The burden and characteristics of nosocomial infections in an intensive care unit: A cross-sectional study of clinical and nonclinical samples at a tertiary hospital of Nepal

Manisha Karn, Dipak Bhargava, Binod Dhungel, Megha Raj Banjara, Komal Raj Rijal, Prakash Ghimire

ABSTRACT

Background: Patients at intensive care units (ICUs) are vulnerable to acquiring nosocomial infections. The main objective of this study was to explore and characterize the burden of nosocomial infections from an ICU of National Medical College and Teaching Hospital (NMCTH), Birgunj, Nepal.

Methods: A prospective hospital-based study was conducted between April and December 2018 at NMCTH, Birgunj, Province 2, of Nepal. A total of 374 specimens including clinical specimens (n = 190) from patients admitted in an ICU and animate and inanimate environmental samples (n = 184) from the ICU were collected. Collected specimens were cultured in specific microbiological media, and microbial isolates were identified and subjected to antibiotic susceptibility test.

Results: Altogether, 374 specimens (190 clinical specimens and 184 nonclinical) of an ICU were analyzed. Out of 190 clinical specimens, 51% (97/190) showed bacterial growth. Isolated bacteria were Staphylococcus aureus (33%; 32/97), Escherichia coli (20.6%; 20/97), Klebsiella spp. (15.5%; 15/97), Pseudomonas spp. (11.3%; 11/97), and Acinetobacter spp. (11.3%; 11/97). Out of 184 nonclinical specimens, 51.6% (95/184) of the samples showed microbial growth. Among the isolates, Klebsiella spp. predominated (30.6%; 26/85) the growth, followed by S. aureus (22.4%; 19/85), Acinetobacter spp. (21.2%; 18/85), and Pseudomonas spp. (17.6%; 15/85). Among all clinical and nonclinical isolates, 61.9% (60/97) of the clinical specimens and 65.9% (56/85) of the nonclinical specimens showed multidrug resistance (MDR).

Conclusion: Two-thirds of the specimens from both clinical and nonclinical specimens showed MDR. Urgent actions are required to address the augmented rate of nosocomial infections and MDR bacteria among ICUs in Nepal.

Key Words: Intensive care units, methicillin-resistant, microbial sensitivity tests, multiple drug resistance, staphylococcus aureus

INTRODUCTION

An intensive care unit (ICU) offers treatment for severe illnesses and injuries that require around-the-clock monitoring and life support. A nosocomial infection is defined as an infection that develops after 48–72 h of
hospital stay, and the incidence of nosocomial infections in an ICU is about two to five times higher than in general inpatient hospital wards.\[2\]

Nosocomial infections are typically exogenous – the source being any part of the hospital ecosystem – including people, objects, food, water, and air in the hospital.\[3,4\] Common bacteria involved in nosocomial infections are Pseudomonas aeruginosa, Acinetobacter spp., Staphylococcus aureus, Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., Proteus spp., and others.\[5\] Gram-positive and Gram-negative bacteria are able to survive up to months on dry inanimate surfaces, with longer persistence under humid and low-temperature conditions.\[6,7\] Antibiotics are the most frequently prescribed drugs in ICUs.\[8\] Bacteria have developed resistance due to irrational use of antibiotics, thereby giving rise to the global problem of antimicrobial resistance (AMR). The burden of AMR has been disproportionately higher in low- and middle-income countries (LMICs) with further complications such as multidrug resistance (MDR), extensively drug resistance, and pandrug resistance.\[9,10\]

Similar to other LMICs settings around the globe, poor health infrastructure, hygiene, water, and sanitation are attributed to high burden of infectious diseases in Nepal.\[9,10\] Poor hygiene in health-care settings is one of the major sources of high nosocomial infections, and Nepal has a disproportionately high burden of nosocomial infections among developing countries.\[11\] Antibiotic stewardship is a systematic, measurable, and remedial effort toward appropriate use of antibiotics. Studies have strongly corroborated the benefits of antibiotic stewardship program for optimization of antibiotic use. Anticipated outcomes of such programs include reduction in treatment failures, length of hospital stays, and pace of emergence and spread of drug-resistant strains.\[12\] Unfortunately, there are no systematic antibiotic stewardship programs in ICUs of Nepal. ICUs in Nepal use some of the broad-spectrum antibiotics to treat infections in patients, which fails to curb the vicious cycle of re-infections and instead adds pressure to these drugs resulting in development of AMR.\[9,13\] AMR is emerging in a rapid pace and continues to pose significant threat to the current options for treatment of common infections in community and hospital settings.\[14\]

Several studies in the past have investigated the prevalence of nosocomial infections among ICU patients and its surrounding environment.\[11,14,15\] Studies have shown the varying prevalence of nosocomial infections in ICUs. For instance, the prevalence was 11.83% in an ICU at National Trauma Center, Kathmandu; 34% in Tribhuvan University Teaching Hospital, Kathmandu;\[16\] and 21.08% in Grande International Hospital, Kathmandu.\[17\] Another study reported 27.49% nosocomial bacterial infections among inpatients at a tertiary care hospital in Kathmandu.\[18\] Increasingly in recent years, more studies have been carried out to explore the prevalence of nosocomial infections in Nepal. Nevertheless, all these studies were conducted in tertiary settings within capital city, Kathmandu. To our knowledge, this is the first study reporting the prevalence of nosocomial infections from outside the capital city where the resources are significantly constrained. The main objectives of this study were to explore the prevalence of nosocomial infections, types of bacterial infections, and antimicrobial susceptibility patterns in bacterial isolates from clinical and nonclinical samples of an ICU at National Medical College Teaching Hospital (NMCTH), Birgunj. Furthermore, our study also explores the similarities and differences based on the occurrence patterns, cultural characteristics, and antimicrobial susceptibilities of bacterial isolates from clinical and nonclinical samples.

**METHODS**

This cross-sectional hospital-based study was conducted between July 1, 2018, and December 30, 2018, at the NMCTH, Birgunj, Province 2, of Nepal.

**Specimen collection**

All the clinical specimens (blood, wound swab, urine, pus, sputum, catheter tip, and tracheal aspirate) were collected from the ICU patients. Patients admitted to the ICU of hospitals for more than 48 h and those developing clinical evidence of infection (after the admission at ICU) were included in the study. Samples from the patients admitted in the hospitals for <48 h were excluded from the study. All the collected clinical specimens were processed on the basis of standard methods of microbiological techniques.\[18,19\]

For the animate and inanimate environmental specimens that included samples from hands of health care workers (doctors, nurses, sweepers, visitors, and staffs working in the ICU) were collected.\[20,21\] The specimens from the hand were collected by taking a swab moistened with sterile normal saline. Swabs were rotated on the surface of hands including webs between the fingers. Samples from fabrics/clothes (bedsheet, gown, and curtains) that are regularly used or are in close contact with patients, ICU-HCWs, and the visitors, were sampled to determine the presence of microorganisms.

Samples from different inanimate objects such as bedside table, medicine table, IV stand, door handles, telephone, ventilator, light, suction machine, nebulizer, pulse oximeter, ECG (Electrocardiogram), oxygen masks, stethoscope, floor, wall, warmer/heater, nurse station, weight machine, surgical drum, surgical instrument, syringe pump, and arterial blood gas machine in an ICU were collected.\[20,21\]
Specimen culture
All the collected clinical and nonclinical specimens from an ICU were subjected to culture in microbiological media. Clinical specimens (except blood which was preinoculated in the BHI broth) were inoculated on the MacConkey agar (MA), blood agar (BA), and chocolate agar (CA).

The CA plates were then incubated at 37°C for 24 h in candle jar, whereas BA and MA plates were incubated under aerobic condition at 37°C for 24 h.[18,19] Nonclinical specimens from the ICU were further incubated with normal saline for 24 h at 37°C. These specimens were further streaked and labeled into MA, BA, and Mannitol salt agar and then incubated at 37°C for 24 h.[18,19]

Identification of isolates
Bacterial isolates were identified by Gram staining and various biochemical tests.[18,19]

Antibiotic susceptibility test
All identified bacterial isolates were tested for susceptibility testing against penicillin (10 μg), ampicillin (10 μg), azithromycin (15 μg), cefoxitin (30 μg), cotrimoxazole (25 μg), gentamicin (10 μg), vancomycin (30 μg), tigecycline (15 μg), linezolid (15 μg), levofloxacin (5 μg), cefazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), meropenem (10 μg), piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), nitrofurantoin (300 μg), and amoxiclav (30 μg) (HiMedia India Pvt. Ltd) following Kirby–Bauer method on Mueller-Hinton agar (MHA, HiMedia India Pvt. Ltd). Using a sterile loop, four to five different colonies of a test organism were mixed with 2 mL of sterile saline and vortexed to create a smooth suspension. The turbidity of this suspension was adjusted to a 0.5 McFarland standard, with an approximate bacterial concentration of 150 million/mL. A sterile swab is then dipped into the suspension, firmly pressed to remove excess fluid, and plated on MHA. Antibiotic discs were then applied on MHA plates and incubated at 37°C for 18–24 h. After incubation, zone of inhibition (ZOI) was measured and interpreted using the standard chart and organisms were reported as susceptible, intermediate, or resistant based on the CLSI 2016 guidelines.[20] Those isolates which were not susceptible (either resistant or intermediate) to three or more antibiotic classes were considered multidrug resistant.[21]

Associating clinical and environmental isolates
On the basis of similarity in occurrence pattern, colony, morphology, pigmentation, biochemical characters, and antibiotic susceptibility pattern, an association between the clinical and nonclinical isolates was explored. Isolates from patients and an ICU environment that included specimens from HCWs; clothes/fabrics being used by HCWs, visitors, and patients; and other inanimate objects were recorded, and the sources of microorganisms (that may be contaminating, colonizing, or may be in fact infecting the patient) in the clinical isolates were identified.[22]

Quality control test
Control strains of E. coli (ATCC 25922) and S. aureus (ATCC 25923) were used for quality control. Piperacillin-tazobactam showed 24–30 mm of ZOI against control strains E. coli (ATCC 25922) and 27–36 mm ZOI against S. aureus (ATCC 25923).

Data management and statistical analysis
Data were entered and analyzed using IBM SPSS Statistics for Windows, version 24.0, Armonk, NY, USA: IBM Corp. Descriptive and inferential statistics were analyzed. Statistical analysis included Chi-squared test to explore the association between the clinical isolates and nonclinical (environmental) isolates from various animate and inanimate objects. Association was considered significant if \(P \leq 0.05\). A correlation between MDR isolates from clinical and nonclinical samples was calculated using the Pearson correlation coefficient (r-values).

RESULTS

Specimen size and type
A total of 374 specimens were obtained, including clinical specimens from ICU patients (\(n = 190\)) and nonclinical environmental specimens (\(n = 184\)) from both animate and inanimate surfaces. Among 190 clinical specimens, blood (\(n = 97\)), wound swab (\(n = 34\)), urine (\(n = 23\)), pus (\(n = 19\)), sputum (\(n = 13\)), catheter tip (\(n = 3\)), and tracheal

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**Table 1: Distribution of bacterial isolates from clinical specimens of ICU patients**

| Specimens       | Growth | S. aureus | E. coli | Klebsiella spp | Pseudomonas spp | Acinetobacter spp | CoNS* | Streptococci | Enterococci |
|-----------------|--------|-----------|---------|---------------|----------------|------------------|--------|--------------|-------------|
| Blood (\(n = 97\)) | 41     | 42        | 20      | 62.5          | 2              | 10               | 5      | 33.3         | 1           |
| Wound swab (\(n = 34\)) | 22     | 23        | 7       | 21.8          | 8              | 40               | 4      | 26.7         | 2           |
| Urine (\(n = 23\)) | 8      | 8         | 1       | 3.2           | 4              | 20               | 1      | 9.1          | 1           |
| Pus (\(n = 19\)) | 15     | 16        | 3       | 9.3           | 6              | 30               | 1      | 6.6          | 2           |
| Sputum (\(n = 13\)) | 7      | 7         | 1       | 3.2           | 0              | 3                 | 20     | 1            | 27.2        |
| Catheter tip (\(n = 3\)) | 3      | 3         | 0       | 0             | 2              | 13.4             | 1      | 9.1          |             |
| Tracheal aspirate (\(n = 1\)) | 1      | 1         | 0       | 0             | 0              | 0                 | 1      |               |             |
| Total (\(n = 190\)) | 97     | 32        | 20      | 15            | 11             | 11               | 4      | 3            | 1           |

*CNS = Coagulase Negative Staphylococci*
aspirate (n = 1) were included. Among 184 nonclinical specimens, hand swabs (n = 46), clothes/fabrics from health-care workers (n = 21), inanimate objects (n = 59), and medical instruments in the ICU (n = 58) were included in the study.

Prevalence of bacterial isolates
A total of 190 ICU patients (age range: 6 months to 90 years) were included in the study. Out of 190 ICU patients, 54.2% (103/190) were male and 45.8% (87/190) were female. Out of 190 clinical specimens, 51% (97/190) showed growth of bacteria. Among the isolates, 33% (32/97) were *S. aureus*, 20.6% (20/97) were *E. coli*, 15.5% (15/97) were *Klebsiella* spp., 11.3% (11/97) were *Pseudomonas* spp., and 11.3% (11/97) were *Acinetobacter* spp. [Table 1].

### Table 2: Microbial investigation of non-clinical samples from an ICU

| Samples                        | Growth on cultural Media |  |  |  |  |
|--------------------------------|--------------------------|---|---|---|---|
|                                | Yes | % | No | % | Total | % |
| Hand swab Samples              |     |   |    |   |       |   |
| Doctor hand swab before washing| 2   | 2.1 | 1 | 1.1 | 3 | 1.6 |
| Doctor hand swab after washing | 0   | 3 | 3.4 | 3 | 1.6 |
| Nurse hand swab before washing | 3   | 3.2 | 2 | 2.3 | 5 | 2.7 |
| Nurse hand swab after washing  | 0   | 5 | 5.7 | 5 | 2.7 |
| Sweeper hand swab before washing| 3   | 3.2 | 2 | 2.3 | 5 | 2.7 |
| Sweeper hand swab after washing| 0   | 5 | 5.7 | 5 | 2.7 |
| other HCWs swab before washing | 1   | 1.1 | 4 | 4.5 | 5 | 2.7 |
| others HCWs swab after washing | 0   | 5 | 5.7 | 5 | 2.7 |
| Visitors hand swab before washing| 4   | 4.2 | 1 | 1.1 | 5 | 2.7 |
| Visitors hand swab after washing| 0   | 5 | 5.7 | 5 | 2.7 |
| Sub total                      | 13  | 13.8 | 33 | 37.5 | 46 | 25 |
| Fabrics/clothing samples       |     |   |    |   |       |   |
| Doctor’s gown samples          | 2   | 2.1 | 2 | 2.3 | 4 | 2.2 |
| Patient’s gown samples         | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| Visitors gown Samples          | 3   | 3.2 | 1 | 1.1 | 4 | 2.2 |
| Patient’s bed sheet samples    | 6   | 6.3 | 0 | 0 | 6 | 3.6 |
| Curtains samples               | 2   | 2.1 | 3 | 3.4 | 5 | 2.7 |
| Sub total                      | 14  | 14.8 | 7 | 7.9 | 21 | 11.4 |
| Inanimate Surfaces             |     |   |    |   |       |   |
| Patient’s trolley              | 2   | 2.1 | 0 | 0 | 2 | 1.1 |
| Medicine trolley               | 6   | 6.3 | 1 | 1.1 | 7 | 3.8 |
| Door handle                    | 3   | 3.2 | 3 | 3.4 | 6 | 3.3 |
| Floor                          | 5   | 5.3 | 2 | 2.3 | 7 | 3.8 |
| Nurse station                  | 8   | 8.4 | 3 | 3.4 | 11 | 6 |
| Surgical drum                  | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| IV Stand                       | 1   | 1.1 | 3 | 3.4 | 4 | 2.2 |
| Oxygen Cylinder                | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| Light                          | 2   | 2.1 | 3 | 3.4 | 5 | 2.7 |
| Wall                           | 5   | 5.3 | 2 | 2.3 | 7 | 3.8 |
| Patient’s Board                | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| Bed site monitor               | 0   | 2 | 2.3 | 2 | 1.1 |
| Basin Tap                      | 0   | 2 | 2.3 | 2 | 1.1 |
| Sub total                      | 35  | 37.1 | 24 | 27.2 | 59 | 32.1 |
| Instrument/Machines surfaces of ICU |     |   |    |   |       |   |
| Nebulizer mask                 | 1   | 1.1 | 2 | 2.3 | 3 | 1.6 |
| Ventilator                     | 2   | 2.1 | 1 | 1.1 | 3 | 1.6 |
| Ventilator vent                | 0   | 2 | 2.3 | 2 | 1.1 |
| Oxygen Mask                    | 4   | 4.2 | 1 | 1.1 | 5 | 2.7 |
| Suction Machine                | 9   | 9.5 | 1 | 1.1 | 10 | 5.4 |
| Oxymeter pulse                 | 1   | 1.1 | 3 | 3.4 | 4 | 2.2 |
| Stethoscope                    | 1   | 1.1 | 4 | 4.5 | 5 | 2.7 |
| Electrocardiogram              | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| Warmer                         | 1   | 1.1 | 0 | 0 | 1 | 0.5 |
| Ambubag masks                  | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| Weighing machine               | 1   | 1.1 | 0 | 0 | 1 | 0.5 |
| Phototherapy                   | 1   | 1.1 | 0 | 0 | 1 | 0.5 |
| Syringe pump                   | 1   | 1.1 | 1 | 1.1 | 2 | 1.1 |
| Surgical instruments           | 5   | 5.3 | 3 | 3.4 | 8 | 4.3 |
| ABG machine                    | 4   | 4.2 | 2 | 2.3 | 6 | 3.3 |
| Isolated room oxymeter         | 0   | 1 | 1.1 | 1 | 0.5 |
| oxygen hood                    | 0   | 1 | 1.1 | 1 | 0.5 |
| X-ray                          | 0   | 1 | 1.1 | 1 | 0.5 |
| Sub total                      | 33  | 35.2 | 25 | 28.1 | 58 | 31.5 |
| Total                          | 95  | 89 | 184 |     |     |   |

Out of 184 nonclinical specimens sampled from the ICU environment, 25% (46/184) were hand swab samples, 11.8% (21/184) were fabrics/clothing swabs,
32.2% (59/184) were inanimate surface swab samples, and 31.2% (58/184) were instrument/machine surface swabs of ICU [Table 2]. Out of 184 nonclinical samples sampled from ICU environment, 51.6% (95/184) of the samples showed microbial growth, of which 85 pathogenic bacterial isolates were confirmed [Table 3]. Out of 85 bacterial isolates, 30.6% (26/85) showed growth of Acinetobacter spp., followed by S. aureus (22.4%; 19/85), and Pseudomonas spp. (19.2%; 18/85), Acinetobacter spp. (17.6%; 15/85) [Figure 1].

### Antibiotic susceptibility pattern of bacterial isolates isolated from clinical specimens and environmental specimens in intensive care unit

Out of 32 S. aureus isolated from clinical specimens, 87.5% (28/32) were methicillin-resistant S. aureus (MRSA), whereas 94.4% (18/19) were MRSA in S. aureus isolated from nonclinical specimens in the ICU. Vancomycin was found effective for both the S. aureus isolates isolated from clinical specimens and nonclinical specimens in the ICU [Table 4].

### Multidrug resistance profile in bacterial isolate

Among the total of 97 bacterial isolates from clinical specimens, 61.9% (60/97) were found to be MDR, whereas 65.9% (56/85) were found to be MDR in nonclinical specimens from the ICU environment. The highest MDR strains were detected in Acinetobacter spp. (90.9%; 10/11) in clinical isolates followed by nonclinical specimens (94.4%; 17/18) [Figure 2].

### Comparisons between clinical and environmental isolates

Comparisons between clinical and nonclinical isolates found that five types of clinical isolates matched their biotype with nonclinical samples. In addition, antibiogram typing for antibiotic susceptibility patterns was compared.

In nonclinical specimens of S. aureus, 7 types of antibiotic patterns were found, whereas from clinical isolates, 11 patterns were observed. Among them, the most repeating patterns found from ICU patients were pattern 1, pattern 3, pattern 4 (six times), and pattern 11 (four times) [Table 5].

In the case of nonclinical specimens E. coli, three types of antibiotic pattern were found. Similarly, from clinical E. coli, four antibiotic patterns were found. Among them, pattern 1 was found most consistently (six times) from pus specimens, followed by pattern 3 (four times), pattern 4 (four times), and pattern 2 (two times). Acinetobacter spp. from nonclinical samples showed three different types of antibiotic pattern. Among clinical Acinetobacter spp., both pattern 1 and pattern 2 were consistently found in ICU patient’s specimens [Table 6].
Klebsiella spp. from nonclinical samples showed six types of antibiotic pattern. Klebsiella spp. from clinical specimens showed nine types of antibiotic pattern; among them, most of the patterns were similar to antibiotic pattern 3 and pattern 4. Pseudomonas spp. from nonclinical samples showed eight different types of antibiotic patterns. Pseudomonas spp. isolated from clinical specimen showed five different types of antibiotic patterns; all of them were 100% similar to some of antibiotic patterns of environmental Pseudomonas spp. [Table 7].

**DISCUSSION**

More than half of the clinical and nonclinical samples from the ICU of NMCTH hospital showed microbial growth. In addition, there was a high prevalence (>60%) of MDR bacteria in those clinical specimens. In this study, the most commonly isolated Gram-positive bacteria from both the specimens (clinical and nonclinical) were S. aureus followed by Gram-negative bacteria (E. coli, Klebsiella spp., Pseudomonas spp., and Acinetobacter spp.). Our findings echoed the previous studies conducted in different clinical settings of Nepal.\(^{[5,15,22]}\) Patterns of bacterial genera identified in this study were similar to a study conducted in Iran.\(^{[24]}\) The most common infections in ICUs/hospital-acquired infections were bacteremia, surgical site infections, and urinary tract infections. The presence and predominance of particular potential pathogens in this study may have been affected by the types of patients, their illnesses, device utilization rates, and the empirical antibiotic usage patterns in addition to the prevailing flora in ICUs.\(^{[1]}\)

**Role of environmental factors (nonclinical specimens) for transmission of microorganism in intensive care units**

Environmental factors such as hands of health-care workers; different types of fabrics or clothing materials used by the health-care workers, visitors, and patients; and different inanimate surfaces such as surfaces of medical devices, door handle, bed rails, and tables might harbor some forms of microorganisms through contamination from exogenous or endogenous sources. In this study, hand swabs were taken from doctors, HCWs, other staffs, and visitors regularly present in the ICU. Swab specimens were taken before and after handwashing and sanitization. Half of the hand swab samples showed bacterial growth. However, there was no bacterial growth in hand swab samples collected after handwashing and sanitization. Inanimate surface contaminations lead to nosocomial infections either through direct contact of patients or indirectly through the hand of HCWs that become contaminated after touching contaminated surfaces and should be disinfected every 4 h.\(^{[24]}\) Poor hygiene and sanitation conditions in LMICs’ health-care settings are one of the main precursors of high nosocomial infectious diseases. Maintaining hygiene through simple handwashing alone was found to decrease the use of antimicrobials by 40%.\(^{[25]}\)

This study showed that handwashing and sanitizing procedure was a prudent step to reduce the burden of pathogens in the hands. In this study, two-third of the cloth/fabric samples had microbial growth. In the case of visitor’s gown and patient’s bedsheet, all the tested samples had had microbial growth. Nosocomial pathogens can thrive for a long duration on medical fabrics or clothing materials such as aprons, gowns, privacy curtains, and bedsheet and may serve as an active source of nosocomial infections.\(^{[20]}\)

In our study, two-third of the inanimate surfaces of objects such as patient’s trolley, medicine trolley, floor, door handle, nurse station, surgical drum, basin tap, oxygen cylinder, light, wall, and patient’s board were contaminated with microorganisms. Inanimate hospital environment surfaces often become contaminated with nosocomial pathogens either through contaminated hand contact or through contact...
with other environmental sources. Once the organisms contaminate the inanimate surfaces, it can persist and remain viable for a long time.\(^6\)

Various equipment surfaces such as bedside monitor, ventilator, and stethoscopes are the objects that come in frequent contact with HCWs. Therefore, contaminated hands of HCWs could be the source of pathogens in an ICU. In our study, half of the instruments in the ICU were contaminated with microorganisms.

**Antibiotic susceptibility patterns of bacterial isolates from the intensive care unit and its environment**

In this study, antibiotic susceptibility pattern of Gram-negative isolates showed susceptibility toward tigecycline, piperacillin-tazobactam, gentamicin, and nitrofurantoin. Most of the strains were resistant to ampicillin, ceftriaxone, cefotaxime, and ceftazidime. The findings of our study resonate with a study from Iran;\(^1^\) National Institute of Neurological and Allied Sciences, Kathmandu, Nepal;\(^2^\) Tribhuvan University Teaching Hospital, Kathmandu, Nepal;\(^3^\) and ICUs of Military Hospital in Iran.\(^4^\) Our study found a high prevalence of MDR bacteria in clinical specimens and environmental specimens. The findings of our study are consistent with the study reported from Beasat Hospital of Hamadan, West of Iran.\(^5^\) The higher prevalence of MDR bacteria in clinical specimens was also reported from the study conducted in Everest Hospital, Kathmandu;\(^6^\) International Friendship Hospital, Kathmandu;\(^7^\) 

### Table 5: Comparison of antibiotic pattern between non-clinical and clinical isolates of *Staphylococcus aureus*

| Antibiotics  | Environmental isolates pattern | Clinical isolates pattern |
|--------------|--------------------------------|--------------------------|
|              | I     | II    | III   | I     | II    | III   |
| Penicillin   | R     | R     | R     | R     | R     | R     |
| Azithromycin | R     | R     | R     | R     | R     | R     |
| Cefotaxime   | R     | R     | R     | R     | R     | R     |
| Ceftriaxone  | R     | R     | R     | R     | R     | R     |
| Gentamicin   | R     | R     | S     | R     | R     | R     |
| Levofloxacin | R     | R     | S     | S     | S     | S     |
| Meropenem    | R     | R     | R     | R     | R     | R     |
| Piperacillin | S     | S     | S     | S     | S     | S     |
| Nitrofurantoin | S     | S     | S     | S     | S     | S     |
| Cotrimoxazole| R     | S     | R     | R     | R     | R     |
| Tigecycline  | R     | S     | S     | S     | S     | S     |
| Linezolid    | R     | S     | S     | S     | S     | S     |

### Table 6: Comparisons of antibiotic pattern between non-clinical and clinical isolates of *E. coli* and *Acinetobacter spp.*

#### E. coli

| Antibiotics  | Non-clinical isolates pattern | Clinical isolates pattern |
|--------------|------------------------------|--------------------------|
|              | I     | II    | III   | I     | II    | III   |
| Penicillin   | R     | R     | R     | R     | R     | R     |
| Gentamicin   | R     | R     | S     | R     | R     | R     |
| Levofloxacin | R     | R     | R     | R     | R     | R     |
| Meropenem    | R     | R     | R     | R     | R     | R     |
| Amoxycillin  | R     | R     | R     | R     | R     | R     |
| Piperacillin | S     | S     | S     | S     | S     | S     |
| Ceftriaxone  | R     | R     | R     | R     | R     | R     |
| Tigecycline  | R     | S     | S     | S     | S     | S     |
| Linezolid    | R     | S     | S     | S     | S     | S     |
| Nitrofurantoin | R     | S     | S     | S     | S     | S     |
| Cotrimoxazole| R     | S     | S     | S     | S     | S     |
| Percentage of similarity of environmental isolates with clinical isolates | 92% with III | 83% with III | 90% with I | 83% with I |

#### Acinetobacter spp.

| Antibiotics  | Non-clinical isolates pattern | Clinical isolates pattern |
|--------------|------------------------------|--------------------------|
|              | I     | II    | III   | I     | II    | III   |
| Penicillin   | R     | R     | R     | R     | R     | R     |
| Gentamicin   | R     | R     | S     | R     | R     | R     |
| Ceftriaxone  | R     | R     | R     | R     | R     | R     |
| Tigecycline  | R     | S     | S     | S     | S     | S     |
| Nitrofurantoin | R     | S     | S     | S     | S     | S     |
| Percentage of similarity of non-clinical isolates with clinical isolates | 100% with I | 100% with II |

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International Journal of Critical Illness and Injury Science | Volume 11 | Issue 4 | October-December 2021
Table 7: Comparisons of antibiotic pattern of non-clinical and clinical bacterial isolates *Klebsiella* spp. and *Pseudomonas* spp.

| Antibiotics | Non-clinical isolates pattern | Clinical isolates pattern |
|-------------|------------------------------|--------------------------|
|             | I   | II  | III | IV  | V   | VI  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| PIP         | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   |
| GEN         | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| CAZ         | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   |
| LVX         | R   | R   | S   | R   | S   | R   | S   | R   | S   | R   | S   | S   | R   | S   | S   |
| MEM         | R   | R   | R   | S   | R   | R   | R   | R   | S   | R   | S   | S   | R   | S   | S   |
| AMC         | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   |
| TZP         | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| CTR         | R   | R   | S   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   |
| COT         | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| TGC         | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| NIT         | R   | R   | S   | R   | S   | R   | S   | R   | S   | R   | S   | S   | R   | S   | S   |
| CXI         | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   |

Percentage of similarity of environmental isolates with clinical isolates

- of V
- of II
- of IV
- of II

### Antibiotics

- PIP (Piperacillin), Genamicin (GEN), Ceftazidime (CZA), Levofloxacin (LVX), Meropenem (MEM), Amoxyclyvanuate (AMC), Piperacillin-tazobactam (TXP), Ceftriaxone (CTR), Cotrimoxazole (COT), Tigecycline (TGC), Nitrofurantoin (NIT), Cefoxitin (CXX), Ceftazidime (CAX)

Universal Medical College, Bhairahawa;[30] Nobel Medical College, Biratnagar;[31] Alka Hospital, Lalitpur;[32] and Padma Nursing Hospital, Pokhara,[33] Nepal.

In Gram-negative pathogens, 90% of *Acinetobacter* spp. (70% *Acinetobacter baumannii*) isolated from both clinical and environmental specimens were found MDR, followed by *Klebsiella* spp., *Pseudomonas* spp., and *E. coli*. High MDR strains of *Acinetobacter* spp. showed less resistance against tigecycline. This particular finding was similar to a study reported from B. P. Koirala Institute of Health Sciences, Dharan, Nepal.[34] The variation in susceptibility patterns of antibiotic depends upon the use and misuse of antibiotics in different hospitals. Drug resistance in *Acinetobacter* spp. is increasing in a rapid pace.[35,36] The profile of antimicrobial agents used to treat *A. baumannii* infections has changed according to the patterns of resistance colistin is currently the most frequently administered antimicrobial agents, whereas the use of carbapenems has markedly reduced since 2000 reflecting on the high resistance over the years.[37] All Gram-positive isolates were fully sensitive toward tigecycline and vancomycin. However, emergence of MRSA was high. In our study, more than 85% isolates of *S. aureus* were MRSA in clinical and nonclinical specimens. Our findings are consistent with previous studies from ICUs and community settings of Nepal.[5,38-42]

### Antibiotic resistance profile of clinical and environmental bacterial isolates

In this study, there were several similarities in the antibiotic susceptibility pattern of bacterial isolates (70%–90%) in clinical and nonclinical specimens. *Acinetobacter* spp. showed a greater similarity in resistance pattern, suggesting the cross-contamination between clinical and nonclinical samples in the ICU and the likelihood of having a common origin. Our findings are consistent with the previous studies from ICUs in Iran[43] and Kenya.[44] This study found a significant correlation between MDR bacteria from clinical samples and MDR bacteria from nonclinical samples, which may indicate that the drug-resistant bacteria might have been transmitted from clinical specimens to ICU’s environment via hands of health-care workers, and other inanimate objects present in ICU. Previous systematic reviews and studies have clearly established the correlation between hand hygiene and reduction in infectious disease.[9,25,45] As high as 40% reduction in antimicrobial prescription was attributed to handwashing by clinicians.[25]

### Strengths and limitations

This is the first study reporting the prevalence of infectious agents in an ICU from a tertiary hospital of Nepal outside Kathmandu that explores the correlation between the clinical and nonclinical samples. Exhaustive inclusion of samples from clinical and nonclinical specimens from the ICU allowed the study to draw a robust conclusion.
This study will be a useful reference for future studies to explore causative agents with biotyping, antibiogram typing, serotyping, and genotyping and studies for homogeneity of causative agents of nosocomial infections. Since our study was based on phenotypic detection of drug-resistant bacteria (often superbugs) from clinical and nonclinical specimens, genotypic characterization with phylogenetic analysis is recommended in future studies.

CONCLUSION

This study showed a high prevalence of MDR bacteria from both the clinical and nonclinical samples from the ICU of NMCTH. A high correlation between clinical and nonclinical samples found in this study suggests a high cross-contamination and ongoing transmission which may augment the risk of infections, irrational use of antimicrobials, and development of MDR. Therefore, prompt actions are suggested to maintain the hygiene and aseptic conditions at ICUs in Nepal.

Availability of data and materials
All data pertaining to this study are within the manuscript.

Author’s contributions
All the authors made substantial contribution to the study. MK, BD, KRR, and PG conceived and designed the study. MK collected samples and investigated and recorded the laboratory findings. DB, BD, KRR, MRB, and PG advised and formulated the methodology for the study. MK and KRR drafted initial draft of the manuscript. KRR and BD are responsible for reviewing several versions of manuscript. All authors read and approved the final manuscript.

Research quality and ethics statement
This study was approved by the Institutional Review Board / Ethics Committee at the Nepal Health Research Council (Approval # 460/2018; Approval date July 23, 2018). The authors followed the applicable EQUATOR Network (http://www.equator-network.org/) guidelines during the conduct of this research project.

Acknowledgments
We would like to express our sincere gratitude to all the staffs and faculties of the Central Department of Microbiology, Tribhuvan University, Kirtipur, and NMCTH, Birgunj, for their support. We would like to thank ICU patients for their patience and participation.

Financial support and sponsorship
This research was supported by the Departmental Fund of Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu.

Conflicts of interest
There are no conflicts of interest.

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