INTRODUCTION

Staphylococcus aureus is recognized as the causative agent of skin, soft tissue, and systemic infections. It frequently associated with the pus formation in different body sites infections. It is also more common nosocomial as well as opportunistic pathogens [1]. It causes different types of diseases such as skin infections, rhinitis, otitis media infection, mastitis, suppurative wounds osteomyelitis, urinary tract infections, and septic arthritis with life-threatening invasion diseases such as pneumonia, septicaemia, endocarditis, bacteremia, and toxic shock syndrome [2].

Origin of methicillin-resistant S. aureus (MRSA) made serious problems for the treatment and selection of therapeutics available alternatives for staphylococcal infections [3]. Earlier staphylococcal infections have been treated by the use of erythromycin since three–four decades and become resistant against it has been reported since long [4]. The increase of resistant staphylococcal infection with MRSA has led to renewed interest in the use of macrolide-lincosamide-streptogramin B (MLSB) antibiotics for the treatment of such infections [5]. These family MLSB antibiotics with clindamycin are the preferred antibiotic due to its best pharmacokinetics property [6].

Macrolides (erythromycin, clarithromycin, and roxithromycin) resistance may be constitutive and inducible in the presence of a macroclide inducer being the most common erythromycin [7]. It is the best inducer for the synthesis of erythromycin ribosome methylase (erm) enzyme. In inducible clindamycin resistance S. aureus (iMLSB), erm enzyme only produced in the presence of erythromycin whereas in constitutive phenotype (constitutive clindamycin-resistant S. aureus [cMLSB]) the erm enzyme naturally produced without the presence of inducer. When this enzyme produced through erm gene induced confers cross resistant to other members of group including lincosamide and streptogramin B [8].

The double disk diffusion test (D-test) is used to distinguished inducible clindamycin resistance from constitutive clindamycin-resistant S. aureus among erythromycin-resistant isolates to determine therapeutic for clindamycin to be used as a therapeutic option [9].

In this present research, we aimed to determine the prevalence of MLSB and MLSBc among hospital-based isolates of S. aureus in the form of MRSA and methicillin-sensitive S. aureus (MSSA).

METHODS

This research included 2000 different clinical samples; pus, blood, urine, sputum, throat swab, pleural fluid, synovial fluid, ascitic fluid, ear swab, cerebrospinal fluid, stool, and high vaginal swab and processed in National Medical College and Teaching Hospital, Birgunj, Nepal from February 2017 to December 2017. Among these, samples 310 (15.5%) S. aureus was isolated by growth on blood agar, nutrient agar, and mannitol salt agar. These isolates were confirmed by use catalase and coagulase test as per the standard operative procedure CLSI [10].

MRSA was identified by the oxacillin disk diffusion method. In this method, 0.5 MacFarland standard inoculum of S. aureus was inoculated on Muller-Hinton agar (MHA) media through swabbing and left for 1 h. The oxacillin disc (1 µg) was now put on inoculated MHA media and incubated at 37°C for 24 h. By the measurement of the zone of diameter,
≥13 mm, 11–12 mm, and ≤10 mm around oxacillin disc was considered oxacillin susceptible, intermediate, and resistant, respectively [10].

iMLSB and cMLSB were identified by the use of erythromycin (15 μg) and clindamycin (2 μg) disks through D-test. In this test 0.5, MacFarland standard inoculums of *S. aureus* were swabbed on the surface of MHA media. The erythromycin and clindamycin disks were placed at a distance of 15–20 mm distant edge to edge from each other incubated at 37°C for 24 h. Through the observation as flattening of the zone of inhibition around the clindamycin proximal to erythromycin disk (D shaped zone of inhibition) indicated as iMLSB phenotypes. There no formation of any zone of inhibition around both antibiotic disks that was defined as cMLSB phenotypes [11].

### RESULTS

Two thousand different clinical samples were processed and the patient samples distribution shown in Fig. 1. Out of total samples, 310 (15.50%) *S. aureus* were isolated and identified from different samples (Table 1). Among *S. aureus*, MRSA, MSSA, iMLSB, and cMLSB were selected as 242 (78.06%), 64 (20.64%), 106 (34.19%), and 72 (23.22%), respectively (Fig. 2). MRSA and cMLSB were found more in blood. MSSA and iMLSB were higher in pus than other clinical samples (Fig. 3). Out of total MRSA, 106 (43.80%) strains were iMLSB and 65 (26.85%) were cMLSB whereas among MSSA, 26 (40.62%) *S. aureus* were iMLSB and 7 (10.93%) were cMLSB (Fig. 4).

### DISCUSSION

Empirical therapy for staphylococcal infection has become more limited due to the emergence and the prevalence of MRSA. Among options available for the treatment of MRSA and MSSA, clindamycin is one of the good alternatives [6]. However, therapeutic failure caused by iMLSB strains is now being reported commonly. We found a high prevalence of 78.06% and 20.64% of MRSA and MSSA among all staphylococcal isolates. A study conducted in Nepal, the prevalence of MRSA was observed as 19% [12]. Majumder et al., from Assam found 52.9% MRSA [13]. In our study found 34.19% iMLSB isolates than 23.22% cMLSB. A similar study conducted in Turkey observed a prevalence of iMLSB as 21.9% [14]. Ravisekar et al., found 21% iMLSB isolates [15].

It was also observed that the prevalence of iMLSB and cMLSB was higher among MRSA (43.80% and 26.85%, respectively) as compared to MSSA (40.62% and 10.93%). This was in concordance with Yilmaz et al. who found 24.4% iMLSB among MRSA and 14.8% among MSSA [16]. Rahabar and Hajia reported 22.6% iMLSB among MRSA and 4% in MSSA [17].

In one study, all iMLSB shown resistance against macrolides-lincosamide-streptogramin B and constitutive clindamycin-resistant *S. aureus* among methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus* were isolated and identified from different samples (Table 1). Among *S. aureus*, MRSA, MSSA, iMLSB, and cMLSB were selected as 242 (78.06%), 64 (20.64%), 106 (34.19%), and 72 (23.22%), respectively (Fig. 2). MRSA and cMLSB were found more in blood. MSSA and iMLSB were higher in pus than other clinical samples (Fig. 3). Out of total MRSA, 106 (43.80%) strains were iMLSB and 65 (26.85%) were cMLSB whereas among MSSA, 26 (40.62%) *S. aureus* were iMLSB and 7 (10.93%) were cMLSB (Fig. 4).
resistance to MLS antibiotics are now common to clinical practice, especially in developing countries. There should be cautious for using the clindamycin in a severe case of infections due to making constitutive mutants during the course of clindamycin therapy in patients with iMLSB [19].

At present, some authors suggested less or no use of clindamycin for serious infections with high burden bacteria such as abscess or osteomyelitis [20]. Conversely, all erythromycin-resistant S. aureus was labeled as clindamycin resistant would prevent the use of clindamycin in infections caused by clindamycin-susceptible isolates. Clindamycin is now mostly used in non iMLSB or less severe form of infections caused by S. aureus.

CONCLUSION

Although the prevalence rate may differ from hospital to hospital, this study reflects characterization and prevalence of MRSA, MSSA, iMLSB, and cMLSB strains through oxacillin disk diffusion and the D-test at tertiary care hospital. Microbiologists should conform for theses mutants strains among S. aureus isolates.

ACKNOWLEDGMENT

The authors wish to acknowledge to the administrative peoples as well as laboratory workers of the Department of Microbiology of National Medical College and Teaching Hospital, Birgunj, Nepal, for their valuable suggestions and cooperation.

AUTHORS’ CONTRIBUTIONS

Amrullah Shidiki was involved in concepts, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, manuscript preparation, and manuscript editing and was a manuscript review guarantor. Bijay Raj Pandit was supported in concepts, design, definition of intellectual content, literature search, clinical studies, data analysis, manuscript preparation, and manuscript editing. Ashish Vyas was helped in concepts, definition of intellectual content, experimental studies, data analysis, and manuscript editing.

CONFLICTS OF INTEREST

The authors have not declared any conflicts of interest.

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