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Phenotypic Detection of Inducible Clindamycin Resistance among Clinical Isolates of Staphylococcus aureus in Bharatpur Hospital

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ABSTRACT

Background: Clindamycin is regarded as a reserve drug in the treatment of staphylococcal infections. Among few therapeutic alternatives available for treatment of erythromycin-resistant *Staphylococcus aureus* infections, clindamycin has several advantages but major limitation in its use is the development of resistance resulting in treatment failure. Routine clindamycin susceptibility test may fail to detect such inducible resistance which can be detected by Double disc diffusion test (D-test). The present study was undertaken to determine the incidence of inducible clindamycin resistance among clinical isolates of *S. aureus* in a tertiary care hospital in central Nepal.

Methods: A cross-sectional study was carried out among the patients visiting Bharatpur Hospital from September to November 2019. A total of 1279 clinical samples were examined for the identification of *S. aureus* by standard microbiological procedures. Antibiotic susceptibility testing of the isolates was done by Kirby-Bauer disc diffusion method and all the erythromycin-resistant isolates were subjected to D-test for the phenotypic detection of inducible clindamycin resistance according to CLSI guidelines (2016).

Results: *S. aureus* was recovered from 4.5% (58/1279) samples of which 35 isolates were Methicillin-Resistant *Staphylococcus aureus* (MRSA) and 23 were multi-drug resistant (MDR). Tetracycline was found to be the most effective antibiotic whereas erythromycin was the least effective. D-test revealed that 39.7% isolates showed iMLSB phenotype, 3.5% showed cMLSB phenotype and 56.8% showed MS phenotype. The percentage of inducible and constitutive resistance was seen higher amongst MRSA isolates compared to Methicillin-Sensitive *Staphylococcus aureus* (MSSA) isolates. Incidence of *S. aureus* was found higher among females and in the age group 20-30 years and in pus samples ($p<0.01$).

Conclusions: Routine testing of inducible clindamycin resistance is suggested among the clinical isolates of erythromycin-resistant Staphylococci to avoid treatment failure.

Keywords: Clindamycin resistance; cMLSB, iMLSB; MRSA; *S. aureus.*

INTRODUCTION

Staphylococci are the most abundant colonizer of human body which include coagulase positive *Staphylococcus*, mainly *Staphylococcus aureus* and Coagulase Negative *Staphylococci* (CoNS) group which are recognized as the most common cause of nosocomial and community-acquired infections in every region of the world.[1] *S. aureus* colonizes almost all parts of body and it is believed that almost 20-30% of the population are carriers of *S. aureus*.[2] The increasing prevalence of Methicillin resistance among staphylococci (MRSA) is a global problem with addition of resistance towards other drugs such as cephalosporins, cephems and other beta-lactams.[3] In recent years, community-acquired MRSA (CA-MRSA) strains are rapidly increasing and they are now showing resistance to vancomycin, which was presumed to be the drug of choice for MRSA.[4,5]

Clindamycin is mostly used in the treatment of MRSA but can also be used in the case of MSSA infections.[6] Clindamycin, a semisynthetic drug and protein synthesis inhibitor, developed in 1966, is capable of decreasing toxin production and increase microbial opsonization and phagocytosis at subinhibitory concentrations. It is used in treatment of skin and soft tissue infections, abscesses, decubitus ulcers, osteomyelitis, head and neck, pleuropulmonary, abdominal and pelvic infections besides being an alternative in penicillin allergic patients.[6,7] Macrolide resistance in *S. aureus* occurs due to active efflux mechanism coded by *mecA* gene which confers resistance to macrolide and streptogramins type B (MLSB) or

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due to *erm* genes which encode enzymes capable of conferring inducible (iMLSB) or constitutive (cMLSB) resistance to all the three group of drugs via methylation of the *23S* rRNA.[8] Clindamycin suppresses production of Panton-Valentine leucocidin and other virulence factors in MRSA.[9] The enzyme encoded by erm gene called as *23S* rRNA methylase renders ribosomes incapable of binding the MLS antibiotics and low levels of erythromycin act as the most effective inducer.[8]

Staphylococcal phenotypes observed in one study found an apparent inverse correlation between the resistance observed and the use of erythromycin in each hospital. Greatest erythromycin use yielded the lowest incidence of cMLSB and vice-versa.[10] D-test is recommended by CLSI for detection of inducible clindamycin resistance which can be categorized as MS, iMLSB, cMLSB.[11] Constitutive resistance can be detected by routine disc diffusion method but it fails to detect inducible resistance (iMLSB), which appears sensitive to clindamycin on routine testing, resulting in institution of inappropriate clindamycin therapy but inducible resistance cannot be detected by broth or agar dilution methods.[8] The current study was carried out to know the incidence of clindamycin resistant phenotypes among clinical isolates of *S. aureus.*

METHODS

Study design and setting

A cross-sectional study was conducted among the patients visiting Bharatpur Hospital over 3 months from September to November 2019. Data were collected using a semi-structured questionnaire. Chi-square test ($\chi 2$) was used to determine significant associations between various attributes including age and gender of the patients with the prevalence rate of *S. aureus* infection.

Sample size, inclusion and exclusion criteria

A total of 1279 clinical samples were collected from all suspected patients. Samples showing mixed growth and non-significant growth on culture were rejected and only the samples showing significant growth were included in the study. Samples with inappropriate labeling and leaked samples were also excluded from the study.

Sample processing and identification

Clinical specimens were streaked on 5% sheep Blood Agar and Mannitol Salt Agar (MSA) and incubated at 37°C for 24 h aerobically. Bacterial colonies showing typical characteristics of *S. aureus* (Beta-hemolytic on Blood Agar and golden yellow pigmentation on Mannitol Salt Agar) were subcultivated on Nutrient Agar and subjected to Gram staining and biochemical tests such as catalase and coagulase. DNase test was further performed to confirm *Staphylococcus aureus* isolates. All the tests were performed in duplicates in order to ensure that the growth was not due to contamination.

Antibiotic susceptibility test (AST) of the isolates

Antibiotic sensitivity testing was performed by Kirby Bauer disc diffusion method and methicillin resistance was determined by using cefoxitin (30 µg) disc and interpreted as per CLSI guidelines (2016).[12] Resistance to at least one drug from 3 different antibiotics of different structural classes was considered MDR as described elsewhere.[13] Erythromycin resistant *S. aureus* isolates were further studied for detection of inducible and constitutive clindamycin resistance by D-test according to CLSI guidelines (2016).[12] Briefly, a 0.5 MacFarland suspension was prepared in normal saline for each isolate and inoculated on Muller-Hinton Agar plate. A 2 µg clindamycin and 15 µg erythromycin discs were placed 15 mm apart edge to edge. Following overnight incubation at 37°C, three different phenotypes were observed and interpreted as follow:

1. MS phenotype: Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin (D-test negative).
2. Inducible MLS_B ($iMLS_B$) phenotype: Staphylococcal isolate showing resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D-shaped zone of inhibition around clindamycin with flattening toward the erythromycin (D-test positive).
3. Constitutive MLS_B ($cMLS_B$) phenotype: Staphylococcal isolates that showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition around clindamycin.

Quality control

For quality control of biochemical tests, purity plate was used. Similarly, for the standardization of the culture and antimicrobial susceptibility testing, *S. aureus* (ATCC 25923) was used as a control strain.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) software (Version 16.0). Chi-square test was used to compare categorical data. All tests were performed in duplicate, and $p < 0.05$ was considered as statistically significant.

RESULTS

Among the 1279 samples examined, 314 (24.5%) showed at least one bacterial growth. Out of these

314 samples, 125 belonged to staphylococci species. Prevalence of *S. aureus* among total samples was 4.5% (58/1279). Prevalence of *S. aureus* was observed high in females with 74.1% cases whereas the males accounted for 25.9% only. *S. aureus* infection was seen more prevalent in the age group 20-30 (34.5%) and least prevalent in the age group 40-50 (6.8%). Bhramin/Chhetri were found to be more (51.7%) infected with *S. aureus* and Muslims the least (3.4%). Pus sample showed the maximum growth (79%) among all types of samples examined. Incidence of *S. aureus* was found to have a significant association with the gender, age-group as well as with the type of samples (Table 1).The antibiotic susceptibility test revealed that tetracycline was the most effective drugs with 93.9% sensitivity; whereas erythromycin was the least effective drug with only 7.5% sensitivity. Linezolid and chloramphenicol showed 86.3% and 84.5% sensitivity against the test isolates. Cefoxitin was effective against only 39.7% isolates (Table 2). Out of 58 *S. aureus* isolates, 39.7% were MDR, 60.3% were MRSA and 39.7% were MSSA. Among 58 *S. aureus* isolates, 39.7% showed iMLSB phenotype, 3.5% showed cMLSB phenotype and comparatively higher proportion (56.8%) showed MS phenotype (Table 3).

DISCUSSION

Staphylococci are considered as one of the abundant skin colonizing agents and are important causes of nosocomial infections and community associated skin infections. Macrolide resistant *Staphylococcus* sp. may show constitutive or inducible resistance to clindamycin or may be resistant to only macrolides.[14] However, there has been increase in inducible clindamycin resistance among erythromycin resistant clinical isolates of *Staphylococcus* sp.[6]

Table 1. Association of S. aureus incidence and some variables.

Variables	Category	Growth	No Growth	Total	p-value
Gender	Male	15	521	536	0.011*
	Female	43	700	743	
Age Group	0-10	8	327	335	
	10-20	8	124	132	
	20-30	20	235	255	
	30-40	7	205	212	0.021*
	40-50	4	170	174	
	50-60	5	74	79	
	60+	6	86	92	
	Brhamin/Chhetri	30	641	671	
Ethnicity	Janajati	14	343	357	
	Dalit	9	195	204	0.335
	Madhesi	3	28	31	
	Muslim	2	14	16	
Samples	Urine	2	631	633	0.000*
	Pus	46	81	127	
	Blood	5	386	391	
	Throat Swab	1	11	12	
	Vaginal Swab	2	21	23	
	Sputum	1	70	71	
	Body Fluids	1	21	22	

Table 2. Antibiotic Resistance Pattern of S. aureus

S.N.	Antibiotics	Sensitive	Intermediate	Resistance
1	Tetracycline (30µg)	93.9%	4.4%	1.7%
2	Erythromycin (15µg) 27.5%	27.5%	8.6%	63.9%
3	Cefoxitin (30µg)	39.7%	0	60.3%
4	Linezolid (30µg)	86.3%	12%	1.7%
5	Chloramphenicol (30µg)	84.5%	10.3%	5.2%

Table 3. Phenotypic distribution of S. aureus by D-Test.

Phenotype	MRSA	MSSA	Overall
iMLSB	12(34.3%)	11(47.8%)	23(39.7%)
cMLSB	2(5.7%)	0	2(3.5%)
MS	21(60%)	12(52.2%)	33(56.8%)
Total	35	23	58

The prevalence of *S. aureus* in 1279 clinical specimens was 4.5% in the current study. A similar study in India showed a prevalence of 11.2% in 7172 samples.[15] In another similar study in North India, growth rate was found to be 8.9%.[6] The variation in isolation of *S. aureus* among different studies could be due to many factors like differences in personal hygiene, lifestyle, population characteristics, direct skin to skin contact, crowded living environment, hospitalization, sharing of individual objects, etc.[16] In the current study, majority of the isolates were recovered from females (74.1%). A study conducted in Kathmandu, Nepal also showed a higher incidence of *S. aureus* in females (60%) compared to their male counter parts (40%).[17] In contrast, a study in northern area of Jordan found that 57.2% of *S. aureus* were isolated from male and 42.8% from female.[18] Probable reason behind these findings maybe because of the habit of underestimating skin abrasions which may lead to the infection and other facts such as less knowledge of personal hygiene. In this study, age group 20-30 was found more infected (34.5%) by *S. aureus* than the other age groups. A similar study in a tertiary-care hospital in Nepal, a high proportion (24.1%) of *S. aureus* isolates was obtained in the age group 21-30.[19] Another study showed a different result with a higher (34.4%) prevalence in the age group 0-10.[20] Higher rate in this age group may because of active involvement of this age group in various fields and thus more likely to get community-acquired infections.

In the current study the higher proportion of *S. aureus* and MRSA isolates was recovered from pus sample (71.3%). Higher incidence of *S. aureus* in pus sample was statistically significant ($p<0.05$). Higher prevalence of *S. aureus* (54.4%) was also noted in an identical study.[17] *S. aureus* is considered as the he most common pus-forming pathogen in skin and systemic infections, which might be the reason for its higher incidence.[21] In the current study, a total of 35(60.3%) MRSA isolates were obtained. This result is similar to another study done by Gupta and Sinha in Uttar Pradesh where it was 76.7%.[18] Saxena and his associates showed 18.1% MRSA prevalence.[22] Different studies in different countries show variations in prevalence of MRSA. In this study, tetracycline was found to be the most effective drug which was sensitive to 93% isolates. In a study done in Jordan, tetracycline was one of the effective drugs to which 81.3% isolates weresusceptible.[23] But, in a particular study in Ujjain India, 41% of *S. aureus* isolates were found resistant to tetracycline.[24]

In the current study, D-Test of erythromycin-resistant isolates revealed l23(39.7%) inducible phenotypes (iMLSB), 2(3.5%) constitutive phenotypes (cMLSB) and 33(56.8%) MS phenotypes. The percentage of different phenotypes obtained in our study is similar to a study performed in Atlanta.[19] Another study from North India showed 16.9% iMLSB phenotype whereas cMLSB phenotype was 33.1%, much higher than our study.[6] Among 35 MRSA isolates, 34% showed iMLSB phenotype whereas only 6% showed cMLSB phenotype and 60% showed MS phenotype. Other studies such as one in India showed 32% iMLSB phenotype and36% MSSA phenotype among the MRSA isolates.[6] Mittal and his colleagues found 8.6% cMLSB phenotype among MRSA which is almost similar to our study (6.4%) but MS phenotype in his study was 13.3% which is 60% in the current study.[25] Several studies done across the country have reported that constitutive and inducible MLSB strains are seen more in MRSA than in MSSA strains.[26,27]

CONCLUSIONS

In the context of changing antibiotic resistance patterns of *Staphylococcus aureus*, their treatment has always been challenging. Hence, a simple D-test must be performed for every erythromycin-resistant *Staphylococcus* isolate beside the routine clindamycin susceptibility testing for the proper treatment of the patient.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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