



Detection of Oxacillin (Methicillin)-resistant *Staphylococcus aureus* Isolated from a Tertiary-care Hospital, Georgetown, Guyana

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Authors' contributions

This work was carried out in collaboration among all authors. Author PC designed the study, provided the study protocol and wrote the final draft of the manuscript. Authors DS and TR managed the literature searches, conducted the laboratory testing, performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a major problem globally. Previous data had suggested that the prevalence of MRSA infections in the tertiary hospital setting was 51%. The aim of this study was to conduct a point prevalence survey of MRSA infections occurring at a tertiary-care hospital in Georgetown, Guyana, and to determine to what extent methicillin-resistance was occurring among *Staphylococcus aureus* isolates utilising the minimum inhibitory concentration (MIC) data.

Study Design: This study was based on a prospective, analytical design.

Place and Duration of Study: Microbiology department, Georgetown Public Hospital Corporation (GPHC), and Department of Medical Technology, University of Guyana, between May 2019 and July 2019.

Methodology: A total of 101 consecutive, non-repetitive, laboratory-identified MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates were tested using an oxacillin broth microdilution method.

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Results: We found that 65.4% of *Staphylococcus aureus* were oxacillin (methicillin) resistant with a majority of the isolates being high level oxacillin resistant strains (i.e., MICs > 256 µg/ml) (84.85%). Most of the resistant isolates were collected from patients admitted to medical and surgical wards. **Conclusion:** Methicillin-resistance continues to be a major problem in the hospital setting and conventional techniques are unlikely to identify all of the potentially resistant isolates.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MRSA; MSSA; broth microdilution; Guyana.

1. INTRODUCTION

Staphylococcus aureus is a leading cause of hospital and community-associated infections. In the hospital, *S. aureus* is the most frequent cause of surgical site (skin and soft tissue infection), lower respiratory tract and cardiovascular infections (endocarditis). Furthermore, it is the second most common cause of healthcare-associated pneumonia and bloodstream infections worldwide [1,2]. The mortality rate of *S. aureus* bacteraemia remains approximately 20 - 40% despite the availability of antimicrobials [3].

Historically, β -lactam antibiotics have exhibited potent activity against *S. aureus*, which along with their good safety profiles has traditionally made these agents the first choice for the treatment of staphylococcal infections [4,5]. Since the first reported cases of penicillin resistance one year after this drug was first used to treat staphylococcal infections, there has been a growing appreciation that penicillin is no longer the miracle drug that it once appeared to be [6]. Methicillin, introduced in 1959, was thought of as the drug that would provide a means of overcoming the spread of penicillin resistance, but two years after its introduction, the first methicillin-resistant *S. aureus* (MRSA) was reported. The cause, the expression of an additional penicillin-binding protein (PBP2a), expressed as a consequence of the presence of the *mecA* gene proposed to originate from *Staphylococcus sciuriform* which was resistant to the action of the antibiotic [7]. Recently, an additional *mec* gene, *mecC* which encodes penicillin-binding protein 2c (PBP2c), has emerged as a major contributor to the increasingly documented cases of MRSA in the hospital setting [2].

The growing prevalence of MRSA in both hospital and community-associated infections continues to be a growing concern [4,5]. High rates of isolation (>50%) have been reported in Asia, North American Europe, and Latin America and the Caribbean [8,9].

Dilution methods are used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing [10]. Drug MIC measurements are used as a quantitative measure of drug effectiveness and is an essential tool for resistance surveillance, epidemiological studies of susceptibility and in comparisons of new and existing antimicrobial agents. Additionally, the MICs can be used as a guide for the clinician to the susceptibility of the organism to the antimicrobial agent and aids treatment decisions [10]. However, MIC-type methods are not currently part of the test repertoire of the microbiology department at the GPHC, which limits the interpretation of susceptibility results to simply "resistant" or "susceptible". These results do not indicate the exact concentration of the antimicrobial agent that inhibits the growth of the microorganism. This research aimed to determine the minimum inhibitory concentrations (MICs) of clinical isolates of methicillin-susceptible and methicillin-resistant *S. aureus* at a tertiary-care hospital in Georgetown, Guyana using the broth microdilution method.

2. MATERIALS AND METHODS

2.1 Study Setting

The study was carried out at the Georgetown Public Hospital Corporation, the only public tertiary-care and referral healthcare facility in Guyana, located in the capital city Georgetown. The hospital is a 600-bed facility that serves to provide a wide range of specialized services. The clinical isolates analysed in the study were obtained from the hospital's microbiology laboratory which provides diagnostic services for a number of critical care units as well as a number of inpatient wards. The study was conducted during a 6-week period from May to July 2019.

2.2 Study Design

Clinical Isolates: This study was based on a prospective, analytical design. A total of 101

consecutive, non-repetitive, laboratory-identified isolates of *S. aureus* were obtained from the hospital. Only one isolate per patient was collected in order to prevent the over expression of a particular isolate. Antibiotic susceptibility tests were initially conducted by the microbiology department using the disk diffusion methodology and interpreted in accordance with the Clinical Laboratory and Standards Institute (CLSI) M100-S28 guidelines [11]. Demographic details of patients were not included in this study.

Broth microdilution testing: Antibiotic susceptibility testing using the broth microdilution method was then performed to determine the MICs of those isolates that were previously identified as either methicillin-susceptible or methicillin-resistant *Staphylococcus aureus*. Briefly, the microdilution trays containing oxacillin (Acros Organics, Geel, Belgium) (range 2 to 256 µg/mL) were prepared in accordance with the CLSI guidelines [11,12]. These trays were prepared using cation-adjusted Mueller-Hinton Broth (BD BBL, New Jersey, U.S.A).

Preparation of inocula: Suspensions of the overnight growth of the test organisms, equivalent to a 0.5 McFarland turbidity standard, were prepared in Mueller-Hinton broth. Each well of the microdilution trays was then inoculated with 10 µL of the suspension and incubated at 35 ±2°C for 16-18 hours.

Interpretation of results: Resazurin dye was added to the microdilution trays to aid in the determination of the MICs. Non-fluorescent resazurin (blue) is reduced by active bacterial cells to fluorescent resorufin (pink) [13]. The dye was prepared as previously described by Forester et al. [14]. After incubation, 50 µL of the dye was added to each well and the plates were then incubated for 2 hours at 35±2°C. On completion of incubation, wells with no colour change (blue resazurin colour did not change) were determined to be above the MIC [13]. The MIC for the different test organisms was then interpreted using the CLSI guidelines [11].

2.3 Statistics

Data were analysed using SPSS ver. 20. Descriptive data were presented using graphs. Chi-square test was used to analyse the association between the cefoxitin disk diffusion results and the oxacillin broth microdilution results. The statistical significance was determined using a p value < 0.05.

3. RESULTS

Out of the 101 *S. aureus* isolates processed during the study, 57 (56.44%) and 44 (43.56%) were identified by the hospital microbiology laboratory as being MRSA and MSSA, respectively, by disk diffusion testing using cefoxitin.

All of these isolates were re-tested using an oxacillin broth microdilution method in order to determine their minimum inhibitory concentration (MIC) for this drug, which is similar in activity to cefoxitin and methicillin. MIC-based methods such as the one employed for this study are not currently available in this hospital setting.

All of the 57 laboratory-identified methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were found to be oxacillin resistant by the broth microdilution method with MIC cut-off value of 4 µg/ml. The MIC's for these isolates were ≥256 µg/ml.

Out of the 44 laboratory-identified methicillin-susceptible *Staphylococcus aureus* (MSSA), 35 (79.55%) were oxacillin susceptible with MIC's. There were 9 (20.45%) isolates that were originally identified as susceptible by disk diffusion that exhibited minimum inhibitory concentrations consistent with oxacillin resistance.

The MRSA isolates were recovered from patients in a number of wards across the hospital (Figs. 1 and 2).

Statistically, there was a significant association ($p < 0.001$) between the cefoxitin disk diffusion results and the oxacillin broth microdilution results, which seemed to relate to the congruence between the results obtained from these two methods.

4. DISCUSSION

Staphylococcus aureus is one of the most important human pathogens implicated in nosocomial infections and is responsible for a significant amount of morbidity and mortality worldwide.

In our study, the prevalence of methicillin-resistant *S. aureus* at this tertiary-care hospital during the study period was 65.35%. The prevalence seen in this study was much higher than that of a previous study done by Dozois et

al. [15], who found that the prevalence in 2015 was 51%. Our findings suggest that the prevalence is also higher than in other countries in this region, such as Peru (62%) [16], Brazil (62%) [17], Venezuela (57%) [17], St. Kitts and Nevis (45%) [18] and Trinidad (39.5%) [19]. While the difference in rates of isolation of MRSA in different studies might be due to the differences in locations and time periods of the studies, differences in hygienic conditions maintained in different hospitals, the existence of infection control programmes, and rational use of antibiotics [20], the increasing prevalence observed is alarming. It seems likely that a selection pressure and patient to patient spread via health care workers may be playing a role in the dissemination of resistant clones, but this is still to be investigated. This may be especially true for patients that are transferred to referral centres like this hospital, since research has shown that these patients often receive antibiotics prior to admission, thereby, increasing the likelihood of selective pressure [21,22].

We found a statistically significant relationship between the cefoxitin disk diffusion results and the oxacillin broth microdilution results ($p < 0.001$). Of the 101 *S. aureus* isolates obtained, 57 (56.44%) were identified as resistant using both the cefoxitin disk diffusion method and oxacillin-resistant by the broth microdilution

method. While the agreement between the two testing methodologies was not surprising, what was troubling, however, was that the disk diffusion method misidentified nine isolates (20.45%) as methicillin-susceptible. These isolates were re-categorised as MRSA based on their oxacillin resistant results and CLSI recommendations [11]. This discrepancy in the results may be related to the high sensitivity and specificity of the broth microdilution method as compared to cefoxitin disk diffusion method for the detection of *mecA*-mediated resistance. In the study of Farahani et al. [23], the sensitivity and specificity of the broth microdilution method was 100% and 98.9% respectively while the cefoxitin disk test demonstrated a sensitivity of 98.8% and specificity of 94.7% for MRSA detection. Furthermore, *mecA* homologues, such as *mecC*, pose significant diagnostic problems with the potential to be misdiagnosed as methicillin-sensitive *S. aureus* [24].

The minimum inhibitory concentrations (MIC's) for the 66 (65.35 %) oxacillin-resistant (MRSA) isolates ranged from 8 µg/ml to ≥ 256 µg/ml. There were 60 MRSA isolates with high level oxacillin resistance (MICs >256 µg/ml). It is plausible that these observable variations in the oxacillin MIC's may have been due to *mecA* promoter mutations, which play an important role in determining the level of oxacillin resistance. For example, a study done by Chen et al. [25]

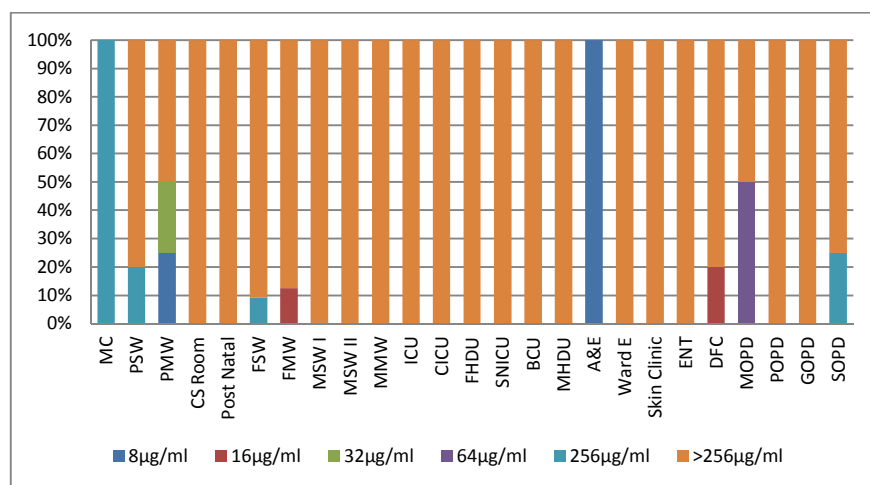


Fig. 1. Showing the distribution of the MICs for the resistant isolates per ward

*Abbreviations: medical clinic (MC), paediatric surgical ward (PSW), paediatric medical ward (PMW), C-section room (CS-room), post-natal ward (post-natal), female surgical ward (FSW), female medical ward (FMW), male surgical ward I (MSW I), male surgical ward II (MSW II), male medical ward (MMW), intensive care unit (ICU), cardiac intensive care unit (CICU), female high dependency unit (FHDU), step-down neonatal intensive care unit (SNICU), burn care unit (BCU), male high dependency unit (MHDU), accident and emergency (A&E), ear, nose and throat clinic (ENT), diabetic foot clinic (DFC), medical outpatient department (MOPD), paediatric outpatient department (POPD), gynaecological outpatient department (GOPD), surgical outpatient department (SOPD)

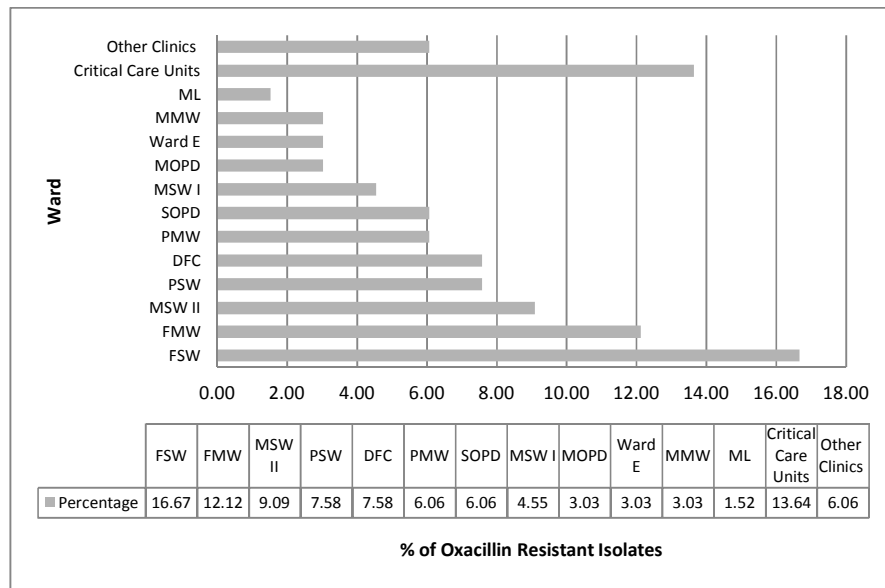


Fig. 2. Showing the percentage of the oxacillin-resistant isolates recovered from each ward

found that the *mecA* promoter mutation G-25A is associated with a high oxacillin MIC (256 µg/ml); G-7T conferred a moderate oxacillin MIC (32 to 64 µg/ml); and strains with the C-33T mutation showed a low oxacillin MIC (4 to 8 µg/ml).

Furthermore, given that so many isolates expressing high level oxacillin resistance had the same MIC values; it is worth considering that these isolates may have been clonally related. According to Alvarez et al. [26], dissemination of *S. aureus* or its antibiotic-resistant forms in hospitals is most often clonal, that is, very few clones are circulating, commonly, in each hospital at the same time.

In our study, most of the resistant *S. aureus* were collected from patients admitted to the female surgical ward (FSW) (16.67%), paediatric wards (paediatric surgical ward [PSW] and paediatric medical ward [PMW]) (13.65%), male surgical ward (MSW) (13.64%), and female medical ward (FMW) (12.12%). Overall, the surgical wards represented the predominant source for MRSA at the hospital. A study by Srinivasa et al. [27] also found that higher numbers of MRSA could be observed from patients in surgical units when compared to those in medical units. This may be related to the increased length of stay in the hospital for patients that tend to be in these wards, which is associated with an increased likelihood of MRSA exposure and infection [27]. Additionally, given that the female wards (FSW & FMW), male wards (MSW I & II), and paediatric wards (PSW & PMW) are located in close

proximity to each other, it seems likely that there may have been cross contamination of these wards with *S. aureus*.

The high prevalence of MRSA among patients at the hospital along with the high oxacillin MIC values observed for these isolates is quite alarming and suggests that more attention should be given to infection control and surveillance programs within the hospital setting. Strategies such as contact precautions [28,29], use of appropriate hand hygiene practices [30] and strict disinfection of furniture and health care equipment [28] have been shown to be effective at limiting MRSA transmission. It is worth noting that in the critical care units (e.g., the intensive care unit (ICU), burn care unit (BCU), and the neonatal intensive care unit (NICU)), the rate of MRSA was lower than all of the other departments. A single isolate was identified in each of the critical care units which displayed an MIC >256 µg/ml. This low frequency of isolation may be due solely, or in part, to existing infection control measures and practices that were implemented for these units. This finding further highlights that implementation of infection control measures can help minimize the spread of MRSA and reduce carriage and infection rates in hospitals where MRSA is endemic [26].

5. CONCLUSION

In concluding, it seems obvious that MRSA continues to be a major problem in our hospital setting. It is equally obvious that more needs to

be done to monitor resistance trends and identify resistance as it is occurring with the aid of testing methodologies that provide information on organism MIC's and their genotypic resistance profiles. Further, there needs to be greater emphasis on compliance with infection control measures (both hygiene-based and decolonisation-based) in order to ensure positive clinical outcomes for key high-risk patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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