INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATES AND ITS CORRELATION WITH MULTIDRUG RESISTANCE: A STUDY FROM NORTH INDIA

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ABSTRACT

CONTEXT

In *Staphylococcus aureus* strains with inducible resistance antibiotic therapy with clindamycin may select constitutive *erm* gene mutants in vivo leading to clinical failure. Therefore, it is imperative to do a simple 'D-test' to detect isolates with inducible clindamycin resistance. Very few studies are available to state whether or not this inducible resistance has any correlation with multidrug resistance.

AIMS

To find out the prevalence of inducible clindamycin resistance amongst *S. aureus* isolates from various clinical specimens and determine its correlation with multidrug resistance.

SETTINGS AND DESIGN

A prospective study done in the Department of Microbiology in a tertiary care hospital for a period of ten months.

METHODS AND MATERIAL

All strains of *S. aureus* isolated from various clinical specimens were subjected to Antibiotic Susceptibility Testing as per CLSI guidelines and methicillin resistance was detected using cefoxitin. All the erythromycin resistant and clindamycin susceptible isolates were further subjected to 'D-Test'.

STATISTICAL ANALYSIS USED

Data was entered in Microsoft excel and analysed using SPSS version 16. Frequencies and proportions were done. Chi square test was the test of significance.

RESULTS

Out of 1153 *S. aureus* strains isolated 49.8% were methicillin resistant and 50.2% were methicillin susceptible. Prevalence of inducible phenotype, MS phenotype and constitutive phenotype was 18.4%, 22.1%, 18.4% respectively. Resistance to penicillin, amoxicillin-clavulanic acid, ciprofloxacin, sulphamethoxazole-trimethoprim was 92.1%, 47.8%, 43.4% and 49.3% respectively, whereas none of the isolates showed resistance to vancomycin, teicoplanin and linezolid. About 22.8%, 41.6% and 82.9% isolates with inducible phenotype, MS phenotype and constitutive phenotype respectively were multidrug resistant.

CONCLUSION

Prevalence of multidrug resistance is least likely with inducible clindamycin resistance than with other phenotypes.

KEYWORDS

Staphylococcus Aureus, Inducible Phenotype, Multidrug Resistance.

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INTRODUCTION

Methicillin resistance in *Staphylococcus aureus* (*S. aureus*) a recognized nosocomial and community acquired pathogen worldwide is an escalating problem today.¹ The Macrolide-Lincosamide-Streptogramin B (MLS_B) family of antibiotics serve as an alternative therapeutic agent to treat such *S. aureus* infections with clindamycin being the preferred agent.²

However, the widespread use of MLS_B antibiotics has led to an increase in the number of staphylococcal strains acquiring resistance to these antibiotics as well.^{3,4} A common mechanism by which staphylococcal strains acquire resistance to MLS_B antibiotics is the target site modification mediated by *erm* gene products.⁵ The *erm* gene can be expressed either constitutively (Constitutive MLS_B phenotype) or inducibly (Inducible MLS_B phenotype), where the gene product is either produced always or only in the presence of an inducing agent like erythromycin respectively. Another mechanism of resistance that affects only macrolides and type B streptogramins is by efflux by *msr A* gene (MS phenotype).^{6,7,8} In vitro, *S. aureus* isolates with constitutive resistance show resistance to erythromycin and clindamycin and isolates with inducible resistance are resistant to erythromycin, but appear susceptible to clindamycin.

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In vivo antibiotic therapy with clindamycin may select constitutive erm gene mutants.⁹ which may lead to clinical failure.^{10,11,12} Staphylococcal isolates with msr A-mediated efflux mechanism also appear erythromycin resistant and clindamycin susceptible by in vitro tests, but such isolates do not become clindamycin resistant in vivo. A simple in vitro test known as disk diffusion induction test (D-Test) can distinguish between the staphylococcal strains with inducible erm-mediated resistance from those with msr A mediated resistance (Figures: 1 & 2).^{2,10,13} In vitro routine susceptibility tests may fail to detect inducible clindamycin resistance, thus necessitating the need to detect such resistance by the 'D-Test' on a routine basis, so as to ensure safe and effective use of clindamycin. Because very few studies have been done correlating inducible clindamycin resistance and multidrug resistance, this study was done to find out the prevalence of inducible clindamycin resistance amongst *S. aureus* isolates from various clinical specimens and determine its correlation with multidrug resistance.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology in a tertiary care hospital for the duration of 10 months and included all isolates of *S. aureus* isolated from various clinical specimens like pus, urine, wound swab, aspirates, blood, respiratory secretions and sterile fluids, etc. (Figures: 3 & 4). The isolates were first identified as *S. aureus* by standard biochemical techniques.¹⁴ and then subjected to Antibiotic Susceptibility Testing by Kirby Bauer's disk diffusion method on Mueller Hinton Agar plates using erythromycin (15µg), penicillin (10U), clindamycin (2µg), vancomycin (30µg), teicoplanin (30µg), amoxicillin-clavulanic acid (20/10µg), ciprofloxacin (5µg), linezolid (30µg) and sulphamethoxazole-trimethoprim (1.25/23.75µg) as per CLSI guidelines.¹⁵ Methicillin resistance was detected using cefoxitin (30µg) on Mueller Hinton Agar supplemented with 2% NaCl followed by incubation at 35°C. An inhibition zone of 21mm or less around Cefoxitin disk indicated methicillin resistance.¹⁵ All the erythromycin resistant and clindamycin susceptible isolates were further subjected to 'D-Test' as per the CLSI guidelines by placing an erythromycin (15µg) disk at a distance of 15mm (Edge to edge) from clindamycin (2µg) disk on a Mueller Hinton Agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at 37°C, if there is (D shaped) flattening of zone of inhibition around clindamycin disk in the area between the two disks, it indicates inducible clindamycin resistance (Figure: 1).¹⁵

RESULTS

A total of 1153 *S. aureus* strains were isolated and included in the study with maximum isolation from pus, wound swabs, tissue and other pyogenic samples 63% (726) followed by blood 29.3% (338) and urine samples 4.7% (54) etc. (Figure: 4). Of these 1153 strains, 49.8% (574) were methicillin resistant and 50.2% (579) were methicillin susceptible. On antibiotic susceptibility testing, 59% (680) isolates were erythromycin resistant which were then subjected to D-Test, where 212 (18.4%) isolates showed Inducible Phenotype, 255 (22.1%) isolates showed MS Phenotype and 212 (18.4%) isolates showed Constitutive Phenotype. Resistance pattern to different antibiotics tested was as follows:

Percentage of isolates resistant to penicillin, amoxicillin-clavulanic acid, ciprofloxacin, sulphamethoxazole-trimethoprim were 92.1%, 47.8%, 43.4% and 49.3% respectively. None of the isolates showed resistance to vancomycin, teicoplanin and linezolid. About 22.8%, 41.6% and 82.9% isolates with Inducible phenotype, MS phenotype and Constitutive phenotype respectively were multidrug resistant based on the resistance of an isolate to three or more than three class of drugs.

Fig. 1: Inducible MLSB Phenotype: Flattening of the Clindamycin Zone of inhibition Adjacent to Erythromycin Disk (D-Test positive)

Fig. 2: MS Phenotype: No Blunting of Zone of Inhibition around Clindamycin adjacent to Erythromycin Disk (D-Test negative)

Fig. 3: Distribution of total samples processed

Fig. 4: Percentage distribution of *Staphylococcus aureus* Isolates from various Clinical Samples

DISCUSSION

S. aureus is an important nosocomial and community acquired pathogen worldwide,¹ which can cause both superficial and deep pyogenic infections as well as a number of toxin mediated illnesses.¹⁶ With the emergence of methicillin resistance in *S. aureus*, MLS_B family of antibiotics serve as an alternative therapeutic agent with Clindamycin being the preferred agent.² erm gene expression leading to ribosomal target site modification leads to resistance to these MLS_B group of drugs including Clindamycin. This erm gene can be expressed either constitutively or inducibly. In vitro, *S. aureus* isolates with constitutive resistance are resistant to Erythromycin and Clindamycin and isolates with inducible resistance are resistant to Erythromycin, but appear susceptible to Clindamycin; however, in vivo antibiotic therapy with Clindamycin may select constitutive erm gene mutants,⁹ which may lead to clinical failure,^{10,11,12} thus necessitating the need to detect such resistance by a simple 'D-Test' on a routine basis, so as to ensure safe and effective use of Clindamycin, only in those patients with truly susceptible strains.

In the present study we included 1153 *S. aureus* isolates, out of which 49.8% isolates were Methicillin resistant and 50.2% were Methicillin susceptible. The findings were in concordance with the observations made by other authors like Deotale et al. (2010).³ Gadepalli et al. (2006).¹⁷ Yilmaz et al. (2007).¹ and Azap et al. (2005).¹⁸ Unlike our study, some authors have reported a higher prevalence of MRSA in their studies like Velvizhi et al. (2011).¹⁹ Fasih et al. (2010).²⁰ In comparison to these studies, the prevalence of MRSA in our hospital appears to be low. The prevalence of *S. aureus* isolates with Inducible phenotype in our study was 18.4%. Similar results were obtained by Lyall et al. (2013).²¹ where they found 17.2% prevalence of *S. aureus* isolates with Inducible phenotype. Other studies by various authors like Yilmaz et al. (2007).¹ Aleksandra et al. (2014).²² Reddy et al. (2012).²³ and Gadepalli et al. (2006).¹⁷ showed the prevalence of *S. aureus* isolates with Inducible phenotype as 19.8%, 16.2%, 20.6% and 21% respectively.

In the present study, we found that the maximum resistance in *S. aureus* isolates was against Penicillin 10U (92.1%) followed by Amoxicillin-Clavulanic acid 20/10µg (47.8%) and Ciprofloxacin 5µg (43.4%).

However, all (100%) the isolates were susceptible to Vancomycin 30µg, Teicoplanin 20µg and Linezolid 30µg. The resistance pattern did not vary with different phenotypes.

But resistance to different antibiotics was significantly more amongst MRSA isolates than MSSA isolates. Similar

results were found in a study done by Reddy et al. (2012).²³ The prevalence of multidrug resistance amongst different phenotypes was as follows: 22.8%, 41.6% and 82.9% in isolates with Inducible phenotype, MS phenotype and Constitutive phenotype respectively. So the prevalence of multidrug resistance was least likely with inducible clindamycin resistance than with other phenotypes. We found 18.4% prevalence of *S. aureus* isolates with inducible phenotype amongst the total *S. aureus* isolates obtained. Labelling all in vitro erythromycin resistant and clindamycin susceptible strains as clindamycin resistant will prevent the use of clindamycin in treating those Staphylococcal infections, which would have responded to clindamycin therapy.

On the other hand, if D-Test is not done to identify these inducible phenotype strains, we would misidentify them as clindamycin susceptible on the basis of in vitro sensitivity testing, ultimately leading to therapeutic failure. Therefore, performing D-Test on routine basis is essentially the need of the hour to differentiate between strains with Inducible phenotype and MS phenotype, so as to prevent irrational use of drugs, thereby preventing drug resistance. Hence, this simple, cheap, easy to perform in vitro D-test can be incorporated as a part of routine antibiotic susceptibility testing, so that the isolates with inducible phenotype can be identified well in time.

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