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Research Article

MICROBIOLOGICAL ASSESSMENT OF HOSPITAL ENVIRONMENT OF NATIONAL MEDICAL COLLEGE &TEACHING HOSPITAL, NEPAL

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ABSTRACT

Objective: This research was carried out from September to March 2023 at the National Medical College and Teaching Hospital with the objective to detect most prominent nosocomial infection causing microorganisms in hospital environment.

Methods: A total of 148 environment samples (66 in air samples and 82 surface samples) from different wards were taken from the hospital environment. All the samples were processed following standard microbiological methods.

Results: Gram-positive cocci were the most prominent ones followed by Gram-positive bacilli and then Gram-negative bacilli. Among Gram-positive isolates, coagulase-negative staphylococci accounted for 28.84% of the isolates followed by *Staphylococcus aureus* with 25.76%, *Streptococci* with 8.23%, and Gram-positive rods with 17.68%. Among Gram-negative isolates, *Acinetobacter* spp. accounted for 8.08% followed by *Escherichia coli* with 1.43% and *Klebsiella* spp with 0.35%. Among fungal isolates, yeasts were found in higher number with 9.59% in comparison to *Aspergillus* spp. All Gram-positive isolates were sensitive to vancomycin. Among Gram-negative isolates, *E. coli* showed the highest susceptibility to amikacin with 85%, *Klebsiella* spp, and *Acinetobacter* spp showed the higher degree of susceptibility to ciprofloxacin with 80% and 74.2% respectively.

Conclusions: The high incidence of similar types of bacterial isolates detection in the hospital environment suggests that the environment surrounding the hospital may be significantly contaminated with nosocomial pathogens and could represent a major mode of transmission to patients.

Keywords: Hospital environment, Nosocomial infections, Coagulase-negative *Staphylococci*, Gram-positive cocci, Gram-negative rods.

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INTRODUCTION

Hospitals are sources of different outdoor and indoor environments that support the growth of different strains of microorganisms [1-3]. The study of the distribution of different microorganisms in the hospital environment is very much crucial. These microorganisms get chance to make different clinical conditions through different modes from environmental source of the hospital. The poor microbiological quality of the hospital environment has been linked with the emergence of different infectious disease outbreaks, such as influenza, measles, and tuberculosis [4-6]. The distributed pathogens in hospital origin make different cases of hospital-based infections called nosocomial infections (NIs) and made one of the major problems in public health [7]. The novel appearance and distribution of multidrug-resistant bacteria and its resistance genes in hospital source made health-related issues globally [8]. The distribution of these resistance strains of bacteria especially in soil [9-11] and water environment [12,13] has been suggested from different reports.

The pathogenesis and epidemiology of infectious diseases in hospital are depending on the distribution of nasal carriage of bacteria among different peoples [14]. Hospital workers make association between hospital and community which work for a cross-contamination of hospital and community-acquired infectious diseases [15]. The most frequent pathogens made hospital-acquired infections are *Staphylococcus aureus*, *Escherichia coli, Micrococcus* spp., *Pseudomonas* spp., *Enterobacter, Bacillus cereus, Aspergillus* spp., *Cladosporium sp.,* and viruses [16-17]. Regular microbiological surveillance of different units of the hospital and Infection Control Unit will work out to reduce NIs.

The work was therefore considered at investigating the degree of indoor and outdoor environment containing different pathogens in wards and units of the National Medical College and Teaching Hospital (NMCTH), Birgunj, Nepal. This relates to their contribution in producing the rate of infection in the hospital.

METHODS

A cross-sectional study involving eight wards/units namely intensive care unit, hemodialysis unit, male surgery ward, female surgery ward, post-operative ward, medical ward, orthopedic ward and operation theatre and health care personals who are working at different departments in NMCTH during the period from September to March 2023. A total of 148 hospital environment samples including 66 indoor air and 82 surface samples (floor, bed bar handle, table, door, handle, tray, instruments like hemodialysis machine, etc.) were collected.

Air samples were collected as per the method of sedimentation technique through using open nutrient agar (NA) and Sobaraud's dextrose agar (SDA) [18]. NA and SDA were exposed to air for 30 min in sterilized wards and 5–10 min in unsterilized wards. The plates were immediately transported to the working laboratory. The exposed NAs and SDA were incubated at 37°C for 24 h and 28°C for 3–5 days, respectively. Surface samples from different wards were collected through the use of sterile cotton swabs. The collected swab sample was sealed in a tube and immediately transported to the laboratory. Swabs were then inoculated into blood agar and MacConkey agar. The isolates were identified according to standard procedures [19,20]. The identified bacteria were subjected to Antimicrobial susceptible test (AST) as per the Clinical and Laboratory Standards Institute [21]. The antibiotic used was ampicillin (10 μg), penicillin (10 μg), cotrimoxazole

(25 µg), ciprofloxacin (5 µg), gentamycin (10 µg), amikacin (10 µg), cefotaxime (30 µg), ceptriaxone (30 µg), nitrofurantoin (100 µg), erythromycin (15 µg), methicillin (5 µg), and vancomycin (30 µg).

Statistical analysis
The data thus obtained were analyzed by simple menu value, percentage, and test of significance using Chi-squares [22].

Ethical statement
The study was approved by the institutional research committee of NMCTH, Birgunj, Nepal. Under ethics codes of F-NMC/603/079-080.

RESULTS

Bacteria distribution
148 hospital environment samples (66 air samples and 82 surface swabs) from different wards were studied. The percentage distribution of bacterial growth among samples is shown in Table 1. Among samples, 119 (84.40%) had shown growth of bacteria. The growth of bacteria was found more in surface swab samples 63 (76.82%) than air sample 56 (84.84%). The highest bacterial growth rate (100%) was observed in the medical ward, female surgical ward, male surgical ward, orthopedic ward, post-operative ward, followed by hemodialysis unit, intensive care unit (ICU) respectively, and the least growth rate (39.13%) in samples from operation theaters (OT).

Bacterial load
The load of bacterial growth in samples from different sources of the hospital was expressed by counting the number of colonies in the form of colony forming unit (cfu). The load of cfu among different sampling sites is shown in Fig. 1. Our result revealed that the bacterial load was found highest in the medical ward followed by surgical, orthopedic, post-operative, intensive care unit, OT, and hemodialysis unit. A total of 1379 cfu were counted in samples from different sites. The percentage distribution of different microorganisms in samples has been shown in Fig. 2. Our result found rate of Gram-positive cocci was more than that of Gram-positive rods, followed by Gram-negative organisms and yeasts.

Percentage distribution of bacteria
The percentage distribution of bacteria in the form of Gram-positive and Gram-negative isolates from wards is shown in Figs. 3 and 4. The result revealed that coagulase-negative Staphylococci (CONS) were found more 403 (28.84%), followed by *S. aureus* 360 (25.76%), *Streptococci* spp.115 (8.23%) and Gram-positive rods 247 (17.68%). Among Gram-negative isolates, *Acinetobacter* spp. were observed higher 113 (8.08 %), followed by *E. coli* 20 (1.43 %) and *Klebsiella* spp. 5 (0.35%).

Microbiological assess of air of hospital environment
Among 66 air samples (33 NA plates and 33 SDA plates exposed) were collected from eight different sampling sites of the hospital. Table 2 shows the growth positivity rates of microorganisms among air samples in different wards. Our result showed that 56 (84.84%) showed the positive growth in both used media. Among different wards sampled, all the wards showed 100% growth positivity in plates exposed except for the ICU (68.75%) and OT (37.5%). The percentage distribution of different microorganisms isolated from air samples is shown in Fig. 3. The result showed that a higher number of isolated bacteria was Gram-positive cocci (64.39%), followed by Gram-positive rods (17.27%), yeast (10.64%) and Gram-negative rods (7.67%). Among Gram-positive cocci, the higher number was *S. aureus* (47.69%), followed by CONS (40.37%) and *Streptococci* sp. (11.92%). Similarly, among Gram-negative rods, the highest number of identified bacteria was *Acinetobacter* sp. (95.45%), followed by *E. coli* (2.27%) and *Klebsiella* sp. (2.27%). The result of positive growth in 33 SDA-exposed plates at different wards of the hospital showed that 84.84% growth in plates. Fungal growth was obtained in 100% of used SDA plates in wards except in ICU and OT. Among identified fungi, *Aspergillus* sp. was

Table 1: Distribution of microorganism in environmental samples of hospital wards

Sampling sites	Number of samples taken	Bacterial growth	%
Intensive care unit	38	24	63.15
Hemodialysis unit	7	6	85.7
Surgery ward (male)	16	16	100
Surgery ward (female)	16	16	100
Postoperative ward	16	16	100
Medical ward	16	16	100
Orthopedic ward	16	16	100
Operation theatre	23	9	39.13
Total	148	119	80.40

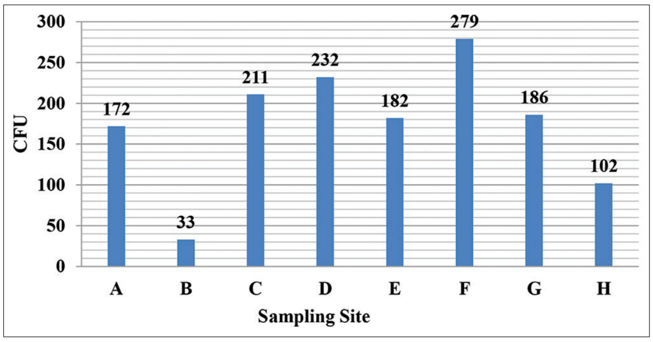

Fig. 1: Microbial load in different wards of the hospital. (A) Intensive care unit, (B) Hemodialysis unit, (C) Surgery (male), (D) Surgery (female), (E) Post-operative, (F) Medical, (G) Orthopedic, (H) Operation theatre

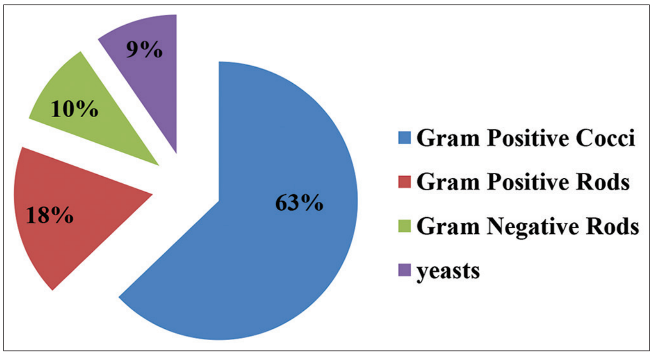

Fig. 2: Percentage occurrence of microorganisms in environment of hospital

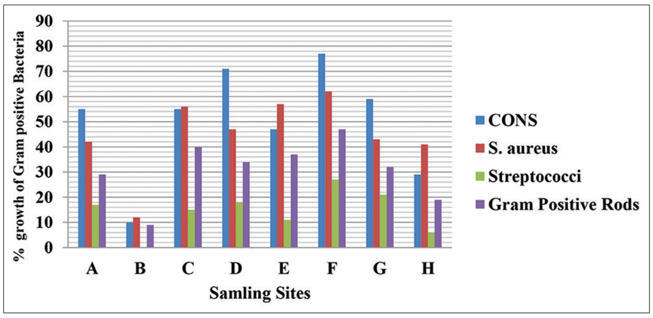

Fig. 3: Occurrence of different Gram-positive bacteria in the hospital environment. (A) Intensive care unit, (B) Hemodialysis unit, (C) Surgery (male), (D) Surgery (female), (E) Post-operative, (F) Medical, (G) Orthopedic, (H) Operation theater

Table 2: Occurrence of microorganisms in air samples of different wards

Wards	Nutrient agar media		SDA media		Total (%)
	Number of media exposed	Growth of organisms (%)	Number of media exposed	Growth of organisms (%)	
ICU	8	5 (62.5)	8	6 (75)	11/16 (68.75)
HD	1	1 (100)	1	1 (100)	2/2 (100)
Surgery (male)	4	4 (100)	4	4 (100)	8/8 (100)
Surgery (female)	4	4 (100)	4	4 (100)	8/8 (100)
POW	4	4 (100)	4	4 (100)	8/8 (100)
Medicine	4	4 (100)	4	4 (100)	8/8 (100)
Orthopedics	4	4 (100)	4	4 (100)	8/8 (100)
OT	4	2 (50)	4	1 (25)	3/8 (37.5)
Total	33	28 (84.84)	33	28 (84.84)	56/66 (84.84)

Table 3: Occurrence of microorganism in surface swabs from different wards

Wards	Number of swabs	Growth positive % (n)
ICU	22	59.09 (13)
HD	5	80 (4)
Surgery (Male)	8	100 (8)
Surgery (Female)	8	100 (8)
POW	8	100 (8)
Medical	8	100 (8)
Orthopedics	8	100 (8)
OT	15	40 (6)
Total	82	76.82 (63)

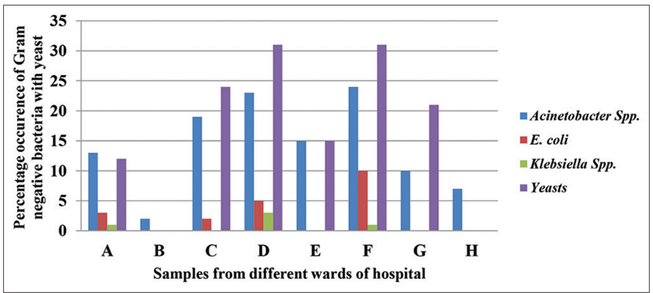

Fig. 4: Occurrence of Gram-negative bacteria with yeast in the hospital environment. (A) Intensive care unit, (B) Hemodialysis unit, (C) Surgery (male), (D) Surgery (female), (E) Post-operative, (F) Medical, (G) Orthopedic, (H) Operation theater

found higher and followed by *Penicillium* sp., *Alternaria* sp., *Mucor* sp. and *Cladosporium* sp.

Microbiological assess of surface swabs of the hospital environment

63 (76.82%) surface swabs were showed the positive growth of bacteria among 82 surface swabs samples collected from different wards of the hospital. The percentage growth of bacteria from surface swabs is shown in Table 3. The higher growth of bacteria (100%) was shown in swabs collected from medical ward, male surgery ward, female surgery ward, post-operative ward, and orthopedic ward. The least growth of bacteria (40%) was found to be in the swabs of OT followed by 59.09% in ICU and 80% was found to be in the hemodilysis unit. A load of positive growth of bacteria was found in count of 842 cfus as shown in Fig. 4. Gram-positive cocci were found higher in number counting for 509 (61.77%), followed by Gram-positive rods 148 (17.96%), Gram-negative rods 94 (11.40%) and yeasts 73 (8.85%). Among Gram-positive cocci, 254 (49.90%) were found to be CONS, 184 (36.14%) were *S. aureus* and 71 (13.94%) were found to be *Streptococci sp.* Among Gram-negative isolates, Gram-negative isolates, 71 (75.53%) were found to be *Acinetobacter* sp., followed to be *E. coli* 19 (20.21%) and *Klebsiella* Sp. 4 (4.25%).

Antibiotic susceptibility profile of bacterial isolates

The bacterial isolates (*S. aureus*, CONS, *E. coli*, *Klebsiella* sp., and *Acinetobacter* sp.) from samples of hospital were subjected to antibiotic sensitivity testing. The sensitivity profile of both Gram-positive and Gram-negative bacterial isolates has been shown in Tables 4 and 5 respectively. The result of the antibiotic sensitivity profile of Gram-positive bacteria revealed that all the isolates of *S. aureus* showed sensitivity against vancomycin with value of 100% and followed by 90.25% against amikacin, 75.6% against ceftriaxone, 36.6% against methicillin and 14.6% against penicillin. Similarly, for isolates of CONS, all these isolates showed susceptibility against vancomycin (100%), followed by 92.85% isolates against amikacin, 83.4% against gentamycin, 30.95% against methicillin, and 9.5% against penicillin.

The result of the antibiotic susceptible profile of Gram-negative bacteria was revealed that the isolate *E. coli* was showed the highest susceptibility against amikacin with value of 85%, followed by cefotaxime (75%), ciprofloxacin (75%) and nitrofurantoin (10%) respectively. Against *Klebsiella* sp., ciprofloxacin, and cefotaxime were found to be the most effective drugs with each having a sensitivity rate of 80% followed by amikacin (60%). All the isolates were showed resistance to ampicillin and nitrofurantoin.

DISCUSSION

NIs occur worldwide and affects both developed and resource-poor countries. It is the single largest factor that adversely affects both the patients and hospital, causing mortality and increased morbidity among hospitalized patients. A prevalence study conducted under the auspices of the WHO showed the frequency of NIs to be 2.4% [23]. The pathogens caused NIs showed resistance to many antibiotics. It is because of the selective pressure with frequent use of broad-spectrum antibiotics in hospital [23-25]. The antibiotic-resistant pathogens found in different sources of hospital environment namely air, dust, clothes, and inanimate surfaces and equipment, which caused diseases, particularly to the immunocompromised patients [26-28]. Among 148 environment samples taken from different sites of hospital, 80.40% of the sample showed growth. For air sample, growth was found in 84.84% of the samples whereas for surface swabs growth was found in 76.82% of the samples. A similar type of result was found in one research [28]. The predominant isolates from environmental samples were found to be Gram-positive cocci. Similar results were obtained in other studies [28,29]. CONS were most frequent followed by *S. aureus* and *streptococci*. Among Gram-negative isolates, *Acinetobacter* spp. was found higher in comparison to *E. coli* and *Klebsiella* spp. In regards to our result, one study reported the predominant organisms were CoNS (30.3%) followed by *S. aureus* (26.1%), yeast (13.9%), Micrococci (13.7%), *Streptococci* (7.2%), Gram-positive bacilli (6.8%), and Gram-negative bacilli (2.4%) [28]. In a surveillance study carried out in a tertiary care hospital based in Lahore, Pakistan, to assess the level of bacterial contamination of air, surface, and equipment in OT and ICUs, it was reported that CONS, *S. aureus, Bacillus* spp., *Streptococcus* spp.,

Table 4: Antibiotic susceptibility pattern of Gram-positive isolates

Antibiotics	Organisms			
	Staphylococcus aureus (n=41)		CONS (n=42)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Penicillin	6 (14.6)	35 (85.4)	4 (9.5)	38 (90.5)
Methicillin	26 (63.4)	15 (36.6)	29 (69.05)	13 (30.95)
Ceftriazone	31 (75.6)	10 (24.4)	28 (66.6)	14 (33.4)
Amikacin	37 (90.25)	4 (9.75)	39 (92.85)	3 (7.15)
Ciprofloxacin	28 (68.3)	13 (31.7)	35 (83.4)	12 (28.6)
Gentamycin	28 (68.3)	13 (31.7)	35 (83.4)	7 (16.6)
Erythromycin	30 (73.2)	11 (26.8)	27 (64.3)	15 (35.7)
Vancomycin	41 (100)	0	42 (100)	0

Table 5: Antibiotic susceptibility pattern of Gram-negative isolates

Antibiotics	Organisms					
	Escherichia coli (n=20)		*Klebsiella* (n=5)		*Acinetobacter* (n=31)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	3 (15)	17 (85)	0	5 (100)	4 (12.9)	27 (87.1)
Amikacin	17 (85)	3 (15)	3 (60)	2 (40)	18 (58.1)	13 (41.9)
Ceftriaxone	12 (60)	8 (40)	3 (60)	2 (40)	18 (58.1)	13 (41.9)
Cefotaxime	15 (75)	5 (25)	4 (80)	1 (20)	20 (64.5)	11 (35.5
Ciprofloxacin	15 (75)	5 (25)	4 (80)	1 (20)	23 (74.2)	8 (25.8)
Gentamycin	13 (65)	7 (35)	3 (60)	2 (40)	19 (61.3)	12 (38.7)
Cotrimoxazole	9 (45)	11 (55)	2 (20)	3 (60)	16 (51.6)	15 (48.4)
Nitrofurantoin	2 (10)	18 (90)	0	5 (100)	0	0

Aspergillus spp., and various Gram-negative rods (*Pseudomonas* spp., *E. coli, Klebsiella* spp.) were predominant [30].

Gram-negative isolates, though isolated in small number in this study, are significant as they are more pathogenic. In this study, *Acinetobacter* spp. was predominant Gram-negative isolates followed by *E. coli* and *Klebsiella* spp. *Acinetobacter* spp. is normal environmental colonizer. The distribution of MDR *Acinetobacter* spp. has made a serious global problem in NIs over the past few years [31-34].

Antibiotic sensitivity testing of environment isolates showed a high degree of resistance towards different antibiotics. One study has reported a relatively lower resistance rate among environment isolates [28]. The *S. aureus* and CONS were all sensitive to vancomycin followed by amikacin. Gentamycin was more effective against CONS than *S. aureus*. Resistance toward ceftriaxone, ciprofloxacin, and erythromycin was higher in both groups of organisms. The Antibiotic sensitivity test (AST) of Gram-negative isolates revealed a higher sensitivity towards ciprofloxacin, cefotaxime, amikacin, gentamycin, and ceftriaxone and a higher resistance towards cotrimoxazole, nitrofurantoin, and ampicillin. One research supported the activity of ciprofloxacin against all Gram-negative isolates [35]. Similar to our study, one study showed a higher rate of resistance in Gram-negative against all used antibiotics [36].

CONCLUSION

This work provides knowledge about the distribution of high bacterial contamination load around the hospital environment exposing to patients and visitors may be significantly infected with nosocomial pathogens and that surface could represent an important reservoir in the transmission. Contaminated surfaces contribute to transmission of health-associated pathogens by serving as sources of hand or glove contamination among healthcare workers and by direct spread of pathogens to susceptible patients. Gram-positive bacteria built up the major portion of hospital flora. The occurrence of bacterial and fungal flora in large quantities alarms the need of effective housekeeping and supervision.

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AUTHORS' CONTRIBUTIONS

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

CONFLICTS OF INTEREST

The authors have not declared any conflicts of interest.

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