Detection of β-Lactam Resistance PER-1 Gene in Multidrug Resistant Isolates of 
Acinetobacter baumannii Isolated from Urinary Tract Infections in Diyala City

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Abstract: The detection of β-lactam-resistant A. cinetobacter baumannii in Diyala city and their resistance genetic mechanisms was undertaken. We studied the extended-spectrum beta-lactamase genes, particularly the PER-1 gene, among β-lactam-resistant A. baumannii isolates from patients suffering from urinary tract infections in teaching Baquba hospital and Al-Betul hospital in Diyala city. From April to October (2015), 250 urine samples were collected. And the results of bacterial culture on media of MacConkey agar, blood agar and biochemical tests and confirm the diagnosis using Api 20 E system and Vitek 2 compact system showed that 8 isolates are belonging to bacteria of Acinetobacter baumannii. Fresh subculture samples were tested for antimicrobial susceptibility against (11) antibiotics using disk diffusion method, the results revealed that all isolates were variable resistance to ampicillin, aztreonam, ceftazidium, cefotaxime, cefepime, ciprofloxacin, piperacillin, meropenem, nalidixic acid and amikacin (62.5, 50, 75, 75, 50, 50, 75, 37.5, 50 and 25%) respectively. Whereas all isolates were sensitive to antibiotic imipenem. Total genomic DNA was extracted from each isolate and further used for Polymerase Chain Reaction (PCR). The results of detection of β-lactam resistance gene by molecular technique PCR revealed that of these 8 isolates, 6 were found to harboring PER-1 gene.

Key words: Acinetobacter baumannii, multidrug resistant, PER-1 gene, antibiotics, technique, culture

INTRODUCTION

Acinetobacter baumannii has appeared as an main nosocomial pathogen. This organism has become endemic in some of hospital outbreaks have been described from various environmental areas. The role of the environmental corruption in the transmission of nosocomial infections in general and in A. baumannii infections in real is fine documented (McConnell et al., 2013; Sengstock et al., 2010). A. baumannii is Gram negative bacterium and is a typically short, almost round, rod-shaped (coccobacillus). It can be an opportunistic pathogen in peoples with compromised immune systems (Antunes et al., 2014). A. baumannii is the best described among the genus Acinetobacter and most often associated with human diseases and fatalities and viewed as an opportunistic pathogen, mostly targets liable hosts where it causes ventilator-associated pneumonia, Urinary Tract Infections (UTIs), skin, soft tissue and wound infections, secondary meningitis and bacteremia (Perez et al., 2007; Safari et al., 2013). In the 1970s, A. baumannii was liable to most antimicrobial causes. It has now become a major cause of nosocomial infection global because of its incredible ability to acquire resistance determinants to various kinds of antimicrobial means (Dijkshoorn et al., 2007). A major increase in the number and severity of cases of A. baumannii infections from hospital outbreaks as well as sporadic community-associated and wound-associated cases has been observed and consequently. In latest A. baumannii has developed as a major cause of nosocomial infections linked with significant morbidity and mortality, particularly in severely compromised individuals, ICU patients and military personnel suffering from traumatic injury and in a prevalence study of infections in ICUs showed among 75 countries of the five continents. A. baumannii was found to be the fifth most common pathogen (Vincent et al., 2009). Additionally and because the progress resistance to most antimicrobial agents, many A. baumannii infections can be quite severe with mortality rates ranging from 26-68%. Moreover, Multi-Drug Resistant (MDR) strains have recently emerged globally, accentuating the need for new therapeutic attitudes for the treatment of Acinetobacter infections (McConnell et al., 2013). Gram-negative bacteria have developed increasingly resistant to antimicrobial means. They have developed several mechanisms by which they can withstand to antimicrobials, these mechanisms include the production of Extended Spectrum β-Lactams (ESBLs) and carbapenemases (El Salabi et al., 2013).

Penicillins and cephalosporins efficiently hydrolyzes via PER-1 β-lactamase and is susceptible to clavulanic acid inhibition. PER-1 was first noticed in P. aeruginosa
isolate from a Turkish patient in France (Nordmann et al., 1993; Neuhauser et al., 2003) and later in *S. enterica* serovar Typhimurium and *Acinetobacter* isolates as well (Vahaboglu et al., 1997). In Turkey, as many as 46% of nosocomial isolates of *Acinetobacter* spp. and 11% of *P. aeruginosa* were established to produce PER-1 (Vahaboglu et al., 2001). PER-2 which dividends 86% homology to PER-1 has been noticed in *S. entericaserovar Typhimurium, E.coli, K. pneumoniae, P. mirabilis* and *V. cholera* O1 El Tor . PER-2 has only been established in South America, thus far (Bauernfeind et al., 1996).

While PER-1-producing organisms have been predominantly found in Turkey, a *P. aeruginosa* outbreak in Italy occurred with no apparent associates with Turkey (https://aac.asm.org/content/40/3/616.short 2001). Also PER-1 gene has also been found in *P. mirabilis* and *Alcaligenes faecalis* in Italy (Pereira et al., 2000). *P. aeruginosa* isolates producing PER-1 have been identified in France, Italy and Belgium (De Champs et al., 2002). Moreover, a high incidence of PER-1 in *Acinetobacter* spp. from Korea has been distinguished (Kwon et al., 2002). Also, the prevalence and genetic variety of extended-spectrum β-lactamase genes and their resistance genetic mechanisms, particularly the PER-1 gene, among carbapenem-resistant *A. baumannii* strains from patients at a tertiary care hospital in Riyadh, Saudi Arabia (Aly et al., 2016).

**MATERIALS AND METHODS**

**Bacterial samples:** A total El Salabi et al. (2013) *A. baumannii* isolates were isolated from UTIs by cultivating urine sample on MacConkey agar, after 24 h of incubation at 37°C, the suspicious colonies which were non lactose fermentative and were pale on MacConkey agar, traditional biochemical tests were used for final identification of bacterial isolates and the confirmed identifications to species level were also carried out by using Api 20 E system (Enterobacteriaceae identification system, BioMerieux, France) and by Vetek 2 system (BioMerieux, France)(MacFadden, 2000).

The susceptibility of *A. baumannii* isolates against Ampicillin (30 µg), aztreonam (30 µg), cefazidium (10 µg), cefotaxime (5 µg), cefepine (30 µg), ciprofloxacin (5 µg), piperacillin (30 µg), meropenem (10 µg), nalidixic acid (30 µg) and amikacin (30 µg) were tested using the standard disk diffusion method on Mueller Hinton (MH) agar plates and using the breakpoints defined by Clinical and Laboratory Standards Institute (2012) (CLSI, 2012).

**Detection of β-lactam resistance PER-1 gene:** Total DNA was extracted from all 8 isolates by using DNA extraction kit (Bioneer, Korea) and 3 µL of the isolated DNA was subjected to PCR with specific primers, PCR amplification of the PER-1 gene was with primers PER-1 (forward) 5-ATGAATGTCATTATAAAAAGC-3 and PER-1 (reverse), 5-AATTTGGGCTTAGGGCCAGAA-3, yielding 926-bp product. The quantity and quality of the extracted DNA were evaluated using a Nano Drop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA concentration and the ratio of the optical density at 260/280 nm to evaluate the purity of the DNA samples were calculated simultaneously. Conventional PCR reactions with genomic DNA were achieved in a 15 µL mixture according to the manufacturer's protocol for the Maxima SYBR Green/GoTaqPCR Master Mix (Thermo Fisher Scientific).

PCR conditions were 10 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, followed by a final extension for 10 min at 72°C. Agarose gel electrophoresis was carried out in conventional Tris-Borate-EDTA (TBE) buffer with agarose 1% W/V for PER-1-PCR product. A 100 bp DNA ladder mix (Promega/USA) was used to provide molecular size markers. The gels were stained with ethidium bromide and observed under UV transillumination.

**RESULTS AND DISCUSSION**

Disk diffusion test was employed to determine antibiotic susceptibility of *A. baumannii* isolates on MHA following the Clinical and Laboratory Standards Institute (CLSI, 2012) guideline. The results revealed that all isolates were variable resistance to ampicillin, aztreonam, cefazidium, cefotaxime, cefepine, ciprofloxacin, piperacillin, meropenem, nalidixic acid and amikacin (62.5, 50, 75, 75, 50, 50, 75, 37.5, 50 and 25%), respectively. Whearase all isolates were sensitive to antibiotic imipenem. Traditionally, infections were treated with imipenem or meropenem but a steady rise in carbapenem-resistant *A. baumannii* has been noted (Su et al., 2012). Prevention methods in hospitals focus on increased hand-washing and more diligent sterilization actions (Anonymous, 2013). The current study with agreement with previous Iraqi studies (Al-Muhamma, 2006) make study about *Acinetobacter* spp. And find that all isolates were sensitive to imipenem (Al-Muhamma, 2006) and Al-Ajeeli also find all *A. baumannii* isolates were sensitive to imipenem (Al-Ajeeli, 2014). Whearase study by Al-Baglan revealed that 50% of *A. baumannii* isolates were resist to meropenem and imipenem (Al-Baljari, 2015).

The development of resistance to the carbapenem group belong to produce carbapenemases, also Siroy et al. (2005) observed that the lost of 29 kDa protein in the outer membrane of bacteria that defined as Car O which have relation with the resistance to meropenem and
imipenem (Siroy et al., 2005). Also, the current study detect that 6(75%) isolates of A. baumannii were found to be resist to cefazidium, ceftoxime and piperacillin and that in agreement with studies by Al-Bajlany (2015) and Al-Masoudi et al. (2015).

The PCR amplification results of nine isolates of A. baumannii revealed that the extended-spectrum β-lactamase blaPER-1 gene was present in six isolates. PCR amplified blaPER-1 gene showed a molecular weight of 925 bp, Fig. 1 the study by Kim et al. (2008) exposed that 42 Multidrug-Resistant (MDR) A. baumannii isolates were attained throughout outbreaks in a Korean hospital. The co-carriage of blaOXA-23, blaOXA-51, blaPER-1 and armA was observed in 23 isolates (Kim et al., 2008; Sacha et al., 2012) detected that PCR technique did not approve the presence of the blaPER-1 gene in any of the A. baumannii strains observed in their hospital. A. baumannii strains reveal significant resistance to many groups of antibiotics (Sacha et al., 2012). The current study in agreement with study by Aly et al. (2016) genotyping results of PER-1-like genes showed that 384/503 (76.3%) were positive among MDR Acinetobacter isolates (Aly et al., 2016). Whereas Cao et al. (2009), observed that among the 64 MDRA isolates 39.1% (Al-Bajlany, 2015) with blaPER-1 gene.

CONCLUSION

The prevalence of multidrug-resistant A. baumannii occurred at 2 hospitals with the prevalence of the PER-1 resistance gene among them in percentage (75%).

REFERENCES


