Original Article

A Comparative Study of Conventional Phenotypic Methods for the Detection of MRSA.

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ABSTRACT

Aim: Methicillin-resistant Staphylococcus aureus (MRSA) were first reported in 1961 and have since become a major nosocomial pathogen worldwide. It is axiomatic that the sooner an MRSA infection is diagnosed, and the susceptibility to antimicrobial agents established, the sooner appropriate therapy and control measures can be initiated. Health care workers and infection control personnel depend on the laboratory for the reliable detection of MRSA in clinical specimens. To compare conventional phenotypic methods for the detection of methicillin resistance in Staphylococcus aureus in routine laboratory practice.

Materials and Methods: This study was conducted on a selection of 100 isolates of Staphylococcus aureus from different clinical specimens from the patients attending CAIMS, Karimnagar. The organism was isolated and identified using standard conventional techniques, tested for antibiotic susceptility by Kirby–bauer disc diffusion method and subjected for methicillin resistance using four methods ; resistance to oxacillin, cefoxitin, E-Test and Hichrom agar.

Results: Oxacillin discs, Cefoxitin discs, E- strips and Hichrom agar, all these methods showed 100% sensitivity. Comparatively E-strips have shown highest sensitivity and specificity. However, Cefoxitin disc was the preferable method for detecting Methicillin resistance, as it is easy to perform and cost effective.

Conclusion: Surveillance of Methicillin-resistant Staphylococcus aureus (MRSA) Locally, Nationally, and Globally is dependent on accurate laboratory tests. In the absence of availability of molecular techniques, the cefoxitin disc was the best predictor of methicillin resistance in Staphylococcus aureus from among the techniques tested.

Keywords: Methicillin resistant staphylococcus aureus.

INTRODUCTION

Methicillin resistant Staphylococcus aureus (MRSA) has been recognised as an important and universal hospital acquired pathogen causing endemic and epidemic infections world wide. MRSA is a leading cause of gram positive bacterial infections and produces a wide spectrum of diseases , ranging from minor skin infections to fatal necrotizing Pneumonia [1]. It is one of the significant pathogens known for sporadic infections and has been recognised as an important nosocomial pathogen because of increasing resistant strains [2].

In 1995, Staphylococcus aureus was recovered from 13%

of infections and ranked as the common nosocomial infections causing morbidity and mortality[3].

It is widely known for its ability to acquire resistance to antibiotics , most notably ß lactams such as Penicillin and Methicillin[4].

Penicilliniase resistance was first developed by staphylo coccus aureus in 1940s[5]. Methicillin ,a Penicillinase resistant Penicillin was introduced in 1959 to combat Penicillin resistance. Methicillin resistant strains were first identified in 1961 soon after its introduction[6].

Methicillin, a penicillinase, structural homologue of Penicillin inhibits cell wall synthesis[7].

MRSA has type IV staphylococcal cassette chromosome (SCC), a genetic element which encodes Mec-A gene , tends to be resistant for ß lactams[8].

In recent years, the increase in the number of bacterial strains that show resistance to Methicillin (MRSA) has become a serious clinical and epidemiological problem, because this antibiotic is considered as the first option in the treatment of staphylococcal infections, and because resistance to this antibiotic implies resistance to all ß-lactam antibiotics. For these reasons, accuracy and promptness in the detection of methicillin resistance is of key importance to ensure correct antibiotic treatment in infected patients as well as control of MRSA isolates in hospital environments, to avoid them spreading.

The main objective of this study was to evaluate four different methods in relation to the detection of Methicillin resistance and to compare and contrast their suitability as routine methods for detecting MRSA isolates in clinical microbiology laboratories.

MATERIALS AND METHODS

The present study was carried out from July 2012 – September 2012 in the department of microbiology , CAIMS, Karimnagar. A total of 100 staphylococcal strains were isolated from different clinical specimens including sputum , urine , wound swabs and blood from patients attending CAIMS , were included in the study. Organism was confirmed by using standard conventional techniques , tested for antimicrobial susceptibility by Kirby – Bauer's disc diffusion method and simultaneously tested for Methicillin resistance by four different methods.

The following antibiotics were used for testing antimicrobial susceptibility:

Ampicillin (10µg) , Erythromycin (15µg) , Vancomycin (30µg), Linezolid (30µg), Amoxyclav (20 / 10µg), Piperacillin / Tazobactam (100 / 10µg), Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (10µg), Cefoxitin (30µg), Ofloxacin (5µg), Clindamycin (2µg), Oxacillin (1µg).

Zone diameters were measured at 24 and 48 h following NCCLS criteria.[9]

Methicillin resistance was detected by four different methods:

1.Oxacillin disc method

2.Cefoxitin disc method

3. E-Test

4.CHROM agar

Oxacillin disc method

A lawn culture of Staphylococcus aureus was made on Mueller-Hinton agar , Oxacillin disc (1µg) was placed in the center of the plate and was incubated at 30^0C overnight.

Cefoxitin disc method

A lawn culture of staphylococcus aureus was made on MH agar , Cefoxitin disc (30µg) was placed along with other antibiotics , incubated at 37^0C overnight.

E – TEST

A lawn culture was made on MH agar, E-strips coated with Cefoxitin and Vancomycin was placed on it , incubated at 37^0C overnight along with the Control strain ATCC – 29213.

HI CHROM agar

Staphylococcus aureus was streaked on freshly prepared plates of CHROM agar with supplement of Cefoxitin , incubated at 37^0C overnight and observed for growth.

RESULTS

A total of 100 Staphylococcal strains were isolated from different clinical specimens.The percentage of Staphylococcus aureus isolated from sputum was 48% followed by woundswabs - 26% ,Urine – 21% and Blood – 05%.

Table.1 : Percentage of Staphylococcus aureus from clinical specimens.

S.No	Specimen	%
01.	Sputum	48
02.	Wound swabs	26
03.	Urine	21
04.	Blood	05
	Total	100

Out of 100 strains isolated the percentage of Methicillin resistant strains were 32%.

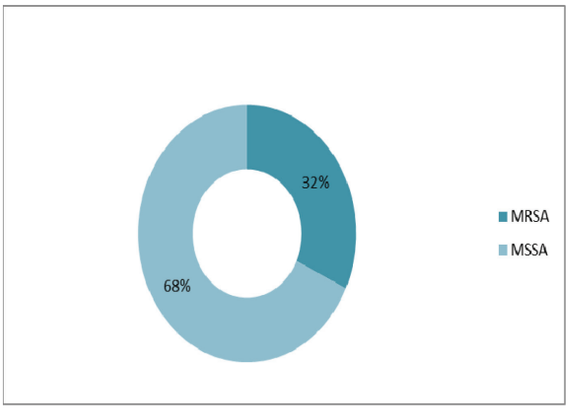


Figure 1:Percentage of Methicillin resistant strains isolated.

Table 2: Comparison of conventional phenotypic methods for the detection of MRSA.

Test method	Sensitvity (%)	Specificity (%)
Disc diffusion method		
Oxacillin (1µg)	100	90
Cefoxitin (30µg)	100	95
E - Test	100	100
CHROM agar	100	97

Out of four methods evaluated for detection of methicillin resistance following are the advantages and drawbacks.

All the four methods were equally sensitive with slight variability in their specificity.

Drawbacks

Oxacillin disc method

A separate culture media plate has to be inoculated and incubated separately at different temperature (30^0C).

E-Strip method:

1. Only two antibiotics can be tested at a time.

2. Expensive

CHROM agar:

1. Every time freshly prepared culture media has to be used.

2. Expensive.

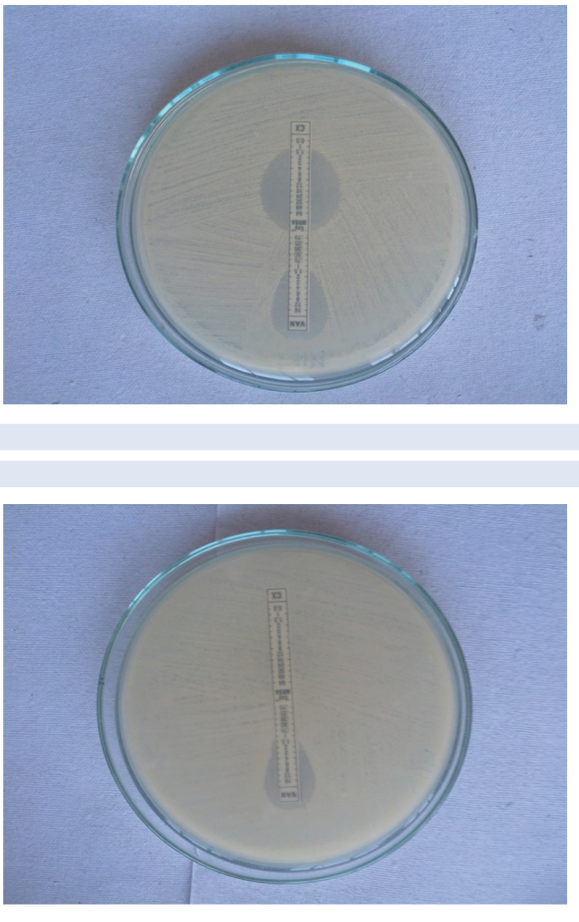
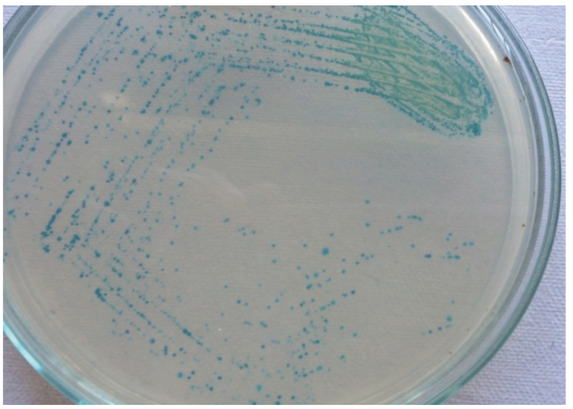


Figure 2: E-TEST showing zones of inhibition for Cefoxitin and Vancomycin.

DISCUSSION

Increase in incidence of infections due to Staphylococcus aureus is partially a consequence of advances in patient care and also of the pathogens ability to adopt to a changing environment. The accurate diagnosis of MRSA in the laboratory is vital for



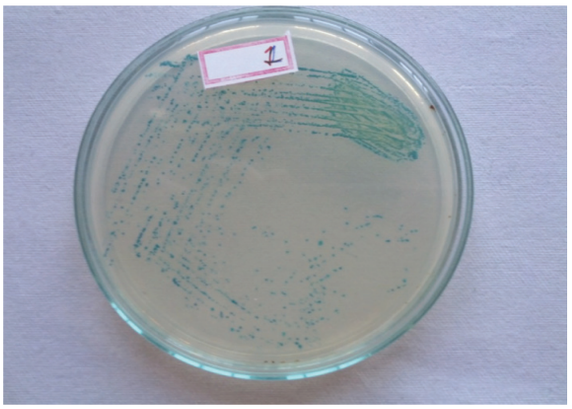


Figure 3: CHROM Agar showing green colour colonies of Methicillin resistant Staphylococcus aureus.

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 and interpretation of breakpoint MIC values.[10,11,12] We have isolated total of 32% of MRSA isolates, whose sensitivity was higher (100%) which is in accordance with the previous studies done by P U Krishnan et al.[13] CHROM Agar method has also yielded high sensitivity and specificity in the detection of MRSA isolates which is similar to the studies done by David Velasco et al.[14] As discussed above in the results about the advantages and drawbacks , the preferable method for routine testing of MRSA in laboratories is Cefoxitin disc method, as it can be tested along with other antibiotics in the same culture plate by Kirby-Bauer disc diffusion method , most importantly it is cost-effective and equally specific and sensitive as other three methods evaluated.

CONCLUSION

As laboratories rise to help meet the challenge of control of MRSA in the healthcare facilities and now in the community, rapid diagnostic methods are needed. It is axiomatic that the sooner an MRSA infection is diagnosed, and the susceptibility to antimicrobial agents established, the sooner appropriate therapy and control measures can be initiated. Laboratory diagnosis and susceptibility testing are crucial steps in treating, controlling and preventing MRSA infections.

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