Original Article

Investigation of Carbapenem-Resistant Acinetobacter baumannii Resistance Rate in Clinical Specimens of Newborns at Imam Khomeini Hospital in Tehran

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Abstract

Background: Carbapenem-resistant Acinetobacter baumannii (CRAB), as one of the important causes of hospital acquired infections, poses a serious problem for the newborns at neonatal intensive care units (NICU). The present study was conducted to evaluate the prevalence of infections with CRAB at NICU at Imam Khomeini hospital in Tehran. **Materials and Methods:** The blaOXA-51-like gene was targeted among the isolates using PCR to identify the isolates organism at the species level. Then, susceptibility of isolates to different antibiotics was assessed using disc diffusion method and broth micro dilutions to determine the minimum inhibitory concentrations (MICs). Pulsed field gel electrophoresis (PFGE) was used for typing of isolates. **Results:** Totally, 10 CRAB infections were isolated during the 6 months study period, and all of them were positive for blaOXA-51-like gene in PCR assay. They were resistant to all tested antibiotics, except colistin, polymyxin B, and tigecycline. CRAB isolates had a high MIC values for imipenem, cefotaxime, and amikacin at NICU. Based on the results of PFGE, 3 pulsotypes including clone A (7%), clone B (2%), and clone D (1%) were seen in the 10 CRAB isolates. Clone A was a dominant clone and spread in different wards of the hospital, especially in other ICUs and the emergency ward. **Conclusion:** Based on the results of this study, CRAB infection, with a high resistance rate, has the ability to enter into important wards such as NICU, and thus it is highly important to control the presence of these isolates in different parts of the hospital. Moreover, the similarity between the pulsotypes showed the ability of transferring CRAB infection from different wards of the hospital to the NICU.
Keywords: CRAB; NICU; blaOXA-51 like ; MIC; Clonal Relationship.

Introduction

The infants who are cared at NICU in hospitals are at high risk for various types of nosocomial infections due to insufficiency of the immune system (1). Acinetobacter spp. is a non-fermentative, gram-negative, opportunistic, aerobic, polymorphic, and non-motile bacterium.

Acinetobacter baumannii (A. baumannii) is the most important cause of nosocomial infections worldwide and is ranked second after Pseudomonas aeruginosa among different bacteria with hospital infection ability (2, 3, 4). The Centers for Disease Control and Prevention (CDC), reported A. baumannii in about 80% of Acinetobacter infections in the USA in 2004.

A. baumannii is able to enter the body through open wound, catheter, and respiratory tract, causing major diseases such as pneumonia, meningitis, endocarditis, urinary tract infections, and burn infections, especially in the intensive care units (ICUs) and surgery ward, and among burn and dialysis patients (3, 5-7). Although A. baumannii is known as a nosocomial pathogen in adults, it is also highly important in developing these infections in newborns (6). More than 25% of people have this bacterium as a normal flora on their skin surface, and this bacterium can spread through the infected hands of the hospital staff between different parts of the hospital (7). The extensive dissemination ability of A. baumannii in hospital environments by persisting on hospital equipments in dry and wet environments, such as soaps, anti-infectious and antiseptic agents, and also its ability to produce biofilms and use various metabolic sources can lead to its long-term survival (2, 8, 14). Another important reason for the establishment and incidence of bacterial infections in important wards, such as NICU, is the ability of this bacterium to acquire resistance to multiple antibiotics, in addition to its intrinsic resistance. Over the past 2 decades, multidrug resistant A. baumannii (MDR A. baumannii), which is resistant to at least 3 antibiotic classes including cephalosporins, fluoroquinolones, and aminoglycosides, has been developed due to the overuse of broad-spectrum antibiotics in hospitals. Now a days, resistance to this bacterium is defined in various classes of MDR, extensively drug resistant (XDR), which is resistant to all available antimicrobial drugs other than colistin and polymyxin B, and pan-drug resistant (PDR), which is practically resistant to all antibiotics (9,10, 11). Until 1991, carbapenems were the preferred drug for the treatment of MDRAB (12, 13). CRAB isolates are developed by several factors including enzymatic inactivation by beta-lactamases, loss of outer membrane purins, changes in penicillin-binding protein (PBP), and specific drug delivery pumps. Seven phylogenic subgroups of oxalocylinases with carbapenem ligation activity were identified by CHDLs (Class D Ampler) (14, 15).

Septicemia induced by AB is one of the major causes of infant mortality in developing countries in NICUs. In India, the incidence of neonatal septicemia was 30 per 1000 live births (5). Several studies have been done on CRAB in hospitals, but limited studies have been conducted on the presence of this bacterium in the NICU and its resistance to various antibiotics at this ward. Therefore, in this study, the multi drug resistance of CRAB to antibiotics was investigated at NICU of Imam Khomeini hospital, Tehran, Iran.

Methods

Clinical Specimens. Different clinical specimens (blood, wound, urine, stool, and sputum) were collected from the NICU of Imam Khomeini hospital during 9 months. To ensure the accuracy of selecting A.baumannii isolates, the followings assays were performed: microscopic examination and culture in the eosin methylene blue; and biochemical tests (including aerobic and anaerobic OF test, lactose, triple Sugar iron agar (TSI), and IMVIC (Indole (SIM), movement (SIM), methyl red (MR), and Voges-Proskauer (VP), and simon citrate) (Merck, Germany)). Then, CRAB isolates were randomly selected from A. baumannii isolates (16).

Identification of the bla$_{OXA-51- like}$ Gene Using PCR. PCR method was used to confirm the identification of the isolates. For this purpose, DNA of CRAB isolates was extracted by boiling method; moreover, 5'-TAA TGC TTT GAT CGG CCT TG-3 ' and 5'-TGG ATT GCA CTT CAT CTT GG-3 ' forward, and reverse primers (353bp) (Sina gene, Iran) were, respectively, used as specific primers to detect blaOXA-51 like gene. A. baumannii NCTC 12156 reference strain was used as a positive control for the blaOXA-51-like gene. Furthermore,1% agarose gel (Fermentas) was used for electrophoresis of PCR products. After identification, isolates were store at - 70 ° C in the trypticase soy broth and glycerol until used (15, 17).

Determining the Susceptibility of Isolates to Antibiotics by Disc Diffusion (Kirby-Bauer) Method

Inoculums samples equivalent to 0.5 MC Farland turbidity were prepared and cultured on the surface of the solid medium with a swab. Kirby Bauer disk diffusion method was used to assess the susceptibility of isolates to various classes of antibiotics including gentamicin (10 µg), amikacin (30 µg), ampicillin (10 µg), ampicillin/sulbactam (20 µg),

trimethoprim/sulfamethoxazole (25 μg), piperacillin/tazobactam (100/10 µg), tetracycline (30 µg), ticarcillin (75µg), ticarcillin/clavulanic acid (75/10 µg), tobramycin (10 µg), netilmicin (30 µg), cefepime (30 µg) cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ofloxacin (5 µg), meropenem (10 µg), imipenem (10 µg), doripenem (10 µg), doxycycline (30 µg), minocycline (30 µg), tigecycline (10 µg), colistin (10 µg), and polymyxin B (300 unit) (MAST, Merseyside, U.K). The plates were incubated for 16 to 18 hours at 37° C. The diameters of inhibitory zones were measured according to the CLSI guidelines. To control the quality of the discs on the Muller Hinton Agar, Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Klebsiella pneumoniae (ATCC 13883) were used (18).

Table1: Resistant of CRAB isolates to different antibiotics

Antibiotics	Sensitive	Intermediate	Resistant
Gentamicin	0	0	10
Tobramycin	0	0	10
Amikacin	0	0	10
Netilmicin	0	0	10
Imipenem	0	0	10
Meropenem	0	0	10
Doripenem	0	0	10
Ciprofloxacin	0	0	10
Levofloxacin	0	0	10
Ofloxacin	0	0	10
Piperacillin/tazobactam	0	0	10
Ticarcillin	0	0	10
Ticarcillin/clavulanic	0	0	10
Cefotaxime	0	0	10
Ceftriaxone	0	0	10
Ceftazidime	0	0	10
Cefepime	0	0	10
Trimethoprim/sulfamethoxazole	0	0	10
Ampicillin	0	0	10
Ampicillin/Sulbactam	0	2	8
Colistin	10	0	0
Polymyxin B	10	0	0
Tetracycline	0	0	10
Doxycycline	0	0	10
Minocycline	0	2	8
Tigecycline	8	2	0

Table2: The MIC50 and MIC90 Values of CRAB

Antibiotics	MIC 50 µg/ml	MIC 90 µg/ml	Range µg/ml	MODE µg/ml
Imipenem	1280	1280	0.625-1280	1280
Meropenem	80	320	0.625-1280	40
Cefotaxime	640	1280	0.625-1280	640
Amikacin	1280	1280	0.625-1280	1280
Colistin	0.3	0.83	0.15625-1280	0.16
Ampicillin/ Sulbactam	80	160	0.625-1280	80

Table3: The relationship of PFGE clones and different cliniclal specimens.

clones \ cliniclal specimens	Blood	Trachea
Clone A	5	2
Clone B	-	2
Clone D	1	-

Determining the MIC of Antibiotics by Micro broth Dilution Method. The powder of amikacin, ampicillin-sulbactam, imipenem, meropenem, and colistin (Sigma Aldrich, Germany) were used. At first, antibiotic storage solutions were prepared based on the potency of antibiotic powder using the following formula:

$$Weight(W) = Concentration(C) \times Volume(V) \times \frac{1000}{(potency)}$$

A spectrum of 0.625- 1280 for amikacin and ampicillin/sulbactam, and a spectrum of 0/15625-1280 for imipenem and meropenem were used for colistin. Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC 25922) were used as control microorganisms (18).

MIC$_{50}$ and MIC$_{90}$ Detection. The concentration of MIC, which inhibits 50% and 90% of isolates, was considered as MIC50 and MIC90, respectively. For this purpose, the obtained MICs of antibiotic powders were arranged from low to high using the following formulas.

$$\frac{50 \times n}{100} \text{ and } \frac{90 \times n}{100} \text{ for MIC50 and MIC90,}$$

respectively (number of samples = n) (18).

Pulsed Field Gel Electrophoresis. Typing of the randomly collected CRAB from different wards of the hospital was done using Durmazet al. methods using Field Inversion Gel Electrophoresis (FIGE). The ApaI (Thermo scientific) endonuclease was used for digestion of pure genomic DNA. DNA of lambda phage ladder (New England Biolab) was used as a marker, and Gel Compare II Version 6.5 was used to analyze the clones (19, 20).

Results

During this study, 10 samples of CRAB were collected from the NICU of Imam Khomeini hospital. All isolates were cultured from female patients, 5 samples from trachea and 5 from the blood. All CRAB

isolates were resistant to the used antibiotics except to colistin, polymyxin B, and tigecycline (table 1). According to Table 1, all isolates of CRAB were MDR and XDR, and none was PDR.

According to table 2, colistin and ampicillin/sulbactam and meropenem showed the lowest MIC levels, but, cefotaxime, ampicillin, and amikacin showed the highest. The PFGE results revealed that 7 isolates belonged to clone A, 2 to clone B, and 1 to clone D, respectively. The most isolates of clones were collected from trachea and the single isolate in clone D was isolated from blood (table 3). All isolates were susceptible to colistin and polymyxin.

Discussion

In the present study, 10 CRAB isolates were collected at NICU of Imam Khomeini hospital in Tehran, Iran, from September 2015 to June 2016. Touati et al. stated that A. baumannii isolates, which are responsible for NICU infection, are often resistant to imipenem, and they further revealed that the presence of these isolates cause problems for other therapeutic pathways. Studies have shown that CRAB isolates are responsible for the incidence of this bacterium compared with sporadic cases (14). Several studies have reported that the use of respirator and intravenous catheters in NICU cause high persistence of these bacteria at NICU (7). Touati et al. have stated that the mortality rate due to A. baumannii infections in neonates is 13.9% to 38% and Wei et al. reported that the mortality rate due to MDR A. baumannii sepsis was 20.34% from 59 neonatal patients in a hospital in Taiwan, signifying the importance of the infection with A. baumannii isolates in the NICU (7, 14). At present study the blaOXA-51-like gene was detected among all the studied CRAB isolates. Turton et al. (2006) stated that finding blaOXA-51-like in A. baumannii isolates is a reliable way to identify the organism at species level (15). In this study, NICU samples were obtained from blood and respiratory tract of infants. Wei et al., in their study on MDR A. baumannii found that 56.72%, 88.23%, 10.45%, 4.48%, and 2.99% of the isolates were collected from blood, sputum, pus, ascites, spinal cord, and fluid, respectively. Moreover, they indicated that one of the

isolates that belonged to plural fluid showed the highest number of isolates and was recovered from the blood and the respiratory tract (7). Several studies demonstrated that the risk factors for CRAB infection in NICU include low gestational age, preterm birth, low birth weight infants, infants with less than 7 days age, hospitalization time of more than 7 days, use of invasive devices (such as vascular catheters and artificial respiration), and use of imipenem (1, 7, 14, 21). Due to the inherent ability of this bacterium to remain on the surfaces and equipment of the hospital, CRAB can also gain the ability to be distributed in different wards of the hospital. According to PFGE analysis of the NICU, the collected isolates belonged to clones A, B, and D (7%, 2%, and 1%). Isolates within Clone A have also been found at other ICUs and emergency ward of Imam Khomeini Hospital, indicating that these bacteria can spread to different wards. In Michigan (2008- 2009), McGrath et al. studied 6 low weight infants with PFGE. They stated that the employees who had contacts with infants could transfer the CRAB to the NICU (22). Melamed et al., in their study of A. baumannii outbreak at NICU with PFGE, concluded that a rapid investigation for outbreak of A. baumannii is essential to find the source of infection (23). In a study conducted by a control team at one of the hospitals in China, the areas that have a higher risk for NICU deployment of MDR A.baumannii including NICU doors, incubator windows, incubator control panels, artificial respiration, electrocardiogram monitoring, device buttons suction, computer keyboard, ultrasound apparatus for breast, and bed infants were investigated (7). The results of a study in France revealed that the preparation of the antibiotic susceptibility pattern for all newborns who have developed A. baumannii is essential to prevent the spread and incidence of infections in all NICUs in the hospital (14). Thus, in this study, the CRAB isolates resistance pattern to different antibiotics was studied. According to the results of this study, the most effective antibiotics were colistin, polymyxin B, and tigecycline. Moreover, all CRAB isolates obtained from the NICU were sensitive to colistin and polymyxinB, 8 isolates were susceptible to tigecycline, and all isolates were resistant to the rest of the used antibiotics. Also, among the 6 antibiotics used in the MIC in this study,

colistin and ampicillin/sulbactam and meropenem had the lowest levels of MIC values, and cefotaxime, ampicillin, and amikacin had the highest. These results indicated that CRAB isolates were even resistant to highest doses of these antibiotics.

In this study, all CRAB isolates were MDR and XDR, and none of the isolates were PDR. Several studies have revealed that previous use of third-generation cephalosporins, fluoroquinolones, and carbapenems are effective in the development of MDR A. baumannii strains (21, 24). Also, in Taiwan (2010 to 2013), Wei et al. investigated the microbial resistance of A. baumannii isolates at the NICU ward, and they found that from 67 studied isolates, all were MDR A. baumannii, and most isolates were just sensitive to colistin and tigecycline (7). Zarrilli et al. in a study in Italy (2012) on XDR A. baumannii isolates of NICU concluded that A. baumannii isolates were resistant to aminoglycosides, quinolones, and beta-lactams, but they were only sensitive to colistin and tigecycline (21).

Jaroush et al. (2004) studied the resistant patterns of A. baumannii isolates that were diverted from 579 blood samples of 2 NICUs in 2 hospitals in Gaza, Palestine. They found that A. baumannii isolates were resistant to antibiotics that were commonly used in those hospitals, while their sensitivity to meropenem, imipenem, ciprofloxacin, gentamicin, and ceftriaxone was 92.5%, 90%, 75%, 5.75%, and 50%, respectively. Moreover, in the present study, A. baumannii isolates were resistant to commonly used antibiotics in Imam Khomeini hospital, but the resistance patterns of A. baumannii isolates in Palestine were completely different from those of the present study.

In fact, their results indicated that imipenem and meropenem were effective antibiotics against A. baumannii isolates. However, sensitivity of the third and fourth genera and ciprofloxacin to cephalosporin was different, which is why despite the fact that ciprofloxacin is not prescribed for treatment of neonates; these hospitals have to use this antibiotic due to lack of suitable substitutes. They also found that 37.5% of MDR A. baumannii, which are caused by the uncontrolled consumption of antibiotics, are used to prevent and treat infants (1).

Based on the results of this study, to prevent the presence of highly resistant A. baumannii isolates, especially in important wards such as NICU that provide very little treatment, implementing monitoring programs, such as evaluating multiple antibiotic resistance of isolates and calculating antibiotic MIC levels, are of paramount importance.

Conflicts of Interest

All authors have no conflicts of interest to express.

Acknowledgment

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