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Comparison Of Susceptibility Test Methods For Determining Oxacillin Resistance In Clinical Isolates

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ABSTRACT

To compare conventional phenotypic methods for the detection of methicillin in *Staphylococci aureus* in routine laboratory practice with reference to an established molecular method. This study was conducted on a selection of 30 isolates of methicillin resistant *Staphylococci aureus* (MRSA) from clinical specimens. The Kirby- Bauer disc diffusion tests and oxacillin screen ager method were performed on all isolates using the presence of penicillin binding protein (PBP2a) as the reference standard. A commercial latex agglutination test (Oxoid, UK) was assessed for the detection of penicillin binding protein 2a (PBP2a), the Mec A gene product. Twenty of 30 isolates were positive to PBP2a and concomitant manifest resistance to (oxacillin) was confirmed using Kirby-Bauer diffusion test. All the thirty isolates were resistant using disk diffusion method. The specificity and sensitivity of this method, in comparison with PBP2a was 100% in our examined strains, whereas the specificity and sensitivity for oxacillin agar screen was 80% and 73.3% respectively. The specificity of routine laboratory tests for MRSA detection was variable mec A gene detection, the "gold standard" to confirm ambiguous results is difficult to perform in routine diagnostic laboratories. The Oxoid kit for the detection of PBP2a is an alternative that could be used in most laboratories.

INTRODUCTION

In recent years, the most common infectious agents have been *Staphylococcus* sp. They are frequently isolated from clinical specimens, where they may be only a contaminant or the cause of infections. *S. aureus* is known as a major pathogen in nosocomial infections[1,2]. Methecillin -resistant *Staphylococcus aureus* (MRSA) has been recognized as an important and universal hospital acquired pathogen causing endemic and epidemic infections in health care centres world wide[3]. Health care workers and infection control personnel depend on the laboratory for the reliable detection of MRSA in clinical specimens. This has implications for the treatment of invasive infections, perioperative prophylaxis and infection control procedures. Surveillance of MRSA locally, nationally and globally is also dependent on accurate laboratory reporting. The purpose of our study was: to compare several phenotypic methods; including a commercial latex agglutination kit that detects the MecA gene product (penicillin binding protein 2a, PBP2a), for the detection of methicillin resistance in *S. aureus* with reference to the presence of the PBP2a as the standard.

Materials and Methods

We selected 30 isolates from patients admitted at different units of General Hospital Minna and National Hospital Abuja, Nigeria from January, 2007 to December 2007. These isolates represented clustering of MRSA infections.

Comparison of phenotypic methods for the routine detection of MRSA

All MRSA isolates were identified and susceptibility tested at the Department of Microbiology, National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja using the Kirby-Bauer technique with oxacillin discs (1ug) and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Susceptibility to oxacillin in all 30 strains was carried out in the Department of Microbiology, Federal University of technology, Minna Nigeria using oxacillin screen agar method.

Detection of oxacilin using oxacillin agar screen method

All isolates were tested for oxacillin resistance using oxacillin agar base (Oxoid, UK) supplemented with 9mg/ml of oxacillin. The agar screen test was performed by inoculating 10^6 Cfu into oxacillin agar base supplemented with 9mg/ml of oxacillin injection. After 24hrs incubation at 35^0C, the agar was inspected for growth. The presence of over one colony is indicative of resistance[4].

Detection of PBP2a using a commercial latex agglutination kit

The MecA product (PBP2a) was detected using the Oxoid MRSA kit. This is a commercial kit that detects the PBP2a present in MRSA. A boiled, centrifuged extract of a suspected colony of MRSA was mixed with latex particles sensitized with monoclonal antibody directed against PBP2a; a suspension of unsensitized latex particles was used as the control.

RESULTS

Different methods used for the detection of oxacillin resistance in *Staphycoloccus aureus* are presented in table 1. We studied one hundred and fifty one strains of *S. aureus*, 77 of this number were MRSA. Out of this number a selection of 30 isolates were tested using PBP2a. 20 out of this number were sensitive to PBP2a and concomitant manifest resistance to methicillin (oxacillin) was confirmed using disk diffusion method. All the 30 strains were resistant using disk diffusion method. The specificity and sensitivity of this method, in comparison with PBP2a was 100% (Table 2). The study using oxacillin agar screen tested showed that twenty two positive strains were oxacillin agar screen positive and only 8 PBP2a positive strains were oxacillin agar screen negative (Table 3). Accordingly, specificity and sensitivity of this test, compared to PBP2a as a "gold standard" were 80% and 73.3% respectively Table 2.

Table 1: Susceptibility Test Methods use in the Study

Method	Media	Oxacillin concentration	InoculumSize	Incubation	Interpretive guideline
Disk diffusion	Mueller Hinton agar(Oxoid) with 2% Nacl	1µg disk	Swab, a MacFarland standard equal to 0.5 to 1	35^{O}Cfor 24 and 48h	Susceptible ,zone diameter of e"13mm,Intermediate, zone diameter of 11 to 12mm Resistant, zone diameter of d"10mm
Oxacillin agar screen	Oxacillin agar base (Oxoid)	9mg/ml	Swab, a Macfarland standard equal to 0.5 to 1	35^{O}C for 24 and 48h	Resistant > 1 colony growth

Table 2: Evaluation of Disk Diffusion and Oxacillin Agar Screen Method for Detection of Oxacillin resistance in Clinical Isolates of *Staphylococcus aureus*

PBP2a Result	PBP2a No. of Isolates tested	Disk Diffusion Method		Oxacillin Screen Agar	
		Positive	Negative	Positive	Negative
Positive	20	30	-	22	8
Negative	10	20	10	20	10
Specificity		100%		80.0%	
Sensitivity		100%		73.3%	

DISCUSSION

The accurate diagnosis of MRSA in the laboratory is vital for patient management. It is also essential for the meaningful interpretation of surveillance data. Currently, surveillance data are difficult to interpret because there is no uniformity of testing methods for the detection of MRSA, and laboratories vary in their standard operating procedures[5-7]. In our study the specificity of the Kirby-Bauer method was the highest (100%) among the phenotypic methods studied. Other studies have reported a specificity averaging 80 to 86.2% [8,4] respectively, and in a similar comparative study Khoner *et al*.[9] and Krishnan *et al*. [10] the specificity were shown to range between 41.7% and 58.3% and 50% respectively. Recent studies have compared different phenotypic methods and have shown that, with modifications of test conditions phenotypic methods including the Kirby-Bauer method can reliably detect oxacilli resistance in *S. aureus*[9]. The BSAC has issued new guidelines for the detection of methicillin resistance in *Staphylococci*, which adds another dimension to the laboratory diagnosis of MRSA[11].

Numerous studies have shown that the phenomenon of heterogeneous resistance is an inherent limitation to the accuracy of susceptibility testing for methicillin resistance in *S. aureus* [8,12,13] . Conventional *PCR* methodology is not always suitable for busy diagnostic laboratories. Detection of MecA product, PBP2a, was a highly sensitive and specific technique for the detection of methicillin resistance in *S. aureus*. Several workers have corrobonated the high sensitivity and specificity of MRSA detection with this method even in strains with ambiguous and borderline oxacillin resistances[14-16].

Table 3: Phenotypes and Genotypes of Isolates showing different results by one or more tests (PBP2a Positive Isolates)

Isolates	Source	PBP2a results	Results of MRSA using disk diffusion test	Oxacillin screen agar test
SA*1	Ear	+	-	+
SA2	Ecs	+	+	+
SA3	Sputum	+	+	+
SA4	Stool	+	-	+
SA5	Urine	+	-	+
SA6	Urine	+	-	+
SA7	Urine	+	-	+
SA8	Ecs	+	-	+
SA9	Ecs	+	+	+
SA10	Wound	+	+	+
SA11	Ecs	+	+	+
SA12	Sputum	+	-	+
SA13	Abscesses	+	-	+
SA14	Urine	+	+	+
SA15	Ear	+	+	+
SA16	Ecs	+	+	+
SA17	Umblical cord	+	-	+
SA18	Ear	+	+	+
SA19	Sputum	+	+	+
SA20	HVS	+	+	+
SA21	Wound	-	+	+
SA22	Ecs	-	+	+
SA23	Eye	-	+	+
SA24	Urine	-	+	+
SA25	Urine	-	+	+
SA26	Wound	-	+	+
SA27	Sputum	-	+	+
SA28	Sputum	-	+	+
SA29	Ear	-	+	+
SA30	Ecs	-	-	+

KEY: Ecs- Endocervical swab, Hvs-High vagina swab, MRSA- Methicillin-resistant *Staphylococcus aureus*, *- *Staphylococcus aureus*, (+) – Susceptible, (-) - Resistant

RECOMMENDATION

The following recommendations were observed by Krishnan *et al.* [10].

1. Mec A gene detection is the "gold standard" for methicillin resistant *Staphylococcus aureus* (MRSA) testing but it is difficult to perform in routine diagnosis laboratories.

2. A cost effective option would be to adopt a well standardized phenotypic technique with stringent quality control measures and to retest ambiguous results with a second conventional phenotypic methods.

3. Isolates that give inconsistent results with two different conventional tests could then be tested with the mastalex ^{Tm} kit and sent to a reference laboratory for Mec A detection.

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ABBREVIATIONS:

BSAC -	British Society for antimicrobial Chemotherapy
MRSA -	Methicillin Resistant *Staphylococcus aureus*
NCCLS -	National Committee for Clinical Laboratory Standards
PBP2a -	Penicillin Binding Protein 2a
NIPRD -	National Institute for Pharmaceutical Research and Development

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