

ISOLATION AND IDENTIFICATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS [MRSA] FROM COW DUNG

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Abstract: Methicillin-Resistant Staphylococcus aureus (MRSA) has been perceived as one of the significant microorganisms in both clinic and local area settings. In India, the percentage of nosocomial diseases brought about by MRSA fluctuates somewhere in the range of 20 and 40%. Conventional strategies for the identification of MRSA incorporate oxacillin disc diffusion, oxacillin MIC and oxacillin screen agar methods. In this study, Coagulase test is principally used to separate Staphylococcus aureus (positive) which produce the catalyst coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which don't produce coagulase, for example Coagulase Negative Staphylococcus (CONS). For Antibiotic sensitivity test, Instead of methicillin disks, oxacillin, which has a place with a similar class (penicillinase-resistant penicillins), is preferred. These days, methicillin is neither utilized for treatment nor for testing of gram-positive microscopic organisms. Also, Oxacillin action permits a higher identification of heteroresistant strains than methicillin.

Keywords: Methicillin, Coagulase, Sensitivity

1. INTRODUCTION

Staphylococci are diverse ubiquitous opportunistic colonizers of human epithelia involved in nosocomial infections that cause diseases of major importance in both human and animals, ranging from minor skin infections to life-threatening bacteremia as well as septicemia (Aklilu et al., 2010). Systemic infections include endocarditis, osteomyelitis, meningitis, and toxemic syndromes. It is a highly resistant non-spore forming and might continue to exist in dryness for extended periods, rendering it not possible to remove from the environment. Staphylococcus aureus produces a highly heat-stable enterotoxins that cause gastroenteritis in humans and predominantly incriminated in staphylococcal food poisoning (Weese, 2010).

Methicillin-resistant Staphylococcus aureus (MRSA) has been diagnosed as one of the major pathogens in both health facility and community settings. The primary case of MRSA became remoted way returned in 1961. Because then, there has been an escalating charge of infections resulting from MRSA international ensuing in accelerated mortality and morbidity data. In India, the superiority of nosocomial infections caused by MRSA varies between 20 and 40%. MRSA can be isolated from diverse samples like cow dung, hands of milk handler and different milk products. Traditional methods for the detection of MRSA consist of oxacillin disc diffusion, oxacillin screen agar methods and oxacillin MIC

2. EXPERIMENTAL METHODS OR METHODOLOGY

Sample was collected and subjected to biochemical tests and other analysis. Finally, Antibiotic sensitivity tests were carried out.

□ Collection of sample

Cow dung was collected from a rural area in a sterile paper. From this, By using sterile loop, small loop sample was collected in a sterile distilled water. Here we can also use Saline water instead of distilled water. This sample solution was taken in beaker, later subjected into a sterile test tube for serial dilution.

ISOLATION OF MRSA

Subjected for serial dilution. For this, Various six dilutions were taken and only Two dilutions of 10^{-4} and 10^{-5} were selected and further Isolated in MSA medium

ANTIBIOTIC SENSITIVITY TESTS

After incubation, plates were observed and taken for sub culturing, Subculturing was done in both MSA medium and MHA medium. MHA medium was taken and various eight discs were used for sensitivity test. ATCC positive control was also taken for comparative study

Discs used:

Oxacillin, Pencillin, Vancomycin, Amoxycillin, Chloramphenicol, Tetracycline, Cotrimaxazole, Cefoxitin

3.RESULTS AND DISCUSSION

3.1 GRAM STAINING

Purple color, grape like colonies were found. Hence Its confirmed as Gram Positive Bacteria

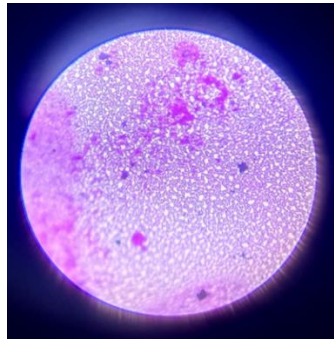


Fig .1 Gram staining

3.2 BIOCHEMICAL TESTS

TESTS	RESULTS
GRAM STAINING	Positive (+ve)
Catalase	Positive (+ve)
Citrate	Positive (+ve)
Coagulase	Positive (+ve)
Gas	Negative (-ve)
Indole	Negative (-ve)
Motility	Negative (-ve)
MR (Methyl Red)	Positive (+ve)
Nitrate Reduction	Positive (+ve)
OF (Oxidative-Fermentative)	Fermentative
Oxidase	Negative (-ve)

Fig. 2. Biochemical test results

3.3 ANTIBIOTIC SENSITIVITY TEST

Methicillin turned into powerful for years for the treatment of staphylococcal infections until resistance emerged. But, that is not the primary motive of its discontinuation. Methicillin is now not commercially to be had because of its side effects such as interstitial nephritis and kidney failure.in recent times, methicillin is neither used for remedy nor for susceptibility testing of gram-positive bacteria Instead, oxacillin, which belongs to the identical class (penicillinase-resistant penicillins), is favored. Oxacillin activity allows a higher detection of heteroresistant strains than methicillin. But, the authentic term MRSA is still used .



Fig 3 ANTIBIOTIC TEST – ISOLATED MRSA

Fig.4 ANTIBIOTIC POSITIVE CONTROL-ATCC

After the tests, Clear zone was observed and comparatively analysed .ATCC Antibiotic Positive control discs were used. Different types of zones were observed.

ANTIBIOTIC DISCS	ZONE OF INHIBITION
Penicillin	19mm
Oxacillin	No Zone
Vancomycin	10mm
Amoxycillin	18mm
Chloramphenical	22mm
Tetracycline	19mm
Cotrimaxazole	19mm
Cefoxitin	30mm

Fig.5 ABST RESULTS

4.CONCLUSION

As per the preceding studies and findings, MRSA organism is isolated, identified and confirmed as well. When the growth in MSA medium was identified, its confirmed that it might be staphylococcus or streptococcus species. As Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identity of Staphylococcus aureus from medical and non-scientific specimens. Whilst Coagulase test was proved as positive, its confirmed the organism is staphylococcus aureus. In India, most of the rural areas, human beings frequently use cow dung mixed water to spray on their ground and roads, for that reason the opportunity to get infected is very excessive as it is fatal and resistance to drugs.

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