ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 14, Issue 5, 2021

Online - 2455-3891
Print - 0974-2441

Research Article

MACROLIDE-LINCOSAMIDE-STREPTOGRAMIN B RESISTANCE AMONG *STAPHYLOCOCCUS AUREUS* IN CHITWAN MEDICAL COLLEGE TEACHING HOSPITAL, NEPAL

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Received: 05 February 2021, Revised and Accepted: 11 March 2021

ABSTRACT

Objectives: *Staphylococcus aureus* is often linked with human infection. Clindamycin is one of the key substitute antimicrobial agents in the treatment of *S. aureus*, especially in methicillin-resistant *S. aureus* (MRSA) infections. Inducible macrolide-lincosamide-streptogramin B (iMLS B) resistance is a crucial factor in antimicrobial susceptibility testing. The intention of the research was to identify *S. aureus* from distinct clinical specimens and investigate the prevalence of inducible clindamycin resistance among them and also study their association with MRSA.

Methods: A descriptive cross-sectional study was accomplished in the Dept. of Microbiology CMC-TH, Nepal from January 2018 to December 2020 with 525 non-repeated *S. aureus* obtained from a different clinical specimen. Antibiotic susceptibility test was performed by Kirby–Bauer disc diffusion method. MRSA was detected using cefoxitin (30 µg) and results were interpreted as stated by CLSI. "D-Test" was done by applying erythromycin (15 µg) and clindamycin (2 µg) as per CLSI guidelines. Data were analyzed using SPSS IBM version 20.

Results: Among 525 isolates, there were 315 (60.00%) MRSA. Results of D test analysis showed that 280 (53.33%) were MLSB sensitive while 245 (46.67%) were MLSB resistant; where 80 (15.24%) iMLSB with D zone, 100 (19.05%) constitutive MLSB (cMLSB) phenotype, and 65 (12.38%) MS phenotype. Of a total of 80 iMLSB, a significant proportion of 64 (80.00%) was MRSA ($p<0.001$). All the isolates were sensitive to vancomycin, teicoplanin, and linezolid. The prevalence of both iMLSB and cMLSB was high among MRSA.

Conclusion: In this study, cMLSB phenotype was predominant (19.05%) followed by iMLSB phenotype (15.25%) and then MS phenotype (12.38%). Inducible iMLS B phenotypes, as well as cMLSB, are higher among MRSA. It is advisable to include "D-Test" as a part of regular antibiotic susceptibility testing to detect iMLSB resistance among *S. aureus*.

Keywords: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, Clindamycin resistance, Erythromycin resistance, D-Test, Inducible macrolide-lincosamide-streptogramin B, Constitutive MLSB, MS phenotype.

 DOI: http://dx.doi.org/10.22159/ajpcr.2021v14i5.41012. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Staphylococci were first identified by Sir Alexander Ogston in 1880, in pus from a surgical abscess in a knee joint; later in 1884, Freidrich Jukius Rosenbach distinguishes *Staphylococcus aureus* from *Staphylococcus epidermidis* [1]. *S. aureus* is small, ovoid, Gram-positive cocci found typically in grape-like clusters or may occur in pairs or singly [2]. It commonly colonizes the skin and noses of healthful individuals. However harmless at these sites, it may get into the body through a crack in the epidermis, for example, erosion, scratch, lesion, surgical procedure, and Foley catheters and give rise to infections [3]. It is accountable for extensive infections including bacterial skin infections, food poisoning, and osteomyelitis; moreover, it is a foremost source of nosocomial infection, extending out of minor skin diseases to a disastrous state such as post-operative wound infection, nosocomial pneumonia, sepsis, and bacterial endocarditis [4,5].

S. aureus infections generally respond to β-lactam and related classes of antibiotics such as macrolide-lincosamide-streptogramin (MLS) group. Nevertheless, because of the evolution of methicillin resistance out of *S. aureus* isolates, the therapeutics of such infections have become difficult [6]. The rise of methicillin-resistant *S. aureus* (MRSA) among staphylococci is inflation trouble and clindamycin is considered as one of the powerful other agents accessible to address this issue [7]. One of the most key reasons for MRSA evolution is redundant and wide ranging antibiotic overuse for less severe infections. Unluckily these MRSA isolates which are susceptible only to glycopeptides antibiotics such as vancomycin are becoming multidrug resistant (MDR) [8]. The rise in the number of MDR strains has led to a renewed curiosity in the utilization of macrolide (e.g., erythromycin)-lincosamide (e.g., clindamycin)-streptogramin B (e.g., quinupristin/dalfopristin) (collectively called as MLSB family) antibiotics to treat *S. aureus* infections with clindamycin being the favorable agent because of its superb pharmacokinetic properties [9].

Staphylococcal strains resistant to MLSB antibiotics have escalated in number after the extensive use of these antibiotics for treating serious staphylococcal infections [10]. The most usual process for such resistance is target site modification mediated by erm genes, which can be demonstrated either constitutively (cMLSB phenotype) or inducible (iMLSB phenotype). The erm genes code for methylase enzyme which methylates and alters the target site of MLSB antibiotics, that is, the 23S ribosomal RNA [11]. Active efflux pump encoded by msr A gene (MS phenotype) is another process of resistance [12]. *S. aureus* with constitutive resistance show resistance to erythromycin and clindamycin on *in vitro* testing, whereas isolates with inducible resistance show resistance to erythromycin but seem susceptible to clindamycin on disc diffusion test. Inducible clindamycin resistance in staphylococci can be detected by D test [13]. It is advocated that an accurate proportion of clindamycin resistance is being misjudged, especially for laboratories where testing for inducible resistance is not routinely done. For this reason, it is recommended that microbiology laboratories must accomplish the D-zone test on all staphylococcal strains that are erythromycin resistant and clindamycin sensitive,

before communicating clindamycin sensitive, to ascertain those strains that perhaps resistant in the course of treatment [14]. Failure to point out iMLSB resistance may accelerate the clinical failure of clindamycin treatment [15].

The incidence of clindamycin resistance varies from place to place and therefore a local data are important to guide empirical treatment [16]. In Nepal, few reports on the prevalence of inducible clindamycin resistance among *S. aureus* have been published [17,18]. Data describing the prevalence of clindamycin resistance among clinical isolates of *S. aureus* and MRSA are lacking in our geographic area. Thus, the present study was accomplished to determine the prevalence of inducible clindamycin resistance among *S. aureus* isolates and also to study their association with MRSA in our set up.

METHODS

The study was conducted in the Department of Microbiology CMC-TH, Nepal, from January 2018 to December 2020 with 525 non-repetitive *S. aureus* isolated from distinct clinical specimens. Antibiotic susceptibility test was performed by Kirby–Bauer disc diffusion method [19,20]. Based on the hospital antibiotic policy, the following antibiotics were used, namely, amikacin (30 µg), amoxiclav (30 µg) (amoxicillin/clavulanic acid 20/10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), cotrimoxazole (25 µg) (trimethoprim/sulfamethoxazole 1.25/3.75 µg), erythromycin (15 µg), gentamicin (10 µg), cloxacillin (5 µg), linezolid (30 µg), penicillin (10 units), piperacillin/tazobactam (100/10 µg), tigecycline (15 µg), tetracycline (30 µg), teicoplanin (30 µg), and vancomycin (30 µg). Sensitivity testing of vancomycin and teicoplanin was done by the minimum inhibitory concentration method. All antimicrobial drugs were obtained from HiMedia Laboratories, India. Screening of MRSA was done using cefoxitin (30 µg). If the cefoxitin (30 µg) zone of inhibition was less than 21 mm, it was reported as MRSA (Fig. 1) [19].

Inducible clindamycin resistance was detected using erythromycin (15 µg) and clindamycin (2 µg) as per CLSI guideline [20]. Three different types of the phenotype were appreciated and interpreted. Inducible MLSB (iMLSB) phenotype: Staphylococcal isolates showing resistance to erythromycin while being sensitive to clindamycin and giving a D-shaped zone of inhibition around clindamycin with flattening toward erythromycin disc. Constitutive MLSB (cMLSB) phenotype: Those staphylococcal isolates, which showed resistance to both erythromycin and clindamycin with the circular shape of the zone of inhibition, if any around clindamycin. MS phenotype: Isolates exhibiting resistance to erythromycin and sensitivity to clindamycin and giving a circular zone of inhibition around clindamycin (Fig. 2).

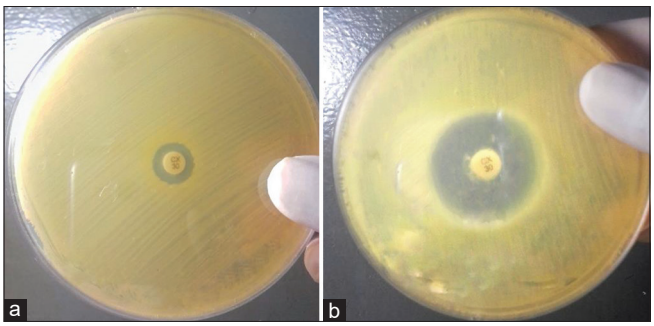

Fig. 1: Detection of methicillin-resistant *Staphylococcus aureus* using cefoxitin disk. (a) Methicillin-resistant *S. aureus*. (b) Methicillin-sensitive *S. aureus*

To verify that the susceptibility result is accurate, control strain of *S. aureus* American type culture collection (ATCC) 25293 was streaked on the prepared media plates and observed for significant growth. Control strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *P. aeruginosa* (ATCC 27853) were used for the standardization of the Kirby–Bauer test and also for the correct interpretation of the zone of inhibition. For MRSA test standardization, *S. aureus* strains ATCC 25923 were used as negative and ATCC 43300 were used as positive controls. Qualities of each agar plate were tested by incubating one plate of each batch on the incubator overnight without inoculating. The collected data were summarized, presented, and analyzed using the software SPSS version 20 (Chicago, USA). Qualitative data were summarized as frequency and percentages. $p<0.05$ was considered statistically significant. Ethical approval was taken from Chitwan Medical College (CMC) – Institutional Review Committee. Informed consent was taken from the patient before their inclusion in the research.

RESULTS

Demographic distribution and identification of bacterial isolates

A total of 525 non-repetitive *S. aureus* were collected from the different specimens. Among them, 420 (80.00%) were from pus, 42 (8.00%) from blood, 26 (4.95%) from sputum, 21 (4.00%) from urine, and 16 (3.05 %) from body fluid and tissue, respectively. Isolation frequency of *S. aureus* was 273 (52.00%) and 252 (48.00%) among males and females, respectively. *S. aureus* was dominant in IPD patient 315 (60.00%) than OPD patient 210 (40.00%). Among IPD patient, the growth of *S. aureus* was predominant in surgical ward 157 (29.90%) followed by medicine ward 82 (15.62%), orthopedic ward 65 (12.38%), medical/pediatric/neonatal ICU and CCU 63 (12.00%), pediatric ward 53 (10.10%), ENT ward 42 (8.00%), gynecology ward 42 (8.00%), and tropical ward 21 (4.00%). Out of 525 *S. aureus*, 315 (60.00%) were MRSA. MRSA isolates were isolated more from pus 257 (48.95%) as compared to other samples. The distribution of MRSA was found to be dominant in IPD patients 195 (37.14%) compared to 120 (22.86%) among OPD patients. Results of D test analysis showed that out of 525 *S. aureus,* 80 (15.24%) showed a D zone; where 280 (53.33%) were MLSB sensitive while 245 (46.67%) were MLSB resistant. In this study, cMLSB phenotype was predominant 100 (19.05%) followed by the iMLSB phenotype 80 (15.25%) and then MS phenotype 65 (12.38%), as shown in Fig. 3.

iMLSB phenotype as well as cMLSB was higher among MRSA 64 (20.32%) and 79 (25.08%) as compared to MSSA 16 (7.62%) and 21 (10.00%), respectively (Chi-square test, $p<0.001$), as expressed in Table 1.

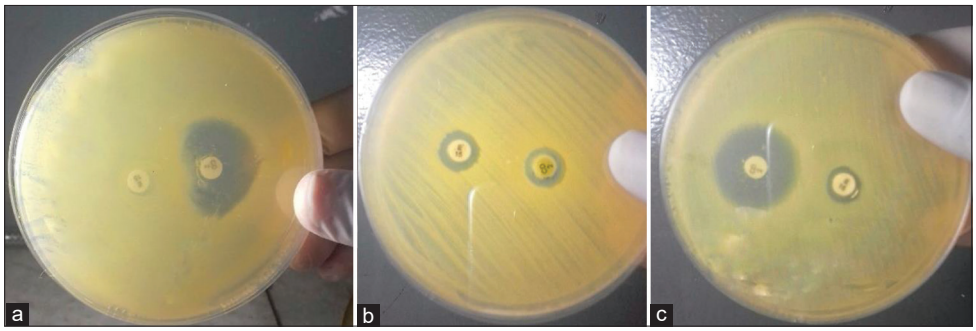

Fig. 2: Different MLSB phenotype. (a) iMLSB phenotype. (b) cMLSB phenotype. (c) MS phenotype

Table 1: Susceptibility pattern against erythromycin and clindamycin among MRSA and MSSA isolates (n=525)

Isolates	iMLSB (%)	cMLSB (%)	MS (%)	MLSB sensitive (%)	Total (%)	p-value
MRSA	64 (12.19)	79 (14.80)	52 (9.90)	120 (22.86)	315 (60.00)	p<0.001
MSSA	16 (3.05)	21 (4.00)	13 (2.48)	160 (30.48)	210 (40.00)	
Total	80 (15.24)	100 (19.05)	65 (12.38)	280 (53.33)	525 (100.00)	

Antibiotic sensitivity profile

β-lactams, sulfonamides, quinolones, aminoglycosides, tetracycline, glycopeptides, macrolide, lincosamide, and oxazolidinones groups of antibiotics were tested against *S. aureus* isolates. All the isolates (100%) were sensitive to vancomycin, teicoplanin, and linezolid. Among antibiotics of β-lactam class, penicillin was least effective with only 14.29% sensitivity. Cloxacillin showed 42.86%, cefoxitin showed 41.41%, and amoxicillin/clavulanic acid showed 40.00% sensitivity. Other antibiotics of the same class showed a sensitivity rate of less than 40%. Another antibiotic with a higher sensitivity rate was amikacin 88.00%. Gentamicin, being from the same class of antibiotics (aminoglycosides), had 48.95% sensitivity. Ciprofloxacin was the least effective with a sensitivity rate of 31.05%. Cotrimoxazole was 58.86% sensitive. Erythromycin and clindamycin both had more than 50% sensitivity rate, that is, 52.95% and 66.10%, respectively. Antibiotic from the group tetracycline was also effective. Tetracycline had 55.05% sensitivity while tigecycline had 44.95% sensitivity. Detail summary of the susceptibility pattern of *S. aureus* isolates is shown in Table 2.

Table 2: Antibiotic susceptibility pattern of *Staphylococcus aureus* (n=525)

Class of antibiotics	Antibiotics used	Susceptibility pattern			
		Resistant		Sensitivity	
		No.	%	No.	%
Aminoglycosides	Amikacin	63	12.00	462	88.00
	Gentamicin	268	51.56	257	48.95
β-lactams	Amoxicillin/ clavulanic acid	315	60.00	210	40.00
	Penicillin	450	85.71	75	14.29
	Cefoxitin	309	58.86	216	41.14
	Ceftriaxone	325	61.90	200	38.10
	Cloxacillin	300	57.14	225	42.86
	Piperacillin/ tazobactam	320	60.95	205	39.05
Glycopeptides	Vancomycin	00	00.00	525	100.00
	Teicoplanin	00	00.00	525	100.00
Lincosamides	Clindamycin	178	33.90	347	66.10
Macrolides	Erythromycin	247	47.05	278	52.95
Oxazolidinones	Linezolid	00	00.00	525	100.00
Quinolones	Ciprofloxacin	362	68.95	163	31.05
Sulfonamides	Cotrimoxazole	216	41.14	309	58.86
Tetracyclines	Tigecycline	289	55.05	236	44.95
	Tetracycline	236	44.49	289	55.05

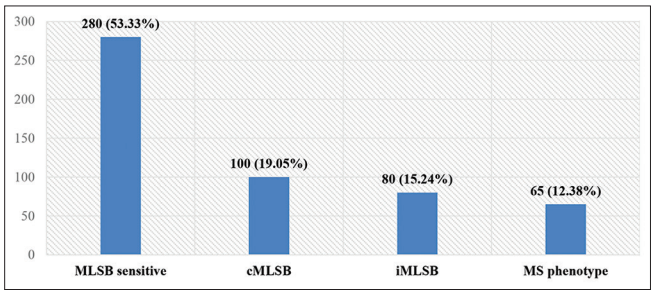

Fig. 3: Susceptibility pattern against erythromycin and clindamycin among *Staphylococcus aureus* isolates (n=525)

DISCUSSION

Screening for antimicrobial susceptibility among the clinical bacterial isolates is important for the best result of the treatment. In our study, of 525 isolates of *S. aureus*, 80.00% were from the pus sample, which signifies their leading role in abscess formation. Antimicrobial resistance has been observed as one of the supreme microbial threats of this century [21]. One of the crucial issues while treating infection caused by *S. aureus* is due to the multidrug resistance plus the emergence of methicillin resistance [22]. Clindamycin is commonly used for the treatment of skin and soft-tissue infections caused by *S. aureus*. However, *S. aureus* with inducible clindamycin resistance is increasing day by day, form such mutant's constitutive resistance can arise spontaneously during clindamycin therapy [23]. Therefore, all the clinical isolates of *S. aureus* must be checked for inducible resistance (D-test) before clindamycin is reported as susceptible and to prevent therapeutic failure [24]. In the present study, the overall prevalence of inducible clindamycin resistance (iMLSB) among the clinical isolates of *S. aureus* was 15.24% which is near to the report of Singh *et al.,* 13.39% [25], Ansari *et al.,* 12.4% [26], and Sah *et al.,* 12.1% [17] from Nepal, Parasa *et al.,* 15.03% from India [27], and Van der Heijden *et al*., 12.3% from Brazil [28]. Different prevalence rates of iMLSB have been reported in other studies; Mohapatra *et al.,* 18.2% [29], Raut *et al.,* 25.6% [30], Shrestha *et al*., 20.6% [31] from Nepal, and Lall *et al.,* 20.30% [32] from India. Higher iMLSB prevalence of 45% from Germany [33] and 62% from the US [34] has also been reported. Constitutive clindamycin resistance (cMLSB) phenotype was found to be 19.50% in the present study, which is low as compared to another study [23,29,31,32]. Such disparity could be because of variation in a study period, group of patients, and geographical site. The present study demonstrated a higher prevalence of both iMLSB and cMLSB among MRSA as compared to MSSA. This finding is consistent with other reports [17,25,29,31,32]. However, Schreckenberge *et al.* [16], Levin *et al.* [35], and Marr *et al.* [36] reported a higher incidence of iMLSB among MSSA. In the present study, MRSA was dominant in male (31.2%) while MSSA was dominant in female (21.1%); a similar study was conducted by Raut *et al*. reported 52.3% and 64.3%, respectively [30]. In our study, MRSA was found to be dominant (47.7%) in the pus sample which is consistent with the reports of other authors, Raut *et al.* (49.2%) [30], Pandey *et al.* (28.73%) [37], and Shrestha *et al.* (82.6%) [31]. However, in the study by Thapa *et al*. [38], *S. aureus* was dominant in blood sample 44.60% while 23.40% was in pus.

This study demonstrated that overall rates of susceptibility pattern of *S. aureus* to the commonly used antibiotics were less than 65%, except clindamycin, amikacin, vancomycin, linezolid, and teicoplanin. A high proportion of isolates, 85.93% were resistant to penicillin. This result was expected as only a few strains of *S. aureus* do not produce beta-lactamases [26]. Ciprofloxacin being cheap and easily available antibiotics it has been widely used to treat against *S. aureus*. In this study, resistance rate of ciprofloxacin was 69.53%, which is comparatively higher than a report of Ansari *et al.*, 63.7% [26]. Some other researchers found a resistance rate of ciprofloxacin to be 32.73% by Sanjana *et al.* [39] and 11% by Baral *et al.* [40]. The rate of resistance of cotrimoxazole in our study was 41.41% which is similar to a study by Shrestha *et al.* [31]. Baral *et al*. found 64% rate of resistance to cotrimoxazole which is higher than in our study [40]. Similarly, gentamicin was 51.56% resistant in our study which is comparable to 54.50% found by Mishra *et al.* [41]. Fortunately, the rate of resistance to amikacin is low at 11.71%. All the isolates (100%) in the present study were susceptible to vancomycin and teicoplanin, this report is consistent with other studies [25,26,39-41], this proves that glycopeptides should be used as empiric therapy for serious staphylococcal infections.

Absolutely susceptibility of this drug in Nepal might be related to the low use of this agent due to its high cost [26]. Linezolid is another drug to have 100% susceptibility which is consistent with the report of Singh *et al.* [25] and Raut *et al.* [30].

CONCLUSION

In this study, cMLSB phenotype was predominant (19.05%) followed by iMLSB phenotype (15.25%) and then MS phenotype (12.38%). Inducible MLS B phenotypes, as well as cMLSB, are higher among MRSA. Clindamycin, which has outstanding bone and tissue penetration along with its ability to accumulate in an abscess, has become one of the beneficial antibiotics to treat *S. aureus* infection. Therefore, D-test should be performed routinely and the clinician should be enlightened regarding the likely failure of clindamycin therapy in infections caused by *S. aureus* harboring iMLSB resistance.

ACKNOWLEDGMENTS

We would like to thank the technician of the Department of Microbiology, CMC-TH, for their assistance and the entire patient for participation in this study.

AUTHORS' CONTRIBUTIONS

NKC: Concept and design of the study, reporting of test results, collection, analysis and interpretation of data, and drafting the final version of the manuscript. RP: Collection, processing of specimens, and preparation of manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest regarding the publication of this article.

AUTHORS' FUNDING

The authors did not receive any funding for this research work.

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