Original Article

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 Salbactum + Cefoperazone. 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, Salbactam, Cefoperazone, 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 health care system specifically for the patients in intensive care units (ICUs). Its survival in hospital environment is because of tolerance the antibiotics and antiseptic 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), housekeeping genes.

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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.[1,2] 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 capability to endure in most of nutrients deficient environments. 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 atmosphere and can affect healthcare workers if not handle properly.[4] A. baumannii, infections has turned into progressively problematic to handle due to the manifestation 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. colistin and olymyxin B; a group of antibacterial medicine which is more poisonous compared to current antibiotics 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 colonizetracheostomy sites and open wounds in ICU patient without causing infection or showing any symptom.[6] A. baumannii can easily infect those patients stay in hospital for long period, which have open wounds and need assistance such as ventilator invasive devices and urinary 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 glt A (citrate synthase), 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 associated 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 facileties 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 Committee.

Specimen Collection

Different samples (blood, urine, tracheal secretion, pus swab, bronchial washing, catheters, intravenous devices 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 processing. Only those patients were identified as one who had acquired infections after 48 h of ICU admission, as per Centers for Disease Control (CDC) guidelines 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 (Cysteine lactose electrolyte deficient) agar, S.S. (Salmonella 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 techniques by recommended conventional methods and standard protocols were used for isolation and identification of nosocomial pathogens.

Antimicrobial Sensitivity Testing

Susceptibility to a panel of antimicrobial agents was tested by the disc 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 –

Bauer using Mueller – Hinton agar.[16] Panel of antibiotics included; AMC (Amoxicilline 10 µg/ Calvulanic acid), TZP (Tazobactam 110 µg), CRO (Ceftriaxone 30 µg), CAZ (Ceftazidime 30 µg), MEM (Meropenem 10 µg), IPM (Imipenem 10 µg), G (Gentamicin 10 µg), AK (Amikacin 10 µg), C (Chloramphenicol 30 µg), SXT (Co-trimoxazole), TOB (Tobramycin 10 µg), SCF (Salbactam 30 µg / Cefoperazone 75 µg), G (Gentamicin 10 µg), AK (Amikacin 30 µg) and Polymyxin disks of 300 units were used for susceptibility testing.

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: GTAGAAATGACTGAACGTCCGATAA while reverse primer: GTAGAAATGACTGAACGTCCGATAA) and gyr B (Forward: GCGCGACGGCAAAGAAGA and reverse: GGAAGCCGGCGAGGTGAG).[18] Master micture for PCR was made by adding buffer 2.5 µl 10x, dNTPs 0.2 mM, Taq DNA polymerase4 Units, $MgCl_2$ 2.5 mM, and DNA template 50ηg, 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 programed 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).

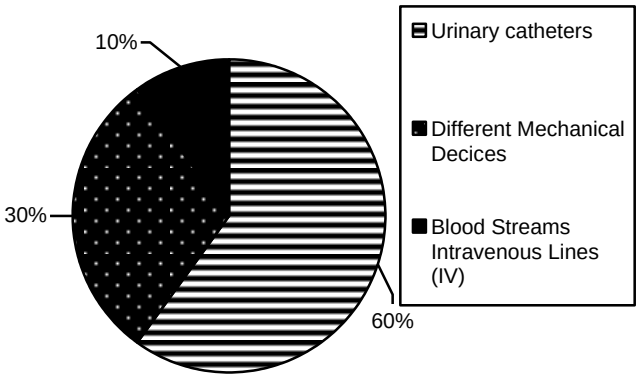

Fig. 1: Frequency of A. baumannii in Patients.

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 Table1.

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 glt A (coded for citrate synthase) and gyr B genes were applied as the objective genes for A. baumannii. Out of seven housekeeping genes of A. baumannii only glt A gene and gyr B 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 glt A and gyr B genes among all seven housekeeping genes for A. baumannii, glt A gene was utilized as reference gene size of generated amplicon was

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

Antibiotics	Resistance of Acinetobacter spp (MDR)	
	n	%
Amikacin	290	92
Amoxicillin + Calvulanic acid	305	97
Piperacillin / Tazobactam	290	92
Ceftriaxone	302	96
Ceftazidime	277	88
Gentamicin	261	83
Ciprofloxacin	284	90
Tobramycin	95	30
Imipenem	287	91
chloramphenicol	290	92
Polymixin B	-	-
Salbactam + Cefoperazone	284	90
Co-trimoxazole	290	92

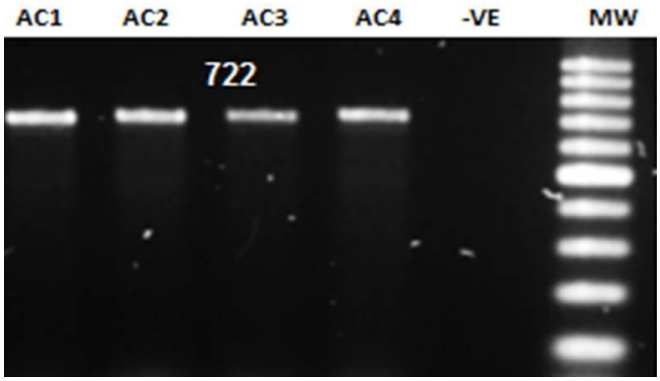

Fig. 2.

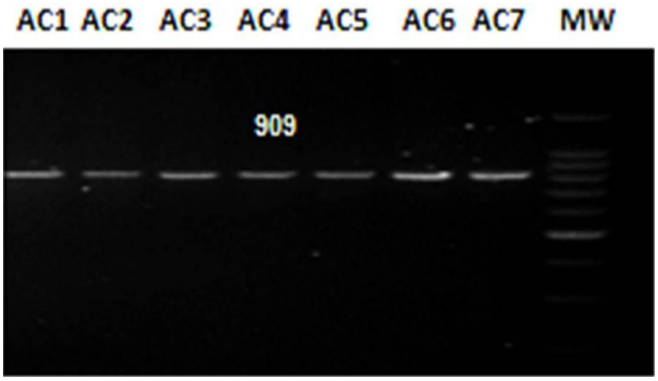

Fig. 3.

722 bp, whereas gyr B was selected as target for DNA gyrase subunit B and the amplicon generated by Gyr B primer was (909 bp). Moreover, rpoDgene was not used in current study because of greater length up to 54 bases of the forward and reverse primer sequences and this may reduce many bands of unspecific nature.[27,28]

According to our study, Ampicillin resistance was found to be most common followed by Cephalothin, Amikacin, Carbencillin, Gentamicin, Amikacin, Chloramphenicol, Tetracycline, Co-trimoxazole, Cirofloxacin 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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