycline had resistance rates of 14% and 23%, respectively. The relatively low resistance of SXT of 16.4%33 and high susceptibility rates of 82.1% and 80.6%28 are comparable to the results from this study.

Gentamicin, lincomycin, and erythromycin had relatively high resistance rates of 56%, 71%, and 77%, respectively. These values are of concern, especially for lincomycin, as it is a relatively new drug that should show increased effectiveness against MRSA. There is concern of the 65% resistance rate for clindamycin, as it has been proven to be an effective drug in treating S. aureus infections. It is well established that inducible clindamycin resistance can decrease its therapeutic efficacy.34,35

In terms of mupirocin resistance, we found a 23% LLR and 18% HLR compared to 30% LLR and 24% HLR reported by Nicholson et al at the UHWI, Jamaica. These figures are high when compared to reports in the literature of 1–13% LLR and 2.4–14% HLR.37 However, the rates at the UHWI are lower than those in Trinidad and Tobago, with 44% LLR and 26% HLR.38 These data are worrying as mupirocin, a topical agent, is widely used in the management of infection and colonization by MRSA, and the inability to effectively clear the nasal colonization of S. aureus will undoubtedly increase the subsequent risk of development of infection by MRSA, in addition to increasing the spread of these pathogens.39 Interestingly, McNeil et al noted a 14.7% mupirocin resistance rate among S. aureus isolates from pediatric patients and that the genetic determinant was more significantly associated with methicillin-susceptible S. aureus (MSSA) than with MRSA.

There have also been a number of reported clindamycin and lincomycin treatment failures in S. aureus infections with iMLS<sub>B</sub> resistance.34,35 This brings into focus the efficacy of clindamycin use in infections caused by erythromycin-resistant S. aureus. Published data indicate that there is significant variability in iMLS<sub>B</sub> resistance among erythromycin-resistant S. aureus isolates and could be related to geographical location, hospital environment, patient age, and clinical samples examined.41 In discussing their finding of 12% inducible resistance, the authors noted that such resistance varied from a low of 11% in Brazil to a high of 35% in India. Of note was the observation that this phenomenon was reduced in MSSA but was more prevalent in coagulase-negative staphylococci. The results of this study appear to be at the lower end of the spectrum as only 8% of erythromycin-resistant MRSA isolates showed iMLS<sub>B</sub> resistance. On the other hand, a number of erythromycin-resistant S. aureus isolates may show true clindamycin susceptibility. Of 52 erythromycin-resistant MRSA isolates obtained, 8 (15%) were clindamycin susceptible, with no indication of inducible resistance. Therefore, the assumption of clindamycin resistance based on actual confirmation of erythromycin resistance and the elimination of clindamycin as a potential therapeutic agent for erythromycin-resistant MRSA infections is problematic.

However, while clindamycin remains useful in the treatment of skin and soft-tissue infections and serious infections caused by S. aureus and MRSA, accurate susceptibility data are important for appropriate treatment decisions. This is because of the fact that susceptibility testing for clindamycin may indicate false susceptibility by the disc diffusion testing with erythromycin and clindamycin discs in nonadjacent positions. If inducible resistance can be reliably detected in clinical isolates, clindamycin can be safely and effectively used in the patients with true clindamycin-susceptible strains.17

An important finding was the high proportion of type IV CA-MRSA among these apparent nosocomial isolates. This could indicate the emergence of a new MRSA lineage in Jamaica, with particular fitness for spread in the community.42 It is clear that particular attention should be paid to the early detection of CA-MRSA strains in hospitals because of the potential for easy transmission of the type IV SCC<sub>mec</sub> element to nosocomial MRSA isolates. The determinants for resistance to multiple antibiotics carried by the types of SCC<sub>mec</sub> elements (types I, II, and III) may be suited for the survival of HA-MRSA, where various antibiotics provide selective pressure, but their large sizes and potentially hazardous arrays of exogenous genes may not be suited to MRSA strains in the community, where selective advantage would make strains more inclined to
have a higher growth rate and to be better able to colonize humans than to have a multidrug resistance phenotype. From this viewpoint, the type IV SCCmec may be one of the fitter SCCmec types that can give β-lactam resistance to community strains of MRSA without compromising their competitiveness among human and other vertebrate hosts. Consequently, the ability of type IV isolates to survive in the hospital setting increases the challenges for control and treatment. However, in this study, we noted that only some of the MRSA isolates produced an SCCmec genotype, having the mecA gene and high minimal inhibitory concentration values against oxacillin. In these isolates without the mecA element, alternate resistance mechanisms, such as overexpressing β-lactamase or altered penicillin-binding proteins (PBPs), could account for this resistance, with or without the concomitant loss of the mecA gene. However, it is possible, albeit remotely, that the new mecC element could be involved.

The eight sets of isolates that showed identical MLVA patterns (same set of bands) proved to be genetically identical; the remaining isolates were unrelated, as they showed a different profile. Furthermore, it was noted that the genetically identical isolates displayed the same SCCmec type. An important factor of MLVA is the differentiation power, defined as the ability to clearly differentiate among unrelated isolates and simultaneously demonstrate the relationship of organisms isolated from individuals infected through the same source. This criterion was fulfilled by MLVA as a typing system as all 18 distinct strains in this study were clearly discriminated. On the other hand, epidemiologically linked, and therefore genetically related, MRSA isolates showed little variation of repeat units. However, an inherent weakness of DNA-based typing methods, which rely on DNA fragment amplification, may fail to type some strains because of differences in the DNA sequence to which primers anneal. This could account for some of the isolates not giving a band.

In conclusion, it is apparent that both HA-MRSA and CA-MRSA are coexisting among hospitalized patients in Jamaica. While some of these isolates are already showing reduced susceptibility to antimicrobial agents not yet licensed for use in Jamaica, many (erythromycin-resistant isolates) display constitutive and iMLS9 resistance. These facts will complicate the treatment of MRSA infections. Hence, continued surveillance and judicial use of antimicrobial agents are warranted.

**Author Contributions**

PDB conceived and designed the experiments, analyzed the data, wrote the first draft of the manuscript, contributed to the writing of the manuscript, including the results and conclusions, developed the structure and arguments for the paper, made critical revisions and approved final version.

**Supplementary Material**

**Supplementary table 1.** Antimicrobial resistance profiles, disc induction phenotypes and resistance mechanisms, and MLVA and SCCmec types of MRSA isolates in this study.

**REFERENCES**

1. Carleton HA, Diep BA, Charlebois ED, Sensabaugh GF, Perederau-Remington F. Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an expanding community reservoir. *J Infect Dis*. 2004;190:1730–1738.
2. Egguia JM, Chambers HF. Community-acquired methicillin-resistant *Staphylococcus aureus*: epidemiology and potential virulence factors. *Curr Infect Dis Rep*. 2003;5:459–466.
3. Shorr AF, Kunkel MJ, Kollef M. Linezolid versus vancomycin for *Staphylococcus aureus* bacteremia: pooled analysis of randomized studies. *J Antimicrob Chemother*. 2005;56(5):923–931.
4. Ryback MJ, LaPlante KL. Community associated methicillin resistant *Staphylococcus aureus*: a review. *Pharmacotherapy*. 2005;25:74–85.
5. Centers for Disease Control and Prevention (CDC). Public health dispatch: outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections, Los Angeles County, California, 2002–2003. *JAMA*. 2003;289:1377.
6. Centers for Disease Control and Prevention (CDC). Methicillin-resistant *Staphylococcus aureus* infections among competitive sports participants, Colorado, Indiana, Pennsylvania, and Los Angeles County, 2000–2003. *MMWR Morb Mortal Wkly Rep*. 2003;52:793–795.
7. Bansal S, Kashyap S, Pal LS, Goel A. Clinical and bacteriological profile of community acquired pneumonia in Shimla, Himachal Pradesh. *Indian J Chest Dis Allied Sci*. 2004;46:17–22.
8. Fey PD, Said-Salim B, Rupp ME, et al. Comparative molecular analysis of community- or hospital-acquired methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2003;47:196–203.
9. Liassine N, Auckenthaler R, Descombes MC, Bes M, Vandenesch F, Etienne J. Community-acquired methicillin-resistant *Staphylococcus aureus* isolated in Switzerland contains the Pantos-Vailette leuconoxin or exfoliative toxin genes. *J Clin Microbiol*. 2004;42(2):825–828.
10. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA*. 2003;290:2976–2984.
11. Oliveira DC, Milheiro C, de Lencastre H. Redefining a structural variant of *staphylococcal* cassette chromosome mec, SCCmec type VI. *Antimicrob Agents Chemother*. 2006;50:3457–3459.
12. Akpaka PE, Kissoon S, Swanson WH, Monteil M. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates from Trinidad & Tobago. *Ann Clin Microbiol Antimicrob*. 2004;5:16.
13. Brown PD, Nengo C. Antimicrobial resistance in clinical isolates of *Staphylococcus aureus* for hospital and community sources in southern Jamaica. *J Infect Dis*. 2007;11:220–225.
14. Orett FA, Land M. Methicillin-resistant *Staphylococcus aureus* prevalence: current susceptibility patterns in Trinidad. *BMC Infect Dis*. 2006;6:83.
15. Rodríguez JL, Vázquez GJ, Bermúdez M, et al. Prospective study using standardized methodology for antimicrobial susceptibility of Gram-positive cocci isolated from the Puerto Rico Medical Center. *P R Health Sci J*. 2002;21:343–347.
16. Chroboczek T, Boisot S, Rasadek JP, et al. Major West Indies MRSA clones in human beings: do they travel with their hosts? *J Travel Med*. 2013;20(5):283–288.
17. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative *Staphylococcus*. *J Clin Microbiol*. 2003;41:4740–4744.
18. Novonna G, Adamkova V, Janata J, Melter O, Spiek J. Prevalence of resistance mechanisms against macrolides and lincomamides in methicillin-resistant coagulase-negative *Staphylococcus* in the Czech Republic and occurrence of an undefined mechanism of resistance to lincomamides. *Antimicrob Agents Chemother*. 2005;49:3586–3589.
19. Sabat A, Krzyzstowie-Russin J, Strzalka W, et al. New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *J Clin Microbiol*. 2003;41:1801–1804.
20. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement*. M100-S22. Vol. 31. Wayne, PA, USA: Clinical and Laboratory Standards Institute (CLSI); 2011:11.
21. Yang JA, Park DW, Sohn JW, Kim MJ. Novel PCR-restriction fragment length polymorphism analysis for rapid typing of staphylococcal cassette chromosome mec elements. J Clin Microbiol. 2006;44:236–238.

22. Kim HB, Jiang HC, Jiang Nam H, et al. In vitro activities of 28 antimicrobial agents against Staphylococcus aureus isolates from tertiary-care hospitals in Korea: a nationwide survey. Antimicrob Agents Chemother. 2004;48:1124–1127.

23. Gade ND, Qazi MS. Fluoroquinolone therapy in Staphylococcus aureus infections: where do we stand? J Lab Physicians. 2013;5:109–112.

24. Reyes J, Hidalgo M, Díaz L, et al. Characterization of macrolide resistance in Gram-positive cocci from Colombian hospitals: a countrywide surveillance. Int J Infect Dis. 2007;11(4):329–336.

25. Roychoudhury S, Catrenich CE, McIntosh EJ, et al. Quinolone resistance in Staphylococci: activities of new nonfluorinated quinolones against molecular target gets in whole cells and clinical isolates. Antimicrob Agents Chemother. 2001;45(4):1115–1120.

26. Beiße L, Yun C, Mengli C, Nan B, Xubong Y, Rui W. Linezolid versus vancomycin for the treatment of Gram-positive bacterial infections: meta-analysis of randomised controlled trials. Int J Antimicrob Agents. 2010;35(1):3–12.

27. Varaldo PE, Cipriani P, Foca A, et al. Identification, clinical distribution, and susceptibility to methicillin and 18 additional antibiotics of clinical Staphylococcus aureus isolates: nationwide investigation in Italy. J Clin Microbiol. 1984;19:838–843.

28. Jung S, Shin DH, Park KH, Shin JH. Antimicrobial susceptibility and clonal relatedness between community- and hospital-acquired methicillin-resistant Staphylococcus aureus from blood cultures. J Microbiol. 2006;44:336–343.

29. Villar M, Marinón JM, García-Arenaza JM, de la Campa ÁG, Ferrándiz MJ, Pérez-Trallero E. Epidemiological and molecular aspects of rifampicin-resistant Staphylococcus aureus isolated from wounds, blood and respiratory samples. J Antimicrob Chemother. 2011;66(5):997–1000.

30. Marais E, Aiththa N, Petrovic O, Oosthuysen WF, Musenge E, Duse AG. Antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus isolates from South Africa. S Afr Med J. 2009;99(3):170–173.

31. Sekiguchi J, Fujino T, Arakae M, et al. Emergence of rifampicin resistance in methicillin-resistant Staphylococcus aureus in tuberculosis wards. J Infect Chemother. 2006;12(3):47–50.

32. Tan CK, Lai CC, Liao CH, Lin SH, Huang YT, Hsuw PR. Increased rifampicin resistance in blood isolates of meticillin-resistant Staphylococcus aureus (MRSA) amongst patients exposed to rifampicin-containing antituberculous treatment. J Antimicrob Agents. 2011;37(6):550–553.

33. Van Grieshuyzen A, Vaart Veen A, Buiting A, Walsh T, Kluystermans J. High percentage of methicillin-resistant Staphylococcus aureus isolates with reduced susceptibility to glycopeptides in the Netherlands. J Clin Microbiol. 2003;41:2487–2491.

34. Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance. J Antimicrob Chemother. 2001;48:315–316.

35. Frank AL, Marcinak JF, Mahgat PD, et al. Clindamycin treatment of methicillin-resistant Staphylococcus aureus infections in children. Pediatr Infect Dis J. 2002;21:530–534.

36. Nicholson AM, Thomas C, Wint H, et al. The detection of mupirocin resistance and the distribution of methicillin-resistant Staphylococcus aureus at the University Hospital of the West Indies, Jamaica. West Indian Med J. 2010;59(5):509.

37. Vasquez JE, Walker ES, Franzus BW, Overbay BK, Reagan DR, Sarubbi FA. The epidemiology of mupirocin resistance among methicillin-resistant Staphylococcus aureus at a Veterans’ Affairs hospital. Infect Control Hosp Epidemiol. 2000;21(7):459–464.

38. Orrett FA. The emergence of mupirocin resistance among clinical isolates of methicillin-resistant Staphylococcus aureus in Trinidad: a first report. Jpn J Infect Dis. 2008;61(2):107–110.

39. Oliveira NE, Cardozo AP, De Andrade Marques E, dos Santos KR, Giambiagi-deMarval M. Interpretive criteria to differentiate low- and high-level mupirocin resistance in Staphylococcus aureus. J Med Microbiol. 2007;56:937–939.

40. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin resistance in Staphylococcus aureus causing recurrent skin and soft tissue infections in children. Antimicrob Agents Chemother. 2011;55(5):2431–2433.

41. Buyal D, Shanshth A, Pal S, Sharma MK, Prakash R, Sharma N. The prevalence of inducible clindamycin resistance among Staphylococci in a tertiary care hospital—a study from the Garwal Hills of Uttarakhand, India. J Clin Diag Res. 2013;7(1):61–65.

42. Francis P, Renzi G, Pittet D, et al. A novel multiplex real-time PCR assay for rapid typing of major staphylococcal cassette chromosome mec elements. J Clin Microbiol. 2004;42:3309–3312.

43. Ma XX, Io T, Tien Saoton C, et al. Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant Staphylococcus aureus strains. Antimicrob Agents Chemother. 2002;46:1147–1152.

44. Brown DJ, Edwards DL, Hawkey PI, et al. Joint Working Party of the British Society for Antimicrobial Chemotherapy; Hospital Infection Society; Infection Control Nurses Association. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRA): J Antimicrob Chemother. 2005;56:1000–1018.

45. Kumurya AS. Loss of the mecA gene during storage of methicillin-resistant Staphylococcus aureus isolates in Northwestern Nigeria. J Public Hlth Epidemiol. 2013;5:410–415.

46. Olajinka BO, Olajinka AT, Obajuluwa AF, Omoalopa JA, Oh-unitola PF. Absence of mecA gene in methicillin-resistant Staphylococcus aureus isolates. Afr J Infect Dis. 2009;12(4):39–56.

47. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends Microbiol. 2014;22(1):42–47.