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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 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 *Acinetobacte 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. Whearase 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 molcular 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-Lactamases (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 establish 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 establish 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 culturing 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 (Enterobacteriacea identification system, Biomrerieux, France) and by Vitek 2 system (Biomerieux, France)(MacFaddin, 2000).

The susceptibility of *A. baumannii* isolates against Ampicillin (30 μg), aztreonam (30 μg), ceftazidium (10 μg), cefotaxime (5 μg), cefepime (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(foreword) 5-ATGAATGTCATTATAAAAGC-3 and PER-1 (reverse), 5-AATTTGGGCTTAGGGCAGAA-3, yielding a926-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 25 μL mixture according to the manufacturer's protocol for the Maxima SYBR Green/ GoTaqPCR Master Mix (Thermo Fisher Scientific).

PCR conditions were10 min at 94C, followed by 35 cycles of 1 min at 94°C, 1 min at55°C and 1 min at 72°C, followed by a final extension for10 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 ë 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, ceftazidium, cefotaxime, cefepime, 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-Muhanna, 2006) make study about *Acinetobacter* spp. And find that all isolates were sensitive to imipenem (Al-Muhanna, 2006) and Al-Ajeeli also find all *A. boumannii* isolates were sensitive to imipenem (Al-Ajeeli, 2014). Whearse study by Al-Bajlani revealed that 50% of *A. boumannii* isolates were resist to meropenem and imipenem (Al-Bajlany, 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

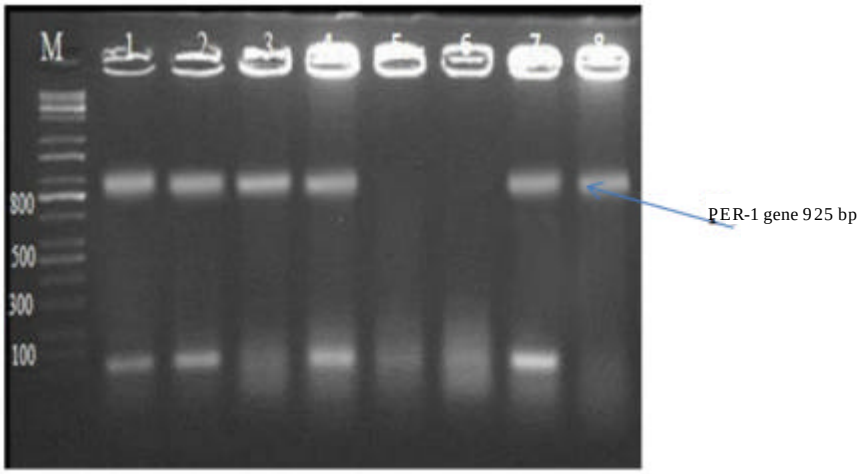

Fig. 1: Agarose gel electrophoresis of PCR amplification products of, blaPER-1 gene (1.5% agarose, TBE buffer (1X), 7 V/cm, 90 min). M: The DNA molecular wight marker (100 bp ladder). All lanes positive amplification of 925 bp for blaPER-1 gene except lane 5 and 6 are negative

imipenem (Siroy *et al*., 2005). Also, the current study detect that 6(75%) isolates of *A. boumannii* were found to be resist to ceftazidium, cefotaxime 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). Wherase 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%).

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