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DETECTION OF VANCOMYCIN RESISTANCE AMONG METHICILLIN RESISTANT STAPHYLOCOCCI ISOLATED FROM DIFFERENT CLINICAL SAMPLES AT TERTIARY CARE HOSPITAL

INTRODUCTION:

Staphylococcus aureus is an increasingly common pathogen in community-acquired and nosocomial infections[1]. Staphylococcus aureus, a major cause of potentially life-threatening infections acquired in health care and community settings, has developed resistance to most classes of antimicrobial agents. A dramatic increase in the number of health care-associated infections due to methicillin-resistant Staphylococcus aureus (MRSA) in the 1990s and the recent emergence of MRSA in community-associated infections highlight the success of this species as a pathogen and its ability to adapt under pressure from antimicrobial agents[2].

Methicillin-resistant Staphylococcus aureus (MRSA) has occurred in many countries since its discovery in 1961. However, in recent years, clinicians have been concerned by the increased frequency of MRSA infections[3]. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant Staphylococcus aureus (VRSA) strains exhibiting two different resistance mechanisms. Initially vancomycin intermediate Staphylococcus aureus (VISA) noted in Japan in 1996 and subsequently in United States in 1997 was believed to be due to the thickened cell wall, where many vancomycin molecules were trapped within the cell wall. The trapped molecules clog the peptidoglycan meshwork and finally form a physical barrier towards further incoming vancomycin molecules. The second, noted in United States in 2002 among Staphylococcus

aureus, was identical to the mechanism seen in vancomycin-resistant Enterococcus. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil, France, United Kingdom, Germany, India and Belgium has confirmed that the emergence of these strains is a global issue[4] .

The emergence of glycopeptide intermediate-resistant Staphylococcus aureus (GISA) and, most recently, vancomycin-resistant Staphylococcus aureus further limits therapeutic options for clinicians[1] .

OBJECTIVES:

1. To isolate and speciate Staphylococcus aureus from various clinical samples.
2. To detect methicillin-resistant Staphylococcus aureus (MRSA) strains.
3. To detect vancomycin resistance among MRSA isolates.

METHODOLOGY:

Bacterial isolates:

Staphylococcus aureus are isolated from various clinical samples at a tertiary care hospital which are collected after obtaining informed consent (Annexure I) over a period of 2 months and these Staphylococcus aureus isolates are identified by colony morphology, Gram stain, catalase test, coagulase test and fermentation of mannitol by conventional methods[4]

Antibiotic susceptibility testing:

The antibiotic resistance profile is determined by Kirby-Bauer disc diffusion method using different antimicrobial agents supplied by manufacturer (HiMedia Laboratories, Mumbai), according to guidelines recommended by Clinical and laboratory Standards Institute (CLSI). The standard Staphylococcus aureus strain ATCC 29213 is used as reference strain for vancomycin resistant Staphylococcus aureus[4].

Determination of Minimum inhibitory concentration (MIC):

Minimum inhibitory concentration (MIC) of vancomycin is determined by using HiComb strip (HiMedia Laboratories, Mumbai) with defined concentration of loaded antibiotic (ranging from 256 to 2 µg and 2.048 to 0.016 µg)[4].

Inoculum suspension is prepared by selecting colonies from overnight growth on nutrient agar plates. The colonies are transferred to sterile saline to produce a suspension that matches the turbidity of a 0.5 McFarland standard. The inoculum is inoculated onto Mueller-Hinton agar plate by doing lawn culture. After drying for 5-10 minutes, HiComb strip is placed onto lawn cultured plate and the plate is incubated at 35^0-37^0 C[4].

After overnight incubation of 18-24 hours, the plate is examined for zone of inhibition which is seen in the form of ellipse[4].

IMPLICATIONS OF THE STUDY:

In Indian hospitals, methicillin-resistant Staphylococcus aureus (MRSA) is one of the common causes of hospital-acquired infections and 30 to 80 per cent methicillin resistance in Staphylococcus aureus has been associated with high morbidity and mortality rates. Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA but

unfortunately, decrease in vancomycin susceptibility of Staphylococcus aureus and isolation of vancomycin-intermediate and resistant Staphylococcus aureus have recently been reported from many countries[4].

The increase of vancomycin resistance among Methicillin-resistant Staphylococcus aureus (MRSA) and excessive use of antimicrobial agents have worsened the sensitivity, which call for further epidemiological studies[4].

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