

Assessing Tetracycline susceptibility in multidrug-resistant *Staphylococcus aureus*: A clinical study in Kathmandu.

Lata Ghimire^{1,2*}, Megha Raj Banjara³, Abdelkodose Mohammed Hussien Abdulla¹

¹ University of Cyberjaya, Malaysia.

² Department of Microbiology, Trichandra Multiple Campus, Tribhuvan University, Nepal

³ Central Department of Microbiology, Tribhuvan University, Nepal

*Corresponding authors: latagk7@gmail.com

Doi: <https://doi.org/10.3126/pragma.v12i02.64198>

Abstract

Introduction: *Staphylococcus aureus*, recognized as an opportunistic pathogen, is frequently implicated in diverse human infections. Managing multidrug-resistant *S. aureus* poses a significant challenge in the clinical sector. Beyond B-lactam antibiotics, healthcare practitioners commonly prescribe Glycopeptides, Tetracycline, Quinolones, and Macrolide-Lincosamide-Streptogramin B antibiotics as alternative treatments for such infections. Consequently, this study was undertaken to evaluate the susceptibility of multidrug-resistant *S. aureus* to tetracycline in clinical samples. **Methods:** The study spanned 15 months, from March 2020 to May 2021, during which clinical samples were collected from three distinct hospitals in Kathmandu- Dirghayu Guru Hospital, Chabahil; Bhaktapur Cancer Hospital, Bhaktapur; and Shankarapur Hospital, Jorpati. Sample processing and laboratory work were conducted in the Microbiology Laboratory of Dirghayu Guru Hospital to assess the prevalence of different multidrug-resistant (MDR) phenotypes among *S. aureus* and their correlation with Methicillin resistance. The study included 266 isolates of *S. aureus*, and Methicillin resistance was determined using the Cefoxitin disc diffusion method. **Results:** Among 2004 clinical samples processed, 266 (13.27%) isolates were *S. aureus*. The multidrug resistant strain isolated from Outpatient department was 56.84% (54/95) and from inpatient department, the data was 43.15% (41/95). Among the 266 *S. aureus*, 28.94% Methicillin resistant *S. aureus* (MRSA) and 71.05% Methicillin sensitive *S. aureus* (MSSA) isolates were detected. Tetracycline resistance was found in 9.8% of total *S. aureus* isolates whereas 27.36% (26/95) among multidrug resistant *S. aureus* isolates. Resistance to Tetracycline was higher in MRSA as compared to MSSA.

Conclusion: The detection rate of MRSA emphasizes the urgency to improve healthcare practices to control the spread of MRSA infections. Tetracycline can be used as a viable alternative antibiotic for combating *S. aureus* infections, particularly in Kathmandu.

Keywords: *Staphylococcus aureus*, MRSA, Tetracycline

Introduction

Staphylococcus aureus is a commensal organism commonly found on the skin and in the nasal passages. It is frequently detected in blood, pus, and urine samples as well. It is

typically a part of the normal flora, but can also act as an opportunistic pathogen. This characteristic has made it one of the most prevalent pathogens in both nosocomial and community-acquired infections. (Ghimire et al., 2020).

The different types of antibiotics are reported effective against the *S. aureus* infection since the discovery of penicillin during 1942. β -lactam antibiotics, tetracycline, vancomycin, and macrolides are widely used antibiotics against *S. aureus*, however, there are reports of resistance to these antibiotics (Nourbakhsh et al., 2016). Antimicrobial resistance poses a significant burden in the context of Nepal, largely attributed to the irrational use of antibiotics. A study conducted in Nepal highlighted that a substantial 78.7% of antibiotic users were uninformed about the potential consequences associated with antibiotic resistance (Rijal et al., 2021).

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 profusely used in clinical practice (Kandel et al., 2020).

Penicillin and Methicillin have historically been the drug of choice for Staphylococcal infections. However, the emergence of Methicillin resistance in this organism has significantly limited the availability of effective therapeutic alternatives for treating these infections. (Adhikari et al., 2017).

Tetracycline has been used as the alternative to Penicillin derivatives. This drug has been used in case of Penicillin allergic reactions as well. In some countries, it is the second most frequently prescribed drug after Penicillin because of its cost effectiveness and broad spectrum nature (Hull et al., 2022).

There are a number of susceptibility testing protocols that suggest to routinely test *S. aureus* strains to understand tetracycline resistance. Basically, tetracycline resistance may be developed in *Staphylococcus* sp 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 (Gholami-Ahangaran et al., 2021). *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 (Aziz & Abdulrahman, 2021).

1.1 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 helps to determine the susceptibility of Tetracycline against multidrug resistant *S. aureus*.

2. Materials and Methods

2.1 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.

Various clinical samples, including pus, wound swab, throat swab, body fluid, urine, and blood, were systematically collected from both inpatients and outpatients seeking medical care at Shankarapur Hospital, Dirghyau Guru Hospital, and Bhaktapur Cancer Hospital.

2.2 Ethics statement

This research study obtained ethical approval from the Nepal Health Research Council (Registration number 114/2020). The patients with the possibility of infection if given informed consent after being provided with comprehensive information about the study were included in the study. On the other hand the individuals who were unwilling to participate and those who had taken antibiotics within the preceding 48 hours were deliberately excluded from the study.

2.3 Sample collection and processing

A total of 266 *Staphylococcus aureus* isolates were isolated from 2004 clinical specimens. Throat swabs and body swabs were precisely collected utilizing sterile cotton swabs. Patients were explicitly instructed to collect midstream urine samples in sterile containers. Furthermore, for blood and body fluid specimens, medical officers were requested to collect the samples directly from the patients following the defined clinical protocols.

The consent form was obtained with the details of patient like name, age, gender, patient type, clinical diagnosis, family history, genetic history, duration of hospital stays and other parameters significant to this study. For the patient less than 18 years of age, the parents or the legal guardian were asked to fill the consent form.

All the test specimen were collected following the aseptic, standardized procedures. The various bacteriological culture media were used to inoculate different samples depending upon the type of the specimen (Ghimire et al., 2022). The specimens were inoculated into various culture media, including Nutrient Agar (NA), Blood Agar (BA), and Mannitol Salt Agar (MSA). Following inoculation, the culture media were placed for incubation at 37⁰C for overnight. After the incubation period, thorough examination of the plates was done to assess and record any observable signs of microbial growth. 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 culture 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 test, oxidase test, coagulase test, and oxidative and fermentative test (Maheshwori, 2019).

The colonial characteristics were studied on the basis of its colony morphology from the nutrient agar. The catalase positive, oxidase negative, slide and tube coagulase positive, fermentative and beta-hemolysis on blood agar confirmed the organism to be *S. aureus*. ATCC 25923 for *S. aureus* was used as a control strain of coagulase positive strain (Olutola et al., 2016).

2.4 Antimicrobial susceptibility testing (AST)

S. aureus was 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°C 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 (2019) were followed to detect MRSA isolates. Other antibiotics used to test antibiotic susceptibility tests were Ampicillin (10 µg), Cotrimoxazole (25 µg), Chloramphenicol (30 µg), Erythromycin (15 µg), Gentamycin (10 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg) and Nitrofurantoin (300 µg) for only urine samples. In accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, the identification of a Multidrug-Resistant (MDR) strain is established by considering a bacterial strain resistant to at least one antibiotic of three or more distinct classes of antibiotics. Adhering to these guidelines enables a standardized and clinically relevant approach to the classification of multidrug resistance in bacterial strains (CLSI, 2019).

3. 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.

4. Results

The table shows the various types of clinical specimens collected, the number of *Staphylococcus aureus* isolates, the prevalence of MRSA and MSSA, and the proportion of isolates demonstrating multidrug resistance. A total of 2004 specimens were analyzed from both the in-patients and out-patients of three selected hospitals. It included 895 urine samples, 348 blood, 342 throat swab/sputum, 228 pus/wound swab and 191 body fluids. Out of 2004 specimens, 899 specimens were collected from male patients and 1105 from female patients. *S. aureus* identified were 266 and among them 95 (35.7%) were multidrug resistant (Table 1)

Table 1. 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(28.9%)	189(71.1%)	95	35.5

Note: U=Urine; Bl=Blood; Th=Throat swab; BF=Body fluid; SA=*S.aureus*; MRSA= Methicillin resistant *S. aureus*; MSSA = Methicillin sensitive *S. aureus*; MDRSA= Multidrug resistant *S. aureus*.

The susceptibility patterns of *Staphylococcus aureus* isolate to various antibiotics has been illustrated in the following table. The data is expressed in terms of the percentage of isolates that are either sensitive or resistant to each antibiotic. 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 2)

Table 2: Antibiogram of *Staphylococcus aureus* (n=266)

Antibiotics	Sensitive	%	Resistant	%
Ampicillin(10mcg)	80	30.08	186	69.92
Cotrimoxazole(25mcg)	144	54.14	122	45.86
Gentamycin(10mcg)	180	67.67	86	32.33
Ciprofloxacin(5mcg)	168	63.16	98	36.84
Chloramphenicol(30mcg)	200	75.19	66	24.81
Erythromycin(15mcg)	206	77.44	60.0	22.56
Cefoxitin(30mcg)	190	71.43	76	28.57
Tetracycline (30mcg)	240	90.23	26.0	33.77
*Nitrofurantoin(300mcg)	51	66.23	27	29.50

*For Urine sample only, which is 77.

The distribution of Tetracycline susceptibility among multidrug-resistant *Staphylococcus aureus* isolates is illustrated in Figure 1. The majority of these isolates remain sensitive to Tetracycline, but a significant proportion has acquired resistance to this antibiotic. For the Tetracycline susceptibility test against MDRSA, 72.63% was detected sensitive from the disc diffusion test.

The following table illustrates the varying levels of antibiotic resistance in *Staphylococcus aureus* isolates, highlighting notable differences between tetracycline-sensitive and tetracycline-resistant groups for each antibiotic tested. The low p-values suggest that these differences are statistically significant. 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 3)

Table 3: Antibiotic resistance pattern of Tetracycline resistant and sensitive *S. aureus*

Antibiotics	Resistance in TSSA isolates (n=240)		Resistance in TRSA isolates (n=26)		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

Note: TSSA=Tetracycline sensitive *S. aureus*; TRSA=Tetracycline resistant *S. aureus*; S=Sensitive; R=Resistant

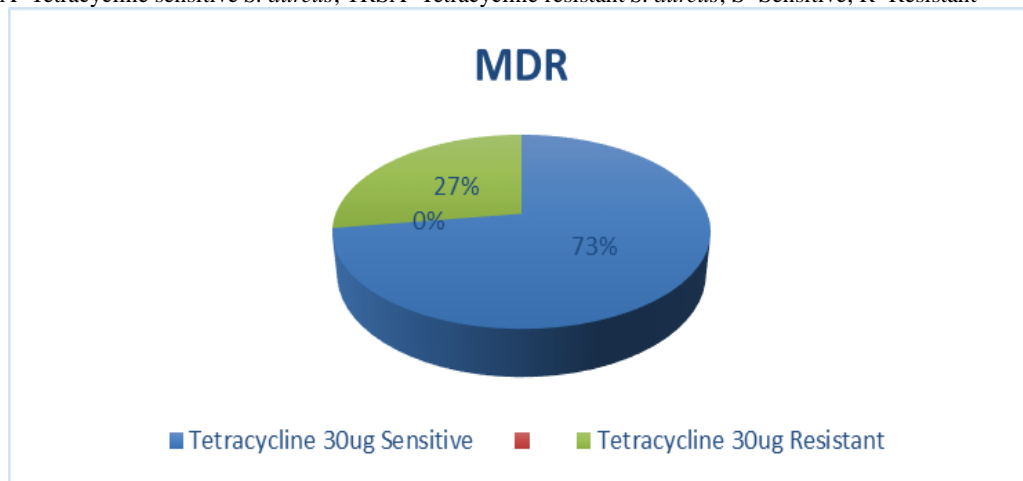


Figure 1: Tetracycline Susceptibility in Multidrug resistant *S. aureus*

The pie chart in figure 1 demonstrates that 27% Tetracycline resistant *S. aureus* were observed multidrug resistant.

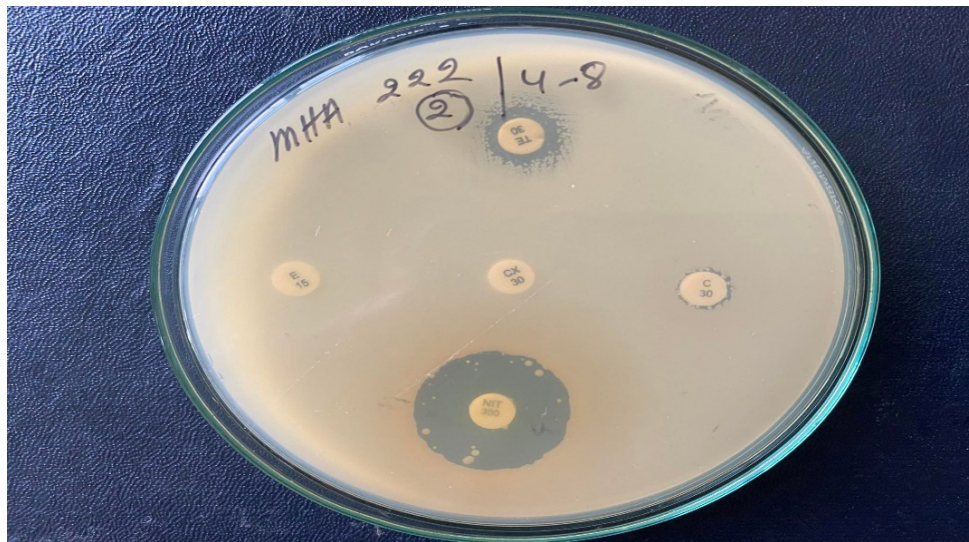


Figure 2: Antibiotic susceptibility testing in MHA

Figure 2 shows the antibiotic susceptibility with zone of inhibition indicating the sensitivity or the resistivity of *S. aureus* in MHA plate towards particular antibiotic.

5. 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% (Thapa et al., 2004). 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 (Raut et al., 2017).

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 (Chaudhary, 2023).

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 (Edan & Alsaimary, 2011).

From the study it was observed that almost one third isolates of *S. aureus* were MDR and among them majority were from the outpatients. 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 infections and other nosocomial infections. The significant resistance was observed in the last decade along with the co-resistance between Erythromycin, Ciprofloxacin and Tetracycline (Lim et al., 2012). However in this study, it was observed that the multidrug resistant strains were 27.36% susceptible towards Tetracycline similar to other studies (Do et al., 2019). 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 it was observed that 72.63% of MDRSA were sensitive towards tetracycline.

6. 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.

7. Acknowledgement

We would like to thank all the hospital and laboratory staffs of the Microbiology Department of Dirghyau Guru Hospital, Bhaktapur Cancer Hospital and Shankarapur Hospital for their kind support in the collection of samples and performing the necessary laboratory tests during the study. A special acknowledgement goes to the individuals whose samples have been taken for this study.

References

- Adhikari, R. P., Shrestha, S., Barakoti, A., & Amatya, R. (2017). Inducible clindamycin and methicillin resistant *Staphylococcus aureus* in a tertiary care hospital, Kathmandu, Nepal. *BMC Infectious Diseases*, 17(1), 4–8. <https://doi.org/10.1186/s12879-017-2584-5>
- Aziz, K. E., & Abdulrahman, Z. F. A. (2021). Detection of Tetracycline tet(k) Gene in Clinical *Staphylococcus aureus* Isolates. *IOP Conference Series: Earth and Environmental Science*, 761(1). <https://doi.org/10.1088/1755-1315/761/1/012128>
- Chaudhary, P. (2023). *PREVALENCE OF MULTIDRUG RESISTANT STAPHYLOCOCCUS AUREUS IN CLINICAL SPECIMENS FROM IPD PATIENTS AT SHAHID GANGALAL NATIONAL HEART CENTRE*. Tribhuvan university.
- CLSI. (2019). *Clinical and Laboratory Standard Institute (CLSI): Performance standards for antimicrobial susceptibility testing; Twenty-Second Informational Supplement*. 1–184.
- Do, K. R. T., Johnson, K. M., & Johnson, D. W. (2019). *Staphylococcus aureus* Antibiotic Susceptibilities in Infections in an Outpatient Dermatology Office on O’ahu. *Hawai’i Journal of Medicine & Public Health*, 78(5), 163–168.
- Edan, I., & Alsaimary, A. (2011). Antibigram and multidrug resistance patterns of *Staphylococcus aureus* (MDRSA) associated with post operative wound infections in Basrah – Iraq. *Medical Practice and Review*, 2(6), 66–72. <http://www.academicjournals.org/MPR>
- Ghimire, L., Banjara, M. R., & Abdulla, A. H. (2022). Tetracycline Resistant Genetic Determinants In MDR *Staphylococcus Aureus* Isolates From Tertiary Care Hospitals ,

- Kathmandu. *Webology*, 19(3), 2715–2723.
- Ghimire, L., Banjara, M. R., Pathak, R., & Abdulla, A. M. H. (2020). Antibiotics susceptibility pattern of Methicillin Resistant Staphylococcus aureus isolated from the clinical samples different tertiary care hospitals of Kathmandu. *Patan Pragma*, 6(1), 39–44. <https://doi.org/10.3126/pragma.v6i1.34390>
- Gholami-Ahangaran, M., Karimi-Dehkordi, M., Miranzadeh-Mahabadi, E., & Ahmadi-Dastgerdi, A. (2021). The frequency of tetracycline resistance genes in Escherichia coli strains isolated from healthy and diarrheic pet birds. *Iranian Journal of Veterinary Research*, 22(4), 337–341. <https://doi.org/10.22099/ijvr.2021.38454.5592> Abstract
- Hull, R. C., Wright, R. C. T., Sayers, J. R., Sutton, J. A. F., Rzaska, J., Foster, S. J., Brockhurst, M. A., & Condliffe, A. M. (2022). Antibiotics Limit Adaptation of Drug-Resistant Staphylococcus aureus to Hypoxia. *Antimicrobial Agents and Chemotherapy*, 66(12). <https://doi.org/10.1128/aac.00926-22>
- Kandel, S., Adhikari, N., Dhungel, B., Shrestha, U., Angbuhang, K. B., Karki, G., Adhikari, B., Banjara, M. R., Rijal, K. R., & Ghimire, P. (2020). Characteristics of Staphylococcus aureus Isolated From Clinical Specimens in a Tertiary Care Hospital, Kathmandu, Nepal. *Microbiology Insights*, 13, 1–6. <https://doi.org/10.1177/1178636120972695>
- Lim, K. T., Hanifah, Y. A., Yusof, M. Y. M., & Thong, K. L. (2012). ErmA, ermC, tetM and tetK are essential for erythromycin and tetracycline resistance among methicillin-resistant Staphylococcus aureus strains isolated from a tertiary hospital in Malaysia. *Indian Journal of Medical Microbiology*, 30(2), 203–207. <https://doi.org/10.4103/0255-0857.96693>
- Maheshwori, U. R. (2019). Phenotypic and genotypic Characterization of Vancomycin resistant Staphylococcus aureus among MRSA isolates in a tertiary care hospital. In *Ayan*. THE TAMIL NADU DR. M. G. R MEDICAL UNIVERSITY.
- Nourbakhsh, F., Namvar, A. E., & Momtaz, H. (2016). Characterization of Staphylococcal Cassette Chromosome Mec Elements in Biofilm-producing Staphylococcus Aureus, Isolated from Hospital Infections in Isfahan. *International Journal of Medical Laboratory*, 3(1), 33–42.
- Olutola, O., Adeyanju, A., & Torimiro, N. (2016). Extended Spectrum β -lactamase Detection and Antibiotic Susceptibility Profile of Staphylococcus aureus Strains Isolated from Surgical Wounds. *British Microbiology Research Journal*, 17(5), 1–7.
- Raut, S., Bajracharya, K., Adhikari, J., Pant, S. S., & Adhikari, B. (2017). Prevalence of methicillin resistant Staphylococcus aureus in Lumbini Medical College and Teaching Hospital, Palpa, Western. *BMC Research Notes*, 10(187), 1–7. <https://doi.org/10.1186/s13104-017-2515-y>
- Rijal, K. R., Banjara, M. R., Dhungel, B., Kafle, S., Gautam, K., Ghimire, B., Ghimire, P., Dhungel, S., Adhikari, N., Shrestha, U. T., Sunuwar, D. R., Adhikari, B., & Ghimire, P. (2021). Use of antimicrobials and antimicrobial resistance in Nepal: a nationwide survey. *Scientific Reports*, 11(1), 1–14. <https://doi.org/10.1038/s41598-021-90812-4>
- Thapa, S., Ghimire, P., & Manandhar, S. (2004). Prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in children visiting Kanti Children hospital. *Journal of Nepal Health Research Council*, 6(1), 38–41.