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# STUDY ON SENSITIVITY PATTERN OF ACINETOBACTER SPP. FROM CLINICAL AND ENVIRONMENTAL SAMPLES OF NATIONAL MEDICAL COLLEGE, BIRGUNJ, NEPAL

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## ABSTRACT

***Introduction:*** *Acinetobacter spp. are saprophytic ubiquitous gram-negative coccobacilli can survive for long periods on dry or moist inanimate surfaces and as commensals on the skin of man and animal which can facilitate its persistence and spread in healthcare facilities. The aim of study is to isolate and evaluate Acinetobacter spp. from clinical and environmental samples for its antibiotic susceptibility profile.*

***Materials and Methods:*** *This cross-sectional study was conducted at National Medical College and Teaching Hospital (NMCTH), Birganj, Nepal. 500 samples were collected from patients and environment of NMCTH. The samples were processed by use of culture media viz. Chocolate agar (CHA), 5% Sheep Blood agar (BA), and MacConkey agar (MA) plates for isolation of Acinetobacter spp. The characterization of antimicrobial susceptibility profile of isolated Acinetobacter spp was studied by use of Modified Kirby-Bauer disc diffusion technique.*

***Results:*** *39.86% Acinetobacter spp were obtained from 276 clinical specimens whereas 48.21% Acinetobacter spp. from 224 environmental samples. The distribution of Acinetobacter spp, more were found in respiratory samples (67.24%), which followed by invasive samples (25.71%) and urinary samples (18.18%). Regarding antibiotic resistance profile, 92.7% Acinetobacter spp showed resistance cefixime, followed by 89% to amoxicillin-clavulinic acid and 86.2% against cefotaxime.*

***Conclusion:*** *The finding of current study suggests the need of direct efforts to reduce hospital-acquired infections and recommend the revision of the treatment protocol for Acinetobacter infections.*

***Keywords:*** *Acinetobacter spp., Antibiotic Resistance, Clinical Specimens, Hospital Environmental-Acquired Infections, Nepal*

## INTRODUCTION

Acinetobacter spp. are saprophytic ubiquitous gram-negative coccobacilli that belong to the Moraxellaceae family. They are normal environmental dwellers and widespread in nature, water and soil[1,2]. They can survive for long periods on dry or moist inanimate surfaces and as commensals on the skin of man and animal which can facilitate its persistence and spread in healthcare facilities.[3,4] They are also been recognized as an important pathogen involved in outbreaks of hospital acquired infection especially in intensive care units (ICU).[5,6] More than 20 species of Acinetobacter have been characterized but only few species including Acinetobacter baumannii, A. calcoaceticus and A. lwoffii play a significant role in infections.[7-9] However, A. baumannii has the greatest clinical significance and identified as the causative agent of the majority of the infections[7,8,9]. A. baumannii can cause a wide range of infections including pneumonia, which is most often related to endotracheal tubes or tracheostomies, endocarditis, meningitis, skin and wound infections, peritonitis in patients receiving peritoneal dialysis, UTI and bacteraemia.[10,11]

Acinetobacter spp. has extremely rapid propensity to develop antibiotic resistance.[12,6] Extensive antibiotic abuse and poor stewardship have contributed to an increase in multidrug-resistant (MDR) strains of this pathogen, which have a marked tendency to develop

multiple resistance mechanisms, resulting in problematic antimicrobial management.[13,14] Therefore, increasing multidrug resistance pattern by Acinetobacter spp. has narrowed range of drugs for treatment. For these reasons, infections caused by MDR A. baumannii represent a major concern for patients admitted to intensive care units (ICU), where inappropriate therapy and limited therapeutic options contribute to the increased mortality and morbidity rates registered in infected patients.[14,15] The accurate identification and reporting of Acinetobacter will help to prevent spread of multidrug resistant organism.

In developed countries many studies and control programs were implemented to prevent transmission of pathogens from hospital environment to the patients.[16,17,18] However, in resource poor countries like Nepal, studies and control programs to identify the Acinetobacter spp. as potential pathogen for hospital acquired infection are scanty. One study reported that antibiotic susceptibility pattern of Acinetobacter spp. may vary widely several geographical areas and between various units of the same hospital at various time points.[19] Therefore, the variations in antibiotic resistant profile of Acinetobacter spp., necessitates a periodic surveillance of these pathogens to achieve appropriate selection of therapy.[20,21] Due to unpredictable multidrug resistance patterns of Acinetobacter spp., it is imperative to know the institutional prevalent susceptibility profiles. Therefore, the aim of the present study was to isolate the Acinetobacter spp. from various clinical and environmental samples of National Medical College and Hospital, Birgunj, Nepal and to determine the antibiotic susceptibility pattern of these isolates.

## MATERIALS AND METHODS

This cross-sectional study was conducted over a period of one year from December 2023 to November 2024 at National Medical College and Teaching Hospital, Birgunj, Nepal to isolate the Acinetobacter spp. from various clinical and environmental samples and to determine the antibiotic susceptibility pattern of these isolates.

### Ethical approval

Ethical approval for the study was taken from the Institutional Ethics Committee on 17th November 2023. (Ref. F-NMC/679/080-081) and the experiment was performed in accordance with the ethical standards of the committee.

A total of 500 isolates were obtained from various clinical specimens and environmental samples and submitted to the Department of Microbiology, National Medical College and Hospital, Birgunj for Acinetobacter spp. characterisation. A total of 276 clinical specimens were obtained from the lower respiratory tract, blood, urine, pus, and other body fluids from the patients admitted in the National Medical College and Hospital, Birgunj during study period according to the guidelines recommended by the American Society of Microbiology[24] (Isenberg 2004). About 224 environmental samples were taken from different locations of hospital environment for Acinetobacter spp. characterisation. 114 surface swabs were taken from wash basin, equipment rack, bed fabric, equipment, etc. 62 hand swabs and 48 air samples were collected.

Microscopic examination of Gram-stained smear of all samples except blood were performed. The specimens were cultured on Chocolate agar (CHA), 5% Sheep Blood agar (BA), and MacConkey agar (MA) plates. Organisms were identified, and their clinical significance was judged following standard microbiological techniques after interpreting microscopic findings, colony morphology, and biochemical properties.[22] Modified Kirby-Bauer disc diffusion technique was used for antimicrobial susceptibility testing of all the isolates following clinical and laboratory standards institute guidelines.[23] E. coli ATCC 25922 was used as controls.

Descriptive statistics were performed for all studied variables. Some were then categorized according to frequency analysis. Chi-square test was performed to assess difference of categorical variables. The level of statistical significance adopted was $p<0.05$.

## RESULTS

Among 500 clinical and environmental samples, 218 (43.6%) samples showed positive growth of Acinetobacter spp. Among 276 clinical specimens (lower respiratory tract, blood, urine, pus, and other body fluids) and 224

hospital environmental samples, 110 (39.86%) showed positive growth of Acinetobacter spp and 108 (48.21%) from environment sample which was shown in Table 1.

**Table 1: Characteristics of specimen samples (n = 250) with Acinetobacter spp.**

| Sample types | | Acinetobacter-positive isolates | | |
|---|---|---|---|---|
| | | n | Positive | % |
| Respiratory | | 116 | 78 | 67.24 |
| Invasive | | 70 | 18 | 25.71 |
| Urinary | | 44 | 8 | 18.18 |
| Soft tissue/wound | | 46 | 6 | 13.04 |
| Environment | Surface swab | 114 | 54 | 47.37 |
| | Hand swab | 62 | 32 | 51.61 |
| | Air | 48 | 22 | 45.83 |

In case of environmental samples; hand swab showed the highest Acinetobacter-positive isolates (51.61%) which followed by surface swab (47.37%) and air samples (45.83%) (Table 1). In case of environmental samples; hand swab showed the highest Acinetobacter-positive isolates (51.61%) which followed by surface swab (47.37%) and air samples (45.83%) (Table 1).

Among the clinical specimens; highest percent of Acinetobacter-positive isolates was found in respiratory samples (67.24%), which followed by invasive samples (25.71%) and urinary samples (18.18%). One research reported out of the total 424 environmental samples; 308 (72.64%) showed growth positive for Acinetobacter spp.

Acinetobacter-positive isolates were detected with high frequency among the male patients (43.59%) than that of female patients (35%). However; the difference was statistically not-significant (Table 2).

**Table 2: Characteristics of study participants (n = 138) with Acinetobacter spp.**

| Sample Type | Acinetobacter-Positive Isolates | | | |
|---|---|---|---|---|
| | n | Positive | % | Chi |
| Male | 156 | 68 | 43.59 | 1.044 (p>0.05) |
| Female | 120 | 42 | 35.00 | |
| Age: ≤40 years | 102 | 32 | 31.37 | 2.428 (p>0.05) |
| Age: >40 years | 174 | 78 | 44.83 | |

The infections were more common in males (58.00%) in comparison with females (42.00%)[25]. It was reported 50.20% more infection in males than others[29]. Similarly, Acinetobacter-positive isolates were high frequency among the participants with age greater than forty years (44.83%); however; the difference was not-statistically significant.

Acinetobacter is one of the notorious nosocomial pathogens and its tendency to develop resistance against antimicrobial drugs is an important rationale for infection control at Health care facility. The antibiotic susceptibility patterns of Acinetobacter spp. were studied in present investigation and compared with previous studies done in Nepal and abroad and the results shown in the Table 3.

**Table 3: Percentage of Antibiotic susceptibility patterns of Acinetobacter spp.**

| | Mwanamoonga et al. 2023 (Zambia) | Panta et al. 2012 (Nepal) | Baral et al. 2019 (Nepal) | Mahto et al. 2021 (Nepal) | Gupta et al. 2015 (India) | Present study |
|---|---|---|---|---|---|---|
| Amikacin | 2 | 64.93 | 65.3 | 55.4 | 71 | 67.9 |
| Amoxicillin-clavulinic acid | | 57.17 | 95.9 | | | 89.0 |
| Ampicillin | | 62.98 | | 81 | | 84.4 |
| Cefipime | 60 | | 81.8 | | 68 | 80.7 |
| Cefixime | | | 97.6 | | | 92.7 |
| Cefoperazone-Sulbactum | | | 48.4 | | | 61.5 |
| Cefotaxime | 22 | 47.4 | 97.1 | 76.5 | 79 | 86.2 |
| Ceftazidime | 65 | | 83.5 | 74.8 | 61 | 84.4 |
| Ceftriaxone | 23 | 42.21 | 88.2 | 77.3 | 64 | 82.6 |
| Ciprofloxacin | 57 | 9.74 | 67.6 | 53.8 | 81 | 73.4 |
| Cotrimoxazole | | | 80.6 | | | 78.0 |
| Gentamycin | 53 | 22.72 | 74.1 | 51.9 | | 61.5 |
| Meropenem | | | 38.2 | 45.4 | | 44.0 |
| Piperacillin-Tazobactum | 20 | | 63 | 46.3 | 55 | 63.3 |
| Polymixin B | | | 18.8 | 8.2 | | 12.8 |

The highest resistance was to cefixime (92.7%), followed by amoxicillin-clavulinic acid (89%) and cefotaxime (86.2%). Both ampicillin and ceftazidime had 84.4% resistance. Ceftriaxone, Cefipime, Cotrimoxazole and Ciprofloxacin had 82.6%, 80.7%, 78% and 73.4%, respectively. The lowest resistance was observed on Polymixin B (12.8).

## DISCUSSION

Several studies have demonstrated the highest isolation rate of Acinetobacter spp. was found from hospital environmental surfaces near patients, mainly from

beds and mechanical ventilation devices. Acinetobacter spp. was found to be a strong ability to form bio films, resist desiccation and sterilization, and thus persist in hospital environments. One research showed 308 (72.64%) Acinetobacter spp were isolated from hospital environment sample.[6] In another study in Manipal Teaching Hospital, Western Nepal reported that 35.19% specimens showed Acinetobacter spp. Among the clinical specimens; highest percent of Acinetobacter-positive isolates was found in respiratory samples (67.24%), which followed by invasive samples (25.71%) and urinary samples (18.18%) which is consistent with previous studies.[24]

One research reported out of the total 424 environmental samples; 308 (72.64%) showed growth positive for Acinetobacter spp. which was much higher than the finding of the present study.[6]

Acinetobacter-positive isolates were detected with high frequency among the male patients (43.59%) than that of female patients (35%). Our results were also consistent with several studies.[2526,27, 28]

Acinetobacter-positive isolates were high frequency among the participants with age greater than forty years (44.83%). One recent study stated that the maximum number of Acinetobacter isolates were noted from 45-64 years age group.[28]

A study in Kathmandu, Nepal reported that ciprofloxacin was the most effective followed by cotrimoxazole and gentamycin while the other aminoglycoside, amikacin was found to be least sensitive[6]. One study observed highest resistance to tetracycline (98%) and co-trimoxazole (70%) while the lowest resistance was seen in imipenem (17%), tobramycin (20%) and cefotaxime (22%).[2] One recorded high incidence of resistance for piperacillin (55%), followed by ceftriaxone (46%) and ceftazidime (46%).[19] One study showed that cephalosporins had highest resistance to the isolates followed by gentamicin.[28] One reported that percentage resistance of Acinetobacter spp. towards polymyxin B was found to be quite low (18.8%).[24] This indicates that Acinetobacter spp. has intrinsic and/or easily acquired mechanisms of resistance against many of the available antimicrobial agents making this pathogen one of the most significant microbial challenges for the current period.

## CONCLUSION

The present study has observed percentage of Acinetobacter spp. growth originated from clinical specimens and environmental samples, which is of public health and clinical significance. The findings of the present study also exhibited the likeness of Acinetobacter species isolated from clinical and environment, signifying that patients could acquire infections from the hospital environment. The results of this study had also revealed that Acinetobacter spp. was resistant to the most commonly used antibiotics. The finding of current study suggested the need of direct efforts to reduce hospital-acquired infections and recommend the revision of the treatment protocol for Acinetobacter infections.

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