STUDY OF ANTISEPTIC RESISTANCE GENES AND ANTIBIOTIC RESISTANCE IN STAPHYLOCOCCI ISOLATED FROM HOSPITAL STAFFS AND ENVIRONMENT AT A TERTIARY CARE HOSPITAL, NEPAL

Laxmi Kant Khanal,[1] Shusila Khadka,[1] Ram Prasad Adhikari,[1] Anil Kumar Sah,[2] Abhinab Acharya,[1] Shiba Kumar Rai[1]

[1]Department of Microbiology, Nepal Medical College Teaching Hospital, Gokarneshwor-8, [2]Department of Research, Annapurna Neuro Hospital, Maitighar, Kathmandu, Nepal

ABSTRACT

Non-susceptibility of bacteria to disinfectants/antiseptics has been reported in various countries, however, such study has not been conducted in Nepal yet. Therefore, we aimed to determine prevalence of antiseptic resistance genes (qacA/B, smr), pattern of antibiotic resistance, and association of antiseptic resistance gene with antibiotic resistance in Staphylococci isolated from hospital staffs and environment. A total of 275 non-repetitive swabs (total of 120 from health staffs: 80 from hands and 40 from nares and total 155 from hospital environment: 120 pre-swabs collected before sterilization of surfaces and 35 post-swabs collected after sterilization) were collected and subjected for culture and sensitivity. Obtained data were analyzed using Microsoft Excel. Among hospital surveillance swabs, prevalence of Staphylococci was 9.09% (25/275). Among them, 40.0% (10/25) of isolates were methicillin resistant and all methicillin resistant isolates were multidrug resistant. Prevalence of antiseptic resistance gene such as qacA/B was 48.0% and smr was 64.0%. The proportion of antibiotic resistance among positive antiseptic resistance was 62.5% which is significantly associated (p=0.003; Fisher exact test). Judicious use of disinfectants for cleaning hospital surfaces is recommended and cleaning should be done with detergent water on regular basis followed by periodic cleaning with disinfectant for most of the hospital surfaces. Regular hospital surveillance along with study of antiseptic resistance genes among isolates could be helpful for formulating policy on "antiseptic use" in Nepalese hospitals.

KEYWORDS

Antibiotic, antiseptic, resistance, genes

***Received on**: February 13, 2025*
***Accepted for publication**: March 16, 2025*

CORRESPONDING AUTHOR

Dr. Laxmi Kant Khanal
Associate Professor,
Department of Microbiology,
Nepal Medical College Teaching Hospital,
Attarkhel, Gokarneshwor-8, Kathmandu, Nepal
Email: khanallk2017@gmail.com
Orcid No: https://orcid.org/0000-0003-0498-0117
DOI: https://doi.org/10.3126/nmcj.v27i1.77542

INTRODUCTION

Various disinfectants/antiseptics are used to kill the bacteria in hospital environment and mainly, quaternary ammonium compounds (QACs) are recommended for this purpose globally.[1] In addition to antibiotic resistance, hospital environmental bacteria have developed tolerance against these various disinfectants/antiseptics also in different countries leading to spread of these bugs in hospital which directly affects treatment of patients.[2-5] This has created substantial health hazard along with financial burden to the patients and ultimately to the country.

Among various hospital environmental bacterial isolates, Staphylococci has been found to be responsible for common health problems related to hospital environment in our set-up according to our routine hospital surveillance findings. Development of non-susceptibility to these commonly used QAC antiseptics such as lyzol, sani-hygiene (brand names) etc. by this hospital environmental isolate has been reported in other countries long back.[2-5] According to literatures, antiseptic resistance genes such as qacA/B, qacG, qacH, qacI, and smr have been found to be responsible for resistance to these commonly used quaternary ammonium compound antiseptics/disinfectants.[2] Among these various antiseptic resistance genes, qacA/B, and smr have been reported in neighboring and other Asian countries among Staphylococci commonly (>70%).[2,4,5]

Moreover, chance of transmission of these antiseptic resistance genes among either same or different bacterial community also remains a great challenge leading to transmission of antiseptic resistance bugs in hospital set-up and even in the community.[6,7] So, findings of our study could be the milestone for preventing spread of such resistant bug in our hospital set-up.

In addition to antiseptic resistance, the other main hurdle of antibiotic resistance exists as big challenge to us and among which Staphylococci related infections only lead to >20,000 deaths per year in USA only.[8-10] Evaluation of antibiotic resistance pattern of this hospital environmental isolate could be helpful to the clinicians for selection of suitable antibiotics against this resistant pathogen and ultimately will be beneficial to the patients in our Nepalese context.

QACs are being used as disinfectants in hospital environment in Nepalese hospitals. Resistance against these QACs in other various countries have been reported.[2-4] However, no study on it for its efficacy evaluation has been conducted in Nepal yet. For our knowledge, this will be the first research study in Nepal aimed to evaluate the efficacy of commonly used QAC disinfectants against hospital environmental bacterial isolates in a Nepalese hospital set-up by detecting antiseptic resistance genes, mainly ^{bla}qacA/B and ^{bla}smr among Staphylococci.[2-5] Therefore, this study is aimed to determine prevalence of antiseptic resistance genes and their association with antibiotic resistance. We believe, findings of this study will give view on association of antiseptic resistance genes with antibiotic resistance among hospital environmental isolates and also could be helpful for policy making on 'antiseptic use in Nepalese Hospitals'.

MATERIALS AND METHODS

An analytical cross-sectional study was conducted at Nepal Medical College Teaching Hospital (NMCTH). Samples were processed at Clinical Microbiology Laboratory after collecting from hospital environment of NMCTH. Molecular work for gene detection was done in molecular lab of Annapurna Research Centre, Kathmandu. Ethical approval was obtained from Nepal Medical College Institutional Review Committee. Study was conducted between July-December, 2023 with the grant support of University Grants Commission (UGC). A total of 275 swabs obtained from hospital environment for hospital surveillance and swabs from hands and nares of health workers working in high care units (Doctors and Nurses in OT, ICU, NICU and PICU); (total 120 from health staffs; 80 from hands and 40 from nares and total 155 from hospital surfaces; 120 pre-swabs collected before sterilization of surfaces and 35 post-swabs collected after sterilization of surfaces) were included in the study.

Bacterial identification, antibiotic susceptibility test, and gene detection: Collected swabs were inoculated on blood agar and MacConkey agar and Staphylococci were isolated according to standard Microbiological technique. Suspected colonies on MacConkey agar were processed for Gram stain, catalase test and coagulase test. Gram positive cocci (GPC) in cluster and positive catalase test indicated the organism as Staphylococci. Species differentiation was done by coagulase test. *Staphylococcus aureus* were considered for positive coagulase test and coagulase negative Staphylococci (CoNS) for negative coagulase test.[11] Isolated Gram-negative bacteria were

also processed for identification according to standard Microbiological technique.[11] Isolated Staphylococci were processed for antibiotic susceptibility test according to standard Microbiological technique using Kirby-Bauer's disc diffusion method.[12] Antiseptic resistance genes were detected by using multiplex PCR.

For gene detection; bacterial DNA was extracted using standard guideline.[13] PCR was performed using forward and reverse primers for qacA/B and smr genes.[14,15] PCR products were run in gel electrophoresis and genes were detected through gel documentation system according to standard guidelines.[14,15]

Primers used for detection of qacA/B and smr genes were as follows:[16]

qacA/B (***F)***: GCA GAA AGT GCA GAG TTC G

qacA/B (***R):*** CCA GTC CAA TCA TGC CTG (for *qacA/B* gene; 361 bp)

and

smr (***F):*** GCC ATA AGT ACT GAA GTT ATT GGA

smr (***R):*** GAC TAC GGT TGT TAA GAC TAA ACC T (for *smr* gene; 195 bp).

Positive controls for qacA/B and smr were taken by primer optimization from known sequences.

Data analysis was done using Microsoft Excel. Fisher Exact test was applied for evaluating association between presence of antiseptic resistance genes and antibiotic resistance.

RESULTS

A total of 275 swabs from hospital surfaces and health staffs (doctors and nurses) working in high care units were collected and searched for prevalence of hospital environmental Staphylococci. No pathogen was isolated from health staffs' hand and nasal swabs. However, few were reported from hospital surfaces.

Hospital environmental pathogens isolated were as follows:

Staphylococci-25 (*S. aureus* = 4, CoNS = 21)
Escherichia coli-3
Klebsiella pneumoniae-4
Acinetobacter spp.-1

Prevalence of Staphylococci: 25/275 = 9.09%

Prevalence of methicillin resistant Staphylococci: 10/25 = 40.0%

Antibiotic resistance was common among MRSA isolates compared to MSSA. All MRSA were multidrug resistant (MDR) too (Table 1).

Prevalence of antiseptic resistance genes was found to be: qacA/B = 12/25 (48.0%) and smr = 16/25 (64.0%).

Table 1: Antibiotic resistance rate among Staphylococci

Name of antibiotics	Antibiotic resistance rate among methicillin resistant Staphylococci (n=10)	Antibiotic resistance rate among methicillin sensitive Staphylococci (n=15)
Ampicillin (Amp)	9 (90.0%)	2 (13.3%)
Cloxacillin (Cox)	10 (100.0%)	0 (0.0%)
Cephalexin (Cn)	10 (100.0%)	0 (0.0%)
Gentamycin (Gen)	0 (0.0%)	1 (6.6%)
Ciprofloxacin (Cip)	5 (50.0%)	1 (6.6%)
Ofloxacin (Of)	6 (60.0%)	1 (6.6%)
Cotrimoxazole (Cot)	5 (50.0%)	1 (6.6%)
Erythromycin (E)	6 (60.0%)	4 (26.6%)
Clindamycin (CD)	8 (80.0%)	1 (6.6%)
Vancomycin (Va)	0 (0.0%)	0 (0.0%)
Teicoplanin (Tei)	0 (0.0%)	0 (0.0%)
Linezolid (Lz)	0 (0.0%)	0 (0.0%)

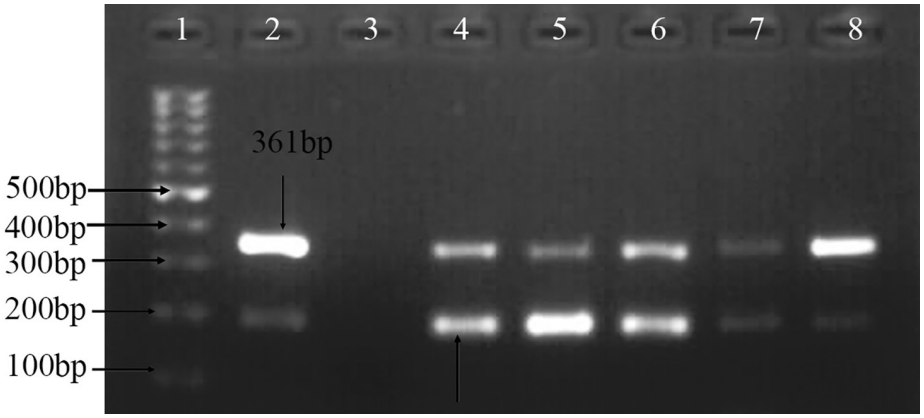

Fig. 1: Gel image showing antiseptic resistance genes

Association of antiseptic resistance with antibiotic resistance: The proportion of antibiotic resistance among positive antiseptic resistant genes is 62.5%, which is significantly associated (P=0.003; Fisher exact test).

DISCUSSION

Hospital environment contains various microbial pathogens among which the most common are *Staphylococcus, Enterococcus, Escherichia, Klebsiella, Acinetobacter, Pseudomonas* etc.[17] In our study, we found Staphylococci as major hospital environmental pathogen followed by *Klebsiella*, *Escherichia,* and *Pseudomonas* which match with findings of other various previous studies.[18-20] Many previous studies have shown Staphylococci as predominant hospital environmental isolate.[17-20] We also reported Staphylococci (*S. aureus* and CoNS) as major hospital environmental pathogen (9.09%) out of 12.0% growth of pathogens in hospital surveillance sample in our study. This corresponds with findings of previous other various studies.

Resistance against antibiotics exhibited by bacteria has become common nowadays and this is not exception for Staphylococci too. Resistance to methicillin shown by Staphylococci is being frequently reported globally nowadays.[21] Various research findings have shown prevalence of MRSA to be 14.6-81.6% among clinical samples[22-24] as detected by MecA gene.[24] Similarly, the prevalence rate among hospital environmental samples such as hospital surfaces and hospital staffs was found to be high (45.9%) in previous study.[18] In our study, we also found high prevalence of MRSA (40.0%) among hospital environmental samples. This high prevalence among hospital environmental samples could be due to existence of more drug resistant bacteria in the hospital environment compared to community due to widespread use of antibiotics in hospitals.[25]

According to WHO, antibiotic resistance has become a great global threat and it will be biggest therapeutic challenge by 2050.[26] Staphylococcal resistance to various antibiotics is also very common and is a big challenge to clinicians. In our study, 13.3% of MSSA and 90.0% of MRSA were resistant to ampicillin. Similarly, 6.6% of MSSA were resistant to gentamycin, however, no MRSA isolate was resistant to it. Total 50.0% MRSA and 6.6% MSSA were resistant to ciprofloxacin and co-trimoxazole. Similarly, 60.0% MRSA and 26.6% MSSA were resistant to erythromycin and 80.0% MRSA and 6.6% MSSA were resistant to clindamycin. However, all MRSA and MSSA isolates were susceptible to vancomycin, teicoplanin and linezolid. Resistance to various antibiotics by MRSA was high compared to that by MSSA. Similarly, all of the MRSA isolates were multidrug resistant (MDR) also which was not shown by even single MSSA isolate. Similarity in findings have been reported in previous studies too.[18,22] This indicates great therapeutic threat for infections due to these hospital environmental MRSA isolates in future.

Nowadays, bacteria have started developing resistance to commonly used disinfectants.[2-4] Various previous study reports have shown that Staphylococci (the most common hospital environmental pathogen) has developed resistance to quaternary ammonium compounds (one of the commonly used disinfectant in hospital).[2-4] This study on antiseptic resistance also showed high prevalence of antiseptic resistant genes (qacA/B = 48.0%, smr = 64.0%) among hospital environmental Staphylococci indicating development of resistant to QACs by Staphylococci. High prevalence of antiseptic resistant genes among this pathogen could be due to inclusion of samples in study from high care units of hospital such as OT,

ICU, NICU, PICU etc. where frequent use of disinfectants is common and this might have led to selective pressure for bacteria and they might have started developing resistance to those disinfectants. As role of environmental cleaning in control of hospital acquired infections is very high, this should be followed according to standard guideline.[27] This suggests need of antiseptic policy development. Reduced and judicious use of disinfectants for cleaning hospital surfaces and cleaning with detergent water only in most of the hospital areas except high care units has been recommended recently internationally too.[28] This guideline should be followed in Nepalese hospitals also which could significantly play role on reducing antiseptic resistance.

Association of antibiotic resistance with antiseptic resistance is unknown as no such association has been analyzed in various previous studies.[2-4] In this first Nepalese study, we found proportion of antibiotic resistance with positive antiseptic resistance genes to be 62.5%. This indicates there is significant association between antiseptic resistance and antibiotic resistance. This shows necessity for judicious use of disinfectants in hospitals to control antiseptic resistance as well as antibiotic resistance.

In conclusion, judicious use of disinfectants in hospital is recommended for reducing prevalence of antiseptic resistance among hospital environmental pathogens. Regular hospital surveillance along with study of antiseptic resistance genes will give view on prevalence of antiseptic resistance and hence could be helpful for formulating policy on use of antiseptics in hospitals.

ACKNOWLEDGEMENTS

This research study was supported by University Grants Commission, Sanothimi, Bhaktapur, Nepal under UGC SRDIG grant 079/80- (HS) 01. Investigators are thankful to UGC- Nepal for providing financial support for the study. Authors are also thankful to Nepal Medical College Teaching Hospital and Annapurna Research Laboratory for their allowance and co-operation to successfully conduct the study.

Conflict of interest: None

Source of research fund: University Grants Commission [SRDIG grant 079/80- (HS) 01], Sanothimi, Bhaktapur, Nepal

REFERENCES

1. Practical Guidelines for Infection Control in Healthcare Facilities. World Health Organization (Dec. 2003).
2. Ignak S, Nakipoglu Y, Gurler B. Frequency of antiseptic resistance genes in clinical Staphycocci and Enterococci isolates in Turkey. *J Antimicrob Resist Infect Control* 2017; 6: 88. DOI: 10.1186/s13756-017-0244-6.
3. Zhang M, O'Donoghue MM, Ito T, Hiramatsu K, Boost MV. Prevalence of antiseptic-resistance genes in *Staphylococcus aureus* and coagulase negative Staphylococci colonising nurses and the general population in Hong Kong. *J Hosp Infect* 2011; 78: 113–7.
4. Alam MM, Ishino M, Kobayashi N. Analysis of genomic diversity and evolution of the low-level antiseptic resistance gene smr in *Staphylococcus aureus*. *Microb Drug Resist* 2003; 9: 1–7.
5. Liu Q, Liu M, Wu Q, Li C, Zhou T, Ni Y. Sensitivities to biocides and distribution of biocide resistance genes in quaternary ammonium compound tolerant *Staphylococcus aureus* isolated in a Teaching Hospital. *Scand J Infect Dis* 2009; 41: 403–9.
6. TripathiA, Shukla SK, Singh A, Prasad KN. Prevalence, outcome and risk factor associated with vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* at a tertiary care hospital in Northern India. *Indian J Med Microbiol* 2016; 34: 38-45.
7. Antimicrobial Resistance Report-2021. Centre for Disease Control and Prevention (CDC), USA.
8. Chemaly RF, Simmons S, Dale C *et al.* The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment. *Ther Adv Infect Dis* 2014; 2: 7990. DOI: 10.1177/2049936114543287.
9. Khanal LK, Adhikari RP, Guragain A. Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) and antibiotic susceptibility pattern in a tertiary care hospital in Nepal. *J Nepal Health Res Counc* 2018; 16: 172-4.
10. Pradhan P, Rajbhandari P, Nagaraja SB *et al.* Prevalence of methicillin-resistant *Staphylococcus aureus* in a tertiary hospital in Nepal. *Public Health Action* 2021; 1: 46-51. DOI: 10.5588/pha.21.0042.
11. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology.14th ed. London: Churchill Livingstone press 2007; 263-73.
12. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial

susceptibility testing. 22nd Information supplement. Wayne, PA: Clinical and Laboratory Standard Institute 2012. (CLSI document no. M100-S22).

13. Qiagen, genomic DNA resource centre, Qiagen. Available at: https://www.qiagen.com/us/service-and-support/learning-hub/molecular-biology-methods/dna/ (Accessed on: February 2024).
14. Kapil A. Ananthnarayan and Paniker's Textbook of Microbiology. 9th ed. Hyderabad: Universities Press 2013; 49.
15. Chakraborty P. A Textbook of Microbiology. 2nd ed. Kolkata: New Central Book Agency 2010; 51-8.
16. Noguchi N, Hase M, Kitta M, Sasatsu M, Deguchi K, Kono M. Antiseptic susceptibility and distribution of antiseptic resistance genes in methicillin resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* 1999; 172: 247–53.
17. Odoyo E, Matano D, Tiria F *et al*. Environmental contamination across multiple hospital departments with multidrug-resistant bacteria pose an elevated risk of healthcare-associated infections in Kenyan hospitals. *Antimicrob Resist Infect Control* 2023; 12: 22. doi: 10.1186/s13756-023-01227-x.
18. Mukhiya RK, Shrestha A, Rai SK *et al*. Prevalence of methicillin-resistant *Staphylococcus aureus* in hospitals of Kathmandu Valley. *Nepal J Sci Technol* 2012; 13: 185-90.
19. Suleyman G, Alangaden G, Bardossy AC. The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. *Curr Infect Dis Rep* 2018; 20: 12. DOI: https://doi.org/10.1007/s11908-018-0620-2.
20. Bonadonna L, Briancesco R, Coccia AM. Analysis of Microorganisms in Hospital Environments and Potential Risks. In: Capolongo S, Settimo G, Gola M (eds): Indoor Air Quality in Healthcare Facilities. Springer Briefs in Public Health. Springer, Cham 2017. DOI: https://doi.org/10.1007/978-3-319-49160-8_5.
21. Almutairi H, Albahadel H, Alhifany AA *et al*. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) at a maternity and children hospital in Saudi Arabia: a cross-sectional study. *Saudi Pharm J* 2024; 32: 102001. DOI: 10.1016/j.jsps.2024.102001.
22. Shrestha LB, Syangtan G, Basnet A, Acharya KP, Chand AB, Pokhrel K. Methicillin-resistant Staphylococcus aureus in Nepal. *J Nepal Med Assoc* 2021; 59: 518-522. DOI: 10.31729/jnma.6251.
23. Rai JR, Amatya R, Rai SK. Hand and nasal carriage of Staphylococcus aureus and its rate of recolonization among healthcare workers of a tertiary care hospital in Nepal, *JAC-Antimicrobial Resistance* 2022; 4: dlac051. DOI: https://doi.org/10.1093/jacamr/dlac051
24. Rai JR, Suzuki T, Amatya R, Rai SK. Detection of mecA gene in *Staphylococcus aureus* isolated from health care workers at a tertiary care center in Kathmandu. *Nepal Med Coll J* 2024; 26: 179-83.
25. Struelens MJ. The epidemiology of antimicrobial resistance in hospital acquired infections: problems and possible solutions. *British Med J* 1998; 317: 652-4. DOI: 10.1136/bmj.317.7159.652.
26. WHO Global Strategy for Containment of Antimicrobial Resistance. Geneva, 2001. Available at: http://www.who.int/drugresistance/WHO_Global_Strategy.htm/en/ (Accessed on: January 2014).
27. Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. *J Hosp Infect* 2019; 73: 378 – 85.
28. JIPMER, (Jawaharlal Institute of Postgraduate Medical Education and Research). Disinfection policy-2019. Hospital Infection Control Committee, JIPMER, Pondicherry, India 2019.