Tetracycline susceptibility in multidrug resistant *Staphylococcus aureus* isolated from clinical samples in tertiary care hospitals of Kathmandu, Nepal.

Lata Ghimire[1]

Megha Raj Banjara[2]

Abdelkodose Mohammed Hussen Abdulla[3]

Abstract

Staphylococcus aureus, being an opportunistic pathogen, is frequently associated with various infections in human. The clinical sector feels challenge regarding the management of multidrug resistant S. aureus. Besides the B-lactam antibiotics, Glycopeptides, Tetracycline, Quinolones, Macrolide-lincosamide streptogramin B family of antibiotics are also commonly prescribed to deal with such infections as an alternative drug. Therefore, this study was conducted to assess tetracycline susceptibility in multidrug resistant S. aureus in clinical samples.

This study was conducted over the period of 15 months from March 2020–May 2021. The clinical samples were collected from the three different tertiary care hospitals of Kathmandu, Dirghayu Guru Hospital, Chabahil, Bhaktapur Cancer Hospital, Bhaktapur, and Shankarapur Hospital, Jorpati. The sample processing and laboratory work was carried out in the Microbiology Laboratory of Dirghyau Guru Hospital to determine the proportion of different phenotypes of MDR among S. aureus and their association with methicillin resistance. Two hundred sixty six isolates of S. aureus were included in the study. Methicillin resistance was detected by cefoxitin disc diffusion method.

Among 2004 clinical samples processed, 266 (13.27%) isolates were S. aureus. The multidrug resistant strain isolated from OPD was 56.84% (54/95) and from inpatient was 43.15% (41/95). Among the 266 S.aureus, 28.94% MRSA and 71.05% MSSA isolates were detected. Tetracycline resistance was found in 9.8% of total S. aureus isolates whereas 27.36% (26/95) among MDR S. aureus isolates. Resistance to Tetracycline was higher in MRSA as compared to MSSA. Detection rate of MRSA in

---

1   Mr. Ghimire is Lecturer at Tri-Chandra Multiple Campus, Department of Microbiology, Tribhuvan University.

2   Dr. Banjara is Associate Professor at Central Department of Microbiology, Tribhuvan University.

3   Dr. Abdulla is Associate Professor at Faculty of Medicine, University of Cyberjaya, Malaysia.

this study shows the necessity to improve healthcare practices to control MRSA infections. Tetracycline can be used as an alternative antibiotic against the S. aureus infection.

Keywords: staphylococcus aureus, MRSA, Tetracycline

Introduction

Staphylococcus aureus is one of the commensal organisms of skin and the nasal carrier. They are also most commonly detected from the blood, pus and urine. It acts as the opportunistic pathogen, hence reported as one of the most common nosocomial and community-acquired pathogens (1).

The different types of antibiotics are reported effective against the *S. aureus* infection since the discovery of penicillin during 1942. Beta lactam antibiotics, tetracycline, vancomycin, and macrolides are widely used antibiotics against *S. aureus*, however, there are reports of resistance to these antibiotics (2). In the context of Nepal, antimicrobial resistance carry a high load. The resistance is due to irrational and inadequate dose and duration of the antibiotics. A study in Nepal reveled 78.7% of the antibiotic users were unaware of the consequences due to antibiotic resistance. (3)

Antibiotic resistance has become a big challenge in the medical science. Among other many antibiotic-resistant bacterial species, methicillin-resistant *S. aureus* (MRSA) is one of the most important causes of treatment failure, morbidity and mortality of the patients. MRSA strains created a havoc globally because of its resistance to most of the antibiotics that are commonly used in clinical practice (4).

The most widely used antibiotics for Staphylococcal infection were penicillin and methicillin, but resistance to methicillin of this organism has given the limited therapeutic alternatives to treat the infections (5).

Tetracycline has been used as the alternative to penicillin derivatives. This drug has been used in case of penicillin hypersensitivity cases. In some countries, it is the second most frequently prescribed drug after penicillin because of its cost effectiveness and broad spectrum nature (6).

There are a number of susceptibility testing protocols that suggest to routinely test *S. aureus* strains to understand tetracycline resistance. Basically tetracycline resistance may develop in *Staphylococcus* spp is due to active efflux resulting from acquisition of the plasmid-located genes, *tetK* and *tetL,* and ribosomal protection mediated by transposon-located or chromosomal *tetM* or *tetO* determinants. *S. aureus* with only

tetK have been described as resistant to tetracycline, but susceptible to minocycline. On the other hand, the *tetM* gene is believed to deliberate resistance to all tetracycline group of antibiotics, including tetracycline and minocycline (7).

Objective of the study

The study aims to understand the distribution of MRSA and MSSA among the *S. aureus* isolated from various samples collected in the hospitals. The antibiotic susceptibility test profile help to determine the susceptibility of tetracycline against multidrug resistant *S. aureus*.

Materials and Methods

Study design and Population

The cross-sectional hospital-based study was carried out over the period of 15 months from March 2020 to May 2021 in the Microbiology laboratory of Dirghyau Guru Hospital, Kathmandu, Nepal. The samples were collected from three different tertiary care hospitals of Kathmandu valley to determine the incidence of different phenotypes of tetracycline resistance among *S. aureus* from clinical samples and their association with methicillin resistant strains.

The clinical samples as pus, wound swab, throat swab, body fluid, urine and blood were collected from both inpatients and outpatients visiting Shankarapur Hospital, Dirghyau Guru Hospital and Bhaktapur Cancer Hospital.

Ethics statement

This study received ethical approval from Nepal Health Research Council (Regd no.114/2020). **Inclusion Criteria:** The patients visiting the hospitals having the possibility of infection were considered as the sample population. For this the informed consent was mandatory to be included in the study. **Exclusion Criteria:** The patients who were unwilling to participate in the study and do not give the consent were not included in the study. Further, if the patient had taken antibiotic within 48 hours were excluded from the study.

Sample collection and processing

A total of 266 *S. aureus* were isolated from the 2004 clinical samples. Throat swabs, body swabs were collected using sterile cotton swab. The midstream urine was taken for urine sample. For the blood and body fluid, the clinicians were requested to get the samples from the patients.

The various bacteriological culture media were used to inoculate different samples depending upon the type of the specimen (1) MacConkey agar, blood agar and nutrient agar were primarily used culture media for the bacterial growth. The aseptically collected specimens were inoculated and incubated at 37ºC for 24 hours (8). The microscopic observation of Gram-positive cocci in cluster was the indication of possible *S. aureus*. Those organisms were inoculated into the mannitol salt agar as the selective medium. The yellow colonies as a result of mannitol salt fermentation were further sub-cultured in nutrient agar and the colonies were subjected to catalase, coagulase, and oxidative and fermentative test(9)

The colonial characteristics were studied on the basis of its colony morphology from the nutrient agar. The catalase positive, slide and tube coagulase positive, and beta-hemolysis on blood agar confirmed the organism to be *S. aureus.*

S aureus ATCC 25923 was used as a control strain of methicillin-sensitive and coagulase positive strain.

Antimicrobial susceptibility testing (AST)

S. aureus were tested for the antimicrobial susceptibility. The bacterial broth culture was standardized using 0.5 McFarland turbidity standards before inoculation in the Muller Hinton Agar. The carpeted organism was cultured at 37^0C for 24 hours along with various antibiotic discs. Cefoxitin (30µg) disk using modified Kirby Bauer disk diffusion method was used to identify Methicillin resistant *Staphylococcus aureus*. The Clinical and Laboratory Standard Institute (CLSI) guidelines (2014) were followed to detect MRSA isolates. Other antibiotics used to test antibiotic susceptibility tests were Ampicillin (10 µg), Cefotaxime (30 µg), Cotrimoxazole (25 µg), Chloramphenicol (30 µg), Meropenem (10 µg), Erythromycin (15 µg), Gentamycin (10 µg), Tetracycline(30 µg), Ciprofloxacin (5 µg),Vancomycin (30 µg) and Nitrofurantoin (300 µg) in case of urine samples (10).

The bacterial strain that is resistant to three or more different class of antibiotics were considered as the multidrug resistant.

Data analysis

The data analysis was done using SPSS 21.0. Chi-square test was used for analyzing categorical variables and $P < 0.05$ was considered significant.

Results

A total of 2004 specimens were processed from both the in-patients and out-patients. It

included 895 urine samples, 348 blood, 342 throat swab/sputum, 228 pus/wound swab and 191 body fluids. Out of 266 *S aureus* isolates, 899 samples were collected from male patients and 1105 from female patients.

266 *S. aureus* were isolated among them 95 (35.7%) were multidrug resistant (Table 1)

Table1.Specimen distribution with the isolation of MRSA and MSSA.

Total Specimen	U	Bl	Th	Pus	BF	Tot SA	MRSA	%	MSSA	%	MDRSA	%
2004	895	348	342	228	191	266	77	28.94	189	71.05	95	35.5

Among 95 MDRSA, 54 were from the outpatients whereas 41 cases were from inpatients. Out of 95 MDR isolates, 26(9.8%) were Tetracycline resistant. (Table 2)

Table 2: Antibiotic resistance pattern of tetracycline resistant and sensitive isolates of *S. aureus*

Antibiotics	Resistance in tetracycline sensitive isolates (n=240) (TSSA)		Resistance in tetracycline resistant isolates (n=26) (TRSA)		p-value
	S	R	S	R	
Ampicillin	79	161	1	25	<0.001
Cotrimoxazole	141	99	3.0	23	<0.001
Gentamycin	174	66	6	20	<0.001
Ciprofloxacin	167	73	1	25	<0.001
Chloramphenicol	195	45	5	21	<0.001
Erythromycin	192	48	14	12	<0.001
Cefoxitin	186	54	5	21	<0.001
Tetracycline	240	0	0	26	<0.001
Nitrofurantoin	48	20	3	7	0.01

Tetracycline was found to have better action as compared with other antibiotics. Tetracycline was found effective against 90.2 % of *S. aureus* isolates. Most of the *S. aureus* isolates were found resistant to previously used antibiotics like Ampicillin. (Table 3).

Discussion

Various MRSA cases have been reported from different hospitals of Kathmandu. The prevalence of MRSA in this study showed 28.94% which is similar with the previous study in Nepal that was 29.23% (11). The emergence of MRSA has increased

worldwide since last two decades and the frequency varies prominently across different countries and among different health centers of the same nation. Due to the differences of the circulating clones or due to the variations of disease preventive practices and tendencies of usage of antibiotics in different hospital setting might have resulted such type of variations (12).Trends of using antibiotics in clinical ground, inadequate dose and duration might be the reasons behind such variations.

Because of the poor policies for the purchase and prescribing antibiotics in Nepal, there exists the unnecessary and irrational use of antibiotics. The incomplete dosing of antibiotics and the poor antimicrobial surveillance are another reason of the emergence of antibiotic resistance .(13)

The sufficient information of microbiological and epidemiological information would guide the medical personnel in selecting the most suitable antimicrobial drug for the treatment of infection. The report of MRSA shows greater risk to the infection due to its resistance to most other antimicrobial agents (7).

Almost one third isolates of *S. aureus* were MDR and among them majority were from the outpatients. This might be the result of over use of antibiotic.

The drug resistance was found to be highest in MRSA especially to ampicillin (94.81%)

In this study, the prevalence of tetracycline resistance among *S. aureus* was found to be 9.8% which was found 27.36% among 95 MDR isolates. Use of tetracycline, ciprofloxacin and erythromycin is still very common in various hospitals of different countries for the treatment of respiratory tract and other nosocomial infections. The significant resistant was observed in the last decade along with the co-resistance between erythromycin, ciprofloxacin and tetracycline(14). However in this study, it was observed that the multidrug resistant strains were 27.36% susceptible towards tetracycline similar to other studies (15). Interestingly, in the study, it was found that all *S. aureus* including both the MRSA and MSSA showed very good susceptibility against broad-spectrum antibiotic Tetracycline. Further the tetracycline was found 72.63% sensitive towards the MDRSA. For the Vancomycin, 3.75% were identified resistant from MIC test.

Conclusion

Multidrug resistant *S. aureus* has appeared as a significant health issue both in community and health care settings.

Regular monitoring and observation of infection pattern and the AST pattern of MDRSA may be helpful in reducing the incidence of these infections as well as effective management of infections.

Tetracycline is still effective antibiotic against *S. aureus* infection.

Acknowledgements

References:

Ghimire L, Banjara MR, Pathak R, Abdulla AMH. Antibiotics susceptibility pattern of Methicillin Resistant Staphylococcus aureus isolated from the clinical samples different tertiary care hospitals of Kathmandu. Patan Pragya. 2020;6(1):39–44.

Nourbakhsh F, Ebrahimzadeh A, Namvar, Momtaz H. Original Article Characterization of Staphylococcal Cassette Chromosome Mec Elements in Biofilm-producing Staphylococcus Aureus , Isolated from Hospital Infections in Isfahan. Int J Med Lab. 2016;3(1):33–42.

Rijal KR, Banjara MR, Dhungel B, Kafle S, Gautam K, Ghimire B, et al. Use of antimicrobials and antimicrobial resistance in Nepal: a nationwide survey. Sci Rep [Internet]. 2021;11(1):1–14. Available from: https://doi.org/10.1038/s41598-021-90812-4

Orth D, Grif K, Erdenechimeg L, Battogtokh C, Hosbayar T, Strommenger B, et al. Characterization of methicillin-resistant Staphylococcus aureus from Ulaanbaatar, Mongolia. Eur J Clin Microbiol Infect Dis. 2006;25(2):104–7.

Adhikari RP, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. BMC Infect Dis. 2017;17(1):4–8.

Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG. Expression of resistance to tetracyclines in strains of methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother. 2000;45(6):763–70.

Fluit AC, Florijn A, Verhoef J, Milatovic D. Presence of tetracycline resistance deter-minants and susceptibility to tigecycline and minocycline. Antimicrob Agents Chemother. 2005;49(4):1636–8.

Cheesebourg M. District Laboratory Practice in Tropical Countries Part 2. Second. Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo: Cambridge University Press; 2006.

Zhang K, Sparling J, Chow BL, Hussain Z, Church DL, Daniel B, et al. New Quad-riplex PCR Assay for Detection of Methicillin and Mupirocin Resistance and Simultaneous Discrimination of Staphylococcus aureus from Coagulase-Negative Staphylococci New Quadriplex PCR Assay for Detection of Methicillin and Mupirocin Resistance an. J Clin Microbiol. 2004;42(11):4947–4955.

CLSI. Clinical and Laboratory Standard Institute (CLSI): Performance standards for antimicrobial susceptibility testing; Twenty-Second Informational Supplement. In 2019. p. 1–184.

Thapa S, Ghimire P, Manandhar S. Prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in children visiting Kanti Children hospital. Tribhu-van University; 2004.

Chambers HF, DeLeo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol [Internet]. 2009;7(9):629–41. Available from: http://dx.doi.org/10.1038/nrmicro2200

Sakoulas G, Moellering, Jr. RC. Increasing Antibiotic Resistance among Meth-icillin-Resistant Staphylococcus aureus Strains . Clin Infect Dis. 2008;46(S5):S360–7.

Lim KT, Hanifah YA, Yusof MYM, Thong KL. ErmA, ermC , tetM and tetK are essen-tial for erythromycin and tetracycline resistance among methicillin-re-sistant Staphylococcus aureus strains isolated from a tertiary hospital in Malaysia. Indian J Med Microbiol. 2012;30(2):203–7.

Theos KR, Johnson KM, Johnson DW. Staphylococcus aureus Antibiotic Suscepti-bilities in Infections in an Outpatient Dermatology Office on O'ahu. Hawaii J Med Public Health. 2019;78(5):163–8.