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STUDY OF SPECIAL RESISTANT DETERMINANTS OF INDUCIBLE CLINDAMYCIN RESISTANCE (ICR) AND METHICILLIN RESISTANCE (MRSA) IN STAPHYLOCOCCAL ISOLATES

Sarita Ugemuge

Assistant Professor Dept. of Microbiology Jawaharlal Nehru Medical College, Data Meghe Institute of Medical Sciences Sawangi (Meghe), Wardha

ABSTRACT

Background: Staphylococci have developed resistance to several antibiotics, leaving clinicians with little treatment choices. As a result, reliable drug susceptibility data is critical for a clinician to make an informed clinical decision.

Aims & objectives: To study prevalence of inducible clindamycin resistance (D test) & to assess the frequency of methicillin resistance staphylococci aureus (MRSA).

Material & methods: A total of 85 clinical Staphylococci isolates were obtained from various samples. The Coagulase Test was carried out on a slide. Kirby Bauer Method was used to measure the antimicrobial resistance of the strains collected.

Results: 80 of the 85 Staphylococci isolates were coagulase positive, while only 5 were coagulase negative. Methicillin Resistant Staphylococci (MRSA) made up 14 (17.50%) of the 80 coagulase positive Staphylococci, while Methicillin Sensitive Staphylococcus aureus made up 66 (82.5%). 19 (23.75%) of the 80 isolates of Coagulase-positive Staphylococci were D-test positive, indicating inducible clindamycin resistance. In addition, four of the 19 ICR isolates (05.00 percent) were found to be MRSA.

Conclusion: The organism must be isolated from clinical specimens and its antimicrobial susceptibility pattern studied. It is therefore essential to assess the various factors and methods by which it acquires antimicrobial resistance.

Keywords: staphylococcal isolates, clindamycin resistance, Methicillin resistance

INTRODUCTION:

Penicillin is ineffective against Staphylococcus aureus. The cell wall gives it rigidity, and structural integrity causes inflammatory cytokines to be released[1]. Opsonisation is inhibited by capsular polysaccharides that surround the cell wall. Cocci adhere to the host cell surface thanks to the teichoic acid portion of the cell wall, which protects them from complement-mediated opsonization. Prone to the antibiotic methicillin MRSA (Methicillin-resistant Staphylococcus aureus) is a bacterium that causes a number of difficult-to-treat infections[2]. Oxacillin-resistant Staphylococcus aureus is another name for it (ORSA). Methicillin-resistant staphylococcus aureus has evolved resistance to beta-lactamase antibiotics such as penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and cephalosporins3 by natural selection. Methicillin-resistant Staphylococcus aureus, or MSSA, are bacteria that are resistant to these antibiotics[3]. MRSA (methicillin-resistant Staphylococcus aureus) is a major source of concern in both hospital and community environments. MRSA is a common cause of nosocomial infections in hospitals around the world. They are also immune to the majority of other antibiotics, leaving vancomycin as the only choice in many cases. MRSA is not only confined to the hospital environment; evidence suggests that it may also cause infections in the community, which is concerning. Infections

**Corresponding author: Sarita Ugemuge |*

caused by Staphylococcus aureus (VISA) strains have been identified in some parts of the world. Staphylococci have developed resistance to several antibiotics, leaving clinicians with little treatment choices[4]. As a result, reliable drug susceptibility data is critical for a clinician to make an informed therapeutic decision. As a result, prior to administering an adequate medication, it is critical to consider the risks and benefits of each agent[5]. Staphylococci resistance to Macrolide, Lincosamide, Streptogramin b (MLSb) antibiotics is once again a major source of concern. Clindamycin, a lincosamide, is used as an alternative to penicillin in penicillin-allergic patients due to its high penetration in soft tissue[6]. If the organism is sensitive to erythromycin, a macrolide, then clindamycin, a lincosamide, can be provided empirically; however, if the organism is resistant to erythromycin, then the organism's sensitivity to clindamycin must be checked along with its sensitivity to erythromycin, using the D-zone test[7]. This is because there are two forms of clindamycin resistance in Staphylococci. msrA confers constitutive MLSb resistance, while ermA and ermC confer inducible MLSb resistance[8]. The diversity of pathways that confer MLSb antibiotic resistance illustrates both the complexity of resistant phenotypes and the clinical situation. The susceptibility test can show the strain to be responsive by disc diffusion method in the presence of inducible clindamycin resistance, but the resistance will manifest only on induction. In order to treat Staphylococcal infections with clindamycin, it is essential to recognise inducible clindamycin resistance that confers resistance to MSLb antibiotics[9].

Aims & objectives: To study prevalence of inducible clindamycin resistance (D test) & to assess the frequency of methicillin resistance staphylococci aureus (MRSA).

Material & methods:

During the duration of 1 February 2015 to 28 February 2015, a total of 85 clinical isolates of Staphylococci were collected from various samples at Vishakha Clinical Microbiology Laboratory (VCML), Nagpur for this research. For this analysis, the ATCC culture S. aureus 25923 was used as the norm. Normal identification procedures such as colony morphology, Gram stain reaction, catalase test, and urease test were used to identify the strains. Before conducting Antimicrobial Susceptibility Testing, all of the strains were screened for Coagulase activity. The slide coagulase test was used to validate the results of the tube coagulase test. The Kirby Bauer Method (disc diffusion method) was used to measure the antimicrobial susceptibility of the collected strains using discs of Amikacin, Amoxyclav, Ampicillin, Cefuroxime, Cephalexin, Ciprofloxacin, Clindamycin, Erythromycin, Gentamycin, Pristinomycin, Rifampacin, and Vancomycin. All of the strains were tested for Methicillin resistance using the standard disc diffuson method described above. According to NCCLS guidelines, all strains were tested for Inducible Clindamycin resistance using the standard D-Zone Test. At the conclusion of the analysis, the findings were interpreted.

Results:

Out of 85 Staphylococcal isolates, the special resistant determinants of Methicillin Resistance and / or Inducible Clindamycin Resistance were seen only with Coagulase Positive Staphylococci. Inducible Clindamycin Resistance was seen more frequently i.e. in 23.75% cases. (Table 1)

Table 1: Shows prevalence of Methicillin Resistance and Inducible Clindamycin Resistance in Staphylococci.

	n)	METHICILLIN RESISTANT		INDUCIBLECLINDAMYCIN RESISTANCE	%
COAGULASE +VE STAPH	0	14	7.50	19	3.75
COAGULASE NEG STAPH		0		0	

The association of two Resistant Determinants viz. Methicillin Resistance and Inducible Clindamycin Resistance showed that they co-exist in about 5 per cent of the isolates. Their association is shown in Table 2.

Table 2: Shows Simultaneous and individual presence of Methicillin Resistance and Inducible Clindamycin Resistance in Coagulase Positive Staphylococci.

RESISTANT DETERMINANT	NO. POSITIVE	PERCENT
Only MR	10	12.50
Only ICR Positive	15	18.75
MR & ICR Positive	4	05.00
MS & ICR Negative	51	63.75
Total	80	100

Discussion:

Antimicrobial agents such as erythromycin (a macrolide) and clindamycin (a lincosamide) inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells. Gram-positive bacteria are among the most common pathogens that cause skin and soft tissue infections[10]. Clindamycin is a good alternative to penicillin for these infections in penicillin-allergic patients. Resistance to both of these antimicrobial agents may develop in staphylococci through methylation of their ribosomal target site. Erm genes are usually involved in such resistance[11]. The erm gene produces a ribosome methylase that is usually under-expressed. These strains are erythromycin resistant since erythromycin induces the development of this methylase, but mutations in the promoter region of erm enable methylase production without an inducer[12]. These mutants are erythromycin and clindamycin resistant for a long time. Since erythromycin resistance can be caused by a variety of mechanisms (including efflux pumps and enzymatic modification), identifying inducible resistance that could lead to mutational clindamycin constitutive resistance is critical[13]. Macrolide resistance can also be caused by efflux, which is usually regulated by the msrA gene. Another resistance mechanism, chemical inactivation of lincosamides (mediated by the inuA gene), appears to be uncommon. Resistance to erythromycin, clindamycin, and streptogramin B15 is caused by the target site alteration process, also known as macrolide-lincosamide-streptogramin B (MLSB) resistance[14]. This process may be constitutive, in which rRNA methylase is generated all of the time, or inducible, in which methylase is produced only when an inducing agent is present. Clindamycin is a poor inducer, but erythromycin is an efficient inducer[15].

Staphylococcus aureus isolates with constitutive resistance are immune to both erythromycin and clindamycin in vitro, whereas isolates with inducible resistance are resistant to both erythromycin and clindamycin. Clindamycin therapy can select for constitutive erm mutants in vivo, resulting in clinical failure. In vitro tests show that isolates with msrA-mediated efflux are both erythromycin resistant and clindamycin susceptible; however, such isolates seldom become clindamycin resistant during therapy[16]. Clindamycin has had a few clinical failures due to the development of resistance. Clindamycin has also been reported to be effective in treating patients with D-test-positive isolates. Clinical failures have been recorded, as well as the emergence of resistance[17]. It would be useful to know the prevalence of inducible resistance in clindamycin-erythromycin discordant bacteria in order to prevent poor clinical results while maintaining the efficacy of clindamycin. Geographic location, patient age, bacterial species, and bacterial susceptibility profile all affect the prevalence[18].

The D-test was designed to detect potential clindamycin resistance such that potentially unsuccessful therapy is not initiated when

traditional tests indicate clindamycin MICs within the susceptible range (0.5 g/ml). The erm gene has molecular markers, but they are expensive and cumbersome to use on a regular basis. The D-test is simple to administer and interpret, as well as reproducible and inexpensive, but it is still not widely used[19]. Clindamycin is a popular treatment for skin and bone infections due to its tolerability, low cost, oral shape, and good tissue penetration. Clindamycin clinical failures are uncommon due to the high prevalence of D-test positivity. It could take time for a mutant strain to emerge, and the virus could already be under control thanks to the immune system[20]. Clindamycin may be used less frequently now that new agents active against gram-positive bacteria have been created. Finally, although a D-test-positive isolate can mutate during treatment, the rate of mutation in clinical infections is uncertain and may be uncommon[21].

Another significant feature of drug resistance in Staphylococci is the presence of MRSA. MRSA was found in 14 (17.5%) of the S. aureus isolates in this study. MRSA has a wide range of prevalence. Our MRSA prevalence rate matches that of Majumdar from Assam, Vidhani from Delhi, and Anupurba from Uttar Pradesh, who all recorded prevalence rates from this subcontinent. It is critical to classify MRSA strains because treatment options for MRSA vary greatly, and it is also critical to eliminate the strain because it is likely to be a problematic nosocomial pathogen[22]. Inducible clindamycin resistance, as measured by the D-test, was found in 19 (23.75 percent) of the 80 Staphylococcus aureus isolates tested in this study. Inducible clindamycin resistance in Staphylococci has been identified by Ajantha et al. at 63 percent, Yilmaz et al. at 21.09 percent, and Feibelkorn et al. at 50 percent. It is critical to identify inducible clindamycin resistance in staphylococcal isolates that are immune to erythromycin; otherwise, patients can be given clindamycin unnecessarily, with little therapeutic benefit[23]. The D-test used in this study is simple, straightforward, and cost-effective, and it can be performed in any laboratory with a moderate level of equipment[24]. As a result of the findings in this report, it appears that a variety of factors play a role in conferring antimicrobial resistance in Staphylococcus, either alone or in combination. As a result, the organism must be isolated from clinical specimens and its antimicrobial susceptibility pattern studied[25]. It is also essential to assess the various factors and mechanisms by which it acquires antimicrobial resistance in order to choose the most effective antimicrobial agent for therapy and develop a strategy for the eradication of drug-resistant problematic Staphylococci strains.

Conclusion:

17.50 percent of Coagulase positive Staphylococci are MRSA, while none of the Coagulase negative Staphylococci is coagulase negative. According to D-Test, inducible Clindamycin resistance is found in 23.75 percent of isolates. All of the staphylococci strains are Coagulase positive. Methicillin resistance and Inducible Clindamycin resistance were found together in 4 (5.00%) isolates, suggesting that such strains can be problematic if they cause infections, especially hospital-acquired infections, as they will be resistant to a wide range of antibiotics. As a result, the organism must be isolated from clinical specimens and its antimicrobial susceptibility pattern studied. It is therefore essential to assess the various factors and methods by which it acquires antimicrobial resistance.

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