Susceptibility Pattern of *Acinetobacter* Isolates in quantitative tracheal aspirates

Ausaf Ahmed, Mubarak Zeb, Madiha Jilani, Syed Bilal Tanvir, Ali Shariq, Badar Jahan Farooqi

**Abstract**

**Background:** *Acinetobacter* is a gram negative, multi drug resistant organism which is contributing to a significant amount of morbidity and mortality in hospitalized patients, especially in ICU patients worldwide including Asian countries. *Acinetobacter* is associated with nosocomial pneumonia and Ventilator associated pneumonia. Research was conducted to determine resistance pattern of *Acinetobacter* isolates in quantitative tracheal aspirates and to see what antibiotics can still be used safely.

**Methodology:** Prospective cross sectional study was conducted in a tertiary care hospital of Karachi, Pakistan (Ziauddin University Hospital) from 1st Jan 2015 to 30th June 2015. Ninety three consecutive samples of quantitative tracheal aspirates containing *Acinetobacter* were selected via Convenient sampling. SPSS version 17 was used for data analysis.

**Results:** 93 consecutive samples of *Acinetobacter* isolates in a quantitative tracheal aspirate were taken and data was analyzed via SPSS. 56% of the patients were males and 44% were females. Age distribution of these sample showed majority of the patients above 40 years of age. *Acinetobacter* isolate showed very high resistance to all commonly used antibiotic including Ceftizoxime, Imipenem, Meropenem Ofloxacin, and Ciprofloxacin reaching upto 94%. Amikacin, Co-trimoxazole resistance reached up-to 89%, resistance to Gentamicin was 83% resistance to Levofloxacin was 90% and Tazobactum-Piperacillin showed 91% resistance.

Colistins and polyoxins showed 0% resistance and had 100% sensitivity. Cefoperazone-sulbactum had sensitivity of 57%.

**Conclusion:** Based on our data we found out *A. baumannii* to be XDR (resistance to 3 classes of antimicrobial plus Carbapenem) showing a progressively higher pattern of resistance to all antibiotics except Colistin and Polymyxin when compared with previous studies. Hence, stricter implementation of infection control practices should be carried out along with judicious usage of antibiotics paired with both advanced and conventional phenotyping methods to prevent an outbreak beforehand.

**Key words:** Susceptibility Pattern, *Acinetobacter*, Multidrug resistance.

**Introduction**

Hospital-acquired infections are a substantial obstacle to patient well-being. Approximately 4.5 patients out-of 100 admissions develop hospital-acquired infections during their hospital stay. Mortalities directly related to nosocomial infections approximated to be 99,000 per year, making Hospital-acquired infections are the sixth leading cause of death in the US. invasive procedures and invasive medical devices put patients at increased risk of getting Hospital-acquired infections. U.S. National Healthcare Safety Network data signifies that 30% of all hospital-acquired infections are caused by gram negative bacteria, of these gram Negative bacteria, *Acinetobacter* species is emerging as common multidrug-resistant organism. *Acinetobacter* is a gram-negative organism, catalase-positive, oxidase-negative, cocobacilli. Large study from the US showed, 5 to 10 percent of ICU-acquired pneumonia were due to *Acinetobacter* species. *Acinetobacter* species is part of skin flora in human body, Epidemiological studies showed colonization of mucous membranes and skin with *Acinetobacter* species reaches up to 43% in non-hospitalized individuals compared to 75% in hospitalized individuals. Rates of colonization in patients tends to increase during their hospital stay. *Acinetobacter* is a significant cause of nosocomial pneumonia and substantial causative agent of ventilator-associated pneumonia (VAP). It is highly resistant to conventionally used antibiotics that infects patients with debilitated immunity like ICU patients. Prolonged Hospitalization,
Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (MHA) medium (Oxide Ltd., England) using modified Kirby Bauer’s double disk diffusion method according to clinical and laboratory standard institute (CLSI) guidelines. A 0.5 McFarland equivalent suspension of organism was inoculated onto a MHA plate. The antimicrobial discs were used, Amikacin 30 ug, Trimethoprim 25ug, Cefotaxime 30ug, Gentamicin 10 ug., Ofloxacin 5 ug, Imipenem 10 ug, Meropenem 30 ug, Tazobactam 110 ug, Carbimicilin 100 ug, Tigecycline 15ug. American Type of Culture collection (ATCC) Controls will be used to check the quality of media and antibiotic disc. *E.coli* ATCC 25922 (Beta-lactamase negative) strains were used as a control organism. All the gram-negative bacteria isolated from these clinical samples were tested for ESBL production by using four disks (concentration in μg) Ceftazidime (30), Ceftazidime/clavulanic acid (30/10), Cefotaxime (30), and Cefotaxime/Clavulanic acid (30/10) and interpreted as per NCCLS guidelines [15].

Data Analysis

Data was analyzed by using Statistical Package for Social Sciences (SPSS) software version-17.0. The resistant pattern and antibiotics susceptibility percentages were calculated and express in graphs.

Results & Discussion

*Acinetobacter* is a strictly aerobic organism, It is gram negative on staining, it is oxidase negative, nonfermenting, nonmotile bacteria and is also catalase positive [16]. It is regarded as one of the most troublesome pathogens, which have recently emerged in different healthcare settings across the world. In the recent era it has been observed that there has been an increasing resistance pattern of this pathogen worldwide to all kinds of antimicrobials. This organism is reported mostly to affect those patients who have been hospitalized for a long period of time or who are gravely ill and have breaks in their skin integrity and airway protection [17].

In the recent years it has been a swift increase in the global emergence of *A.baumannii* strains which are completely resistant to all Beta-lactams including broad spectrum Carbapenems, which is proof enough for how rapidly this pathogen adapts to environmental stress [18, 19]. Although owing to the complexity of this pathogen, there are multiple mechanism by which it resists the beta-lactam group of drugs, but the most prevalent type of mechanism by which resistance occurs is by enzymatic degradation by beta-lactamases [20, 21]. The most important beta-lactamases responsible for development of resistance to carbapenems comprises of serine oxacillinase and metallo Beta-lactamases [22]. Other nonenzymatic mechanisms comprises of structural changes to outer membrane protein [23] and multidrug efflux pumps and affinity for changes in expression of penicillin binding proteins [24, 25].

Resistance to aminoglycosides are caused by important enzymes named as aminoglycoside-modifying enzymes such as phosphotransferases, nucleotidyltransferases, acetyltransferases [25, 26]. These enzymes alter the capability of aminoglycosides to bind to the its target site, hence rendering them useless [27]. Apart from aminoglycosides, resistance to fluoroquinolones is developed by alterations to DNA gyrase or topoisovertase IV in gyrA and parC genes.
These genetic mutations cause a change in the target binding sites, hence rendering the fluoroquinolones useless against it, moreover added resistance is also facilitated by presence of multidrug efflux pumps.

93 consecutive samples of *Acinetobacter* isolates in quantitative tracheal aspirate were taken and data was analyzed via SPSS 17. Data showed that sample isolates containing Acinetobacter were mainly received from ICU setup reaching up-to 41% this includes medical, surgical and pediatrics ICU. Almost 44% of samples were from different branches of Ziauddin University Hospital, exact location of patient in hospital is not known for these patients.

12% of samples were received from different hospitals and only 2% of samples were obtained during clinical visit as seen in Fig.1. According to our data out of 93 samples 56% were males and 44% were females represented in, Fig.2. Age distribution of these sample showed 43% of patients were above age of 60, 31% of patients were between age of 40 to 60 years, 17% of patients were between age 20 to 40 years. There were 8% of patients below 20 years this group also included neonates and infants as portrayed in Fig.3.

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**Fig 1:** *Samples were received from different branches of Hospital plus from out side Hospital setup*

**Fig 2:** *Gender distribution of Samples*
Based on our data, *Acinetobacter* isolate showed a very high resistance pattern to all commonly used antibiotics (Table 1) including Ceftixone, Imipenem, Meropenem Ofloxacin, and Ciprofloxacin reaching up to 94%. Amikacin, Co-trimoxazole resistance reached up to 89%, Resistance to Gentamicin was 83%, Resistance to Levofloxacin was 90% and Tazobactum-Piperacillin showed 91% resistance. Colistins and Polymixins showed 0% resistance and had 100% sensitivity. Cefoperazone-sulbactum had sensitivity of 57%.

According to a research conducted by Vahdani P in 2011 in Tehran *Acinetobacter baumannii* strains had a comparatively low resistance pattern to commonly used antimicrobials i.e. 95% to Ceftriaxone, 58% to Amikacin, 68% to Gentamicin, 85% to both Co-trimoxazole and ciprofloxacin and 9% to Imipenem [31].

Another research conducted by Akan OA in Turkey in 2003 showed that *Acinetobacter* was 44% resistant to Sulbactam/ceferazone, 53.6% to Imipenem, 59.8% for Amikacin, 74% for Ciprofloxacin, 78% for Gentamicin, 82.3% for Cotrimoxazole and 87.3% for Tazo/Piper. Which is also considerably lower pattern of resistance as compared to our research [32].

A similar research carried out in Saudi Arabia by Abdalla NM et al. in 2013 ascertained the *Acinetobacter baumannii* to be 100% sensitive to Imipenem and Colistin, 83% resistant to Ceftriaxone, 77% and 75% to both Gentamicin and Amikacin and 70% resistance to Ciprofloxacin. The results of this research also shows that the resistance pattern is considerably lower in isolates when compared with our data [36].

### Table 1. Resistance and sensitivity pattern of *Acinetobacter baumannii* to antimicrobials

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>sensitive</th>
<th>resistant</th>
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<tbody>
<tr>
<td></td>
<td>Percentage</td>
<td>Percentage</td>
</tr>
<tr>
<td>Amikacin</td>
<td>11%</td>
<td>89%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>6%</td>
<td>94%</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>11%</td>
<td>89%</td>
</tr>
<tr>
<td>Colistin</td>
<td>100%</td>
<td>0%</td>
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<tr>
<td>Gentamicin</td>
<td>17%</td>
<td>83%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>6%</td>
<td>94%</td>
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<tr>
<td>Levofloxacin</td>
<td>10%</td>
<td>90%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>6%</td>
<td>94%</td>
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<tr>
<td>Oflox/Cipro</td>
<td>6%</td>
<td>94%</td>
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<tr>
<td>Polymixins</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Tazo/Piper</td>
<td>9%</td>
<td>91%</td>
</tr>
<tr>
<td>Cef/Sul</td>
<td>57%</td>
<td>43%</td>
</tr>
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*Acinetobacter* Isolates were classified based on their resistance pattern in to MDR, XDR and PDR. Based on our data XDR (resistance to 3 classes of antimicrobial plus Carbapenem) was most common resistance pattern in *Acinetobacter* isolates estimated to be 91%. 6% of Isolates were sensitive to all antimicrobials and 3 percent were MDR which is clearly depicted in Fig 4.
Conclusion

Acinetobacter baumannii is emerging as one of the most resistant pathogen responsible for a wide range severe nosocomial infections in patients who are admitted to the ICU for a prolonged period of time. In our research we found out A. baumannii to be XDR(resistance to 3 classes of antimicrobial plus Carbapenem) showing a progressively higher pattern of resistance to all antibiotics except Colistin and Polymyxin when compared with previous studies. The major factors promoting the resistance of A. baumannii are inadequate infection control practices, non judicious use of antibiotics along with inability to recognize the outbreak strain. Therefore, stricter implementation of infection control practices should be carried out along with sensible usage of antibiotics and moreover phenotyping typing methods such as antibiotic susceptibility patterns, biochemical profiles (biotyping) and serotyping should also be utilized in developing countries to identify the outbreak causing strains, where advanced molecular typing methods are not available, which would enable the healthcare institutions to take appropriate steps and precautions to contain an outbreak before it occurs.

References


