Current Pattern of Antibiotic Resistance in Clinical Isolates of Acinetobacter Baumannii from Intensive Care Units of Tertiary Care Hospital

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Abstract

Purpose: To determine the pattern of antibiotic resistance in clinical isolates of Acinetobacter baumannii from ICU’s of tertiary care hospital in Karachi.

Study Design: A case control study.

Methods: Three hundred and fifteen clinical isolates of Acinetobacter baumannii collected from different ICUs were evaluated during 1 year period. The isolates were identified by morphology, growth and biochemical characteristics, susceptibility to a panel of antimicrobial agents in disc diffusion assay and molecular characterization by PCR using glt A and gyr B genes.

Results: 94% of Acinetobacter spp were detected as multi drug resistant (MDR) and sensitive to Polymixin B only. About 6% Acinetobacter spp were also MDR but sensitive to Polymixin B, Meropenem and Sulfactam + Ceftoperazone. All tested isolates exhibited differing resistance representation, as establish by medium incorporation – replica method, against different tested antibiotics, as follows: Amoxicillin – Calvulanic acid, Tazobactam, Ceftriaxone, Ceftazidime, Meropenem, Imipenem, Gentamicin, Amikacin, Chloramphenicol, Co-trimoxazole, Tobramycin, Sulfactam, Ceftoperazone, Gentamicin and Amikacin. All the isolates of Acinetobacter baumannii were PCR positive for glt A and gyr B.

Conclusions: Acinetobacter baumannii is the most frequently isolated and alarming pathogen in the healthcare system specifically for the patients in intensive care units (ICUs). Its survival in hospital environment is because of tolerance the antibiotics and antisepsic pressures. Multi drug resistance made this pathogen the lethal pathogen of this century to infect debilitated patients. There is a strict need to monitor the surveillance of global clones at institutional and or intra-institutional level for accurate treatment, precise prevention and batter control.

Key Words: Multidrug resistance (MDR), house-keeping genes.

Introduction

Acinetobacter baumannii is the most common isolate, among all nosocomial pathogens causing infectious epidemics in critically ill patients and prominent cause of high mortality and morbidity. Currently Acinetobacter considered as the most harmful organism and a...
major threat in clinical settings, reasoned its multi-
drug resistance, permissiveness to varied degree of pH,
saline nature and moisture, and its inimitable cap-
ability to endure in most of nutrients deficient environ-
ments. The burden of Acinetobacter baumannii is
highest in hospital environments and the bacterium
colonizes very effectively in hospitalized patients.3

Acinetobacter species are aerobic gram – negative
rods which can persist for longer time in the atmos-
phere and can affect healthcare workers if not handle
properly.4 A. baumannii, infections has turned into
progressively problematic to handle due to the man-
ifestation of multi-drug resistance among strains or
frequently recommend antibacterial drugs.5 Such
multi-drug resistant (MDR) strains of Acinetobacter
are commonly sensitive to polymyxins alone i.e. colis-
tin and olymyxin B; a group of antibacterial medicine
which is more poisonous compared to current antibi-
tics in use and now-a-days drug of choice against
Acinetobacter in developing countries.

A. baumannii causes a various type of illnesses
that includes pneumonia, septicemia or localized
wound infections. A. baumannii can colonizetracheo-
stasy sites and open wounds in ICU patient without
causing infection or showing any symptom.6 A. bau-
mannii can easily infect those patients stay in hospital
for long period, which have open wounds and need
assistance such as ventilator invasive devices and uri-
inary catheters.7 A. baumannii may be transmitted to
healthy individuals by person-to-person interaction or
through unhygienic surfaces as well as by health care
workers.8

Epidemic trigger by MDR A. baumannii have
been encountered in hospitals globally; now a days,
they have developed a crucial challenge in different
hospital settings.9 For the molecular identification of 7
house – keeping genes consist of gld A (citrate syn-
thase), gyr B (DNA gyrase subunit B), gdh B (glucose
dehydrogenase B), recA (homologous recombination
factor), cpn60 (60-kDa chaperonin), gpi (glucose-6-
phosphate isomerase), rpoD (RNA polymerase sigma
factor), are commonly used for identification of A.
baumannii isolates.10,11 Owing to the high risk asso-
ciated with A. baumannii infection in hospitals, the
current study was carried to test the occurrence of A.
baumannii in different ICUs of a tertiary care hospital
of Karachi during the period from December 2010 to
January 2012. There is little or no data concerning the
prevalence of A. baumannii among health care facil-
eties located in this region.12,13

Material and Methods
Selection of Cases
Three hundred and fifty patients were selected from
the different ICUs of tertiary care hospital such as;
medical ICUs, medical high dependency unit (HDU)
and surgical ICUs (male and female) were included in
this study. The consent for the participation in study
was received from all the patients through a questioner
prepared as per the approval of Institute Ethics Com-
mittee.

Specimen Collection
Different samples (blood, urine, tracheal secretion, pus
swab, bronchial washing, catheters, intravenous devi-
ces and tips) were collected from the patients who
were admitted to the ICUs. Data on the date and site
of infection, patient demographic information and the
devices used were collected for microbiological pro-
cessing. Only those patients were identified as one
who had acquired infections after 48 h of ICU admis-
sion, as per Centers for Disease Control (CDC) guida-
lines for diagnosis, were included in the study.14

Culture and Growth Requirements
Basic microbiological testing was done by standard
conventional methods. Routine culture media were
used as Blood agar, MacConkeys’s agar, CLED (Cys-
teine lactose electrolyte deficient) agar, S.S. (Salmo-
nella and Shigella)agar, Chocolate agar, SIM (Sulpher,
indole and motility) medium, TSI (Triple sugar iron)
agar, Simmon citrate agar and urease medium. The
organisms were maintained at 4°C on agar slants and
at −20°C in glycerol stocks for further analyses.

Isolation of Pathogens and Cultural
Characteristics
All standard microbiological and biochemical tech-
niques by recommended conventional methods and
standard protocols were used for isolation and identi-
fication of nosocomial pathogens.

Antimicrobial Sensitivity Testing
Susceptibility to a panel of antimicrobial agents was
tested by the disk diffusion technique reference to the
national committee guidelines for Clinical Laboratory
Standards Institute (CLSI).15 Susceptibility pattern was
established by the disk diffusion technique of Kirby –
DNA Extraction

Bacterial DNA was extracted using Qiagen DNA mini (Spin Column) kit according to the manufacturer’s instruction. Briefly, bacterial cells were pelleted from 5 ml of an 18 hours broth culture. Broth was centrifuged for 10 minutes at 1000 X g and re-mixed with phosphate – buffered saline with 100 μg of lysostaphin (Sigma) per milliliter, then laced in incubator at 37°C for half hour or until viscous. DNA from all preparations was subsequently extracted with Qiagen DNA protocol. DNA specimens were dissolved in TE Buffer as 10 mM Tris chloride-1mM EDTA (pH 8.0).17

Polymerase Chain Reaction (PCR)

The presence of glt A gene and gyr B gene was used to confirm A. baumannii by PCR. Specific primers were used as: glt A (forward primer: GTGAAATGACTG-AACGTCGATAA while reverse primer: GTGAAAGTACTGACGTCCGATAA) and gyr B (Forward: GCAGCCGACGGCAAAGAAGA and reverse: GGAAGCGCCGAGGTGAG).18 Master mixture for PCR was made by adding buffer 2.5 μl 10x, dNTPs 0.2 mM, Taq DNA polymerase4 Units, MgCl₂ 2.5 mM, and DNA template 50ng, 1.5 μl of glt A and gyr B primers respectively. Total volume was brought up to 25 μl by adding 2.5 μl of ultra-pure water. Perkin Elmer thermocycler was used for DNA amplification programmed as: preliminary denaturing at 94 C for 4 minutes was after that 35 continuous repeated sequences as denature at 94°C for 45 seconds and anneal at 58°C for 45 seconds and elongation at 72°C 60 seconds termination for an elongation at 72°C for 2 minutes. Finally PCR products, aliquot (10 μl) loaded on an ethium bromide gel (0.5 μg/ml; Sigma Ltd. USA) 1% agarose (Sigma) in Tris Borate EDTA (TBE) buffer, with known markers and run at 90V for one hour before visualization under Ultra Violet Biodoct digital imaging system (UVP, Inc. Cambridge UK) for the presence of 722 and 909 base pair PCR products.

Results

Frequency of Acinetobacter baumannii isolates in different types of specimen collected from the hospitalized patients:

A. baumannii was isolated from 60% of patients with urinary catheters, 30% with different mechanical devices and 10% with central intravenous lines (Fig. 1).

Susceptibility Pattern of Acinetobacter Baumannii

94% of A. baumannii were multi drug resistant (MDR) and only sensitive to Polymixin B, 6% A. baumannii were also MDR but sensitive to Polymixin B, Meropenem and Salbactum + Cefoperazone. All the isolates presented varied resistance sequences (created by medium incorporation – replica method) regarding different antibiotics listed in Table 1.

PCR

PCR analysis showed the presence of glt A gene in all the 305 tested isolates. The glt A specific PCR products of 722 and gyr B specific 909 base pairs were seen in all Acinetobacter baumannii strains (Fig. 2 and 3).
Discussion

Acinetobacter baumannii are able to survive for very long period in hospital environment and via health care workers on their hands, gloves gowns and other surfaces. These organisms can cause serious illness in hospitalized patients specifically in ICUs. Patients in ICUs need special care and precautions when they diagnosed for MDR Acinetobacter. Presently gltA (coded for citrate synthase) and gyrB genes were applied as the objective genes for A. baumannii. Out of seven housekeeping genes of A. baumannii only gltA gene and gyrB were obtained as the most appropriate genes to target A. baumannii in present study.18

The most important and foremost issue with this pathogen remained its resistance to multiple antibiotics.19,20 Antimicrobial resistance against these drugs has delayed treatment strategies, generated growing concern globally.21-23 Emergence of nosocomial or community-acquired infections of Acinetobacter baumannii is a result of high adaptability to adverse environmental conditions, an ability to be persist for several days in dry and harsh environments such as hospital environment, the increased use of broad spectrum antibiotics, the vulnerability of individuals or patients and rapid transformation of organisms that results in increased multidrug resistance.24

Different methods has been introduced to diagnose Acinetobacter and to check the out breaks of this pathogen and to identify out break causing strains from non out breaks strains, for this purpose different tools has been used like; phenotypic identification different biochemical based reactions and antibiogram typing, phage typing and protein profiles were methods of choice but due to limitations these tools were replaced by genotypic methods.25,26 Based on this we have selected gltA and gyrB genes among all seven housekeeping genes for A. baumannii, gltA gene was utilized as reference gene size of generated amplicon was

Table 1: Antibiotic resistance pattern of Acinetobacter baumannii (total 315).

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance of Acinetobacter spp (MDR)</th>
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<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Amikacin</td>
<td>290</td>
</tr>
<tr>
<td>Amoxicillin + Calvulanic acid</td>
<td>305</td>
</tr>
<tr>
<td>Piperacillin / Tazobactam</td>
<td>290</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>302</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>277</td>
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<tr>
<td>Gentamicin</td>
<td>261</td>
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<tr>
<td>Ciprofloxacin</td>
<td>284</td>
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<tr>
<td>Tobramycin</td>
<td>95</td>
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<tr>
<td>Imipenem</td>
<td>287</td>
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<tr>
<td>chloramphenicol</td>
<td>290</td>
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<tr>
<td>Polymixin B</td>
<td>-</td>
</tr>
<tr>
<td>Salbactam + Cefoperazone</td>
<td>284</td>
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<tr>
<td>Co-trimoxazole</td>
<td>290</td>
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</tbody>
</table>
According to our study, Ampicillin resistance was found to be most common followed by Cephalothin, Amikacin, Carbencillin, Gentamicin, Amikacin, Chloramphenicol, Tetracycline, Co-trimoxazole, Ciprofloxacin and Cefoperazone. Present study found 94%, A. baumannii as multi drug resistant, which were only sensitive to Polymixin B, remaining 6% were also MDR but the only difference was their resistance towards Polymixin B. Additional confirmation was done by polymerase chain reaction (PCR) of two housekeeping genes, which confirmed Acinetobacter baumannii. Acinetobacter is still a threat for causing nosocomial and health care associated infections especially in ICUs, so there is a need to move forward and help suffering patients as well as set standards.

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Conflict of Interest: None.

References

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