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Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates from regional hospitals in Trinidad and Tobago☆

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Summary
Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA), first reported in a British hospital in the early 1960s, has now reached global proportions. Geographic spread of one or several MRSA clones in a city, country, and even among countries and continents has been identified by molecular techniques. We sought to determine whether clonal spread of MRSA has occurred in Trinidad and Tobago from all MRSA isolates collected between 2000 and 2001.
Methods: Clinical isolates of MRSA from three major hospitals in Trinidad and Tobago were identified by standard laboratory methods and analyzed using multiplex polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE) after *Sma*I digestion.
Results: There was a 12.8% prevalence of MRSA in three major regional hospitals in Trinidad and Tobago. All 60 randomly selected MRSA strains from these hospitals produced similar PFGE banding patterns, suggesting a genetic relatedness among strains and that they belonged to a single clonal family. All isolates were negative for the Panton—Valentine leukocidin gene (*pvl*). These strains shared a PFGE banding pattern approximately (96%) the same as a Canadian strain called CMRSA-6 in the Canadian National Microbiology Laboratory database.
Conclusions: We conclude that only one major PFGE genotype of MRSA clone is circulating among the three major regional hospitals in Trinidad and Tobago suggesting one of three possible scenarios of microevolution: (1) all were from the dissemination of a single epidemic MRSA clone prevailing in these hospitals in Trinidad and Tobago; or (2) MRSA in Trinidad and Tobago is evolving

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more slowly than in other countries; or (3) that if other MRSA clones have been present in Trinidad and Tobago, they have not persisted.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasing problem in healthcare units and communities around the world since it was first reported among hospitalized patients in a British hospital in the early 1960s.[1,2] Several reports of outbreaks and spread in hospitals, communities, nursing homes and long-term care institutions have been documented.[3–8] Epidemiological links of MRSA isolates occurring in different hospitals, countries, and regions have been demonstrated in several places.[9,10] Transmission of a clone may occur through transfer of patients or healthcare personnel between hospitals, and the elucidation of the mechanism of geographic spread of such clones has been greatly facilitated by molecular techniques.[11] Although the prevalence rate of MRSA in Trinidad and Tobago has been reported,[12] there are no molecular epidemiology reports available. A knowledge of the molecular epidemiology of MRSA is crucial for assessing and implementing infection control measures in any healthcare institution or country.[13]

This study was carried out to delineate and document the clonal relatedness of all MRSA clinical isolates encountered at three major regional hospitals in Trinidad and Tobago by pulsed-field gel electrophoresis (PFGE) testing.

Materials and methods

MRSA isolates used for this study were from routine clinical specimens submitted to the microbiology laboratories of three major regional hospitals (from the south, San Fernando General Hospital; from the northwest, Port of Spain General Hospital; and from north-central, Eric Williams Medical Sciences Complex) in Trinidad and Tobago from January 2000 to December 2001. These isolates were identified morphologically and biochemically by standard laboratory procedures.[14] No duplicate isolates from a single patient were included, and there was no history of MRSA outbreak during this period.

Isolates were identified by latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd, Dartford, UK), tube coagulase test (Becton, Dickinson & Co, Sparks, MD, USA), and detection of DNase (DNase agar; Oxoid Ltd, Basingstoke, Hampshire, UK). The methicillin-resistant *Staphylococcus aureus* screen latex agglutination test (Denka Seiken Co. Ltd, Tokyo, Japan) was performed according to the manufacturer's instructions. Pure isolates of MRSA were stored in brain heart infusion (BHI) broth with 20% glycerol at −70 °C until required for further analysis. All results were validated using American Type Culture Collection (ATCC) quality control strains: methicillin-resistant *S. aureus* ATCC 43300, methicillin-sensitive *S. aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228.

Antimicrobial agents and minimum inhibitory concentration (MIC) testing

Susceptibility tests were performed by the standard disk diffusion method according to the Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines.[15] The following antimicrobial agents were tested: ampicillin, amoxicillin/clavulanic acid, ceftriaxone, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, meropenem, oxacillin, penicillin, tetracycline, trimethoprim–sulfamethoxazole, rifampin, and vancomycin (Oxoid Ltd, Basingstoke, Hampshire, UK). Methicillin (oxacillin) MICs were determined with the E-test system (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions, with resistant isolates having a breakpoint of $\geq$2 mg/l.

Detection of *pvl*, *mecA* and *nuc* genes by multiplex PCR

The presence or absence of *pvl*, *mecA* gene and the presence of *nuc* gene were determined for all MRSA strains by multiplex PCR as described elsewhere.[16–18]

Restriction enzyme digestion and pulsed-field gel electrophoresis (PFGE)

PFGE was used as the genotyping method in this study and was performed as previously described with some modifications.[19] The pulsed-field gel electrophoresis was performed using a contour-clamped homogeneous electric field apparatus (CHEF DRIII, Bio-Rad Hercules, CA, USA). Gel images were captured on the Gel Doc imaging system using Quantity One software version 4.4.1 (Bio Rad Laboratories, Hercules CA, USA). The resulting band patterns were analyzed by visual inspection according to previously established criteria.[20,21] Gel analysis was done using Bionumerics version 3.5 (Applied Maths, Austin TX, USA), and cluster analysis was achieved using DICE and UPGMA.

Results

Of the 1912 *S. aureus* isolates received from the three major hospitals, 244 (12.8%) were MRSA. Most of these MRSA isolates (96.7%) were from hospitalized patients and thus represented healthcare-associated isolates; 86.9% of the isolates were recovered from wound swab specimens.

Half (50%) of the MRSA isolates were from the San Fernando General Hospital, 48% from Port of Spain General Hospital, and 2% from Eric Williams Medical Science Complex. The majority (62.3%) of the isolates were recovered from the surgical facility of each hospital, while the least (1.6%) were from the obstetrics and gynecology facility.

As previously reported, 14% of the MRSA isolates had oxacillin MIC values of >64 mg/l, while 86% had MIC values

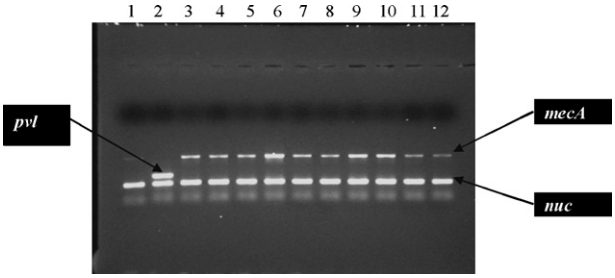

Figure 1 Agarose gel electrophoresis of PCR-amplified products of MRSA clinical isolates from Trinidad and Tobago, 2000–2001. Lane 1 is the ATCC 43300 (MRSA) *pvl* negative, *mecA* and *nuc* positive; lane 2 is the MSSA (ATCC 25923) *pvl* and *nuc* positive, *mecA* negative. Lanes 3–12 are representative of MRSA isolates from Trinidad and Tobago, with all MRSA *mecA* and *nuc* positive, *pvl* negative.

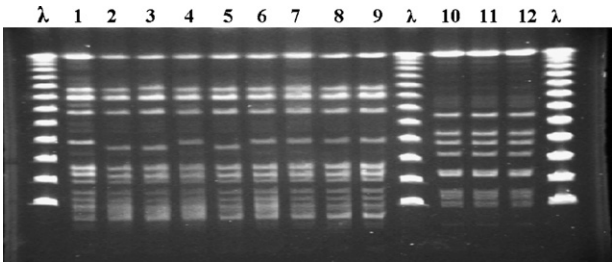

Figure 2 *Sma*1 PFGE profiles of MRSA isolates from three major regional hospitals in Trinidad and Tobago, 2000–2001. Lanes 1–9 are representative of MRSA isolates from Trinidad and Tobago regional hospitals; lanes 10–12 from St. Joseph's Hospital, Hamilton, Ontario, Canada. Lambda (λ) DNA PFGE molecular size marker is indicated in the respective lanes.

>256 mg/l, and the antimicrobial susceptibility testing revealed complete resistance to ceftriaxone, erythromycin and gentamicin.[12] All the MRSA isolates were susceptible to vancomycin, rifampin and chloramphenicol, and no antibiotic resistance pattern was found to be specific to a particular ward. Multiplex PCR analysis confirmed the presence of the genes encoding nuclease (*nuc*), and *mecA* in all 244 MRSA isolates and all strains were negative for *pvl* (Figure 1).

The clonal results of the MRSA isolates by PFGE genotyping were striking and showed a similar banding pattern among all the isolates from these hospitals in Trinidad and Tobago (Figure 2). This result supports our opinion that MRSA infections in this study were hospital-acquired, since over 96% of the isolates recovered were from patients who were in hospital, and the remaining isolates (~4%) came from outpatient clinics and could equally be said to be hospital-acquired as these patients had a past history of hospitalization.

Discussion

Molecular typing results from this study demonstrate that there was a single major PFGE genotype of MRSA clone prevailing in the three regional hospitals in Trinidad and Tobago. A similar finding of a single predominant MRSA clone prevailing in various large hospitals in a country has been documented.[22] A single MRSA clone still prevailing in the country means that there has not been any clonal evolution among the MRSA isolates. It is also possible that no new MRSA clones have been recently introduced to these hospitals or if they have been introduced, they have not persisted.

The relative genetic unity of MRSA isolates may be a result of a single introduction of MRSA into Trinidad hospitals or an acquisition of genetic elements such as phages, transposons, or plasmids that integrated into the staphylococcal chromosome as has been reported.[23] The island of Trinidad is 5128 sq km in size with an accessible road network and continuous movement of people and services. There is a continuous inter-referral of patients and movement of healthcare staff between hospitals in the country, and this could have facilitated the spread of a particular MRSA clone as noted elsewhere.[22] Reports from other areas have noted that in many hospitals, the lack of adequate efforts to prevent MRSA spread, lack of strict adherence to infection control precautions, and lack of an antibiotics use policy have all contributed to the spread of MRSA clones.[24,25] All these risk factors are prevalent in these major hospitals in Trinidad and Tobago.

A comparison of the MRSA isolates from these hospitals in Trinidad and Tobago with some strains from Canada revealed that they were approximately (96%) the same in PFGE banding pattern to a Canadian strain called CMRSA-6 in the Canadian National Microbiology Laboratory database. This strain was first identified in the western region of Canada in 1995 with one isolate, but has now increased to 1035 isolates to date. It comprises five PFGE macrorestriction patterns (CDN-type 0068, 0519, 0320, 0347, 0823) with 0068 being the predominant strain; but it does not have any other PFGE name related to those encountered in other countries. The majority of the CMRSA-6 isolates have been encountered in the western region where in 1999 most of the strains were from a single site. Infection associated with the CMRSA-6 isolates was negligible in 1995–1997, but increased to epidemic proportion in 1998 through 2000; in the past five years it has been on the decrease.

The CMRSA-6 strain, which does not have any other PFGE type identified in any other country, is closely related to the single clone seen in Trinidad. It is surprising to note that only one clonal MRSA is circulating in a country that is a major destination for tourists, visitors and returning residents from North America and Europe in the Caribbean. Each year several thousand people visit Trinidad. For example between January 2000 and December 2001 a total of 1 668 465 arrivals by both sea and air of people (visitors, students, returning residents, diplomats, in-transit passengers) to Trinidad and Tobago was recorded.[26] It is generally believed that migration and movement of people from one place to another, whether it is for tourism or other purposes, may have profound effects on the dissemination of microorganisms into different populations. An overall prevalence of MRSA in healthy individuals without known risk factors has previously been documented as 0.24% in San Francisco, 0.26% in healthy children and guardians in New York City, and 0.06% in the large sample of a Portuguese population.[27] With such a minimal prevalence rate from healthy individuals in the city, hospital or an area, the possibility of transfer to others could be low; and this may have been the case in Trinidad and Tobago.

The majority of the isolates were recovered from wound swab specimens of patients predominantly in the surgical

facility in all these major hospitals, and a similar situation has been noted in reports from other hospitals.[28] In the present study, none of the MRSA isolates were *pvl*-positive and similar results have been obtained in isolates from France with all healthcare-associated MRSA.[29] Several publications have strongly associated Panton—Valentine leukocidin (PVL) with community-associated MRSA.[18,30,31] The lack of *pvl* in our isolates supports our conclusion that the majority of the Trinidad and Tobago isolates are healthcare-associated MRSA since they were not obtained from patients (nasal samples) screened prior to their admission to hospital, community strains or countrywide populations.

Unfortunately, there is no national or hospital policy on the use of pre-operative prophylactic antimicrobial treatment or the empirical antimicrobial treatment of surgical site infections in these regional hospitals in Trinidad and Tobago. However, there are plans to formulate such a policy in one of the hospitals, and infection control nurses and an infection control committee are being set up or revived. Since a single clone is responsible for the MRSA in these regional hospitals in the country, there is a need to implement effective infection prevention and control measures that should include continuous staff education, contact isolation for MRSA-positive patients, an antibiotic use policy, and cohort nursing of MRSA-positive and exposed patients in epidemic situations, to limit further transmission.

Conclusions

It was determined by PFGE that only one clonally related MRSA is circulating among the three major regional hospitals in Trinidad and Tobago, suggesting that: (1) all these were from a single epidemic MRSA clone prevailing in these hospitals in Trinidad and Tobago; or (2) MRSA in Trinidad and Tobago is evolving more slowly than in other countries; or (3) no new MRSA clones have been recently introduced to these hospitals or if they have been introduced, they have not persisted. The MRSA strains and their variants from Trinidad and Tobago when compared with Canadian PFGE types revealed that they are genetically closely related, according to the interpretative criteria of Tenover et al.[20] (approximately 96% by RFLP banding pattern), to Canadian strain CMRSA-6 in the Canadian National Microbiology Laboratory database.

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Conflict of interest: No conflict of interest to declare.

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