ORIGINAL ARTICLE

Antibiotic Susceptibility Pattern of *Acinetobacter baumannii* in Clinical Isolates of Tertiary Care Hospital, Rawalpindi.

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Abstract

Background
Acinetobacter baumannii is an important cause of nosocomial infections. It is developing resistance to many drugs including carbapenems, leading to concerns of increased mortality and cost of illness. The objective of the study was to find antibiotic susceptibility pattern of *Acinetobacter baumannii*.

Methods
A descriptive study was conducted in the Microbiology Department, Fauji Foundation Hospital, Rawalpindi, from January 2015 to August 2015. A total of 146 isolates from various clinical specimens were identified as *Acinetobacter baumannii* using standard microbiological techniques and the antimicrobial susceptibility was carried out by using Kirby-Bauer disc diffusion technique as recommended by Clinical and Laboratory Standards Institute (CLSI). Frequency and percentages were calculated using SPSS (Version 21).

Results
Of total 146 isolates, were obtained from clinical specimens of endo-tracheal tube, pus, blood, sputum, endo-bronchial fluid, cannula, urine, cerebrospinal fluid and high vaginal swab. 47.2% isolated were obtained from Intensive care unit and 52.7% were obtained from other wards/OPD of hospital. Resistance to tigecycline was 56.7%, whereas no isolate was found resistant to colistin. Multidrug resistant *Acinetobacter baumannii* was calculated to be 98.5%(n=68) in ICU isolates and was 83.11%(n=64) in non-ICU isolates. The percentage of XDR-*Acinetobacter baumannii* in ICU was 65.21%(n=45) and in non-ICU isolates was of 19.48% (n=15).

Conclusion
Acinetobacter baumanni was identified as a common pathogen in the intensive care units infections with disappointing situation regarding antibiotic resistance.

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Keywords
Antibiotic susceptibility, *Acinetobacter baumannii*, Intensive care unit.

Background
Acinetobacter baumannii is notorious for causing various nosocomial infections and various out breaks in Intensive care units (ICUs).[1,2,3] *Acinetobacter baumannii* poses a global threat.[4] Infections caused by this bacteria include wound infections, respiratory tract infections and urinary tract infections etc.[5] Immuno-compromised state, unscheduled admission to the hospital, respiratory failure at ICU admission are the risk factors related to *Acinetobacter baumannii* infections in hospitals. Similarly, past antimicrobial therapy, history of sepsis in the ICU, prolonged mechanical ventilation and the invasive procedures are the other contributing risk factors.[6]

Acinetobacter baumannii is resistant to many antibiotics including cephalosporins, carbepenems, flouroquinolones and aminoglycosides.[7,8] This has made the choice of safe and effective drug very difficult. Though colistin and tigecycline are effective but it is realized that resistance to these drugs is also coming to surface.[9] The high antibiotic resistance of *Acinetobacter baumannii* led to the evolution of terminologies of Multi-drug resistant (MDR) *Acinetobacter baumannii*, extensively drug resistant (XDR) *Acinetobacter baumannii* and pan-drug resistant (PDR) *Acinetobacter baumannii* in use.[9] The percentage of Multi drug resistant *Acinetobacter baumannii* has regional variations. It is as high as 100 % reported from Pakistan to 67% in Saudi Arabia.[5,7] MDR-*Acinetobacter baumannii* is defined as resistance to more than two antimicrobials classes from the following five drugs classes: flouroquinolones, carbapenems, ampicillin-sulbactam, aminoglycosides and anti-pseudomonal cephalosporins.[4] XDR-*Acinetobacter baumannii* was taken as resistance to all drugs except one or two classes of antimicrobials whereas PDR-*Acinetobacter baumannii* was taken as resistance to all classes of antimicrobials.[10] However, there have been considerable variations in the definitions of terms MDR, XDR and PDR due to lack of international standardization.[10,11] *Acinetobacter baumannii* in the past years has developed resistance to those

drugs which were considered life saving in ICU infections like carbapenems.[12] Strains that are resistant to carbapenems are generally resistant to many antibiotics except colistin and tigecycline.[12] The mechanism of resistance of *Acinetobacter baumannii* to antimicrobials is attributed to up-regulation of innate resistance mechanisms, high ability to acquire resistance from other resistant strains, gene mutations, efflux pumps and inactivating drug by enzymatic activity like beta lactamase enzymes, acetyl transferases or other enzymes.[4]

The study aims to determine the current trends of *Acinetobacter baumanni* antibiotic susceptibility in Fauji foundation hospital and develop guidelines based on local data collected that may help the physicians in managing patients with *Acinetobacter baumanni* infection.

Material and Methods

A descriptive study was conducted at Microbiology laboratory, Pathology Department of Fauji Foundation Hospital, Rawalpindi from January 2015 to August 2015. Formal permission was taken from ethical review and research committee. A total of 146 isolates identified as *Acinetobacter baumannii* were obtained from clinical specimens of endo-tracheal tube, pus, blood, sputum, urine, endo-bronchial fluid**,** cerebrospinal fluid, intravenous cannulas and high vaginal swab. All duplicate samples were excluded from the study. *Acinetobacter baumannii* was initially identified on the basis of non-fermenting growth and by colony morphology on MacConkey agar followed by Gram staining. Furthermore, catalase test, oxidase and motility tests were done and finally species were identified by Analytical profile index-20 non Enterobacteriaceae (BioMerieux, UK). Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method using Muller-Hinton agar. Cefotaxime (30µg), gentamicin (10µg), imipenem (10µg),ciprofloxacin (5µg),amikacin (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), ampicillin (10µg), colistin (10µg), tigecycline (15µg) and doxycycline (30µg) discs by Mast Diagnostics, UK were used. The incubation was done at 35°C ± 2 for 24 hours. The zone diameters around each disk was measured and interpreted as per guidelines of Clinical and Laboratory Standards Institute.[13] Multi-Drug resistant *Acinetobacter baumannii* (MDR) was taken on the basis of resistance to three or more drugs whereas extensively drug resistant (XDR) *Acinetobacter baumannii* was taken as resistance to all drugs tested except colistin and tigecycline.[11] The data was analyzed by SPSS (version 21) software and results were interpreted in terms of percentages and frequencies. (ERCno:FF/FUMC/217-Phy-16)

Results

Of the total sample of 146 isolates, 47.2% (n=69) were collected from ICU, whereas 52.7% (n=77) were collected from other hospital departments including outpatient department, medical and surgical units etc.

Distribution of *Acinetobacter baumannii* from various clinical specimens collected from ICU and departments other than ICU are given in table 1. The mean age of patients with *Acinetobacter baumannii* infections in ICU was 39.4 years whereas mean age in non ICU patients with infections was 36.79 years.

Acinetobacter baumannii in ICU was most frequently isolated from endo-tracheal tube secretions (79.7%, n=55) followed by pus (5.8%, n=4) whereas in non-ICU samples, pus (26.6%, n=28) and urine (33.76%, n=26) were the most common specimens for *Acinetobacter baumannii*.

Table 1. Distribution of *Acinetobacter baumannii* from various clinical specimens collected from ICU and Non ICU patients

Clinical Specimen	ICU n (%)	Non-ICU n (%)
Endo-tracheal tube	55(79.7%)	-
Pus	4(5.8%)	28(36.36%)
Blood	3(4.34%)	3(3.8%)
Sputum	2(2.8%)	16(20.7%)
Endo-bronchial fluid	1(1.44%)	-
Cannula	1(1.44%)	2(2.6%)
Urine	2(2.8%)	26(33.76%)
Cerebrospinal fluid	1(1.44%)	-
High vaginal swab	-	2 (2.6%)
Total	**69 (100%)**	**77 (100%)**

Acinetobacter baumannii detected in clinical isolates of ICU were more resistant than from isolates from other hospital departments. MDR-*Acinetobacter baumannii* was calculated to be 98.5% (n=68) in ICU isolates and was 83.11% (n=64) in non-ICU isolates. Overall, percentage of XDR-*Acinetobacter baumannii* was 41.09% (n=60). The percentage of XDR-*Acinetobacter baumannii* in ICU was65.21% (n=45) and in non-ICU isolates was of 19.48% (n=15). Colistin and tigecycline were tested only for XDR-*Acinetobacter baumannii*. Colistin sensitivity was 100% (n=60) whereas, tigecycline was sensitive only in 43.3% (n=26) of isolates. The antibiotic resistance pattern to the drugs applied to all isolates is calculated in percentages and is shown in Table 2.

Isolates of *Acinetobacter baumannii* from ICU and non ICU were both resistant to all beta lactam drugs tested, with more resistance to carbapenems in ICU isolates than non ICU isolates. Doxycyline in ICU and amikacin in non ICUisolates were the drugs with least resistance.

Discussion

For the past several years *Acinetobacter baumannii* has been a major pathogen of hospital acquired infections especially in

Table 2.Antibiotic Resistance Pattern of *Acinetobacter baumannii:*

Drug	Percentage resistance in ICU isolates (n=69) %(n)	Percentage resistance in Non-ICU isolates (n=77) %(n)
Gentamicin	98.5%(68)	66.2%(51)
Amikacin	85.5%(59)	58.44%(45)
Trimethoprim-sulfamethoxazole	97.1%(67)	87.01%(67)
Doxycycline	57.97%(40)	61.03%(47)
Ciprofloxacin	98.5%(68)	85.71%(66)
Ampicillin	100%(69)	94.8%(73)
Cefotaxime	100%(69)	94.8%(73)
Imipenem	95.65%(66)	72.72%(56)

ICUs, frequently causing out breaks.[1,2,3] Literature from different parts of the world reveals that *Acinetobacter baumannii* infections are higher in ICUs than any other hospital ward. However, the exact percentage varies from one place to other.[4,5,7,14] In this study, conducted in a tertiary care hospital of Rawalpindi, ICUs have the major burden of *Acinetobacter baumannii* infections, almost 46% of all infections occur due to this pathogen. However, the percentage of isolates collected from ICU is less than that reported by Jaggi *et al*, who reported 76.7% of *Acinetobacter baumannii* infections from ICUs among all infections by this bacterium.[15] This is also less than 59% reported by Necati Hakyemez from Turkey.[16] In our study, *Acinetobacter baumannii* was most commonly isolated from endo-tracheal tube specimens in ICU patients. This is consistent with findings of Begum *et al* and Jaggi *et al*.[8,5] In non-ICU samples, *Acinetobacter baumannii* was mostly isolated from pus cultures. This difference can be explained on the level of immunity, age of patients admitted in ICU, difference in setups and different medical procedures in ICU compared to other hospital wards. Antibiotic resistance of all isolates was greater in ICU then in non-ICU isolates. MDR *Acinetobacter baumannii*, was calculated to be 98.5% (n=68) in ICU isolates and was 83.11% (n=64) in non-ICU isolates. MDR *Acinetobacter baumannii* was reported higher in other studies from Pakistan. Begum *et al*, reported 100% prevalence of MDR *Acinetobacter baumannii* among *Acinetobacter baumannii* isolates.[8] Hasan*et al*, has also reported almost 100% Multi drug resistance in *Acinetobacter baumannii* isolates.[7] MDR *Acinetobacter baumannii* reported from Saudi Arabia by Mobarak was 67%.[5]

In our study resistance to ampicillin, cefotaxime, imipenem, trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin approached almost to 100 % in ICU isolates. This itself is alarming as it rules out these major antibiotics as treatment option. Jaggi *et al* from India, reported carbapenem resistance of about 93.2 % in ICU isolates of *Acinetobacter baumannii*, which in our study was 95.5%. Doxycycline and amikacin have lower resistance than other drugs in ICU isolates. The resistance in our ICU setting was more than that reported by a study conducted by Gupta in India.[17]

Gupta and colleagues reported resistance to amikacin of about 29% in non-ICU isolates.[17] Whereas, 76.6 % resistance to amikacin was reported by Hasan *et al*, Bilgari *et al*, reported 62.7% resistance to amikacin in both ICU and non-ICU isolates.[14] In the same study gentamicin resistance was 70.2% as opposed to 66.2% in our study. Resistance to ciprofloxacin in non-ICU isolates was 85.71%. A study conducted in India reported a resistance of just 19%.[17] Whereas, resistance of 73.8% to ciprofloxacin was reported from Pakistan, which is near to our findings.[14] Hasan *et al*, reported 95.5 % resistance to trimethoprim-sulfamethoxazole. On the contrary, in our study resistance was found less than this. Of four Beta lactam drugs tested, 95% resistance to ampicillin and cefotaxime was recorded, the same has been reported from Iran i.e. 97% by Azimi and colleagues. However, carbapenem resistance wasless in our study than found by Azimi *et al* (97% resistance to imipenem).[18] Likewise, in the same study 80% resistance was found against aminoglycosides which is much more than our report. In a review article from Iran 76.5% resistance to imipenem was concluded during 2011-2013, which was said to be a drastic increase in resistance than the previous decade.[19] In our study 72.7% resistance to these carbapenems was found in non ICU isolates.

Colistin and tigecycline were tested only for extremely-drug resistant (XDR) *Acinetobacter baumannii* isolates. Colistin sensitivity was 100% whereas tigecycline was sensitive only in 43.3%(n=23) in our study *Acinetobacter baumannii* is not uniformly reported sensitive to colistin and tigecycline in medical literature. Varying resistance have previously been reported. Jung *et al*, from Korea has concluded 22% resistance to colistin.[20] others have reported a range of resistance from 30% to 75%.[21, 22]

Infection control for *Acinetobacter baumannii* is extremely important and this study endorses, that ICU patients are most vulnerable with endo-tracheal tube as the most common specimen, which signifies the respiratory tract infections. Following strict infection control is the only light of hope because a substantial proportion of this pathogen is resistant to all commonly used antibiotics.

Conclusion

The current situation is disappointing as intensive care units have become single most important place of *Acinetobacter baumannii* infections. Infection control in intensive care units and judicious use of antibiotics are the only ways to control MDR-*Acinetobacter baumannii* infections.

Acknowledgements

The authors are thankful to faculty and staff of Fauji Foundation Hospital, Microbiology Laboratory.

Conflict of Interest

The authors declare no conflict of interest.

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