International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 5, Issue 8 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.08.017

Prevalence and antibiotic resistance pattern of *Pseudomonas* and *Acinetobacter spp.* isolated from blood samples of the intensive care unit (ICU) patients

Md. Jakir Hossain[1], Md. Gahangir Alam[2], Shashwata Biswas[3], Sirajo Ismail Isah[4], Mohammad Shahriar[5] and Sohidul Islam[1*]

[1]Department of Biochemistry and Microbiology, North South University, Dhaka 1229, Bangladesh.
[2]Department of Biochemistry & Molecular Biology, Gono Bishwabidalay, Savar, Dhaka 1344. Bangladesh.
[3]Center for AIDS Research, Kumamoto University, Kumamoto 860-0811, Japan.
[4]Department of Pharmacy, Daffodil International University, Dhaka 1207, Bangladesh.
[5]Department of Pharmacy, University of Asia Pacific, Dhaka 1215, Bangladesh.
[*]**Corresponding author:** Dr. Sohidul Islam. E-mail: *mjh_nsu@yahoo.com*

Abstract

Pseudomonas and *Acinetobacter spp.* are opportunistic pathogens and primarily cause opportunistic nosocomial infections and affects severely immunocompromised patients. In this study, we observed the prevalence and resistance pattern of *Pseudomonas* and *Acinetobacter spp*. from the blood of intensive care unit (ICU) patients.

Isolates of both the organisms were collected during one year period from January to December 2017. MacConkey and Blood agar were used to isolate these bacteria. Different biochemical tests (KIA, MIU, Citrate, and Oxidase tests) were carried out to identify the organisms and polymerase chain reaction (PCR) was performed to correlate and confirm the previous results. After identifying the organisms statistical analysis was done to observe the prevalence based on age and sex. Antibiogram was studied against amikacin (AK), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (CN), imipenem (IMP), meropenem (MEM), doripenem (DOR), and netilmicin (NET). A total of 198 *Pseudomonas spp.* and 57 *Acinetobacter spp*. were isolated from blood of 312 patients. Both the bacteria were most prevalent among male patients of different ages. In antibiotic resistance experiment, *Pseudomonas spp*. was found to have reduced susceptibility (RS) against most of the antibiotics used especially ceftazidime (CAZ) and netilmicin (NET) and 6.76% isolates were found to be resistant against gentamicin (CN). In case of *Acinetobacter spp*., reduced susceptibility (RS) was observed against netilmicin (NET) and 52.63% of isolates were found to have resistance against gentamicin (CN).The presence of *Pseudomonas*, and *Acinetobacter spp*. were in notable range especially among male patients and both the bacteria showed reduced susceptibility (RS) against most of the antibiotics used and total resistance against gentamicin (CN).

Keywords: *Pseudomonas spp.*, *Acinetobacter spp.*, and antibiotic resistance.

Introduction

Infection with *Pseudomonas* and *Acinetobacter spp*. is common in patients with compromised host defense and the most common pathogens isolated from patients who have been hospitalized for more than one week (1). Both the organisms are considered nonpathogenic to healthy persons and persist in the hospital environment. These organisms cause severe life-threatening infections in immune-compromised patients. The spectrum of antibiotic resistance of these organisms together with their survival capabilities makes them potential threats to the hospital as documented by recurring outbreaks (2).

The development of antibiotic resistance among these isolates in Bangladesh has created a new problem for the management of nosocomial illness. The organisms are usually resistant to commonly used antimicrobials and the infections are associated with a bad prognosis. Routine antimicrobial treatment of uncomplicated nosocomial infections caused by these organisms has been discouraged (3). One of the biggest issues with treating *Pseudomonas* and *Acinetobacter spp.* is that these bacteria are naturally resistant to a number of antibiotics, and able to attack the immune compromised patient especially intensive care unit (ICU) patient (4).

Therefore, the aim of this study was to find out the prevalence of *Pseudomonas* and *Acinetobacter spp*. in the blood samples of intensive care unit (ICU) patients, and range of antibiotic resistance pattern against commercially available antibiotics that are often prescribed to treat the infections caused by these bacteria.

Materials and Methods

Sample collection

Blood samples were collected from different hospitals (Dhaka Community Medical College Hospital, Shishu Hospital, Sir Salimullah Medical College Hospital, Dhaka Hospital, Dhaka Medical College Hospital, Ad-din Maa-o-Shishu Hospital, and Green ICU center) located in Dhaka city, Bangladesh. 312 samples were collected from patients of different age and sex.

Isolation of organisms

Pseudomonas and *Acinetobacter spp*. were isolated using MacConkey, and Blood agar medium following the standard laboratory protocol (5).

Biochemical test

Standard laboratory protocol of different biochemical test (Kligler's Iron Agar (KIA), Motility Indol Urea (MIU), Oxidase, and Citrate utilization) was carried out to confirm the suspected colonies (6,7, 8).

Polymerase chain reaction (PCR)

All biochemical tests positive isolate was subjected to molecular detection by PCR method. In this case, *Pseudomonas* and *Acinetobacter spp.* specific primers were used according to standard PCR laboratory protocol (9, 10) (table 1).

Table 1 : Primers used to identify *Pseudomonas* and *Acinetobacter spp.* in PCR.

Organism	Primer	Sequence (5' to 3')
Pseudomonas spp.	Forward	GACGGGTGAGTAATGCCTA
	Reverse	CACTGGTGTTCCTTCCTATA
Acinetobacter spp.	Forward	GAGTAATGCTTAGGAATCTGC
	Reverse	GGTAACCGCCTCTTTG

Determination of antimicrobial susceptibility

In this study, Modifief Kirby-Bauer method was followed to determine the susceptibility of *Pseudomonas* and *Acinetobacter spp.* against antimicrobial agents (11). Disc diffusion method was applied to evaluate the resistance pattern of *Pseudomonas* and *Acinetobacter spp.* bacteria (Spilker et al., 2004). In this case, commercially available antimicrobial discs (amikacin (AK), ceftazidime(CAZ), ciprofloxacin(CIP), gentamicin(CN), imipenem(IMP), meropenem(MEM), doripenem(DOR), netilmicin (NET), ceftriaxone(CRO)) were used. The turbidity of both cultures was adjusted to a McFarland 0.5 standard. Bacteria were cultured on Mueller-Hilton agar plate and the antibiotic disk was put on them. After 18 hours of incubation, the zone of inhibition was measured according to the antibiotic sensitivity index (12).

Results

Biochemical test

Pseudomonas and *Acinetobacter spp.* showed specific biochemical reactions (table 2). A total of 198 *Pseudomonas spp.* and 57 *Acinetobacter spp.* showed the positive reaction from 312 samples.

Table 2: Positive results found from different biochemical test to identify *Pseudomonas* and *Acinetobacter spp.*

Organisms	Name of the test							
	Kligler's Iron Agar (KIA)			Motility Indol Urea (MIU)			Citrate	Oxidase
	Butt	Slant	H_2S	Motility	Indole	Urea		
Pseudomonas	N/C	K	-ve	+ve	-ve	-ve	+ve	+ve
Acinetobacter	N/C	K	-ve	-ve	-ve	-ve	+ve	-ve

Here, N/C = No change, K= Alkaline, +ve= Positive, and –ve= Negative.

Polymerase chain reaction (PCR) for molecular detection

A total of 198 *Pseudomonas spp.* and 57 *Acinetobacter spp.* were taken for molecular identification using PCR (figure-1). All the results of the biochemical test were identical and correlated with the results of PCR for each of the organisms.

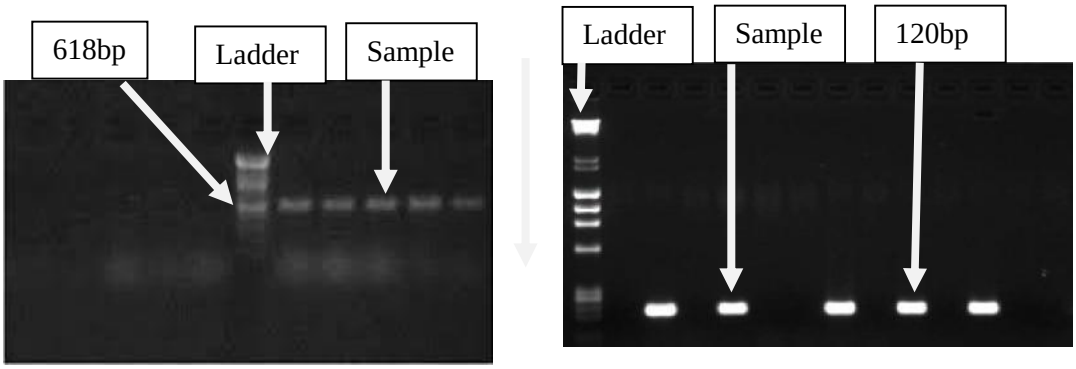

Figure 1: Molecular identification of *Pseudomonas*, and *Acinetobacter spp.* by PCR method. (a) Agarose gel image showing the positive *Pseudomonas spp*. (b) And gel image of *Acinetobacter spp*. positive samples.

Monthly distribution of *Pseudomonas spp.*

This analysis was done based on samples collected in the year 2017. Out of 312 samples, 198 found to have *Pseudomonas spp*. positive. This study showed that highest number of positive sample was in May (n=22) and the number was less (n=8) in February (table 3).

Table 3: Monthly distribution of *Pseudomonas spp.* obtained from blood samples.

Month	Distributions of 198 *Pseudomonas spp.*
January	20(10.10%)
February	8(4.04%)
March	19(9.60%)
April	20(10.10%)
May	22(11.11%)
June	17(8.58%)
July	18(9.09%)
August	12(6.06%)
September	14(7.07%)
October	21(10.60%)
November	18(9.09%)
December	9(4.54%)

The calculation in percentage showed, 3.53% male, and 6.53% female total of 10.1% were positive in the month of January. But only in February, 4.04% positive samples were found to have where the male and female patient's percentage was 2.25 and 1.51 respectively. However, the number of positive samples increased in March and 8.08% male and 1.51% female total of 9.6% sample showed the presence of *Pseudomonas spp*. From total positive samples, 7.07% male and 3.03% female total of 10.10% and 5.55% male and 5.55% female total of 11.11% was found to have in April and May respectively. Similarly, in June, a total of 8.58% samples was found as *Pseudomonas spp.* positive where the male was 6.06% and the female was 2.52%. On the other hand, in July, August, and September the number of male positive patients were 4.54, 4.04, and 4.54% respectively where the female positive patients were 4.54, 2.02, and 2.52% respectively. But the positive patient's number again increased in October where a total of 10.60% samples found as *Pseudomonas spp.* positive and the male and female patient was 6.56, and 4.04% respectively. A number of positive samples again decreased in November and December where a total of 6.06% male and 2.52% female found in November, and 6.06% male and 2.52% female was found as *Pseudomonas spp.* positive (figure 2).

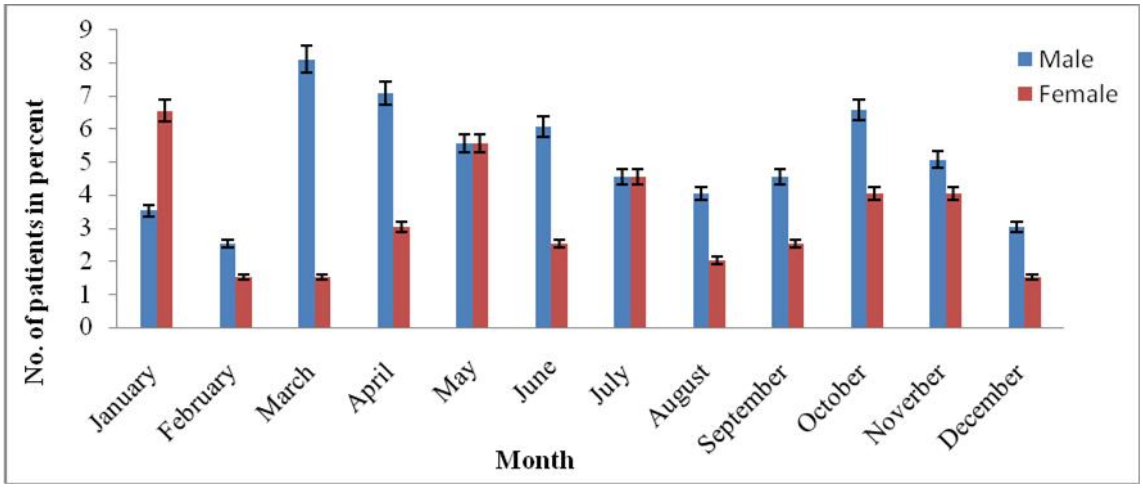

Figure 2: Monthly distribution of *Pseudomonas spp*. from blood samples between male and female.

Distributions of *Pseudomonas spp*. based on age between male and female

Positive samples were then categorized according to the age and sex. In this analysis, it was found that 11.6% male and 6.06% female patients were *Pseudomonas spp.* positive those who belong under 10 ages. On the other hand, the male patient of 0.3% and female patient of 1.51% were found to have within the age group of 10 to 20 whereas 5.55% male patient and 7.57% female patient was found to have in the age group of 21 to 30. The same positive result also found in the male patient (4.54%) those who belong the age of 31 to 40 and 41 to 50. But the female number increased from 1.51% to 6.56% within these two groups. Age group of 51 to 60 showed 14.64% positive result for male, and 5.05% for female. 7.07% male and 6.56% female patients found as *Pseudomonas spp.* positive among the age 61 to 70 and the number was less (male 4.04%, female 2.02%) in the age group of 71 to 80. Only 3.03% male and 2.52% female was positive those who were above 80 years of age (figure 3).

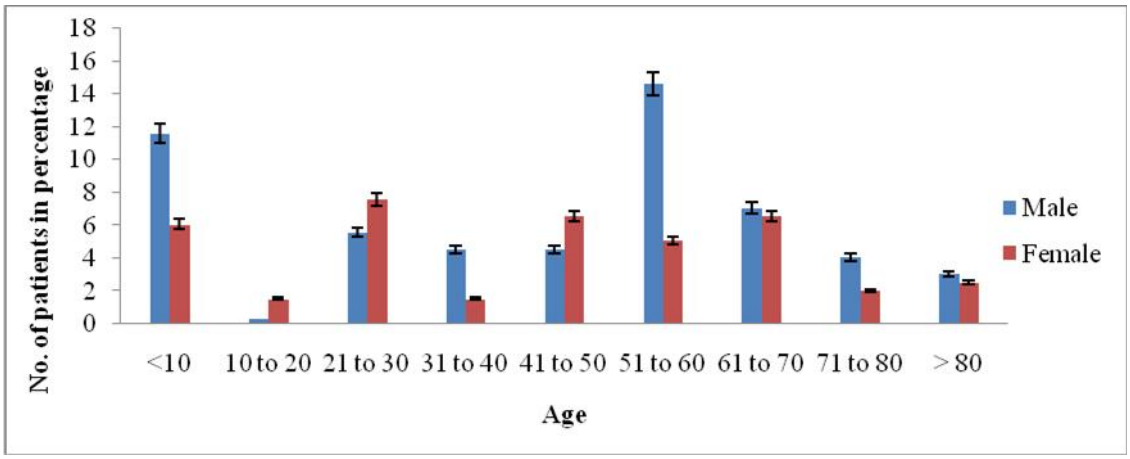

Figure 3: Distribution of *Pseudomonas spp.* among male and female by age. The graph is showing isolates were most prevalent in case of male patients of all ages of people.

Antibiogram results of *Pseudomonas spp.*

The diameter of the complete zone of inhibition of bacterial growth was assured in millimeter and named as susceptible, intermediate and resistant by comparing with an interruption table [12] (table 4).

Table 4: Name of different antibiotics and their potency.

No.	Name of antibiotic	Potency (µg)	Zone of inhibition (diameter) in mm		
			Resistant (R)	Intermediate(I)	Sensitive(S)
1	Amikacin (AK)	30	≤14	15-16	≥17
2	Ceftazidime (CAZ)	30	≤14	15-17	≥18
3	Ciprofloxacine (CIP)	5	≤14	15-28	≥19
4	Gentamicin (CN)	10	≤12	15-18	≥15
5	Imipenem (IMP)	10	≤13	13-14	≥16
6	Meropenem (MEM)	10	≤13	14-15	≥16
7	Doripenem (DOR)	10	≤13	13-14	≥15
8	Netilmicin (NET)	30	≤12	14-15	≥16

The antibiogram result showed. 20% of the isolates were sensitivity against amikacin (AK) whereas 42% of them showed intermediate sensitive and 38% showed resistant. Among the isolates, 28% showed sensitive against ceftazidime (CAZ) where 53% were intermediate and 38% were fully resistant. Both the ciprofloxacin (CIP) and ceftazidime (CAZ) showed the similar result. In this case, 61% of isolates were sensitive, 25% was intermediate, and 14% were resistant. However, 77% of the isolates were resistant against gentamicin (CN), 20% were intermediate and only 3% were sensitive. Imipenem (IMP), meropenem (MEM), and doripenem (DOR) showed all most the same results. 45 to 46% of the isolates were the sensitive where, average 40% were intermediate, and 14 to 15% were resistant. But in case of netilmicin (NET), only 7% isolates were sensitive where 50% were intermediate and 43% were resistant (figure 4).

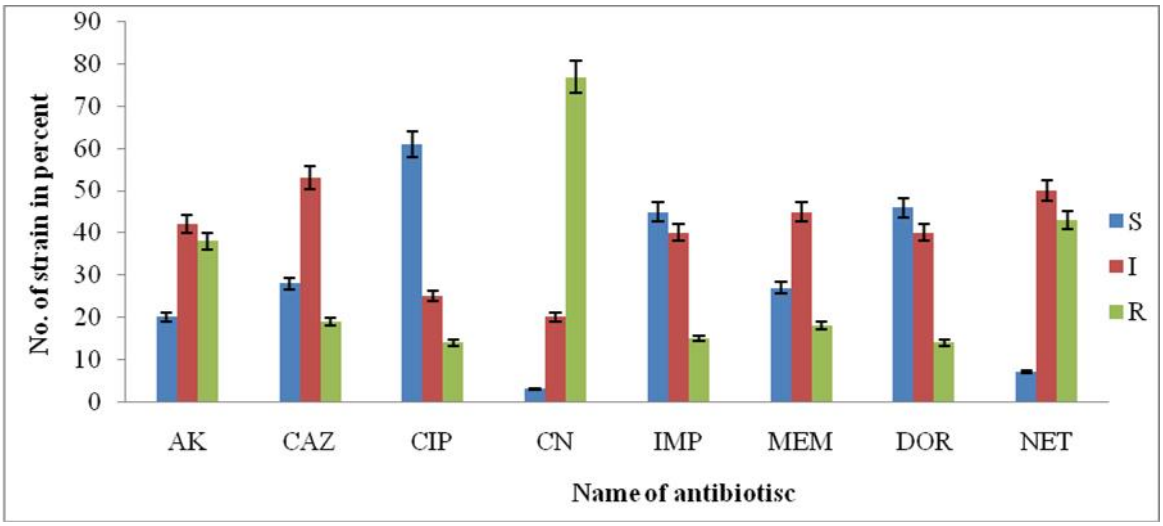

Figure 4: Antibiotic resistance pattern of *Pseudomonas spp*. Here, isolates showed the maximum resistant result (77%) against gentamicin (CN) showed and most sensitive to ciprofloxacin (CIP). Here, sensitive (S), intermediate (I), and resistant (R).

Monthly distribution of *Acinetobacter spp*.

A total of 57 out of 312 patients found to have *Acinetobacter spp*. positive. The highest number of *Acinetobacter spp*. were found to have in October (22.8%) and the number was less in February (1.75%) (table 5).

Table 5: Monthly distribution of *Acinetobacter spp*. obtained from the blood sample.

Month	Distributions of 57 *Acinetobacter spp.*
January	4 (7.01%)
February	1(1.75%)
March	4(7.01%)
April	9(15.78%)
May	6(10.52%)
June	4(7.01%)
July	7(12.28%)
August	2(3.51%)
September	2(3.51%)
October	13(22.8%)
November	2(3.51%)
December	3(5.26%)

The percent calculation showed a total of 7.01% sample was positive in January where the percentage of male and female was equal (3.5%). But in February the positive sample was found only in the male patient (1.75%). However, 5.26% male and 1.75% female positive patients were found to have in March. In April male of 8.77% and female of 7.01%, and in May male of 8.77% and female of 1.57% showed the positive result. The male patient of 5.26% and female patient of 1.75% showed the positive result in June. On the other hand, in July, August, and September the percentage was 7.01, 3.5, and 3.5 for male patients respectively where the percent of female positive patients were 5.26, 0.0, and 0.0 respectively. But in October the male sample was 14.03%, and the female was 8.77%. The number of positive samples decreased in November and December. A total of 3.5% male and no female patients was found November and 5.26% of male and no female patient was found as *Acinetobacter spp*. positive in December (figure 5).

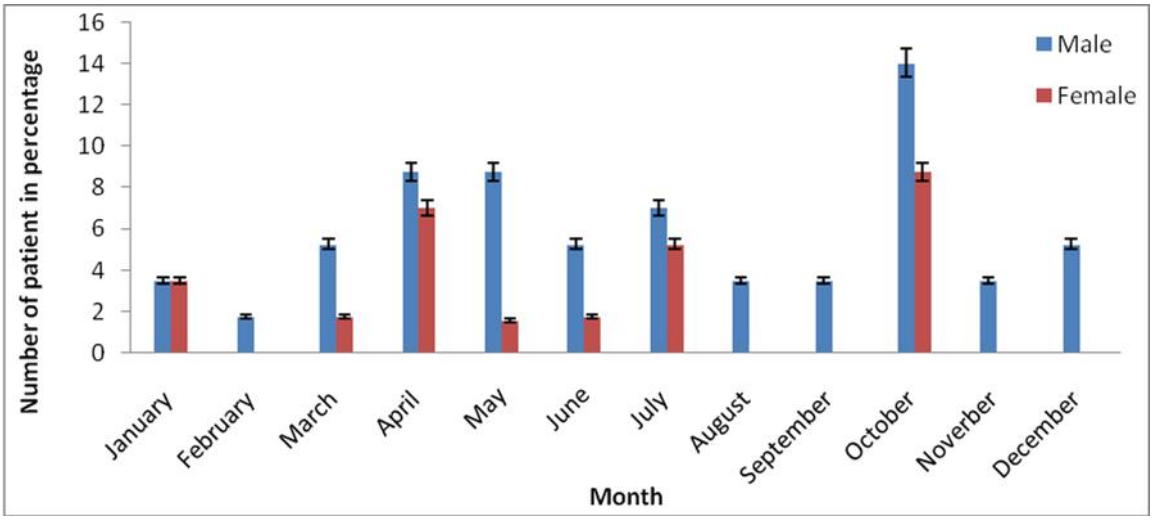

Figure 5: Monthly distribution of *Acinetobacter spp*. from blood samples between male and female. This graph shows that affecting rate of *Acinetobacter spp*. was highest (14.03%) in male and female (8.77%) sample in October and lowest results found on February (Male-1.75% and female 0%).

Distribution of *Acinetobacter spp.* based on age between male and female

Positive samples were again categorized according to the age and sex. A male patient of 29.09% and the female patient of 16.36% were *Acinetobacter spp*. positive those who belong under 10 years of ages. On the other hand, only one male patient (1.75%) was found to have in the age group of 10 to 20. The male patient of 5.45% and female patient of 3.63% were found to have in the age group of 21 to 30 whereas. Only 12.52% of male patients were found to have in the age group of 31 to 40. Age group of 41 to 50 showed 3.63% male, and 1.81% female patient as *Acinetobacter spp*.positive. But the female number increased by 5.45% where the positive male patient was absent in the age group of 51 to 60. However, 10.90% male and 1.81% female patients were *Acinetobacter spp*. positive in the age group of 61to 70. Similarly, 5.45% male and 3.63% female showed the positive result those were over 70 years of age (figure 6).

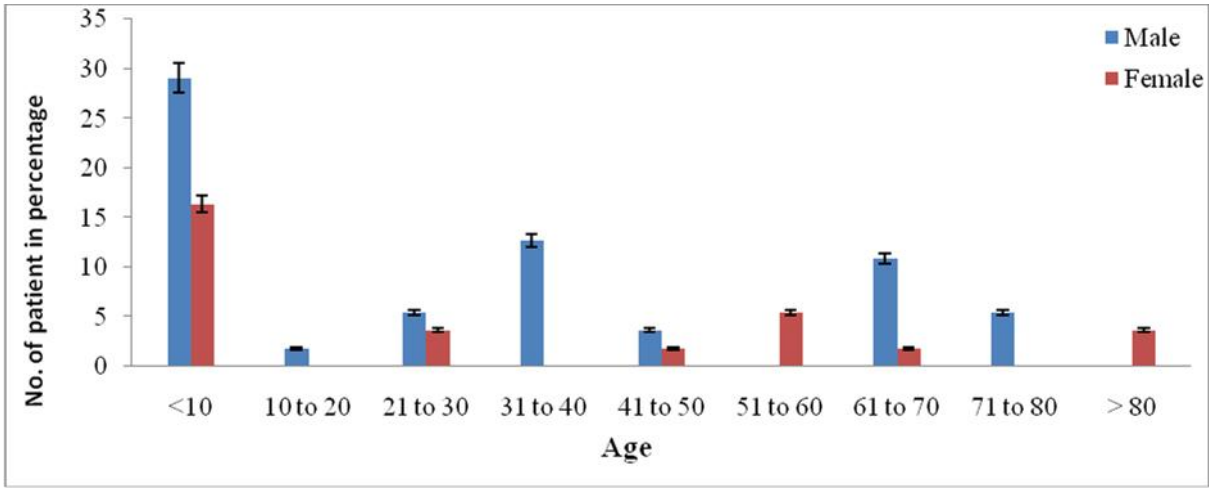

Figure 6: Distribution of *Acinetobacter spp*. among male and female by age. In this study, young children both male and female below 10 years of age showed more prevalence.

Antibiogram result of *Acinetobacter spp*.

The diameter of inhibition of bacterial growth was assured and named as susceptible (S), intermediate (I) and resistant (R) by comparing with an interruption table (table 4). The antibiogram result of *Acinetobacter spp*. showed the sensitivity of 31.40% against amikacin (AK) whereas 30% of them showed intermediate and 38.60% was resistant. 37% of isolates were sensitive against ceftazidime (CAZ) where 25% of them were intermediate and 38% was resistant. In the case of ciprofloxacin (CIP), 21.5% of the isolates were sensitive, 31% were intermediate resistant, and 47.5% of them were resistant. However, 52.65% of the isolates were resistant against gentamicin (CN) where 20% intermediate and 27.35% was sensitive. Imipenem (IMP), meropenem (MEM), and doripenem (DOR) showed all most the same results. 35 to 38% of the isolates were resistant where, average 40% of them were intermediate, and 14 to 15% of them were resistant. But in case of netilmicin (NET), only 34.22% isolates were sensitive where 50% were intermediate and 15.78% were resistant (figure 7).

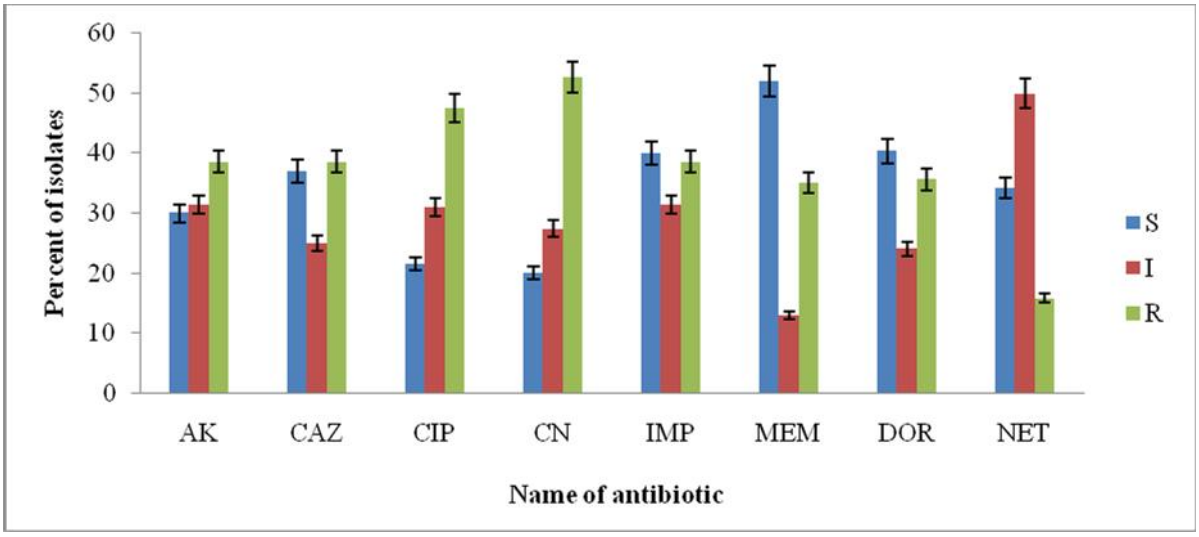

Figure 7: Antibiotic resistance pattern of *Acinetobacter spp*. Here, gentamicin (CN) showed the maximum resistant result (52.63%) and most sensitive to meropenem (MEM). Here, sensitive (S), intermediate (I), and resistant (R).

Discussion

The frequency of *Pseudomonas* and *Acinetobacter spp.* infection associated with ICU patient in Bangladesh was not reported officially in recent past years. In this study, the seasonal distribution revealed that *Pseudomonas* and *Acinetobacter spp.* were prevalent throughout the year 2017. The isolation rate of Pseudomonas was high in March and April and *Acinetobacter* was observed in April and May. However, the rate was less in February for the bacteria.

The resistance of *Pseudomonas* and *Acinetobacter spp.* against antibiotics is increasing day by day both in develop countries and develop countries. In this study, the development of resistance against clinically important drugs was assessed. From the result, it was found that 37.87% of the *Pseudomonas* was resistant against amikacin where 76.76% against gentamicin and 14.14% against doripenem. Most of the *Pseudomonas* showed reduced susceptibility (RS) against ceftazidime (18.68%) meropenem (17.17%), imipenem (14.64%), netilmicin (42.42%) and ciprofloxacin (13.13%). *Acinetobacter* strains were also showed resistant property against most of the antibiotics like ceftazidime (38.59%) meropenem (35.08%), imipenem (38.59%), netilmicin (15.78%), ciprofloxacin (47.36%), amikacin (38.59%), gentamicin (52.63), and doripenem (35.59%). So, based on the results it can be inferred that still Pseudomonal infection can be treated with these antibiotics but it will be very alarming within few years in case of the disease caused by *Acinetobacter*.

In another analysis, it was found that both the *Pseudomonas* and *Acinetobacter spp.* were more prevalent in young children of bellow 10 years. However, *Pseudomonas* strains were most prevalent in male those who belong the age group of 51 to 60. The isolation rate of *Pseudomonas* and *Acinetobacter spp.* was higher in male than female in each age group of people. People belong the age of 11 to 50 years was less susceptible in both cases.

Conclusion

The number of presence of *Pseudomonas spp.* was higher than *Acinetobacter spp*. *Pseudomonas* and *Acinetobacter spp.* were more prevent in young children. However, male patients were more prevalent than female for both the bacteria. Young people were less susceptible in both cases. The antibiotic-resistant pattern study assessed that the majority of *Pseudomonas* and *Acinetobacter spp.* were resistant to more than one drug which is clinically important to treat them. So, it can be documented that the rise in multi-drug resistant nosocomial pathogens especially *Pseudomonas* and *Acinetobacter spp.* continues to threaten hospitalized ICU patients.

Acknowledgments

All sample processing and work was done Community Medical College Hospital, Dhaka.

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DOI:10.22192/ijarbs.2018.05.08.017	

How to cite this article:
Md. Jakir Hossain, Md. Gahangir Alam, Shashwata Biswas, Sirajo Ismail Isah, Mohammad Shahriar and Sohidul Islam. (2018). Prevalence and antibiotic resistance pattern of *Pseudomonas* and *Acinetobacter spp.* isolated from blood samples of the intensive care unit (ICU) patients. Int. J. Adv. Res. Biol. Sci. 5(8): 186-195.
DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.08.017