Multi-Drug Resistant Acinetobacter Species as a Cause of Hospital Acquired Infections

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ABSTRACT
Acinetobacter baumannii is an important opportunistic pathogen that is rapidly evolving toward multidrug resistance and is involved in various nosocomial infections that are often severe. Carbapenems are considered one of the very few antibiotics left to treat infections caused by this organism. The aim of this work is to study the antibiotic resistance pattern of Acinetobacter species isolated from different sites of nosocomial infections. Patients and Methods: Antibiotic resistance pattern for 30 clinical isolates of Acinetobacter was determined by the Kirby Bauer disk diffusion method. Extended spectrum beta-lactamase (ESBLs) production was detected by double disc synergy test. Isolates detected to be imipenem resistant were tested for metallo β-lactamase (MBL) production by E test. Results: Extended spectrum beta lactamase production is high (19/30) 63.3% among Acinetobacter species. Ten (33.3%) isolates are found to be resistant to imipenem and meropenem by the disk diffusion method and 3/30 (10%) of them are found to be MBLs producers. Conclusion: Acinetobacter spp. are resistant to many classes of antibiotics. Production of ESBLs, and MBLs are responsible for the multidrug resistance of these pathogens.

INTRODUCTION
Acinetobacter spp. are opportunistic pathogens with increasing relevance in nosocomial infections. Acinetobacter is ubiquitous and has wide distribution in nature. They are natural reservoirs of and account for up to 35-45% of bacteria isolated from human skin. Acinetobacter baumannii (A. baumannii) has emerged as an important and problematic human pathogen as it is the causative agent of several types of infections including pneumonia, meningitis, sepsis, and urinary tract infections. It ranked second after Pseudomonas aeruginosa among the nosocomial, aerobic, non-fermentative, gram negative bacilli pathogens.

Acinetobacter baumannii is generally considered an opportunistic nosocomial pathogen and there is debate as to its mechanisms of pathogenicity and virulence. The epidemiological profile suggests that it is of low virulence and disease is dependent on significant host immunological impairment. The evidence is now mounting that A. baumannii can no longer be exclusively considered a nosocomial pathogen, and is capable of causing profound clinical disease in the absence of traditional nosocomial risk factors.

Increasing resistance due to extended-spectrum beta-lactamases (ESBLs) and multiple resistance mechanisms in gram-negative hospital isolates restrict the role of beta-lactam antibiotics in empirical treatment of serious infections. As the prevalence of ESBL producing strains and resistance rates to antimicrobial agents can vary in each center, local surveillance studies are required to guide therapy.

Organisms were defined as multidrug resistant if there was resistance to two or more of the following: expanded-spectrum cephalosporins or extended-spectrum β-lactams, quinolones, carbapenems, trimethoprim-sulfamethoxazole, or aminoglycosides.

Acinetobacter spp. have become resistant to many classes of antibiotics. Firstly, Acinetobacter spp. appear to be well suited for genetic exchange and among a unique class of gram-negative bacteria that are described as "naturally transformable." Another study describing the genome sequences of both susceptible and resistant isolates of A. baumannii has shed light on the abundance of resistance genes found in this organism.

Effective treatment is compromised by the high level of resistance to antimicrobials exhibited by hospital strains. Indeed, the emergence of carbapenem resistance in A. baumannii has become of global concern, as these β-lactams are often the only active agents against many multiresistant strains.

The increase in the number of MBLs in A. baumannii is an ominous development in the global emergence of resistance to beta-lactams. MBLs are class B beta-lactamases
that are able to hydrolyze carbapenems as well as every other beta-lactam antibiotic with the exception of aztreonam. They differ from class A and D carbapenemases by having a metal ion in the active site, usually zinc, which participates in catalysis.\(^{12,13}\)

Class B carbapenemases (various IMP-type, VIM-type and SIM-1 metallo-\(\beta\)-lactamases) have been found in \textit{Acinetobacter} spp., but worldwide most \textit{A. baumannii} strains are resistant as a result of the production of OXA-type carbapenemases.\(^{14}\) Four of the eight known clusters of OXA-type carbapenemases have been identified in \textit{A. baumannii}, namely OXA-23, OXA-24, OXA-51 and OXA-58.\(^{15}\)

**AIM OF THE WORK**

The aim of this work is to study the antibiotic resistance pattern of \textit{Acinetobacter} species isolated from different sites of nosocomial infections.

**PATIENTS & METHODS**

**Isolation and Identification of \textit{A. baumannii}**

A total of 30 isolates of \textit{Acinetobacter} species were obtained from various clinical specimens like endotracheal aspirates, sputum, wound swabs, and urine specimens. The strains were inoculated into MacConkey agar and sheep blood agar. Preliminary identification of \textit{Acinetobacter} was done by the Gram stain findings, testing for motility and the oxidase reaction in all the samples. Non-fermenting gram-negative bacilli that were oxidase-negative and non-motile, hemolysis on sheep blood agar, gelatin hydrolysis were identified as \textit{Acinetobacter} spp. All tests were performed at 37°C. Selected isolates were also identified by API 20 E system.

**Antibiotic susceptibility tests:**

Antibiotic susceptibility tests were performed on Mueller-Hinton (MH) agar (Oxoid). Antibiotic disks were obtained from Oxoid. Agar plates were evaluated after 18 hours of incubation at 37°C. The interpretation as sensitive, intermediate or resistant was based on criteria according to Clinical Laboratory Standards Institute (CLSI) guidelines.

**1-Disk Diffusion Test: (Kirby Bauer)**

Antimicrobial susceptibility of all isolates was determined by Kirby Bauer disk diffusion method. Antibiotics tested included amikacin (10 \(\mu\)g), aztreonam (30 \(\mu\)g), ceftazidime (30\(\mu\)g), cefotaxime (30 \(\mu\)g), ceftriaxone (30 \(\mu\)g), ciprofloxacin (\(5\mu\)g), cefepime (30 \(\mu\)g), imipenem (10 \(\mu\)g), meropenem (10 \(\mu\)g), gentamicin (30 \(\mu\)g), piperacillin /tazoparactam (110 \(\mu\)g), Sulphamethoxazole/ trimethoprim (25 \(\mu\)g), colistin sulphate (10 \(\mu\)g).

**2-Screening for ESBL producers:***

Isolates inhibited by at least one of the oxyimino-\(\beta\)-lactam ceftazidime, cefotaxime and aztreonam were considered as putative ESBL producers. The CLSI has proposed disk diffusion methods for screening for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. However, the use of more than one of these agents for screening improves the sensitivity of detection.\(^{16}\)

**3- Confirmatory test for ESBLs producers:***

ESBL production was confirmed by double-disc synergy test (DDST).

DDST was done to determine synergy between a disc of augmentin (20 \(\mu\)g amoxicillin + 10 \(\mu\)g clavulanate) and disc of each of cefotaxime and ceftazidime antibiotics. The test inoculum (0.5 McFarland’s turbidity) was spread onto Mueller-Hinton agar (MHA) by using a sterile cotton swab. A disc of augmentin (20 \(\mu\)g amoxicillin + 10 \(\mu\)g clavulanate) was placed on the surface of the MHA; then, discs of cefotaxime (30 \(\mu\)g) and ceftazidime (30 \(\mu\)g) were kept 16 to 20 mm apart from the augmentin disc (centre to centre). The plate was incubated at 37°C overnight. The enhancement of the zone of inhibition of the cefalosporin disc towards the amoxicillin/clavulanic acid disc was inferred as synergy and the strain was considered as an ESBL producer. For the ESBL confirmation test, isolates were considered positive for ESBL production if zone diameters increased by \(\geq 5\) mm for either cefotaxime, ceftazidime, when tested in combination with CA versus its zone when tested alone, as indicated by the manufacturer or CLSI.\(^{17}\)

**4- Metallo \(\beta\)-lactamase (MBL) detection:**

This was performed by E test using IP/IPI Strips: (AB, Biodisk)

IP coded for the Imipenem (4 -256 \(\mu\)g/ml) and IPI coded for Imipenem EDTA (1-64 \(\mu\)g/ml). \textit{Acinetobacter} strains were streaked evenly across the entire surface of the Muller-Hinton agar plate, left to dry then the strips were applied, and the plate was incubated at 35°C for 16-18 hours in ambient air. When bacterial growth was visible, the IP and IPI MIC values were read where the respective inhibition ellipses intersected the strip. The result was considered positive for MBL when the MIC ratio of IP/IPI was \(\geq 8\). The test was considered negative for MBL when the MIC ratio of IP/IPI
was <8. The test was considered indeterminate if the reading was out of the range of the test.

**RESULTS**

This study included 30 strains of *Acinetobacter* spp. Isolated from different nosocomial infections (Table 1). The antimicrobial resistance profiles of all strains were evaluated using Kirby Bauer disk diffusion method (Table 2). Results of ESBLs and MBLs production by *Acinetobacter* isolates are presented in (Table 3). Multidrug resistance is observed among (25/30) 83.3% of the isolates with different patterns of resistance (table 4).

**Table (1): Number of *Acinetobacter* isolates from different clinical samples.**

<table>
<thead>
<tr>
<th>Nosocomial Infection</th>
<th>Number of isolates (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract infection</td>
<td>5</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>9</td>
</tr>
<tr>
<td>Wound infection</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table (2): Percentage of in–vitro sensitivity of all antibiotics tested by Kirby Bauer for *Acinetobacter* spp**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Percentage of susceptible strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin (AN) 30µg</td>
<td>43.3%</td>
</tr>
<tr>
<td>Gentamicin (CN) 30µg</td>
<td>36.7%</td>
</tr>
<tr>
<td>Aztreonam (ATM) 30µg</td>
<td>60%</td>
</tr>
<tr>
<td>Ceftazidime (CAZ) 30µg</td>
<td>20%</td>
</tr>
<tr>
<td>Cefotaxime (CTX) 30µg</td>
<td>30%</td>
</tr>
<tr>
<td>Ceftriaxone (CRO) 30 µg</td>
<td>33.3%</td>
</tr>
<tr>
<td>Cefepime (FEP) 30µg</td>
<td>36.7%</td>
</tr>
<tr>
<td>Cefuroxime sodium (CXM) 30µg</td>
<td>26.7%</td>
</tr>
<tr>
<td>Imipenem (IPM) 10µg</td>
<td>66.7%</td>
</tr>
<tr>
<td>Meropenem (MEM) 10µg</td>
<td>66.7%</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP) 5µg</td>
<td>30%</td>
</tr>
<tr>
<td>Piperacillin/tazobactam (TZP) 110µg</td>
<td>56.7%</td>
</tr>
<tr>
<td>Colistin sulphate (CT) 10µg</td>
<td>100%</td>
</tr>
<tr>
<td>Sulphamethoxazole/trimethoprim (SXT) 25 µg</td>
<td>16.7%</td>
</tr>
</tbody>
</table>

Only meropenem or imipenem resistant strains were screened for MBL production. Other isolates were not screened for MBL production as they were sensitive to meropenem.

**Table (3): Results of ESBLs and MBLs production as tested by E test.**

<table>
<thead>
<tr>
<th>Total number of <em>Acinetobacter</em> isolates:30</th>
<th>ESBLs production</th>
<th>MBL production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table (4): Multidrug resistance profiles**

<table>
<thead>
<tr>
<th>Number of resistant isolates:25</th>
<th>Multiple drug combination pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CAZ,CTX , FEP,CIP, TZP</td>
</tr>
<tr>
<td>4</td>
<td>CAZ,CTX , FEP, CN,CIP, IMP,MPM, SXT,ATM</td>
</tr>
<tr>
<td>7</td>
<td>CN, ATM, CRO, CXM, TZP, IPM, MEM</td>
</tr>
<tr>
<td>4</td>
<td>AK,CAZ, CRO,FEP,TZP</td>
</tr>
<tr>
<td>8</td>
<td>AK,CAZ,CRO,FEP</td>
</tr>
</tbody>
</table>
Acinetobacter baumannii is a well known but relatively uncommon cause of health-care-associated infections. Because the organism has developed substantial antimicrobial resistance, treatment of infections attributed to A. baumannii has become increasingly difficult. It is believed by some clinicians that the recovery of A. baumannii in the hospitalized patient is an indicator of severe illness, with an associated mortality of approximately 30%. Infections due to Acinetobacter are frequently found in intensive care units (ICUs), where they are implicated as the cause of ventilator-associated pneumonia (VAP), urinary tract infections, and bacteremia. A. baumannii also causes, albeit less frequently, complicated skin and soft tissue, abdominal, and central nervous system infections. A. baumannii has also become a major pathogen found in combat-associated wounds. This is in concordance with the results of our study which revealed that most of Acinetobacter isolates were recovered from samples of wound infection 53.3%, respiratory tract infections 30%, and urinary tract infections 16.7%. It was noticed that most of the strains (22/30) 73.3 % were isolated from the ICU patients. In the present study the Acinetobacter isolates were identified by using the API 20E system which gave probability above 90% for all cases this result is supported by Bergogne-Bézézin & Towner, who accepted that genomic species identification using commercial identification systems is problematic, although use of such systems in routine microbiology laboratories is widespread and is the basis for identification of nosocomial isolates. The API profile gave an excellent identification, with a 99% probability. However, Kropec et al., disagreed this opinion and mentioned that the commercial identification systems, the widely used API 20NE system, are based largely on carbon source assimilation tests and sometimes has problems with sensitivity and reproducibility and the differences between the genomic species are so slight that a reliable identification seems unrealistic. The studied Acinetobacter isolates revealed antibiotic sensitivity pattern by Kirby Bauer method as follow: Colistin showed the highest in-vitro susceptibility (100%) followed by imipenem and meropenem (66.7%) and piperacillin/tazobactam (56.7%). This fact reflect the importance of controlling the use of these antimicrobials in hospitals, to prevent emergence of resistant strains. These observations are in accordance to the results of the study of Cisneros-Herreros et al., in which they reported that colistin is the treatment of choice in A. baumannii pneumonia caused by panresistant strains. The associations of imipenem and rifampin or imipenem and sulbactam may be acceptable alternatives to colistin in infections caused by these strains. Surveillance measures are essential to eradicate this multidrug-resistant pathogen in outbreaks and reduce the number of episodes in endemic situations. In another study by Li et al., Lolans et al., nebulized polymyxin E is increasingly being used for the treatment of respiratory infections caused by multiresistant A. baumannii due to the high concentrations that can be achieved in the lung, and is sometimes combined with intravenous administration, despite concerns of its nephrotoxicity. (Polymyxin B and polymyxin E (colistin, intravenous colistimethate sodium) are peptide antibiotics first isolated in 1947 that have been increasingly used as a "last-resort" treatment of infections caused by multidrug resistant A. baumannii. Bedenic et al., reported that A. baumannii had turned out to be the most resistant Gram-negative bacteria with 81% resistant to ceftazidime, 73% to cefepime, 69% to gentamicin and 71% to ciprofloxacin. Meropenem remains an appropriate antibiotic for the treatment of severe infections caused by Gram-negative bacteria. They added that despite continued use of meropenem, carbapenem resistance is not increasing among species tested, except for A. Baumannii, and suggested that clinicians can still administer carbapenems as a reliable and effective choice in managing serious nosocomial infections. In the present study Acinetobacter showed 80% resistance to ceftazidime, and 63.3% resistance to cefepime. Ceftazidime and cefepime are third generation cephalosporins, so this high pattern of resistance points to the probability of ESBLs production in our strains. Also the resistance to other third generation cephalosporins (Table2) support this inference. On the other hand, 70% of the strains showed resistance to ciprofloxacin, 63.3% resistance to gentamicin, 83.3% resistance to sulfamethoxazole. Nemec et al., mentioned that resistance to aminoglycosides in A. baumannii is mediated principally by aminoglycoside-modifying enzymes (AMEs). Aminoglycoside-resistant isolates from 13
countries were analyzed for the genes encoding AMEs. Seward (33) further demonstrated that similar AMEs are found in unrelated isolates of Acinetobacter spp. and that particular genes are not restricted to specific areas of the world. This finding has been confirmed in Spain, in England, and throughout Europe. (32)

Resistance of A. baumannii to quinolones is often caused by modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance-determining regions of the gyrA and parC genes. (34) These changes result in a lower affinity for the binding of the quinolone to the enzyme-DNA complex. (35) Regarding trimethprim-sulfamethoxazole Van Looveren & Goossens (36) reported that the prevalence of trimethoprim-sulfamethoxazole resistance in A. baumannii is high in many geographic regions.

The resistance to imipenem and meropenem was detected among 33.3% of the isolated strains. Dalla Costa et al. (37) reported that carbapenems have the most extended antimicrobial spectrum of antibacterial activity among all of the β-lactams. However, carbapenem resistance is emerging and increasing in clinical isolates, especially in P. aeruginosa and A. baumannii.

Confirmed ESBL production was done using DDST and 63.3% of the strains were proved to be positive ESBL producers. Thomson and Sanders (38) mentioned that combination disk method using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for detection of ESBL among the members of Enterobacteriaceae. Singhal et al. (39) explained that a five mm or more increase in zone of inhibition for either cefotaxime-clavulanic acid or ceftazidime-clavulanic acid disk compared to the cefotaxime or ceftazidime disk respectively was taken as confirmatory evidence of ESBL production.

In the present study MBLs production was tested using E test among the imipenem and meropenem resistant strains and 3 isolates (10%) were found to be positive. According to Walsh et al. (40) Various criteria for screening for MBL production have been suggested. However, there are no standard guidelines provided by the CLSI for detection of these enzymes in various bacterial species. The MBL Etest has been evaluated in several studies and found to be a sensitive method for detection of MBL production.

Mirroring the spread of other beta-lactamases, IMP MBLs are now found around the world in different genera. In A. baumannii IMP MBLs are usually detected as part of a class 1 integron, as first discovered in the Far East. Although MBLs are not the predominant carbapenemases in A. baumannii, several have been described: IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, and IMP-11. (41) Despite MBLs being less commonly identified in A. baumannii than the OXA-type carbapenemases, their hydrolytic activities toward carbapenems are significantly more potent (100- to 1,000-fold). (42)

The detected multidrug resistant Acinetobacter isolates 83.3% represent a challenge in the clinical practice. Many in vitro studies supported the role of combination therapy with colistin. In particular, colistin in combination with a carbapenem and/or rifampin appears most promising. (43,44) Falagas et al. (43) performed a retrospective cohort study, comparing patients who received colistin only with those who received colistin and meropenem. After adjusting for severity of illness, no difference in outcomes was observed. Whether combination therapy will protect colistin from the emergence of resistance is presently unknown. (44) Combination antibiotic therapy is a strategy often employed in the treatment of multidrug resistant A. baumannii. This approach attempts to achieve synergy, particularly against multidrug resistant strains. (45)

**CONCLUSION & RECOMMENDATIONS**

Acinetobacter spp. Are resistant to many classes of antibiotics. MBL producing Acinetobacter strains have been introduced into the hospital environment, and to prevent the further spread of MBL producers, it is essential for carbapenem resistant isolates to be screened for MBLs. Production of ESBLs, and MBLs are responsible for the multidrug resistance of these pathogens. Knowledge of the resistance pattern of the local pathogens guide the choice of proper antibiotics to avoid the emergence of resistant mutants.

**REFERENCES**


