D-TEST AS A TOOL TO DETECT THE FREQUENCY OF CLINDAMYCIN RESISTANCE IN COMMUNITY ACQUIRED AND HOSPITAL ACQUIRED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS INFECTIONS (MRSA).

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ABSTRACT... Objectives: D-Test as a tool to detect the frequency of clindamycin resistance in community acquired and hospital acquired methicillin resistant Staphylococcus aureus infections. Study Design: A cross-sectional study. Setting: Microbiology department of BMSI, JPMC, Karachi. Period: January 2015 till December 2015. Material & Methods: Pus samples from deep wounds, skin lesions, abscesses, postoperative wounds from surgical, medical wards and OPDs were collected. MRSA testing and susceptibility testing for antibiotics was done according to CLSI2014. The frequency of inducible clindamycin resistance was detected by D-Test of the CA-MRSA and HA-MRSA. Result: In a total of 402 S. aureus isolates, 253 (62.93%) were methicillin-sensitive and 149 (37.06%) were methicillin-resistant. Out of 149 MRSA, 106 (71.14%) were HA-MRSA and 43(28.85%) were CA-MRSA. Among the HA-MRSA, 63(59.8%) were resistant to clindamycin while with D-Test, it increased to 78(73.58%). Out of 43 CA-MRSA, 9 (21.6%) were clindamycin resistant, while with D-Test, the resistance to clindamycin increased to 13 (30.23%). Conclusion: Inducible clindamycin-resistant strains may lead to clindamycin treatment failure in patients with S. aureus infection. Therefore, D-test should be done in priority to detect inducible clindamycin resistance in S. aureus.

Key words: CA-MRSA, Clindamycin, D-Test, HA-MRSA.

INTRODUCTION
Hospital acquired methicillin resistant Staphylococcus aureus (HA-MRSA) is a widespread cause of infection in hospital enrolled patients.¹ Over the years, community acquired methicillin resistant Staphylococcus aureus (CA-MRSA) has also came into view which showed the β-lactam resistance akin to the hospital acquired strains.¹ CA-MRSA which is obtained in the outpatient settings or isolated within 48 hours of hospital admission occurs in a person without a history of prior MRSA infection or colonization. According to CLSI standards, methicillin resistance is defined as zone diameter ≤ 21 mm to a cefoxitin disc diffusion test.² Clindamycin is an important antimicrobial alternative for the cure of Staphylococcus aureus infection.³ This antibiotic has been used as empiric therapy for the intensifying incidence of both hospital and community-acquired methicillin-resistant S. aureus (MRSA).⁴ Resistance to clindamycin in S. aureus is due to its target site modification, which is mediated by erm genes leading to ribosomal methylation.⁵ Resistance may occur either in an inducible or constitutive form. Detection of strains with inducible resistance to clindamycin is difficult in the routine laboratory as erythromycin and clindamycin may show resistance and sensitivity respectively when not positioned next to each other in vitro. Only a small number of clinical laboratories in Pakistan follow suggestions for the detection of inducible clindamycin resistance in MRSA by performing D-Test.⁶

METHODS
After an ethical review statement was given by the relevant committee, this cross sectional study was done. It was conducted in the Microbiology department of Basic Medical Sciences Institute (BMSI) JPMC, Karachi from January 2015 to...
December 2015 after ethical review. Sample size was calculated by Open Epi software https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4864768/. Samples were collected from the medical and surgical departments and OPDs of JPMC after the consent of the patients. Pus from deep wound, skin lesion, abscess, postoperative wounds was sent to Basic Medical Sciences Institute (BMSI), JPMC for processing. Patients with S.aureus infection had at least one of the following risk factors to be included in HA MRSA: (1) the patient is hospitalized in the ward or ICU for more than 48 hours, (2) the patient is hospitalized or had surgery in the preceding year, (3) dialysis in the patient in the previous year, (4) had a cannula or indwelling catheter in the previous year. Persons who do not have any of the HA-MRSA risk factors are considered to have CA-MRSA. Patients on antibiotics or having infection other than S.aureus were excluded.

PROCEDURE
After taking the consent of the patients, samples were collected by using a sterile cotton-wool swab from the infected site and were inoculated onto blood agar, MacConkey agar, mannitol salt agar medium and 37°C incubation temperature for 24 to 48 hours. Isolation of staphylococcus aureus was done from clinical specimens on the basis of Gram staining, morphology, catalase tests, coagulase test, mannitol fermentation. After comparing the bacterial suspension to 0.5 McFarland standard suspension, the antibiotic susceptibility testing was done. Kirby-Bauer disc diffusion method was used to detect methicillin resistance, using cefoxitin (30µg) disc according to Clinical and Laboratory Standards Institutes (CLSI) guidelines. Isolates of MRSA were then tested with erythromycin and clindamycin alone and in combination (induction testing) by disc diffusion by making a lawn culture of bacteria in Muller Hinton Agar (MHA) plates. Clindamycin (2µg) and erythromycin (15µg) discs were put approximately 15 mm apart measured edge to edge. The plate was put in the incubator for 16 to 18 hours at 37°C.

RESULTS
A total of 402 samples of Staphylococcus aureus were taken, in which 253 (62.93%) were methicillin-sensitive and 149 (37.06%) were methicillin-resistant. More males were infected both in the CA and HA S.aureus than females (Figure-1). The mean age of HA-MRSA was higher than CA-MRSA (Figure-2). Out of 149 MRSA, 106 (71.14%) were HA-MRSA and 43 (28.85%) were CA-MRSA. Among the HA-MRSA, 63 (59.8%) were resistant to clindamycin while with D-Test, it increased to 78 (73.58%). Out of 43 CA-MRSA, 9 (21.6%) were clindamycin resistant, while with D-Test, the resistance to clindamycin increased to 13 (30.23%). (Figure-3).

![Figure-1. Gender wise distribution in community acquired and hospital acquired S. aureus infection.](image1)

![Figure-2. Mean age of the patients of staphylococcus aureus infections](image2)
DISCUSSION

Antibiotic susceptibility testing is important for appropriate therapy of infections and for local susceptibility surveillance. For many years, clindamycin was the preferable antibiotic to be used in treating MRSA infections. This study shows that clindamycin might be effective in treating CA-MRSA but should not be used to treat MRSA that are acquired during a hospital stay. True susceptibility results for clindamycin may not be obtained if isolates are not tested for inducible resistance by D-testing. A previous study gave a 70% inducible resistance to clindamycin by D-Test in MRSA. Recognition of this type of resistance is important because treatment of patients who harbor MRSA with inducible clindamycin resistance may lead to constitutive resistance, subsequently leading to therapeutic failure. In our study, 21.6% of S. aureus were CA-MRSA and similar findings were reported by Ahmad which showed 27.8%. HA-MRSA were 52.7% which is less than a study done in Egypt, which showed 76.6% of HA-MRSA. CA-MRSA was more in young patients, with mean age of 29.05 years, which is comparable to a study done in Pakistan. HA-MRSA was seen in older patients, with mean age of 57.7 years. Males had a greater proportion of MRSA, both in the community as well as hospital (72.6% and 67.66%) because of factors like working in overcrowded places, more skin infections and more exposure to the hospital environment. Without performing the D-test, resistance to clindamycin was 21.6% and 59.8% in CA-MRSA and HA-MRSA respectively, which is higher than the study done in Iran which showed 6.4% in CA-MRSA. When D-test was performed, this resistance to clindamycin increased to 30.23% and 73.58% in CA-MRSA and HA-MRSA, showing a 9.30% and 14.15% rise in inducible resistance, respectively, also supported by study conducted in Pakistan. Inducible clindamycin resistance was found to be more among HA-MRSA than CA-MRSA.

CONCLUSION

The present study thus concludes that MRSA with inducible resistance to clindamycin is present in our community as well as in our hospitals. This study has shown the significance of performing the D-test for the presence of clindamycin resistance in MRSA.

RECOMMENDATION

The D-test is simple and low-cost method to detect inducible clindamycin resistance thus preventing treatment unsuccessful. Therefore, this test should be made binding in routine work in the Microbiology laboratory.

ACKNOWLEDGEMENT

We are thankful to the Department of BMSI, JPMC Karachi, Pakistan for the permission of samples collection and their expert technical assistance. Special thanks to Mr. Nisar Usmani and Ejaz Munawer Ali for IT assistance.

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**AUTHORSHIP AND CONTRIBUTION DECLARATION**

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