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Evaluation antibiotic resistance and presence of bla_{OXA-51}, bla_{OXA-58} and bla_{OXA-23} genes in *Acinetobacter baumannii* strains via multiplex PCR

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Abstract: The resistance of *Acinetobacter baumannii* to most antibiotics is increasing. The presence of metallo-beta-lactamase and carbapenemase enzymes has led to the resistance of these bacteria to carbapenems as one of the major classes of broad-spectrum antibiotics and has raised concerns in human societies. This research evaluated the presence of bla_{OXA-51}, bla_{OXA-58} and bla_{OXA-23} genes in *A. baumannii* strains during a 12 months period. One hundred strains were isolated from the patients hospitalized in ICU of Ali Asghar and Shahid Rajaee trauma hospitals in Shiraz. Bacterial identity was determined by biochemical tests and antibiotic resistance was determined by disk diffusion method. The isolated strains were then evaluated in terms of carrying bla_{OXA-23}, bla_{OXA-51} and bla_{OXA-58} genes, using the multiplex PCR method. The results showed that *A. baumannii* was resistant to carbapenems but most strains were susceptible to tigecyclin and colistin. The majority of strains carried the bla_{OXA-23} and bla_{OXA-51} genes, but very few carried the bla_{OXA-58} gene. The results revealed that the antibiotic resistance of *A. baumannii* is increasing, which causes a more outbreak of this organism.

Keywords: *Acinetobacter baumannii,* antibiotic resistance, bla_{OXA} gene, carbapenems, multiplex PCR.

INTRODUCTION

Nosocomial infections are one of the major problems of public health (Boone *et al*., 2021), most of which are caused by Gram-negative bacilli (Kaviani *et al.,* 2020). *Acinetobacter baumannii* is an aerobic, non-fastidious and Gram-negative coccobacillus belonging to the Moraxellaceae family. This bacterium is usually isolated from hospital environments and hospitalized patients, and is an opportunistic infection (Braun and Vidotto, 2004). It is one of the most important nosocomial pathogens, that is recently added to the list of dangerous pathogens of the Infectious Diseases Society of America. *A. baumannii* forms colonies on the human skin, mucous membranes and medical equipment in hospitals (Dijkshoorn *et al*., 2007).

A. baumannii causes a wide range of infections in various anatomical locations, including urinary tract infections, sepsis, pneumonia, as well as skin and soft tissue infections. The prevalence of *A. baumannii* infections is related to the conditions of the hospital, the ward and the patients. This bacterium has low pathogenicity, but in people with risk factors, the severity of pathogenicity is high.

Some of these factors include long time hospitalization, burns, surgery, long-term use of antibiotics, or people with immunodeficiency. In addition to the host's factors, some of the bacteria's own characteristics such as resistance to several antibiotics could play an important role in the pathogenicity of the bacteria. So that some bacteria have quickly developed resistance to selected drugs including beta-lactams and fluoroquinolones and even to selected broad-spectrum drugs such as carbapenems (Salehi *et al*., 2008; Poirel and Nordmann, 2006; Moubareck and Halat 2020).

A. baumannii species has developed resistance against carbapenems due to the production of carbapenem hydrolyzing carbapenemase, a kind of beta-lactamase (Mani *et al*., 2018). Two classes of carbapenemases (class B and D) are found among *A. baumannii* strains. The class D enzymes are the main carbapenemase enzymes worldwide, while metalloenzymes are found in East Asia (Schuertz *et al*., 2018). *A. baumannii* is one of the most common problems in nosocomial infections worldwide due to antibiotic resistance. The clinical relevance of this bacterium, especially over the last 15 years (Espinal *et al*., 2012), has been a serious problem against antibiotic therapies due to its ability to acquire resistance indices. The prolonged resistance to carbapenems has raised concerns in the public health and left few treatment options. Class D enzymes of carbapenem hydrolyzing beta-lactamases (oxacillinase enzymes) are one of the most important mechanisms of carbapenem resistance (Woodford *et al*., 2012).

Carbapenem resistance is mainly attributed to OXA type carbapenemases and metallo-beta-lactamases. OXA beta-lactamases have high hydrolytic activity against oxacillin and cloxacillin and are weakly inhibited by clavulanic acid, playing a significant role in resistance to

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carbapenems. In this study, the presence of three phylogenetic subtypes (*bla*$_{OXA-51}$, *bla*$_{OXA-58}$ and *bla*$_{OXA-23}$) of carbapenemase genes was examined in *A. baumannii* strains.

MATERIALS AND METHODS

Strain collection and detection

This study was conducted for 12 months from April 2019 to April 2020. One hundred clinical and non-recursive *A. baumannii* related isolates were collected from Ali Asghar and Shahid Rajaee trauma hospitals in Shiraz. The strains were identified by hospital laboratories using standard microbiological methods including biochemical tests and by Microgen kits.

Antibiotic susceptibility test

Bacterial susceptibility to antibiotics was determined by disk diffusion method according to CLSI standards (CLSI 2010). Antibiotic discs included imipenem, meropenem, ceftazidime, amikacin, ticarcillin, tetracycline, rifampin, tigecycline, and colistin (MAST Diagnostic Co., Bootle, UK). The standard strain of *Escherichia coli* ATCC 25922 was used as a quality control of antibiotic discs.

Polymerase Chain Reaction (PCR)

Four-five colonies were grown on TSA plates overnight, dissolved in 200µL of PCR-specific distilled water (Sigma), vortexed for 10 seconds and centrifuged at 13,000 rpm for two minutes. The supernatant was directly used as a PCR reaction sample after being stored at -30°C. PCR reactions were used at 30 to 40 cycles at different binding temperatures depending on the primer melting temperatures. The reactions were performed in Micro Amp tubes using an Applied Biosystems 2700 thermocycler. Amplicons were analyzed on 1-2% agarose gel in the presence of ethidium bromide. The gels were scanned and photographed with a Biometra gel documentation system (Biometra, Gottingen, Germany). The primers used to perform multiplex PCR of oxacillinase genes included: OXA-51-likeF: TAATGCTTTGATCGGCCTTG, OXA-51-likeR: TGGA TTGCACTTCATCTTGG, OXA-23-likeF: GATCGGAT TGGAGAACCAGA, OXA-23-likeR: ATTTCTGACCG CATTTCCAT, OXA-58-likeF: AAGTATTGGGGCTTG TGC TG, OXA-58-likeR: CCCCTCTGCGCTCTAC ATAC (Gallego and Towner, 2001; Turton *et al*., 2006).

RESULTS

Deployment of strains

After removing the repetitive and clinically unrelated isolates, a total of 100 *A. baumannii* strains from Ali Asghar hospital (35 isolates) and Shahid Rajaee trauma hospital (65 isolates) were used in this study. The distribution of strains by hospital and sample type is shown in fig. 1.

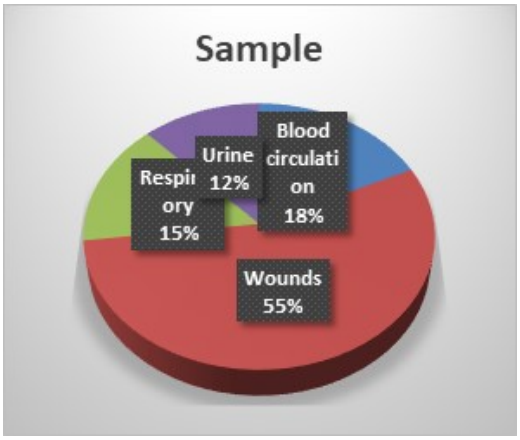

Fig. 1: Clinical specimen distribution *Blood circulation - blood culture + intravenous injection with line and catheter

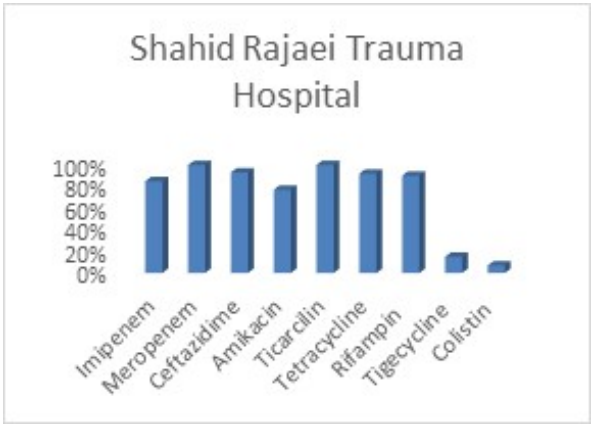

Fig. 2: Resistance pattern in trauma Hospital

Fig. 3: Resistance pattern in Ali Asghar Hospital

Antibiotic resistance

The isolated strains showed high resistance to carbapenem antibiotics. As shown in figs. 2 and 3, Shahid Rajaee trauma hospital strains were slightly more resistant than the strains isolated from Ali Asghar hospital.

Presence OXA genes

The results of multiplex PCR analysis of *bla*$_{OXA}$ genes indicated that the majority of strains carried *bla*$_{OXA-23}$ and *bla*$_{OXA-58}$ genes (fig. 4). According to the fig. 6, 90% of

the strains carried the bla_{OXA-23} gene, 80% the bla_{OXA-51} gene and 2% the bla_{OXA-58} gene. The analysis was performed on both hospitals.

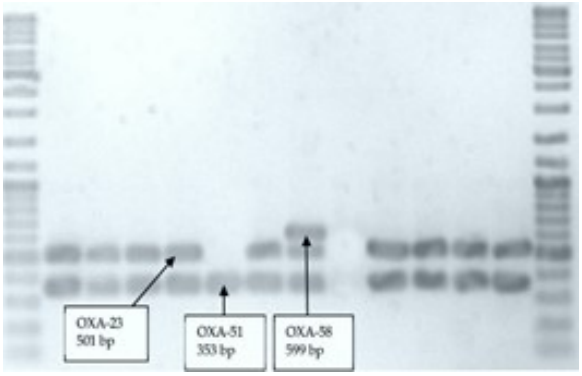

Fig. 4: The results of Multiplex PCR samples

DISCUSSION

Antibiotic resistance exists among the bacteria that cause primary infections in the population. While this is already a serious issue (such as resistance to penicillin and macrolide in *Streptococcus pneumoniae*, pathogens producing ESBLs in the urinary tract - just a few), the situation of hospitals is seriously affected, and the health care is restricted.

Over the past decade, the presence of *A. baumannii* has been recognized as the most important nosocomial pathogen worldwide. The bacteriological results indicated the high prevalence of infection with *A. baumannii* strains in ICU of Ali Asghar (AS) and Shahid Rajaei trauma hospitals in Shiraz. The mortality rate from nosocomial infections with *A. baumannii* has been reported as 19 to 54% in the United States' hospitals. Resistance to carbapenem antibiotics such as imipenem, meropenem and penicillin was very high. Since these antibiotics are considered as the last lines of treatment, these reports are warnings to identify and track the transmission of these bacteria in high-risk environments such as hospitals. Similar results have been obtained in studies conducted in Europe and Asia.

In this study, the highest number of strains was isolated from wound samples. The long-term hospitalization in the ICU is likely to be an important reason for the prevalence of *A. baumannii* in wound samples. A 2014 study in Spain showed that all strains of *A. baumannii* were resistant to piperacillin/tazobactam, gentamicin, impanel, doripenem, and ciprofloxacin (Sepahvand *et al*., 2016). High resistance to cotrimoxazole, tobramycin and ampicillin/ *sulbactam* was reported as well. The prevalence of carbapenem-resistant strains that encode OXA genes is increasing in the world, so that there are reports of outbreaks of nosocomial infections caused by carbapenemase-encoding *A. baumannii* in countries such as Brazil, France, Spain, Turkey, and Korea (Bou and Cervero, 2000; Kanj *et al*., 2018; Da Silva *et al*., 2004; Héritier *et al.,* 2005; Jeon *et al*., 2000; Marqué *et al*., 2005; Sepahvand *et al*., 2017). Therefore, it is important to identify the carbapenems-resistance coding genes.

The organism's remarkable capacity for survival in hospital environment, the acquisition of resistance mechanisms and the emergence of acute infections, especially in critically ill patients that affect mortality, have attracted much attention. The success of this organism in hospitals is probably due to its remarkable adaptation (Gordon *et al*., 2010; Neonakis *et al*., 2010). However, antibiotic resistance has been considered as a means of survival in environments full of antibiotics, such as hospitals. Although increased resistance to therapeutic drugs is described among epidemic strains, significant differences have been shown based on geographical locations (Dijkshoorn *et al*., 1996; van Dessel *et al*., 2006).

The results of this study on antibiotics imipenem, meropenem, ceftazidime, amikacin, *ticarcillin*, tetracycline, rifampicin, tigecycline, and colistin showed that high resistance was observed against meropenem (100%), *ticarcillin* (100%), ceftazidime (93%), tetracycline (92%), rifampin (90%), imipenem (85%), and amikacin (77%). Most strains were susceptible to colistin (7%) and tigecycline (15%). Therefore, the results suggest the treatment with colistin and then tigecycline as a solution among most important antibiotics. In Italy, in 2008, a study on 45 strains of *A. baumannii* showed the lowest resistance to colistin (1%) and then to tigecycline (4%), while the high resistance was revealed to ciprofloxacin (95%), imipenem (59%) and meropenem (50%)[25?]. According to the results of the study in Italy and the present one, resistance to this bacterium is increasing in the world, and the best options for treating this bacterial infection are colistin and tigecycline antibiotics. In the present study, imipenem resistance was 85%, which indicates an increased resistance over time.

The bla_{OXA-51} gene is inherent in this bacterium and the genes bla_{OXA-58} and bla_{OXA-23} are acquired. Mendes *et al* found the class D carbapenemase genes in 70% of the strains, and the bla_{OXA-23} gene was the most common one including 95% of the carbapenemase D coding genes (Mezzatesta *et al*., 2008), which was followed by bla_{OXA-58} gene found in 11.9% of the strains (Mendes *et al*., 2009). In this study, 90% of the strains carried the bla_{OXA-23} gene and 2% had the bla_{OXA-58} gene. The comparison between the two studies showed a high prevalence of the bla_{OXA-23} gene in two geographic regions. The high prevalence of the bla_{OXA-23} is consistent with worldwide reports that revealed a prevalence of 70-100% (Vahaboglu *et al*., 2006; Lee *et al*., 2012; Safari *et al*., 2013; Andriamanantena *et al*., 2010). Most strains carried the bla_{OXA-51} gene that provides resistant to all carbapenems

or one of them. This indicates the prominent role of this gene in resisting or reducing the susceptibility of *A. baumannii* to carbapenems. According to the results of this study, colistin and tigecycline are the most effective antibiotics for *A. baumannii* treatment. The presence of oxacillinase genes in the two hospitals was not significantly different, however, further studies are required in this regard.

CONCLUSION

The results of the present study showed that the resistance of *A. baumannii* strains is increasing. Due to the increasing prevalence of *A. baumannii* MDR isolates in the present study and different geographical areas, identifying and tracking isolates containing oxacillinase genes can be an important step in the treatment of *A. baumannii* infections and control of nosocomial infections.

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