Diversity among Community Isolates of Methicillin-Resistant
Staphylococcus aureus in Australia

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Received 2 February 2004/Returned for modification 15 March 2004/Accepted 5 April 2004

Community methicillin-resistant Staphylococcus aureus (CMRSA) strains are being isolated with increasing frequency around the world. In Western Australia CMRSA are endemic in geographically remote communities and have been found to belong to five different contour-clamped homogeneous electric field (CHEF) electrophoretic patterns. Representatives of each of these CHEF patterns have been compared to CMRSA representative of CHEF patterns from other Australian states and New Zealand. With one exception, all of the isolates were nonmultiresistant and were not resistant to many antimicrobial agents other than the β-lactams. With one exception, which is not believed to be a CMRSA, all of the isolates harbored a β-lactamase plasmid. Erythromycin resistance was associated with a 2-kb plasmid. One of the β-lactamase plasmids was found to be able to acquire additional resistance determinants to become a multiple resistance plasmid. There were 10 multilocus sequence types belonging to eight distantly related clonal complexes of S. aureus. One new sequence type was found. Although most of the CMRSA harbored the type IVa SCCmec, a type IV structural variant was found and two new SCCmec types were identified. Protein A gene (spa) typing revealed two new spa types and, with two exceptions, corresponded to multilocus sequence typing. In contrast to other reports on CMRSA, most of the CMRSA strains studied here did not contain the Pantone-Valentine leukocidin genes. The results also demonstrate that nonmultiresistant hospital strains such as UK EMRSA-15 may be able to circulate in the community and could be mistaken for CMRSA based on their resistance profiles.

Methicillin-resistant Staphylococcus aureus (MRSA) is an important pathogen that has been historically associated with hospitals and health care facilities (22). However, there are increasing reports of MRSA being isolated from the community (10, 27). Two types of MRSA have been isolated from communities: multiresistant (2, 47) and nonmultiresistant (29) MRSA. The former appear to have been transferred from hospitals or health care facilities by patients or health care workers and spread to close contacts (4), whereas the latter have been isolated from people who have had little or no contact with health care facilities or workers (43). We consider these isolates to be true community MRSA (CMRSA). The earliest CMRSA were isolated from people belonging to minority groups and living in disadvantaged communities (8, 15, 25, 56). These strains have since been isolated from the general community, including children (16, 17, 45) and members of close contact sporting groups (6) and from a prison population (7).

In Western Australia (WA) all MRSA that have been isolated since 1982 have been referred to a reference center for epidemiological typing and storage. A report of the first Australian CMRSA was published in 1993. These strains were isolated from people in the remote and sparsely populated Kimberley region of WA, which is located ca. 2,000 km north of the capital city of Perth. These people have little or no contact with urban communities or health facilities. The strains became collectively known as WA MRSA (35, 56). Initially, the Kimberley region had the highest notification rate for WA MRSA, but these organisms are now endemic in most remote communities throughout the state (38). Surveillance data has shown that the isolation of these strains has increased dramatically since 1991 (39).

Like other CMRSA, WA MRSA are typically nonmultiresistant. However, some WA MRSA isolates obtained from people living in the Kimberley region were found to be multiresistant due to the acquisition of a 41.4-kb plasmid that encoded β-lactamase (Bla) production and resistance to mupirocin, tetracycline, trimethoprim, and cadmium (35, 55). The number of WA MRSA that were mupirocin-resistant peaked at 18% of WA MRSA isolates (37) until guidelines restricting the use of mupirocin lowered the rate to 0.3% 3 years later (49).

Nonmultiresistant MRSA have also been reported in the Northern Territory (NT) of Australia, and it was suggested that they might have been WA MRSA that had spread across the border from WA (24). However, the NT strains have been reported to be genetically unrelated to the WA MRSA (21). CMRSA are not generally known to spread in hospitals. However, in 1995 a WA MRSA caused a single-strain outbreak in a large metropolitan hospital in WA. The index strain was isolated from a patient from a remote community and was indistinguishable from the predominant strain found in two
isolated communities in WA where 42% of people in one community and 24% in the other were colonized with this strain (29). There have been few other reports of CMRSA involved in a hospital outbreak (3, 42).

CMRSA have also been isolated in Auckland, New Zealand (NZ), with the majority of isolates coming from people from Western Samoa (1, 28, 54). The isolates have two phage patterns and are referred to as Western Samoan Phage Pattern 1 and 2 (WSPP-1 and -2) (1, 25). The WSPP clone has been reported to have spread to New South Wales (NSW) (9, 14) and Queensland (28) on the eastern seaboard of Australia. A second CMRSA strain, designated the Queensland clone, has also been reported from Queensland (57).

Since 1995 five different contour-clamped homogeneous electric field (CHEF) electrophoretic patterns have been found among WA MRSA, and a recent investigation of some CMRSA from Australia and NZ identified several CHEF electrophoretic patterns that were mainly confined to specific geographical areas (21).

In an effort to better understand the epidemiology of CMRSA in Australia, CMRSA isolates representing the different CHEF patterns were compared by a number of methods. The overall relatedness of isolates has been compared by using multilocus sequence typing (MLST) (11), protein A gene (spa) typing (46), antibiograms, and resistograms. However, isolates with the same overall genetic background can differ as a result of acquiring additional genetic information in the form of cassettes, transposons, and plasmids. The gene for methicillin resistance, mecA, is contained within a mobile element known as the mec region or staphylococcal cassette chromosome mec (SCmec). The SCmec types vary depending on variations in the mecA regulatory region (mec complex), the type of cassette chromosome recombinases (ccr genes) they have, and the resistance determinants they have acquired due to the integration of plasmids and transposons (18). It has been reported that the Panton-Valentine leukocidin (PVL) determinant is common in CMRSA but rare among hospital MRSA and therefore may be a useful marker for the rapid identification of CMRSA (57). Consequently, all of these approaches have been used to compare CMRSA isolates from Australia.

### MATERIALS AND METHODS

#### Strains

The CMRSA isolates used (Table 1) were selected as representative of the CHEF pattern types present in a collection of 425 CMRSA isolates from WA, 32 CMRSA isolates from South Australia (SA), 34 CMRSA isolates from the NT, 36 CMRSA isolates from NSW, and 5 CMRSA isolates from Victoria (VIC). The WA isolates WBG8366, WBG8578, and WBG8404 were nasal carriage isolates from inhabitants of remote WA communities; all of the other isolates were from cases of infection. The criteria for designating the isolates as CMRSA were that they were nonmultiresistant MRSA that were isolated from people from the community. The WA MRSA belonged to five different CHEF patterns. For comparison, the type strains for the NZ WSPP-1 and WSPP-2 isolates were also included. In addition, the plasmids in two isolates belonging to the same CHEF group as WBG7583 were studied in order to elucidate the evolution of multiresistance in these isolates. The recipient for mixed-culture integrations of plasmids and transposons (18). It has been reported that the Panton-Valentine leukocidin (PVL) determinant is common in CMRSA but rare among hospital MRSA and therefore may be a useful marker for the rapid identification of CMRSA (57). Consequently, all of these approaches have been used to compare CMRSA isolates from Australia.

#### Antimicrobial susceptibilities

All isolates were resistant to methicillin and produced Bla. In population analyses, WBG8287, WBG9087, and WBG9093 expressed heterogeneous resistance to methicillin (not shown). In WA a panel of eight antimicrobial agents (gentamicin, erythromycin, tetracycline, trimethoprim, rifampin, fusidic acid, ciprofloxacin, and mucopeptide) is used to provisionally distinguish between multiresistant and nonmultiresistant MRSA (29). Strains resistant less than three of the antibiotics are regarded as nonmultire-

### RESULTS

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### TABLE 1. Genetic and phenotypic properties of CMRSA isolates from Australia and NZ

<table>
<thead>
<tr>
<th>Isolate</th>
<th>plasmid content</th>
<th>Resistance phenotype</th>
<th>ST</th>
<th>MLST CC</th>
<th>CHEF patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSPP-1a</td>
<td>2, 6</td>
<td>oxacin, tetracycline</td>
<td>J1</td>
<td>30</td>
<td>IVa</td>
</tr>
<tr>
<td>WBG9093</td>
<td>2, 6</td>
<td>oxacin, tetacycline</td>
<td>G</td>
<td>258</td>
<td>IV</td>
</tr>
<tr>
<td>WBG9087</td>
<td>2, 6</td>
<td>oxacin, tetracycline</td>
<td>J1</td>
<td>30</td>
<td>IVa</td>
</tr>
<tr>
<td>WBG10201</td>
<td>3</td>
<td>fusidic acid</td>
<td>D2</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>WBG8897</td>
<td>0</td>
<td>none</td>
<td>H</td>
<td>22</td>
<td>B</td>
</tr>
<tr>
<td>WBG8378</td>
<td>2</td>
<td>arsenate</td>
<td>J1</td>
<td>30</td>
<td>B</td>
</tr>
<tr>
<td>WBG8900</td>
<td>2</td>
<td>arsenate</td>
<td>C2</td>
<td>22</td>
<td>B</td>
</tr>
<tr>
<td>WBG8366</td>
<td>2</td>
<td>arsenate</td>
<td>C1</td>
<td>22</td>
<td>B</td>
</tr>
<tr>
<td>WBG8873</td>
<td>2</td>
<td>arsenate</td>
<td>B2</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>WBG8287</td>
<td>2</td>
<td>fusidic acid</td>
<td>B1</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>WBG8378</td>
<td>2</td>
<td>fusidic acid</td>
<td>B1</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>WBG9093</td>
<td>2</td>
<td>fusidic acid</td>
<td>B3</td>
<td>1</td>
<td>B</td>
</tr>
</tbody>
</table>

Plasmid sizes, when given, are in kilobases. Abbreviations: As, arsenate resistance; Bla, Bla production; C, cadmium resistance; Ci, ciprofloxacin resistance; EI, inducible erythromycin resistance; Eb, ethidium bromide resistance; F, fusidic acid resistance; Hg, mercury resistance; ND, not determined; T, tetracycline resistance.
is the case for Australian CMRSA. These results also demonstrate that there is a close correlation between CHEF pattern type, CC type, and spa type and that, generally, CMRSA types are confined to particular geographic areas. WA CMRSA strains, however, are of five distinct types. Four of these, CC1, CC5, CC45, and CC8, belong to four distantly related pandemic genetic lineages (12, 31), and one, CC298, belongs to a smaller clone that includes isolates from Australia, Portugal, and Japan (determined at http://www.mlst.net). CMRSA strains belonging to CC1 and CC298 were found in SA and the neighboring states of WA and VIC. Likewise, CMRSA belonging to CC30 were found in the neighboring eastern states of NSW and VIC. CMRSA isolates belonging to CC5 were found in WA and VIC.

The CC1 strains have an MLST allelic profile and a spa type that are the same as the S. aureus that is the proposed ancestor of MW2, the CMRSA that was responsible for the deaths of four children in the United States (5, 13, 41). Furthermore, as well as being found in the United States (31), CC1 CMRSA strains have been reported in France (57) and Australia (31), indicating that this is a particularly successful clone.

Despite the diversity of CCs, the CMRSA strains were remarkably uniform in their SCCmec. Of the vast majority of CMRSA strains studied thus far, most harbor the Type IVa SCCmec (31), suggesting that, in the Australian community environment, this type is the most-transmissible and best-adapted type of SCCmec. The CC45 isolate WBG8404 and the CC30 isolate WBG10198 had been previously reported to harbor classes Bl and E mec complexes, respectively (21). The present study has now found that they harbor the type 5 cer gene which has also been described in CMRSA isolates (19). The vastly different structures of the WBG8404 and WBG10198 SCCmec would suggest that they have been acquired from different sources on different occasions. However, how, and from where, the SCCmec has been acquired is yet to be determined.

The plasmid content of the CMRSA in CC1 further supports the similarity of the isolates in this CC and suggests that the same strain is found in WA, SA, and VIC, although the state from which it originated cannot be ascertained from these results. Likewise, the plasmid content of the strains in CC298 also indicates that this strain is found in WA and SA. The isolates in CC1, CC298 and the ST258 isolate, WBG9093, contain the same plasmids, suggesting that they have possibly been acquired from the same source. It is interesting that the NSW strain WSPP-1b has the plasmid content of the NZ strain WSPP-2 but the CHEF pattern of WSPP-1a, whereas the VISA isolate in this CC has a different spa type, different plasmids and a different SCCmec, suggesting that it may have originated independently of the other strains.

The present study has provided some insight into how CMRSA strains have acquired additional resistances. Inducible erythromycin resistance was always accompanied by a 2-kb plasmid (Table 1) that appears to be the same as the 2-kb inducible erythromycin resistance plasmid that was cured from WBG8287 and WBG8873 (Table 2). In two cases, WBG8873 and WBG7583 tetracycline resistance is chromosomal. However, it is not known at this stage whether this is due to the integration of a pT181 type plasmid into SCCmec, as has occurred in multiresistant MRSA (18). In the case of WSPP-1a it appears that tetracycline resistance is due to the acquisition of

<table>
<thead>
<tr>
<th>Strain</th>
<th>CC Plasmid size (kb)</th>
<th>Phenotype</th>
<th>EccRI fragment size(s) (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBG8287</td>
<td>1 19.6 Bla, C</td>
<td>14.6, 4.0</td>
<td></td>
</tr>
<tr>
<td>WBG8873</td>
<td>1 19.6 Bla, C</td>
<td>14.6, 4.0</td>
<td></td>
</tr>
<tr>
<td>WBG10200</td>
<td>1 19.6 NA</td>
<td>14.6, 4.0</td>
<td></td>
</tr>
<tr>
<td>WBG9093</td>
<td>75 19.6 Bla, C</td>
<td>14.6, 4.0</td>
<td></td>
</tr>
<tr>
<td>WBG7583</td>
<td>8 28.6 Bla, C</td>
<td>11.5, 10.3, 2.6, 2.3, 1.9</td>
<td></td>
</tr>
<tr>
<td>WBG8320</td>
<td>8 32.1 Bla, C, T</td>
<td>14.3, 11.0, 2.6, 2.3, 1.9</td>
<td></td>
</tr>
<tr>
<td>WBG7569</td>
<td>8 41.4 Bla, C, T, Mup, Tp</td>
<td>14.3, 11.5, 3.7, 3.0, 2.6, 2.3, 2.1, 1.9</td>
<td></td>
</tr>
<tr>
<td>WBG10198</td>
<td>30 36 NA</td>
<td>20.0, 11.0, 3.2, 1.2, 0.6</td>
<td></td>
</tr>
<tr>
<td>WBG10201</td>
<td>30 32.0 NA</td>
<td>19, 13.0</td>
<td></td>
</tr>
<tr>
<td>WSSP-1b</td>
<td>30 25.0 NA</td>
<td>17.3, 5.0, 2.7</td>
<td></td>
</tr>
<tr>
<td>WSSP-2</td>
<td>30 25.0 NA</td>
<td>17.3, 5.0, 2.7</td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: As, arsenate resistance; Bla, production; C, cadmium resistance; E, erythromycin resistance; Mup, mupirocin resistance; NA, not applicable; ND, not determined; Tp, trimethoprim resistance.

DISCUSSION

It has been reported that CMRSA from different parts of the world have different genetic backgrounds (31). The results
a 4.4-kb plasmid of the pT181 type. Analysis of the Bla plasmids in WBG7583, WBG8320, and WBG7569 (Table 2) has demonstrated that the 28.6-kb Bla, cadmium resistance plasmid can acquire tetracycline resistance and also muiprocin and trimethoprim resistance. It is known that the acquisition of tetracycline resistance is due to integration of a pT181-type plasmid, trimethoprim resistance is due to the acquisition of Tn4003, and muiprocin resistance is due to the acquisition of 


The SA isolate, WBG8897, is particularly interesting. It was regarded as a CMRSA, and yet it has the same ST as the English epidemic strain, UK EMRSA-15 (12), and has a CHEF pattern that is 92% similar to that of UK EMRSA-15 (21). Like UK EMRSA-15, it is resistant to ciprofloxacin (30). UK EMRSA-15 is known to be present in hospitals in Austr-

alia (34), and these results suggest that it is now in the commu-

nity in SA.

All of the isolates produced Bla and were resistant to cad-

mium. In every case, except for WBG8897, which is probably UK EMRSA-15, these were plasmid encoded. It is not known why isolates should be resistant to cadmium, but there is a good rationale for why they produce Bla, even though they do not require it for penicillin resistance, since mecA mediates resistance to all β-lactam antibiotics. All of the CMRSA strains studied harbored mec complexes that have their regulatory genes disrupted (23), leaving mecA unregulated. The Bla regu-

lators blaR1 and blaI are better regulators of mecA than mecR1 and mecI, and it is possible that they are regulating the expression of mecA in the CMRSA (40). Also, it has been suggested that the blaI determinant may stabilize mecA in S. aureus genetic backgrounds (20).

It has been suggested that the PVL genes may be a good marker for detecting CMRSA (57), but its absence in all but one CC of the Australian CMRSA isolates investigated sug-

gests that it would not be useful for this purpose in Australia. PVL has been associated with virulence (57), but there was no correlation between virulence and possession of the determin-

ant in the present study. Three of the WA CMRSA strains were carriage isolates, and all of the other isolates, including a hospital outbreak index strain, WBG8287 (29), were from pa-

tients with infections.

The present study emphasizes the diversity of CMRSA found in Australia and the importance of typing in tracing the origin of isolates and in designing antibiotic policies for their control in the community.

**ACKNOWLEDGMENTS**

This study was funded in part by grants from the NHMRC of Australia and the Department of Health in WA.

We thank Bart Currie, Helen Heffernan, P. C. Lee, I. Gosbell, and S. Graves for providing isolates.

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