Inducible Clindamycin Resistance in Staphylococcus Species

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ABSTRACT

Objective: To determine the frequency of inducible clindamycin resistance in clinical isolates of Staphylococcus species by phenotypic D-test.

Study Design: Observational study.

Place and Duration of Study: Ziauddin University Hospital, Karachi, from July to December 2011.

Methodology: Consecutive clinical isolates of Staphylococcus species were collected and identified by conventional microbiological techniques. Antimicrobial susceptibility testing and inducible clindamycin resistance was carried out by performing D-test using CLSI criteria. Methicillin resistance was detected by using Cefoxitin disk as a surrogate marker. Statistical analysis was performed by SPSS version-17.

Results: A total of 667 clinical isolates of Staphylococcus species were obtained during the study period. In these isolates, 177 (26.5%) were Staphylococcus aureus, and 490 (73.5%) were coagulase negative Staphylococci. The total frequency of inducible clindamycin resistance among isolates of Staphylococcus species was 120/667 (18%). Frequency of inducible clindamycin resistance among coagulase negative Staphylococcus group and Staphylococcus aureus group were 18.57% and 16.38% respectively. Median age of patients in D-test positive group was 19.5 (1 - 54) years.

Conclusion: The frequency of inducible clindamycin resistance among Staphylococcus species may differ in different hospital setup. Clinical microbiology laboratories should implement testing simple and effective D-test on all Staphylococcus species. D-test positive isolates should be reported clindamycin resistant to decrease treatment failure.

Key Words: Inducible clindamycin resistance, Staphylococcus. Phenotype, D-test positive staphylococcus isolates.

INTRODUCTION

Staphylococci are Gram-positive bacteria, which form grape-like clusters. Almost 30% of the normal healthy population is affected by Staphylococcus aureus (S. aureus) as it asymptomatically colonizes human hosts.¹ S. aureus is the common cause of various diseases including: mild skin infections (impetigo, folliculitis, etc.), invasive diseases (wound infections, osteomyelitis, bacteremia with metastatic complications, etc.), and toxin mediated diseases (food poisoning, toxic shock syndrome, scaled skin syndrome, etc.). Common superficial infections include carbuncles, impetigo, cellulitis, and folliculitis. Community-acquired infections include bacteremia, endocarditis, osteomyelitis, and pneumonia.² Staphylococcus species are the major cause of hospital-acquired infections. Foreign bodies, such as sutures, indwelling catheters, and implanted joints are extremely susceptible to Staphylococcus epidermidis (S. epidermidis) colonization, and often serve as the point of entry for infection.

By forming biofilms, S. epidermidis is resistant to antibiotics, and can serve as a reservoir for antibiotic resistant genes that can be transferred to other bacteria.³ The first serious emergence of antibiotic resistant Staphylococcus occurred with a specific strain referred to as Methicillin-Resistant Staphylococcus aureus (MRSA).⁴ This strain expressed a modified penicillin-binding protein encoded by mecA gene and is present in 4 forms of Staphylococcus cassette.⁴

Clindamycin is considered as a useful alternate agent in penicillin-allergic patients in the treatment of skin and soft tissue infections caused by S. aureus. It has an excellent tissue penetration, accumulates in abscesses, and no dosage adjustments are required in the presence of renal disease. The good oral absorption of clindamycin makes it an alternative option for use in outpatients or as follow-up treatment after intravenous therapy (de-escalation).⁵

Target site modification is the most common mechanism of acquired resistance to macrolides, lincosamides, and streptogramin B (MLS₉) antibiotics in Staphylococci and confers cross-resistance to the MLS antibiotics.⁶ MLS₉ resistance can be either constitutive or inducible, if it is inducible, bacteria often test resistant to Erythromycin (E) but susceptible to Clindamycin (DA).⁷ When the disk diffusion test is used to determine susceptibility, a distorted “D-shaped” zone of inhibition is observed around DA if an E disk is placed nearby.⁷ Although isolates appear susceptible to DA in the absence of an inducing agent, there is widespread reluctance to prescribe DA for treatment of patients with infections caused by such organisms because of concerns that resistance to DA may cause treatment failure in vivo.⁸
This study was conducted to investigate the frequency of inducible clindamycin resistance in Staphylococcus species to assess the resistant pattern.

METHODOLOGY

This observational study was conducted over a period of 6 months from July to December, 2011 in the Department of Clinical Microbiology of Ziauddin Medical University Hospital, Karachi. Six hundred and sixty seven consecutive clinical isolates of Staphylococcus species were collected from different clinical samples by convenient sampling and included in the study. Sources were blood, respiratory secretions, wound swabs, central venous pressure (CVP) lines tips, and pus. All the duplicate isolates were excluded from the study. Written approval from the institutional ethical committee was obtained. Informed consent was taken from either the patient or any other patient's relative.

Clinical samples were received in a sterile container or in an Amies transport medium supplied from the Microbiology laboratory. These samples were processed and incubated at 37°C in ambient air for 24 - 48 hours, using standard microbiological techniques. Staphylococcus species including S. aureus were identified using conventional techniques (colony morphology, gram staining, catalase test, coagulase test, mannitol salt agar, and DNase test).

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (MHA) medium (Oxoid Ltd., England) using modified Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) 2010 guidelines. Detection of inducible clindamycin resistance was carried out by performing D-test using CLSI 2010 guideline. A 0.5 McFarland equivalent suspension of organism was inoculated onto a MHA plate, the E (15 ug) disk was placed 15 - 26 mm (edge-to-edge) apart from DA (2 ug) disk on this MHA plate. The exact distance between the two disks (edge-to-edge) was 20 mm apart on most of the occasions. The plates were incubated overnight at 37°C in an ambient air incubator. After incubation, isolates showing rounded zones of inhibition with diameter of ≤ 13 mm for E and ≥ 21 mm for DA were interpreted as negative for inducible resistance (D-test negative). Isolates with similar inhibitory zones of inhibition as above but a D-shape zone around DA, were interpreted as positive for inducible resistance (D-test positive) as shown in Figure 1.

A research proforma was used to document the essential data including age and gender. Data analysis was performed by using Statistical Package for Social Sciences (SPSS) version-17. Frequencies and percentages were computed for presentation of all categorical variables like microorganisms, gender and D-test positivity. Median Inter-quartile range (IQR) is reported for median age of patients.

RESULTS

A total of 667 clinical isolates of Staphylococcus species were obtained during the study period. Distribution of isolates of Staphylococcus species from different clinical samples are shown in Figure 2. In these 667 isolates, 177 (26.5%) were S. aureus, and 490 (73.5%) were coagulase negative Staphylococci (CoNS). Predominantly, the isolates were from male patients 361/667 (54.1%), while isolates from female patients were 306/667 (45.9%). Male to female ratio was 1.17:1. In total, the frequency of inducible clindamycin resistance among isolates of Staphylococcus species is 120/667 (18%). Out of these 120 D-test positive isolates, 91 were...
CoNS and 29 were S. aureus. Frequency of inducible clindamycin resistance among CoNS group was 91/490 = 18.57% and S. aureus group was 29/177 = 16.38%. In total 363/667 (54.4%) isolates of Staphylococcus species were resistant to methicillin. Among the isolates of CoNS 239/490 (48.77%) were methicillin sensitive and 251/490 (51.22%) were methicillin resistant. Among the isolates of S. aureus 65/177 (36.72%) were methicillin sensitive and 112/177 (63.27%) were methicillin resistant.

Median age (IQR) of patients in D-test positive group was 19.5 (53) years versus 30 (56) years for patients in D-test negative group.

**DISCUSSION**

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and the emergence of multi-drug resistant organisms. The emergence of resistant to multiple antibiotics among gram-positive cocci has left very little therapeutic options for clinicians.

The increased frequency of Staphylococcal infections, combined with resistant to antimicrobial agents which are used to treat Staphylococcal infections, have caused a great deal of problem in therapeutic treatment of skin, soft tissues as well as other organs infected by Staphylococci. Clindamycin which is a Lincosamide has long been an attractive option because of its efficacy against Methicillin Sensitive Staphylococcus aureus (MSSA) and MRSA for its good bone marrow and tissue penetration and potential antitoxin effects.11 Clindamycin resistance among the isolates of S. aureus, 16 Fasih et al. from Iran documented 35% inducible clindamycin resistance among the isolates of S. aureus.19 These results indicate that inducible clindamycin resistance phenotype may vary in different hospital setups.

With respect to methicillin resistance in S. aureus isolates, the present results are also different from other authors. Vivek et al., Fasih et al., and Seifi et al. reported 32.5%, 36%, and 41.7% methicillin resistance in the isolates of S. aureus respectively.20,18,21 Moreover, Cetin et al. from Turkey reported a very high percentage of methicillin resistance (91%) among the isolates of S. aureus.22 This indicates the overall non-judicious use of cloxacillin in these setups.

There is a possibility that Staphylococcal strains may be inducible resistant to MLSB antibiotics, if erythromycin resistance and clindamycin susceptibility is detected in these strains by standard disc diffusion method. This inducible resistance can easily be missed by clinical laboratory, if erythromycin and clindamycin discs are not placed at adjacent positions by using standard disc diffusion test. One of the therapeutic options in the treatment of MRSA is considered to be clindamycin.23 However, the lower positivity rate of inducible clindamycin resistance in this study by D-test among both S. aureus and CoNS isolates made us think that clindamycin can be considered as an effective treatment option in D-test negative MRSA and CoNS isolates especially in lower respiratory tract isolates in the situation of aspiration pneumonia where anaerobic cover is also required. Clindamycin also has good anaerobic activity. The proper use of clindamycin in methicillin resistant Staphylococcal species will also reduce the non-judicious use of glycopeptides. This can be achieved by accurate results of antimicrobial susceptibility testing including the application of D-test on a routine basis.

**CONCLUSION**

The frequency of inducible clindamycin resistance among Staphylococcus species may differ in different hospital setups. Clinical microbiology laboratories should implement testing simple and effective D-test on all Staphylococcus species. D-test positive isolates should be reported clindamycin resistant to decrease treatment failure.

**REFERENCES**


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