Evaluation of Methods and Costs for Detecting Methicillin-resistant *Staphylococcus aureus* isolates from Clinical Specimens at Regional Hospitals in Trinidad and Tobago

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**ABSTRACT**

**Objectives:** To evaluate and determine the most cost effective, rapid and specific method for detection of methicillin resistance in clinical isolates of *S aureus* in a setting with limited personnel and resources.

**Methods:** Standard laboratory methods were used to identify *S aureus* isolates. The conventional Methicillin Resistance *Staphylococcus aureus* (MRSA) detection methods used included, 1 µg oxacillin disk diffusion, oxacillin salt agar screen (CLSI), penicillin binding protein (PBP 2) latex agglutination test and E-tests oxacillin. Results of conventional tests were compared with a polymerase chain reaction (PCR) method for detecting MRSA isolates. Polymerase chain reaction detection of the meca gene in *S aureus* was used as the “gold standard” for MRSA identification.

**Results:** All methods had 100% sensitivity except for oxacillin disk diffusion and oxacillin-salt agar screening with 98% and 99%, respectively. Specificity was also 100% for all methods except for oxacillin-disk diffusion (99%). Turn around time (TAT) for detection of MRSA was calculated to be within six hours for PCR. The fastest TAT of 1.25 hours was obtained for PBP 2 latex agglutination. Total cost for labour and materials to perform each method was highest for E-test, US$13.76/isolate. The cost for PCR when compared to that of latex agglutination was not statistically significant (US$3.74 vs US$5.91, p = 0.4).

**Conclusions:** All methods presented high sensitivity and specificity, but the latex agglutination test had the advantage of giving a reliable, rapid and most cost effective result that compares well to PCR in this environment.

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Evaluación de Métodos y Costos para la Detección de Aislados de *Staphylococcus aureus* Resistentes a la Meticilina a Partir de Especímenes Clínicos en los Hospitales Regionales de Trinidad y Tobago

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**RESUMEN**

**Objetivos:** Evaluar y determinar el método más específico, rápido y costo-efectivo para la detección de la resistencia a la meticilina en aislados clínicos de *S aureus* en un lugar con personal y recursos limitados.

**Métodos:** A fin de identificar los aislados de *S aureus*, se utilizaron métodos estándar de laboratorio. Los métodos convencionales de detección de SARM usados incluyeron difusión por disco de oxacilina de 1 µg, prueba de tamizaje (“screening”) de oxacilina en agar-sal (CLSI), test de aglutinación en látex para la detección de la proteína 2 fijadora de la penicilina (PBP 2), y la prueba E-Test de oxacilina. Los resultados de las pruebas convencionales fueron comparados con un método de PCR para la detección de aislados SARM. La detección por PCR del gene meca en *S aureus* fue usada como “estándar de oro” para la identificación de SARM.
INTRODUCTION
Accurate and timely detection of methicillin resistant *S. aureus* (MRSA) in the clinical laboratory is paramount for early administration of appropriate antimicrobial therapy to reduce cost and length of hospital stay, to institute timely and appropriate infection control measures that prevent transmission and further spread of MRSA strains (1–2).

The present study was undertaken to evaluate and compare the efficiency of conventional and molecular methods for detection of methicillin resistance in clinical isolates of *S. aureus* and to determine the most cost effective, rapid, specific and reliable method to use in a developing country.

MATERIAL AND METHODS

**Bacterial isolates**
A group of 100 (20 methicillin susceptible and 80 methicillin resistant) isolates of *Staphylococcus aureus* cultured from clinical specimens received at three regional hospitals in Trinidad and Tobago were used for this study. *Staphylococcus aureus* isolates previously identified and stored at -70°C were sub-cultured on sheep blood agar plates and incubated overnight at 35°C prior to further testing.

**Conventional detection methods**

**Oxacillin-salt agar and Oxacillin disk diffusion tests**
The oxacillin (1 µg) disk diffusion and oxacillin salt agar screening tests were carried out on the isolates according to the recommendations of the Clinical and Laboratory Standards Institute, CLSI (formerly National Committee for Clinical Laboratory Standards, NCCLS) (3).

**MRSA-Screen latex agglutination test**
The MRSA-Screen latex agglutination test which uses a monoclonal antibody to detect penicillin-binding protein 2 (PBP 2) in the bacterial isolate was performed according to the instructions of the manufacturer (Denka Seiken Co Ltd, Tokyo, Japan).

**E-test**
Oxacillin minimal inhibitory concentrations (MICs) were determined with the E-test system (AB Biodisk, Solna, Sweden) according to the manufacturer’s instructions. Briefly, Mueller Hinton agar plate supplemented with 2% NaCl was used. An inoculum of the *S. aureus* isolates was applied and an oxacillin (Ox) E-test strip having a gradient range of 0.016 – 256 µg/mL was placed on the plate. After 24 hours incubation at 35°C in an ambient air, the plates were read using a transmitted light source. Isolates having an MIC of $\geq 2$ µg/mL were regarded as resistant as previously reported (4).

**Molecular detection methods**

*Detection of mecA and nuc genes by multiplex PCR*
The presence or absence of mecA gene and the presence of *nuc* gene were determined for all staphylococcal isolates by multiplex PCR as previously described (5, 6). Pure yellow colonies obtained from a fresh subculture on Mannitol Salt 0–6 with 6 mg/L oxacillin were used as the DNA template for PCR amplification. Two in frame fragments 850 bp and 270 bp corresponding to the mecA and *nuc* genes of MRSA respectively were co-amplified. After re-optimizing the conditions, a reaction mix was produced. The mix comprised the followings: 2.5 mM MgCl₂, 1X PCR reaction buffer, 250 µM each of dATP, dCTP, dGTP and 150 µM dTTP (Boehringer Mannheim, Laval, Quebec, Canada); 2.5 pmol each of mecA primers M1 TGG CTA TCG TGT CAC AAT CG-3’; M2 AGT TCT GCA CTA CCG GAT TTG C-3’; 2.5 pmol each of *nuc* primers – N1 GCG ATT GAT GGT CTT GAT CCG TTG AGT TTA TTA ACG N2 AGC CAA GCC TTG ACG AAG TAA ACG (Mobix, McMaster University, Ontario, Canada); 1.25U of AmpliTaq DNA polymerase (Roche Molecular Systems, Branchburg, NJ USA) and 0.625U of Uracil-DNA-glycosylase (UNG) (Boehringer Mannheim) were prepared. Master mixes were dispensed into PCR tubes in 23 µl aliquots after adding enzymes. Test organisms were suspended in 0.45% saline to a density of 60–70% as measured in a Vitek colorimeter and 2µl of each cell suspension was added to a reaction mix aliquot. PCR was performed in Gene Amp PCR system 9700 (PE Applied Bio systems, Mississauga, Ontario, Canada) thermal cycler using the following programme: initial denaturation for 5 minutes at 94°C followed by 35 cycles at 94°C for 15 seconds, 55°C for 15
seconds and 72°C for 30 seconds with a final extension for 5 minutes at 72°C.

Following amplification, 15 µl of the sample was electrophoresed on 1% agarose gels (Promega Corporation, Madison, WI, USA) containing 0.5 mg/l ethidium bromide to detect PCR products. Gel images were photo documented using a Gel Doc 1000 (Bio-Rad Laboratories Ltd, Mississauga, Ontario, Canada) for interpretation.

All PCR runs were controlled using American Type Culture Collection (ATCC) quality control strains: MRSA ATCC 43300, S aureus ATCC 25923 and S epidermidis ATCC 12228. All results were satisfactory.

Definitions

Turn Around Time
The turn around time (TAT) was taken as the time to detect methicillin resistance in the S aureus isolate. The TAT excluded the time for the primary growth and identification of the S aureus isolate, which was approximately the same time (18.5 hours) for all methods.

Cost for the tests
Cost per test method was calculated by determining the cost of materials and labour. This included the cost of each individual item (reagents and expendables) used to perform the test, and labour. The cost for labour was calculated based on the basic monthly salary of the technician time and hands-on duty that was approximately US$5.65/hour ($35.00/hour in Trinidad and Tobago dollars). The cost did not include the capital costs of major equipment such as the thermocycler, gel electrophoresis and gel imaging equipment for PCR and incubators.

The data were analyzed using the Epi Info 3.2 software. Chi-square test was used where indicated to compare data (7).

RESULTS
The results obtained with the conventional – PBP 2 latex agglutination, E-test, oxacillin disk diffusion method, oxacillin-salt agar screening test and the molecular (PCR) detection methods for MRSA revealed all test methods had 100% sensitivity except for oxacillin disk diffusion and oxacillin-salt agar screening methods that had 98% and 99%, respectively. The specificity result for all methods was also 100% except for the oxacillin disk diffusion method that was 99%.

The costs of each test method and the turn around time for a result are summarized in the Table below which reveals that the TAT for detection of methicillin resistance in a S aureus isolate was 6 hours for PCR and 18 hours for E-test and oxacillin salt plate methods, respectively. The fastest TAT was recorded for PBP 2 latex agglutination method as 1.25 hours. The Disk diffusion (modified Kirby Bauer) method had a TAT of 22 hours. The TAT for PCR when compared to that of PBP 2 latex agglutination was not statistically significant (6 hours versus 1.25 hours, p = 0.1).

The results also revealed that the total costs for labour and materials to carry out the different detection tests were highest for E-test with US$13.76 per single isolate. This was followed by oxacillin-salt agar (US$10.34) latex agglutination (US$5.91), oxacillin disk diffusion (US$4.90) and least for PCR test (US$3.74). The cost for PCR when compared to that of latex agglutination was not statistically significant (US$3.74 versus US$5.91, p = 0.4).

DISCUSSION
Detection of the mecA gene by polymerase chain reaction was considered the gold standard and the performance of the different conventional methods was compared to it. This study showed that MRSA screening using PBP 2 latex agglutination was the most rapid conventional test. Results were available in a similar TAT to PCR, with 100% specificity compared to PCR similar to the experience of other investigators (8). Turn around time in our hands appeared better than another MRSA identification assay (Velogene) that was also rapid and easy to perform, providing results in approximately 90 minutes as was noted with other workers.

<table>
<thead>
<tr>
<th>Method</th>
<th>Unit cost in US$</th>
<th>Total cost in US$ (TT$)</th>
<th>TAT hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Materials</td>
<td>Labour</td>
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<tr>
<td>PCR (multiplex)</td>
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<td>1.73</td>
<td>3.74</td>
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<td>Oxacillin disk diffusion Test</td>
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<td>E-Test</td>
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<td>MRSA Screen latex test</td>
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<td>1.05</td>
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<td>37.23</td>
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<tr>
<td>Oxacillin-salt Agar plate</td>
<td>6.31</td>
<td>4.03</td>
<td>10.34</td>
</tr>
</tbody>
</table>

TAT = turn around time. 1US$ = 6.3$ TT (Trinidad & Tobago dollar)
Cost per test was calculated as funds required completing 100 S aureus isolates (80 MRSA, 20 MSSA). Labour cost was calculated based on technician hands-on time, US$5.65 / hour (TT$35.00 / hour).
(9). However, it is significantly more expensive than the PCR method in the present study.

While the MRSA-PBP 2 latex agglutination test was easy to perform and gave excellent sensitive and specificity in results with limited requirements for special equipment, it may not be easily incorporated in a clinical diagnostic laboratory because of its cost. Spending approximately US$6 for a single test would have a huge impact on the limited laboratory resources in a developing country (clinical benefit may outweigh this cost as has been observed in other studies (1, 2).

The E-test is an acceptable system for detecting oxacillin resistance in \textit{S. aureus} isolates as shown by the results, of 100% sensitivity and specificity to the PCR method. Similar results have also been observed elsewhere making E-test a reliable alternative to the conventional agar or broth dilution methods (9, 10). The E-test however is expensive to use as a routine test in a laboratory setting like ours. E-test would cost US$13.76 each to perform and has a lengthy TAT compared to PCR and PBP 2 latex testing.

In this study, the presence of the \textit{mecA} gene was determined by the polymerase chain reaction (PCR) technology. Other investigators have shown that the presence of the \textit{mecA} gene correlates 100% with the detection of methicillin resistance in \textit{S. aureus} when it is compared with the other methods (11). The high cost of DNA probes associated with other hybridization-based molecular methods versus PCR to detect \textit{mecA} gene have prevented their use in routine clinical microbiology laboratory. The current applications and the total workload have to be taken into account when calculating the equipment costs associated with the PCR method. The main advantages of the PCR method over conventional methods is the accuracy of detecting the \textit{mecA} gene and cost effectiveness of obtaining identification by incorporating primers for the \textit{nuc} gene in the reaction mix and a shorter TAT.

It has been shown by other reports that rapid detection of MRSA by standard clinical microbiological procedures is tedious and time consuming since it first requires identification of isolated \textit{S. aureus} colonies within mixed flora samples before assessing their levels of methicillin resistance (12).

In summary, all methods presented high sensitivity and specificity, but latex agglutination test had the advantage of giving a reliable, rapid and most cost effective result that compares very well to the PCR in our environment. The PCR method had the advantage over routine culture methods with its accuracy of detecting the \textit{mecA} gene and a shorter TAT. However, the capital investment for PCR can be prohibitory. The latex agglutination method is offered an alternative.

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