EFFICACY OF LABORATORY TESTS TO DIAGNOSE MRSA: A MULTI-CENTRE STUDY

Gandhiraj D[1], *Wesely E.G[1], Palanisamy A[2]., Sahanapriya.s[3]

[1]Research Scholar,[2]Asst.Professor,[3]Professor,[4]Research Scholar

[1,2] Department of Botany,[3]Department of Microbiology,[4]Department of Microbiology

[1,2] Arignar Anna Govt., Arts College,[3]Muthayammal College of Arts & Science,[4]Dr.NGP Arts and Science college
[1,2]Namakkal,[3]Rasipuram,[4]Coimbatore,TamilNadu,India

Abstract : The genera Staphylococcus are normally present in normal skin or nasal colonizers of human beings. Conventionally the Staphylococcus is divided into two groups on the basis of the coagulase reaction. The coagulase-positive staphylococci is the most commonly isolated pathogenic species S aureus . The coagulase-negative staphylococci (CONS) are now known to comprise over 38 species. The CONS are common commensals of skin, although some species can cause oppartunisitic infections. . Methicillin resistance is indicative of multiple resistance. Methicillin-resistant S aureus (MRSA) causes outbreaks in hospitals among surgical patients and also now isolated from the community as well. Methicillin-resistant Staphylococcus aureus (MRSA) by definition harbors a gene, mecA, for methicillin resistance. The mecA gene codes for penicillin binding protein (PBP) 2a which allows MRSA to continually synthesize its cell wall in the presence of β-lactam antibiotics.Laboratory diagnosis and susceptibility testing are crucial steps in treating and preventing MRSA infections. Therefore, methods used to detect MRSA in clinical samples should have high sensitivity and specificity and, most importantly, the result should be available within a short period of time. The study have used various methods have evolved for rapid detection of methicillin-resistant staphylococcus aureus .

IndexTerms- Staphylococcus Aureus, MRSA, mecA.

I. INTRODUCTION

Staphylococcus aureus remains a common cause of infection in the community for centuries. In the last few decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasingly important cause of healthcare-associated infections. Moreover, these staphylococci that have become resistant to beta-lactam antibiotics can occur in healthy persons without traditional MRSA associated risk factors . Furthermore, since1990s, MRSA has also emerged as cause of infection in the community [1]. The spread of HA-MRSA in resource-limited settings can have devastating consequences because of the lack of microbiology laboratories with outbreak investigation capability and inadequate facilities for bacterial identification and antimicrobial susceptibility testing as well as the high cost of antibiotic drugs required to treat severe HA-MRSA infections [2]. The hospital-associated MRSA infections have predominated in humans and contributed to significant illness and death [3]. Recently, a shift in the epidemiology of MRSA infection has been documented, whereby community- associated (CA)-MRSA infections have become more common [4-8]. CA-MRSA may arise from hospital origin clones that are carried into the community and then transmitted between persons or from de novo development of resistance through acquisition of resistance factors (*mec*A) by methicillin-sensitive strains of *S. aureus* [9]. *Staphylococcus aureus* is an opportunistic pathogen and is the causative agent of infections such as bacteraemia, sepsis, toxic shock syndrome, bone and joint infection and skin and skin-structure infections (SSSIs), which can result in significant morbidity and mortality [10].

Among the staphylococcus species, Staphylococcus aureus is the most virulent species of the genus causing both nosocomial and community acquired infections worldwide [11].

Dramatic changes in the susceptibility of S. aureus to beta-lactam antibiotics particularly to penicillin and cephalosporin in both hospital and community settings have been reported worldwide [12]. Several mechanisms for the development of MRSA have been reported. Among these production of a unique penicillin-binding protein (PBP) that has a low affinity for β-lactam antibiotics and whose effects are determined by several structural genes (e.g., mecR1 and mecI) [13-14], production of the usual PBPs, but with modified affinities for β-lactam drugs, and production of penicillinase enzyme are most important ones [15]. aminoglycosides.12 These strains are seen possessing elevated resistance to a wide range of antibiotics, limiting the treatment options to very few agents such as vancomycin and teicoplanin [16]. Hence, knowledge of prevalence of MRSA and MSSA and their antimicrobial proÞ le becomes necessary in the selection of appropriate empirical treatment
of these infections and controlling nosocomial infection [17] .

II. MATERIALS AND METHODOLGY

A. COLLECTION OF CLINICAL SAMPLES

Pus & Wound clinical samples were collected from different hospitals in western Tamil nadu. The samples were cultured to identify *Staphylococcus aureus.* There are different methods were used for Detecting Methicillin resistance from the identified *Staphylococcus aureus* isolates.

B. IDENTIFICATION USING ANTIBIOTICS

The procured strains will be inoculated in peptone water. The broth culture is incubated at 37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8CFU/ml . To perform this step properly, either a photometric device can be used .Within 15 minutes , the sterile cotton swabs are dipped into the suspension. Take a sterile Mueller-Hinton agar (MHA) plate.Use the swab with the test organism to streak a MHA plate for a lawn of growth. After the streaking is complete, allow the plate to dry for 5 minutes. oxacillin, methicillin, cefoxitin discs can be placed on the surface of the agar using sterilized forceps. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop.Carefully invert the inoculated plates and incubate for 24 hours at 37° C. After incubation, observe the zone of inhibition for each antibiotic used.

C. MeRSA PLATING

The strains were inculated in MeRSA medium containing oxacillin (at a concentration of 6 µg/ml of media) and 4% NaCl

D. AUTOMATED ID AND AST SYSTEM

The Bio merieux Vitek2 Automated System to identify the isolates as MRSA

E. PREPARATION OF INOCULUM

Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. Allow the card(s) to come to room temperature before opening the package liner. Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm test tube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiCHEK Plus Meter. Place the prepared suspensions in the cassette (see section 15, Instrument User Manual). To use the DensiCHEK Plus Meter to read samples: Ensure the instrument is ON and set to the PLASTIC tube setting. Blank the DensiCHEK Plus by filling a test tube with sterile saline and inserting the tube into the instrument. Press the "0" key and slowly rotate the test tube. Ensure one full rotation is completed before the reading is displayed. The instrument will display a series of dashes followed by 0.00. To measure a sample, place a well-mixed organism suspension into the instrument and slowly rotate the test tube. Ensure one full rotation has completed before the reading is displayed. The instrument will display a series of dashes followed by a reading. Remove the test tube after completion of a reading. The instrument will automatically shut off when test tubes are not inserted after one minute. (NOTE: If the instrument flashes 0.00 or 4.00, the suspension is either below 0.0 McF or above 4.0 McF and is not within the reading range. Ensure suspensions are within the appropriate reading range to avoid compromised card results. If necessary, re-calibrate the DensiCHEK Plus instrument after processing each cassette). Insert the straw (in the V2C card) into the inoculated suspension tube in the cassette. Proceed to data entry.

III. EXPERIMETAL RESULTS

A. ANTIBIOTIC SUSCEPTIBILITY TEST

The antibiotic susceptibility testing for identification of MRSA shows oxacillin, cefoxitin, methicillin were resistant.

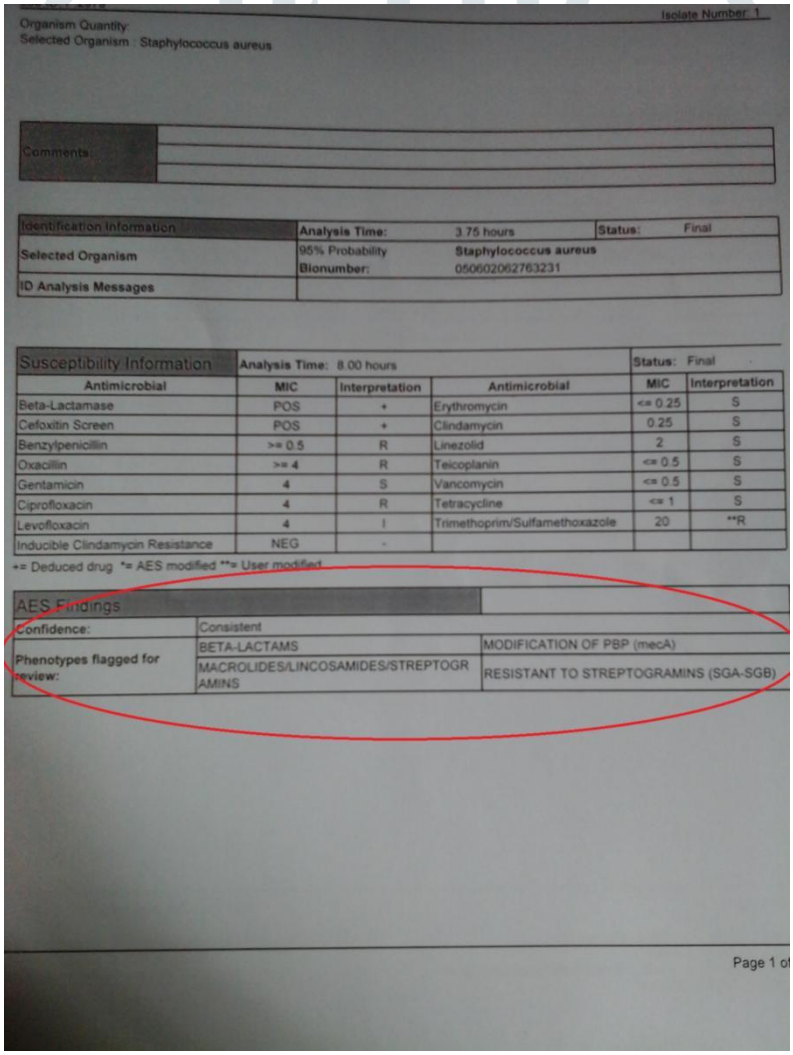
S.NO	ANTIBIOTIC NAME	SUSCEPTIBILITY
1.	Oxacillin	Resistant
2.	Methicillin	Resistant
3.	Cefoxitin	Resistant

B. MeRSA PLATING

The Colonies shows greenish blue colour in MeRSA plate.

C. VITEK RESULTS

The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions). The beta lactamase and the cefoxitin screening shows positive. Penicillin , oxacillin shows resistant report. The results showed the modification of penicillin binding protein (mecA) was detected.



Organism Quantity:
Selected Organism : Staphylococcus aureus

Isolate Number: 1

Comments

Identification Information	Analysis Time:	3.75 hours	Status:	Final
Selected Organism	95% Probability	Staphylococcus aureus		
	Bionumber:	050602062763231		
ID Analysis Messages				

Susceptibility Information	Analysis Time: 8.00 hours			Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Beta-Lactamase	POS	+	Erythromycin	<= 0.25	S
Cefoxitin Screen	POS	+	Clindamycin	0.25	S
Benzylpenicillin	>= 0.5	R	Linezolid	2	S
Oxacillin	>= 4	R	Teicoplanin	<= 0.5	S
Gentamicin	4	S	Vancomycin	<= 0.5	S
Ciprofloxacin	4	R	Tetracycline	<= 1	S
Levofloxacin	4	I	Trimethoprim/Sulfamethoxazole	20	**R
Inducible Clindamycin Resistance	NEG	-			

+= Deduced drug *= AES modified **= User modified

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS	RESISTANT TO STREPTOGRAMINS (SGA-SGB)

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V. SUMMARY AND CONCLUSION

The increasing MRSA prevalence and the presence of methicillin resistant type of drug resistance are constantly evolving and are under ever-changing state. Therefore, it poses a global danger to public health programs. Besides, there is a significant necessity for regular antimicrobial sensitivity surveillance not only for the presence and spread of methicillin resistance genes but for both community and hospitalized patients are more advised and integrated treatment. However, the clinical implication of MRSA infections and its negative attendant effect like rapid propagation within the hospital setting, and community shows difficulty if patients management might prompt the need for urgent periodic surveillance of MRSA strains in our routine microbiological analysis of clinical specimens, and establishment of effective and efficient infection control and prevention measure as early intervention measures by identifying the MRSA strain using a reliable and approved diagnostic methodology.

In this study, we have analysed the staphylococcus aureus strains collected from different hospitals in western tamilnadu and incorporated to find out methicillin resistance. The automated identification system used along with routine conventional methods and found the automated system provides MRSA results within 6-8 hours, where the conventional methods take 24 hrs. Further the automated system renders the presence of methicillin resistance developing gene mecA, that could be taken as a confirmation with minimal cost.

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