Antibiotic susceptibility pattern and risk factors associated with *Acinetobacter* and *Pseudomonas* infection at a tertiary care hospital

Sherchan JB

Jatan Bahadur Sherchan, Associate Professor, Department of Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Kavrepalanchok, Nepal.

**Abstract**

**Background:** Infection due to *Acinetobacter* spp. and *Pseudomonas aeruginosa* is a major worldwide concern these days. Antibiotic resistance and predisposing factors among the patients for acquiring such infection is a major challenge globally and in Nepal.

**Objectives:** To determine antimicrobial susceptibility pattern of *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolates along with predisposing factors.

**Methods:** A total of 9,705 clinical samples were processed in this analytical cross-sectional study from December 2019 to November 2020. Antibiotic susceptibility pattern was determined following Clinical Laboratory Standard Institute guidelines. Patients’ information was obtained after informed consent.

**Results:** *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolates were 92 (0.95%). Fifty-three (57.61%) samples were respiratory samples. Thirteen (20%) *Pseudomonas aeruginosa* and 18 (66.67%) *Acinetobacter* spp. were multidrug-resistant (MDR). Eight (12.31%) *Pseudomonas aeruginosa* strains and 13 (48.15%) *Acinetobacter* spp. strains were sensitive only to Colistin. Twenty-two (95.65%) prolonged hospital stayers had MDR bacteria compared to only nine (13.04%) non-prolonged hospital stayers (p-value <0.001). Sixteen (33.69%) of diabetic patients had MDR bacteria isolates in comparison to only seven (20%) of non-diabetic patients (p-value <0.001). Thirty-one (33.69%) were elderly patients (age ≥65 years) and 61 (66.31%) were of age less than 65 years old. Seventeen (54.84%) of elderly patients had MDR isolates whereas only 14 (22.95%) of patients who are not elderly had MDR isolates (p-value = 0.0047).

**Conclusion:** *Acinetobacter* spp. and *Pseudomonas aeruginosa* strain were isolated from various samples. For effective treatment of infection by such organisms detailed microbiological diagnosis and drug susceptibility testing is needed along with identification of predisposing factors.

**Key words:** *Acinetobacter* spp.; Multidrug resistant; *Pseudomonas aeruginosa*.

**INTRODUCTION**

Infections caused by drug resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* are emerging cause of Hospital Acquired Infection and a significant threat to public health. These bacteria cause a wide spectrum of infections that include pneumonia, bacteraemia, meningitis, urinary tract infection and wound infection. Recent studies done in western and central region of Nepal revealed that the infections caused by drug resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* are associated with prolonged hospital stay and mortality. Globally, *Acinetobacter* spp. and *Pseudomonas aeruginosa* has also been identified as an ESKAPE pathogen (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas*...
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*aeruginosa*, and *Enterobacter species*). The ESKAPE pathogens are a group of pathogens with high rate of antibiotic resistance that are responsible for the majority of nosocomial infections.4

In such situations it is very necessary to conduct research studies on antimicrobial susceptibility of these pathogens along with associated predisposing factors. Hence, this study aims to find out antibiotic sensitivity patterns along with few predisposing factors associated with *Acinetobacter spp.* and *Pseudomonas aeruginosa* infections.

**METHODOLOGY**

The study was an analytical cross-sectional study, which was conducted at Microbiology lab of Kathmandu University Hospital, Dhalulikhel from the month of December 2019 to November 2020. Ethical approval was taken from the Institutional Review Committee of Kathmandu University Hospital before the study was conducted (Ref. 254/19).

Any clinical sample (sputum, tracheal aspirate, endotracheal secretion, pus, wound swab, blood, urine and other body fluids) from which *Acinetobacter* spp. or *P. aeruginosa* was isolated were only included in the study excluding other bacteria. The sample was processed for culture and sensitivity as recommended by Clinical Laboratory Standards Institute (CLSI) 2018.5

Informed consent was taken from the patients from whom *Acinetobacter* spp. or *P. aeruginosa* isolates were detected and clinical information was obtained by history taking and clinical examination by the researcher himself with the help of consultant doctor taking care of the patient as well as by going through the medical and lab records.

The samples were cultured on 5% sheep blood agar (BA) and MacConkey agar (MA) plates. The BA and MA plates were incubated at 37°C for 24 hours in an aerobic atmosphere. All the bacteria were isolated and identified using colony morphology, microscopy, and biochemical tests following standard procedures.5

The isolates were tested for antibiotic susceptibility by modified Kirby-Bauer disc diffusion method in compliance with CLSI 2018 guidelines on Mueller-Hinton agar (MHA) plates.5 All the isolates of *Acinetobacter* spp. were investigated for antimicrobial susceptibility testing (AST) against ciprofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), cefepime (30 μg), cefotaxime (30 μg), cefoperazone-sulbactam (75/30 μg), levofloxacin (5 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), piperacillin/tazobactam (100/10 μg) using Hi-Media India Pvt. Ltd following the Kirby-Bauer method on Mueller-Hinton agar. A suspension of the test organism was prepared in peptone water and matched to 0.5 McFarland standard for AST. With the help of a sterile cotton swab, lawn culture of the suspension was made on a Mueller–Hinton agar plate. Three MHA plates were used and the antibiotic discs were placed on MHA maintaining a 25 mm distance between two discs and were incubated at 37°C for 24 hours. After 24 hours, the zone of inhibition was measured for each antibiotic, and results were interpreted as sensitive, intermediate, and resistant on the basis of CLSI guidelines, 2018. For Colistin susceptibility broth microdilution (BMD) method was done according to CLSI 2018 guidelines in which a susceptibility breakpoint is ≤2 mg/liter.5 *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control organisms for antibiotic sensitivity.

Definition of Multidrug-resistant *Acinetobacter*: Any *Acinetobacter* spp. that has tested either Intermediate (I) or Resistant (R) to at least one drug in at least three of the following six categories:

1. Extended-spectrum cephalosporin (cefepime, ceftazidime, ceftriaxone, cefotaxime)
2. Fluoroquinolones (ciprofloxacin, levofloxacin)
3. Aminoglycosides (amikacin, gentamicin, tobramycin)
4. Carbapenems (imipenem, meropenem, doripenem)
5. Piperacillin/tazobactam 6. Ampicillin/sulbactam.6

Definition of Multidrug-resistant *Pseudomonas aeruginosa*: *Pseudomonas aeruginosa* that has tested either Intermediate (I) or Resistant (R) to at least one drug in at least three of the following five categories:

1. Extended-spectrum cephalosporin (cefepime, ceftolozane/tazobactam, ceftazidime/avibactam)
2. Fluoroquinolones (ciprofloxacin, levofloxacin)
3. Aminoglycosides (amikacin, gentamicin, tobramycin)
4. Carbapenems (imipenem, meropenem, doripenem)
5. Piperacillin/tazobactam.6

Prolonged hospitalisation was defined as length of stay longer than two weeks.7 Patients of age 65 years or older was considered as elderly person.8

Data were analysed by SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, Ill., USA) and p-value <0.05 was considered significant.
RESULTS
Total number of samples (respiratory sample, pus, wound swab, urine, blood, other body fluids) received during the study period was 9,705. Out of this Acinetobacter spp. and P. aeruginosa isolates were 92 (0.95%). Most of the samples from which isolates were found were of respiratory tract origin which was 53 (57.61%). Sixteen (59.26%) of Acinetobacter spp. isolates and 37 (56.92%) if P. aeruginosa isolates were detected in the respiratory sample and the total number of Acinetobacter spp. isolates was 27 (29.35%) and P. aeruginosa isolates was 65 (70.65%). Table 1 as shown below provides the number of each type of clinical sample received along with Acinetobacter spp. and P. aeruginosa isolates from the sample during the study period.

Antibiotic susceptibility pattern of P. aeruginosa showed that, 52 (80%) of the strains were sensitive to Ciprofloxacin, Ceftazidime, and Cefepime; 53 (81.54%) were sensitive to Levofloxacin; 55 (84.61%) were sensitive to Piperacillin-tazobactum, Gentamicin, and Amikacin; 57 (87.69%) were sensitive to carbapenem; and all strains were sensitive to Colistin as shown in Table 2.

Antibiotic susceptibility pattern of Acinetobacter spp. showed that, six (22.22%) of the strains were sensitive to Gentamicin and Amikacin; nine (33.33%) were sensitive to Ciprofloxacin, Levofloxacin Ceftazidime, Cefotaxime, Cefepime; 10 (37.04%) were sensitive to Cefoperazone-salbactum and Piperacillin-tazobactum; 14 (51.85%) were sensitive to carbapenem and all strains were sensitive to Colistin (Table 2).

Thirteen (20%) P. aeruginosa and 18 (66.67%) Acinetobacter spp. were multidrug-resistant. Over all 31 (33.69%) of the isolates were multidrug-resistant.

Eight (12.31%) P. aeruginosa and 13 (48.15%) Acinetobacter spp. were sensitive only to Colistin. Out of 13 patients from whom only Colistin sensitive Acinetobacter spp. was isolated, nine (69.23%) and out of eight patients from whom only Colistin sensitive P. aeruginosa was isolated three (37.50%) were elderly (age ≥65 years)

Table 1: Clinical sample and Acinetobacter/Pseudomonas isolates during study period

| S.N. | Clinical sample | Number of samples received during the study period | Acinetobacter/ Pseudomonas isolates n (%) | Acinetobacter spp. isolates n (%) | Pseudomonas aeruginosa isolates n (%) |
|------|-----------------|-------------------------------------------------|------------------------------------------|-----------------------------------|--------------------------------------|
| 1    | Respiratory      | 1120                                            | 53 (4.73)                                | 16 (30.19)                        | 37 (69.81)                           |
|      | (sputum, tracheal aspirate, endotracheal tube, throat swab, etc.) | | | | |
| 2    | Urine           | 5374                                            | 15 (0.28)                                | 3 (20)                            | 12 (80)                              |
| 3    | Pus/wound swab  | 1088                                            | 18 (1.65)                                | 6 (33.33)                         | 12 (66.67)                           |
| 4    | Blood           | 1973                                            | 1 (0.05)                                 | 1 (100)                           | -                                    |
| 5    | Body fluid      | 150                                             | 5 (3.33)                                 | 1 (20)                            | 4 (80)                               |
|      | (ascitic fluid, bile fluid, etc.) | | | | |

Table 2: Antibiotic sensitivity pattern of Pseudomonas aeruginosa and Acinetobacter species.

| S.N. | Antibiotics | Pseudomonas (n=65) n (%) | Acinetobacter (n=27) n (%) |
|------|-------------|--------------------------|---------------------------|
| 1    | Ciprofloxacin | 52 (80)                 | 9 (33.33)                |
| 2    | Levofloxacin  | 53 (81.54)              | 9 (33.33)                |
| 3    | Ceftazidime   | 52 (80)                  | 9 (33.33)                |
| 4    | Cefotaxime    | -                        | 9 (33.33)                |
| 5    | Cefepime      | 52 (80)                  | 9 (33.33)                |
| 6    | Cefoperazone-salbactum | -         | 10 (37.04)              |
| 7    | Piperacillin-tazobactum | 55 (84.61)          | 10 (37.04)              |
| 8    | Gentamicin    | 55 (84.61)              | 6 (22.22)                |
| 9    | Amikacin      | 55 (84.61)              | 6 (22.22)                |
| 10   | Imipenem      | 57 (87.69)              | 14 (51.85)               |
| 11   | Meropenem     | 57 (87.69)              | 14 (51.85)               |
| 12   | Colistin      | 65 (100)                | 27 (100)                 |
In this study, 23 (25%) of the patients had prolonged hospital stay and 69 (75.00%) of the patients had no prolonged hospital stay (less than two weeks). Number of patients with prolonged hospital stay from whom Acinetobacter spp. was isolated 14 (60.87%) and P. aeruginosa was nine (39.13%). In the present study, 22 (95.65%) multidrug-resistant (MDR) bacteria were isolated from prolonged hospital stayers compared to only nine (13.04%) MDR bacteria isolated from the patients who had no prolonged hospital stay (p-value <0.001). In this study 16 (94.12%) out of the total 17 diabetic patients number had MDR bacteria isolates which was much more in comparison to only 15 (20%) MDR bacterial isolates out of total 75 non-diabetic patients (p-value <0.001). In the current study 31 (33.69%) were elderly patients (age ≥65 years) and 61 (66.31%) were of age <65 years old. Seventeen (54.84%) of elderly patients had MDR isolates whereas only 14 (22.95%) of patients who were not elderly had MDR isolates (p-value <0.0047).

**DISCUSSION**

The present study detected Acinetobacter spp. and P. aeruginosa isolates from various clinical samples and observed the antibiotic susceptibility pattern along with few predisposing factors. Total number of clinical samples received during the study period was 9,705 and out of this the total numbers of Acinetobacter spp. and P. aeruginosa isolates were 0.95%. This finding is much less than the finding in the study conducted by Mirzaei et al. in Iran in which out of 3,248 clinical samples, A. baumannii and P. aeruginosa strains were detected in 9.51% of samples. Most of the sample from which isolates were found was of respiratory tract origin which was 57.61% and 59.26% of Acinetobacter spp. isolates and 56.92% if P. aeruginosa isolates were detected in respiratory samples which is similar but lesser in number in comparison to the finding in the study conducted by Baral et al. in western part of Nepal from 2014 to 2016 in which 74.7% of Acinetobacter spp. and 65.8% of P. aeruginosa were of respiratory tract origin. The reason for present study having lesser percentage compared to that study may be because their study duration was three years and this was only one year. Total number of Acinetobacter spp. isolates was 29.35% and P. aeruginosa isolates was 70.65%. Percentage wise this finding agrees with the finding in the study conducted by Baral et al. in which out of 483 isolates 35.20% were Acinetobacter spp. isolates and 64.80% were P. aeruginosa isolates.

Antibiotic susceptibility pattern of P. aeruginosa showed that, 80% of the strains were sensitive to Ciprofloxacin, Ceftazidime, Cefepime; and 81.54% sensitive to Levofoxacin and 84.61% sensitive to Piperacillin-tazobuctam, Gentamicin and Amikacin. This finding seems quite close with the finding of the study done by Nepal et al. in 2016 in which Gentamicin, Amikacin and Piperacillin-tazobuctam were more than 80% sensitive. In present study, 87.69% were sensitive to carbapenem this agrees with the finding in the study conducted by Mishra et al. in 2008, in which Carbapenem sensitivity was observed more than 80%. Carbenpemems are used as last option for treatment P. aeruginosa infections and other Gram-negative bacterial infections. Carbapenem-resistant P. aeruginosa has become prevalent in our setting too just as globally. Colistin seemed to be the best antibiotic for the treatment of P. aeruginosa infection in present study as all strains included in this study were sensitive to it and it may be useful drug when choices are limited. Colistin might be a useful drug to treat carbapenem resistant strains and this agrees with the findings of Sabuda et al. between 2000 to 2005.

Antibiotic susceptibility pattern of Acinetobacter spp. showed that only 22.22% the strains were sensitive to Gentamicin and Amikacin which correlates with the findings of Baniya et al. Problem of aminoglycoside resistance seems to be a major concern in the current study setting, which agrees with the finding in the study conducted by Moniri et al. Less than 40% of the Acinetobacter spp. were sensitive to Ciprofloxacin, Levofoxacin, Ceftazidime, Cefotaxime, Cefepime, Cefoperazone-salbactum and Piperacillin-tazobuctam which agrees with the findings of Moniri et al., in which more than 60% Acinetobacter spp. were resistant to ceftazidime, ciprofloxacin, levofoxacin, piperacillin/tazobactam. Less sensitivity of Acinetobacter spp. to fluoroquinolones and beta-lactam antibiotics seems another problem in current study setting, similar to other studies conducted in Nepal and outside Nepal. Only 51.85% of Acinetobacter spp. were sensitive to carbapenem which is little less than the findings in the study conducted by Baniya et al. in which it was 56% sensitive, but much more than the findings in the study conducted by Yadav et al. in which carbapenem sensitivity was only around 20%.

Since, all Acinetobacter spp. were sensitive to Colistin it seemed to be the best drug for the treatment of any infection caused by Acinetobacter spp. In the study conducted by Yadav et al. too all strains of Acinetobacter spp. were susceptible to Colistin but in the study conducted in other parts of Nepal by Baniya et al. and Raut et al., Acinetobacter spp. was not fully susceptible to Colistin, but it was 74% and 71.4 % respectively. This
shows that in future there is a high risk that current study setting also might come across these types of strains.

Over all 33.69% of the isolates were MDR and multidrug resistance was seen among much more among Acinetobacter spp. in comparison to P. aeruginosa (66.67% vs. 20.00%). In the study conducted by Baral et al. too, Acinetobacter spp. seemed to be more multidrug-resistant in comparison to P. aeruginosa (75.9% vs. 60.1%). Similar finding was observed in the study conducted by Mirzaei et al. in Iran. Uncontrolled use of broad-spectrum antibiotics including carbapenem might be a major cause for the global rise in MDR Acinetobacter spp. In total, 12.31% P. aeruginosa and 48.15% Acinetobacter spp. were Colistin-only-sensitive. Colistin-only-sensitive P. aeruginosa and Acinetobacter spp. from ICU patients has been reported in several studies. Colistin seems to be effective to treat infection caused by multidrug-resistant P. aeruginosa and Acinetobacter spp. in this setting and elsewhere too.

Of all, 69.23% of the patients were elderly from whom Colistin-only-sensitive Acinetobacter spp. was isolated and only 37.50% of the patients from whom Colistin-only-sensitive P. aeruginosa strain was isolated were elderly.

In this study, 25% of the patients had prolonged hospital stay and 75% of the patients had no prolonged hospital stay. Among them, 60.87% of Acinetobacter spp. and only 39.13% of P. aeruginosa strains were isolated from patients who had prolonged hospital stay. Similarly, 95.65% MDR bacteria was isolated from prolonged hospital stayers compared to only 13.04% MDR bacteria isolated from the patient who had no prolonged hospital stay (p-value <0.001). Hence, in this study prolonged hospital stay might be a predisposing factor for increased incidence of MDR pathogens which agrees with the findings of Baral et al. in which the hospital stay was longer for patients infected with MDR isolate (p=0.001 for Acinetobacter spp. and p=0.003 for P. aeruginosa).

In this study 94.12% of diabetic patients had MDR bacteria isolates and only 20% of non-diabetic patients had MDR isolates (p-value <0.001). Hence, in this study diabetes seems to be another predisposing factor for incidence of MDR pathogens and diabetes mellitus is an important risk factor for colonisation with MDR Acinetobacter spp. as detected in the study conducted by Mody et al. in 2015. In present study, 54.84% of elderly patients had MDR isolates whereas only 22.95% of patients who were not elderly had MDR isolates (p-value = 0.0047). Hence, in current study old age seems to be another predisposing factor for developing antimicrobial resistance and elderly patients have to be taken proper care to prevent this. Similar finding was observed in the study conducted by Pappas et al. in 2009.

CONCLUSION
In this study Acinetobacter spp. and Pseudomonas aeruginosa strains were isolated from various clinical samples and mainly from respiratory samples. Multidrug resistance and predisposing factors such as length of hospital stay, diabetes, and old age have become a major concern. Management of such infection in the community as well as hospital by early investigation and analysis of infection and controlling of risk factors might help to reduce the burden of respiratory tract infection in health care centres and communities.

ACKNOWLEDGMENTS
We express our profound gratitude to the Department of Microbiology and Department of Internal Medicine, Kathmandu University Hospital for their kind support and cooperation throughout this study.

Conflict of interest: None
Source(s) of support: None

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