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Research Article

D-Test Method for Inducible Clindamycin Resistance in Staphylococcus Aureus from Clinical Samples of Children at a Tertiary Care Hospital

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ABSTRACT

Background: Inducible clindamycin resistance in Staphylococcus aureus is an evolving problem, which may cause treatment failure if not routinely detected by the D-test.

Objective: To investigate the frequency of inducible clindamycin resistance in Staphylococcus aureus from various clinical specimens by D-Test.

Methodology: The cross-sectional study was conducted from September 2023 to December 2023 in the Department of Microbiology, Children Hospital and University of Child Health Sciences, Lahore. The samples were taken and cultured on blood agar and MacConkey agar. Gram staining, catalase and coagulase tests were performed for the identification of Staphylococcus aureus (S.aureus). Isolates from all the samples were tested for antibiotic sensitivity by using Kirby Bauer's disc diffusion method. The cefoxitin (30 µg) disc was used to detect methicillin-resistance. D-Test was conducted by putting the discs of Clindamycin and Erythromycin at a distance of 15-26mm (edge to edge) on Mueller Hinton agar and D-Test results were recorded. SPSS-23 was used for statistical analysis. The frequencies and percentages were computed; Chi square test and Fisher's Exact Test were used to find the association with p value of <0.05 considered significant.

Results: Out of 164 Staphylococcus aureus 117(71.3%) were Methicillin resistant Staphylococcus aureus (MRSA) and 47(28.7%) were Methicillin sensitive Staphylococcus aureus (MSSA). Out of 164 Staphylococcus only 14(8.5%) were D-Test positive i.e.inducible clindamycin resistance. Of these 14 D-Test positive 12(85.7%) were MRSA and only 2(14.3%) were MSSA

Conclusion: Inducible clindamycin resistance is seen in Staphylococcus aureus, mostly MRSA. D-Test should be included as part of routine microbiology laboratory practice to get an idea about efficacy of Clindamycin in infections caused by S.aureus.

Keywords: Inducible, Drug Resistance, Methicillin-Resistant Staphylococcus Aureus (MRSA), Staphylococcus Aureus.

INTRODUCTION

Staphylococcus aureus(S.aureus) is a human pathogen that causes a wide range of infections in human, particularly children.[1] The increasing resistance rates of S.aureus, especially, the methicillin resistant S.aureus(MRSA) strains call for the need to effectively employ diagnostic methods for treatment decisions.[2] Among the issues that have been used to treat cases of S. aureus is the development of clindamycin resistance, namely, inducible clindamycin resistance (iMLSB), that may cause a treatment failure in case of its absence.[3,4] It is suggested that Clindamycin is used as an alternative therapeutic agent when beta-lactam antibiotics do not work, but its therapeutic effect can be neutralized by mechanisms of resistance that cannot be identified during routine testing. [5]

The double-disk diffusion (also D-test) has emerged as one of the major tests in the diagnosis of iMLSB in clinical isolates. This method determines the inducible resistance of bacteria isolates, which is concomitant exposure to erythromycin and clindamycin by measuring the typical D-shaped zone of inactivity.[6] A case study undertaken at Bharatpur Hospital, Nepal found the prevalence of iMLSB at 15.24%, which also highlights the need to use the D-test in clinical microbiology laboratories. [3] The inability to identify such resistance may cause the wrong use of clindamycin, which causes amplified morbidity and higher functional expenses. [7]

The aim of the study is to explore the prevalence of inducible resistance to clindamycin in clinical isolates of S. aureus on children in a tertiary care hospital. With the usage of the D-test, we want to clarify the pattern of resistance that could be used in clinical practice and help to provide better patient outcomes and, thus, bridge a significant gap in the current literature on pediatric S. aureus infections. [8,9]

METHODOLOGY

The present descriptive cross-sectional study was carried out in the Department of Microbiology, Children's Hospital and University of Child Health Sciences, Lahore between September 2023 and December 2023. S.aureus strains isolated from pus, wound swabs, cerebrospinal fluid (CSF), blood, nasal swabs, ear swabs, throat swabs and pleural fluid of children were included in the study. Samples were obtained from both admitted patients and those presenting to the outpatient department. A convenient sampling technique was used. The sample size was calculated using the single proportion formula:

$n = Z^2p(1-p)/d^2$

Where Z=1.96

Margin of error d=5%=0.05

Expected prevalence P=12.15%=0.1215[10]

n= 164

Clinical isolates identified as S.aureus from pediatric patients and specimens received in the microbiology laboratory were included in the study. Duplicate samples from the same patient and non-S. Aureus isolates were excluded. Relevant clinical information (ward, and specimen type) was recorded using a structured, self-designed proforma. Samples from wards and OPD were received in the laboratory. All samples were processed at 20-37°C. After getting approval from IRB clinical isolates of Staphylococcus aureus which fulfilled the criteria were included in the study. Clinical data was collected including name, medical record number, ward and specimen (pus, pus swab, wound swab, ear swab, nasal swab, CSF and blood). The specimens were cultured on MacConkey and blood agar and then incubated at 35±2°C for 24 hours. Yellow β-hemolytic colonies were obtained on blood agar but no growth was seen on MacConkey agar. For identification gram staining was performed as follow. A clean glass slide was taken and colony of test organism was emulsified in a drop of saline the smear was air dried and fixed in the hot air oven. Then smear was covered with crystal violet (primary stain) for 60 seconds and rinsed with water, then treated with iodine for few seconds. In the next step, smear was treated with decolorizer for few seconds and rinsed immediately with water. Then safranin was applied as a counter stain for 1.5 minutes and rinsed with tap water. The slide was dried and a drop of oil was placed on the smear. Then it was examined under the microscope by using 100X objective (oil immersion) lens. Gram staining showed purple gram-positive spherical cocci which were in clusters resembling bunch of grapes. For the confirmation of Staphylococcus aureus catalase and coagulase 12 tests were performed. The positive result of the catalase test confirmed Staphylococcus species and the positive result of the coagulase test confirmed Staphylococcus aureus species. The Kirby-Bauer disk diffusion method was used to determine the antibiotic susceptibility of the isolates as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) - 2023. 4-5 isolated colonies that showed similar morphology were taken and suspended in 4-5 ml sterile saline solution adjusted to the turbidity equivalent to 0.5 McFarland standard, suspension was swabbed on the entire Muller Hinton Agar (MHA) plates three times; turning the plate 60° between streaking to obtain even inoculation. With the help of multi-dispenser, commercially available antibiotic discs were added within 15 minutes. Clindamycin and Erythromycin discs were placed manually at the distance of 15-26mm apart(end-to-end) on Mueller Hinton agar and the plate was incubated at 35±2°C for 16-20 hours. Methicillin resistance was determined by using Cefoxitin disc diffusion test as per CLSI guidelines. Next day the zone of inhibition of antibiotics is measured. Flattening of the zone of inhibition of Clindamycin adjacent to Erythromycin was carefully observed. If the

zone of inhibition of Clindamycin had a flattening at the edge towards Erythromycin disc, this was indicative of positive D-Test; Clindamycin resistance is induced by Erythromycin.

The data was entered and analyzed by statistical package SPSS version 26.0. Categorical variables, such as the distribution of Staphylococcus aureus isolates from the different sample types, rate of Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin sensitive Staphylococcus aureus (MSSA), and the D-test results, were expressed as frequencies and percentages. Where the expected number of cases in a cell was <5 Fisher's Exact Test was used. A p-value < 0.05 was considered statistically significant. Results were presented in tabular form for clarity.

Ethical approval for the study was obtained from the Institutional Review Board of School of Allied Health Sciences, The Children's Hospital and University of Child Health Sciences, Lahore (No.1266/SAHS).

RESULTS

Table 1 presents the distribution of S.aureus among various clinical samples. Most of the isolates were from blood (42.1%), pus (28%), wound swabs (12.2%) and ear swabs (9.1%). A few were obtained from nasal swabs, blood, CSF, pleural fluid and axillary abscess.

Table 1: Distribution of Staphylococcus Aureus among Various Clinical Sample

	Specimen	Frequency (%)
1	Pus Swab	46(28%)
2	Wound Swab	20(12.2%)
3	Ear Swab	15(9.1%)
4	Nasal Swab	6(3.7%)
5	Blood	68(42.1%)
6	CSF	3(1.8%)
7	Pleural Fluid	4(2.4%)
8	Axillary Abscess	1(0.6%)

The distribution of (Methicillin Resistance Staphylococcus Aureus) and MSSA (Methicillin Susceptible Staphylococcus Aureus) is shown in Table 2. Of the 164 isolates, 71.3% were MRSA and 28.7% were MSSA.

Table 2: Prevalence of MRSA and MSSA

STRAIN	FREQUENCY (%)
MRSA*	117(71.3%)
MSSA*	47(28.7%)

Table 3 shows the results of D-Test. A mere 8.5% of the isolates showed inducible clindamycin resistance (D-Test positive), while 91.5% are negative.

Table 3: Distribution of Staphylococcus Aureus by D-Test

D-Test	Frequency (%)
Positive	14(8.5%)
Negative	150(91.5%)

Table 4 shows the ward-wise distribution of isolates. The maximum were found from Surgery Ward (18.9%), Emergency Room (14.6%), Outpatient Department (11.0%) and Neonatal Unit (10.4%) with the rest of the wards (ICU, Bone Marrow Transplant, Medical, Cardiac Surgery, Orthopaedic, Nephrology, Gastroenterology, Hematology/Oncology, Plastic Surgery, and Neurology) having relatively fewer numbers.

Table 4: Ward Wise Distribution of Isolates

	Ward	Frequency (%)
1	Medical	10(6.1%)
2	Surgery	31(18.9%)
3	Emergency	24(14.6%)

4	Outpatient Department	18(11.0%)
5	Intensive Care Unit	13(7.9%)
6	Nephrology	5(3.0%)
7	Gastroenterology	6(3.7%)
8	Bone Marrow Transplant	12(7.3%)
9	Neonatal Unit	17(10.4%)
10	Neurology	3(1.8%)
11	Hematology/Oncology	4(2.4%)
12	Orthopaedic	7(4.3%)
13	Plastic Surgery	6(3.7%)
14	Cardiac Surgery	8(4.9%)

Table 5 depicts the cross-tabulation of Staphylococcus aureus strains was done to analyse the relationship between methicillin resistance and inducible clindamycin resistance. Out of the total 116 MRSA isolates, 11 (9.4%) were D-test positive and out of 47 MSSA isolates, 3 (6.4%) were inducible clindamycin resistant. There was a marginally higher percentage of D-test positive in MRSA isolates, but it was not significant. Fisher's Exact Test showed no significant association between methicillin resistance and inducible clindamycin resistance ($p = 0.759$). Likewise, the Chi-square test also did not reveal a significant association ($\chi^2 = 0.391$, $p = 0.532$).

Table 5: Association of MRSA/MSSA with Inducible Clindamycin Resistance (D-Test)

Strain	D-Test Negative N (%)	D-Test Positive N (%)
MSSA	44 (93.6%)	3 (6.4%)
MRSA	106 (90.6%)	11 (9.4%)

Chi-square test: $\chi^2 = 0.391$, $p = 0.532$, **Fisher's Exact Test**: $p = 0.759$

DISCUSSION

The results of the study give the overview of the distribution and antimicrobial resistance of Staphylococcus aureus strains, particularly Staphylococcus aureus methicillin (MLSA)-resistant and inducible clindamycin resistance (iMLSB). The results indicated the isolation of S. aureus from blood (42.1%) and pus (28%) which is in agreement with Chaudhary and Piya who also highlighted the role of blood culture in the diagnosis of S. aureus infections in clinics. [3] It is also well known that pus and wound cultures play an important role in S. aureus, as this is typical of the sites of infection of this organism in hospital patients.[11,12]

MRSA and MSSA have a prevalence of 71.3% and 28.7% respectively. This observation is agreeable to a number of researches done in different geographical areas indicating that MRSA remains a significant issue of public health. In a study conducted by Pandey et al., there were high rates of MRSA in clinical isolates, which indicates an increasing pattern of resistance.[11] MRSA is not very uniform across the different regions, and we find it agreeable that the MRSA has a considerable burden, especially in tertiary care hospitals with potentially suboptimal infection control measures.[13]

The number of isolates with inducible clindamycin resistance is 8.5%. Although this rate is lower than certain reports in which higher cases of iMLSB (up to 24%) are reported[14], it remains a significant issue when it comes to the treatment strategies that involve the use of clindamycin, particularly, as such antibiotics are commonly applied to skin infections and other soft tissue infections. [15] The rate of induced resistance observed suggests that closer attention should be paid to the prescribing practice in relation to antibiotics because the wrong assumptions about the clindamycin susceptibility as the cause of the therapeutic failure have been raised. [16]

Finally, the ward-by-ward distribution of isolates suggests the existence of S. aureus in different clinical environments with the largest percentage in the Surgery Ward (18.9%) and Emergency Room (14.6%). This observation aligns with what has been observed in the literature where surgical wards are usually associated with greater incidences of MRSA infections owing to the invasive conditions of the procedures and the close to dirty areas. [17] The pattern of distribution highlights the fact that critical steps should be implemented in terms of infection control in such high-risk settings in order to reduce the incidence of both MRSA and iMLSB since Sulaiman et al. added

that in their findings, there is a high prevalence of these resistant strains in clinical settings. [18] MRSA strains exhibited a slightly higher inducible clindamycin resistance (9.4%) as compared to MSSA (6.4%) but the difference was not statistically significant, suggesting that methicillin resistance is not related to D-test positive results. Our results are in line with recent reports that have found inducible clindamycin resistance in both MRSA and MSSA with no definite association with methicillin resistance.[19,20]

It has been shown that clonal diversity and resistance genes have a greater impact on the distribution of inducible clindamycin resistance than methicillin resistance. Thus, routine D-testing remains important to guide appropriate clindamycin use in the pediatric patient and prevent treatment failure.

CONCLUSION

In conclusion, the current research indicates a great urgency of continued monitoring of S. aureus resistance patterns especially of MRSA and its effects on children treatment. The inducible clindamycin resistance demonstrated by the D-Test results is relatively low, which is positive but the high prevalence of MRSA demands more stringent infection control interventions and cautious empirical therapy plans to handle such infections. The current study requires more research to clarify resistance trends, treatment outcomes, and optimal practice in the management of infectious diseases among children.

Limitations

Regarding limitations, this study was restricted to single tertiary care hospital and this could limit the generalizability of the results. The sample size is also relatively small and the duration of this study is also small hence limited to observe seasonal variations in resistance. It was used only by phenotypic (D-Test) and there was no molecular evidence of resistance genes. There could also have been selection bias in the form of convenience sampling.

Recommendations

It is recommended that routine use of D-test should be incorporated in diagnostic laboratories to detect inducible clindamycin resistance. These findings are to be confirmed in future studies with bigger multicenter samples and longer period of time. Molecular investigations, including the detection of erm genes, would be required to gain a further insight into the processes of resistance. There should be increased efforts to strengthen antibiotic stewardship programs and inducible resistance made known to clinicians in order to enable rational use of clindamycin in infections in children.

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