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Comparison of different laboratory methods for detection of methicillin resistant *Staphylococcus aureus*

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ABSTRACT: **OBJECTIVE:** The aim of this study was to compare four different methods for detection of methicillin resistant *Staphylococcus aureus* (MRSA).
DESIGN: The clinical specimens including urine, blood, wound, high vaginal swabs, sputum, semen, ear swabs and tracheal tube aspirates ware processed for isolation of *S. aureus*.
SETTINGS: The samples were obtained from patient attending Murtala Muhammad Specialist hospital Kano, Nigeria.
SUBJECTS: 495 Strains of *S. aureus* were testes with four different methods i. e disk diffusion, oxacillin screen agar, E – test and latex agglutination for methicillin resistance.
RESULTS: Of 495tested *S. aureus*: E – test revealed that 128 isolates were MRSA. Oxacillin screen agar showed two fail positive MRSA. The sensitivity and Specificity of oxacillin screen agar methods was 99% and 98% r
Respectively. The sensitivity and specificity for screen latex agglutination method was 100% and 100% respectively. Results of susceptibility testing by disk diffusion methods in comparison with other methods were conflicting.
CONCLUSION: MRSA Latex agglutination Kit is a reliable alternative for detection of MRSA in clinical laboratory ware MIC detection of MRSA or molecular methods are not available. Also Oxacillin screen agar offers an interesting new approach to early detection of MRSA.

Key Words: *Staphylococcus aureus*, MRSA, Oxacillin.

Introduction

The first case of methicillin resistant *Staphylococcus aureus* MRSA was reporting in 1961 the important of MRSA as a nosocomial pathogen is well documented. Because MRSA is often resistant to many antimicrobial agents, injection caused by this organism is difficult to treat. [3, 4]

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Accurate detection of methillin resistance in *Staphylococcus aureus* by routine methods is difficult to Subpopulation of *S. aureus* (i. e one susceptible and other resistant) which may coexit within a culture. All cells in culture may carry the genetics information for resistance but a small numbers can express this kind of resistance in routine susceptibility testing performed in the laboratory. This phenomenon is termed heterogeneous resistance and occurs in Staphylococci resistant to penicillinase – stable penicillin such as oxicillin.[5] The basic of most methillin resistance is the production of an additional penicillin – binding protein, PBP2' or PBP2a, mediated by the mec A gene. Mec A is an additional gene formed in methillin – resistance Staphylococci and with no allelic equivalent in methillin – susceptible staphylococci. There are several additional genes that affect the expression of methillin resistance in *S. aureus*, but these are formed in susceptible as well as resistant strains. [1, 6, 7]

There are many laboratory methods for detection of methillin resistance in *S. aureus*, most laboratory use disk diffusion method for routing test. The gold standard, for antimicrobial susceptibility testing has been the MIC determined by a dilution or E – test method. In recent years MIC methods has been replaced by molecular method that detects mec A gene. However, the use of this assay is largely restricted to reference centers and they are not currently available in most routing diagnostic laboratories. [1, 7 - 10] The aim of this study was to determine the reliability of four different laboratory methods for detection of methillin resistance in a collection of 960 isolates of *S. aureus*.

Material and Methods

Ninety six clinical isolates of S. aureus were obtained from Murtala Muhammad Specialist Hospital and Aminu Kano Teaching Hospital, Kano, Nigeria. These hospitals are the two largest hospitals in Kano. The E – test method for antimicrobial susceptibility is performed as follows. A Mueller Hinton agar medium is moculated with a broth suspension equivalent to 0.5 Mac Farland Standards prepared by directly moculating organism form 24hour old agar medium and then using a cotton swab to apply the suspension. The E – test strips are applied onto the plate and incubated at 35oC for 24hours. After incubation inhibitory concentration are seen as a formation of an elliptical zone of inhibition growth, whose mRrseution are seen as printed on the strip edge and the zone of inhibition is the MIC. MIC d"2µ.ml consider as susceptible and e"4µg/ml resistant. [11, 12]

The mec A product (PBP2a) was detected by using the Mastalex ^{TM}MRSA kit. This is to commercial kit that detects the PBP – 2a present in MRSA. The mastlax MRSA method was used according to the manufactures instruction. Sufficient colonies of S. aureus were suspended in 200µl extraction reagent 1 and heated in boiling water for 3 minutes. Tubes were cooled and 50µl extraction reagent 2 was added. Tubes were of suspension was mixed with 50µl sensitized latex suspension and rotated manually for 3 minute while looking for agglutination. The supernatant was tested simultaneously with a negative control latex suspension. The time at which agglutination was visible by eye was recorded.[8]

Oxacillin screen agar was performed by direct colony suspension method and adjusted to match 0.5 Mac Farland turbidity standards. The suspension inoculated on Muller Hinton agar containing 4% NaCl 6µg/ml oxacillin. Plates were inoculated 24hours at 350C. Any growth on the plate containing oxacillin considered as resistant to methicillin. [11, 12]

Oxacillin disk susceptibility testing was performed according to National Clinical Laboratory Standards. 11,12 Briefly a bacterial suspension adjusted to 0.5 Mac Farland was incolated onto Mullar Hinton agar. A filter paper disk containing 1µg oxacillin was placed on the inculated Mullar Hinton agar. All plates were incubated in 35^{o}C for 24hours. The diameter of zone of inhibition was measured and folloeing criteria were choosen for interpretation of results. [11, 12] susceptibility (e" 13mm) intermediate (11 – 12mm) resistant (d" 13mm)

Results

By using E – test method of 496 isolates 128 isolates were MRSA and 368 isolates susceptible to methicillin oxacillin screen agar showed only two false positive MRSA in comparison with E – test and

hatexagglutination sensitivity and specificity of oxacillin respectively in comparison with the MRSA screen latex agglutination and E – test with sensitivity and specificity of 100% each. The sensitivity and specificity for disk diffusion method was 98% and 98% respectively.

Table 1: Comparison of different laboratory methods for the detection of methicillin resistant *Staphylococcus aureus* (MRSA)

Methods	R	I	S	Sensitivity	Specificity
E-Test	128	–	360	100	100
Oxacillin Screen Agar	125	–	370	99	99
Latex agglutination	128	–	368	100	100
Disk diffusion	124	4	372	98	98

Discussion

The accurate diagnosis of MRSA in microbiology laboratories is vital for patients' management. It is also essential for meaningful interpretation of surveillance data. Currently surveillance data for MRSA are difficult to interpret, because there is no uniform testing method for detection of MRSA, and laboratories vary in their Standard operating procedure and interpretation of breakpoint values.[13] There are many different methods for detection of MRSA phenotypically. The oxacillin disk diffusion test, oxacillin screen agar test, rapid latex agglutination and E-test are four important methods. Amplification test like those based on the polymerase Chain reaction (PCR) detecting mec A gene are gold standard methods. [14]

Disk diffusion method is an easy method for performance in microbiology, laboratories of MRSA. As already reported, the oxacillin disk diffusion test was the least reliable test for detection of MRSA. [15] Among the four methods tested, E – test given MIC results are affected by test condition in a similar way to MIC and diffusion methods. The E – test has advantages over other MIC methods in that it is easy to set up as a disk diffusion method. [10] The oxacillin screen agar test has been evaluated thoroughly, in studies performed since 1990 that used for detection of MRSA was excellent. However two reports noted that when very heteroresistant strains were tested, the sensitivity decreased. Conversely, the specificity among susceptibility strains tested was very good and strains with borderline MIC were included. [14] Our study revealed that latex agglutination test and E – test are the alternative methods wire high sensitivity and specificity for detection MRSA. There have been many recent evaluation of the MRSA – screen latex agglutination test. Most studies reported the sensitivity of this method for detection of MRSA as more than 98%. [14, 16, 17]

As shown in this as well as other studies, disk diffusion method is not reliable enough for detection of MRSA. It is difficult to perform detection of mec A gene in routine diagnostic laboratories and testes costs are relatively high. [3, 18] The latex agglutination ket for diction of PBP2ais an alternative that could be used in most laboratories. This method would be practically useful for urgent confirmation of resistance.

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