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Assessment of Frequency and antibiotic resistance pattern of *Acinetobacter* spp. isolated from traumatic patients in Shahid Rajaee Hospital in Shiraz

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ABSTRACT

Acinetobacter resistant strains have caused medical problems throughout the world. The aim of this study was to evaluate the frequency and pattern of antibiotic resistance of *Acinetobacter* species isolated from traumatic patients in Shahid Rajaee hospital in Shiraz. In this study, 794 samples were isolated from patients in Shahid Rajaee Hospital. Identification of *Acinetobacter* was done by biochemical tests and PCR method. Multi-drug resistant (MDR) *Acinetobacter* were determined using gentamicin, piperacillin, meropenem, colistin, trimethoprim, ciprofloxacin, imipenem, ampicillin, and chloramphenicol antibiotics. In this study, 248 samples of *Acinetobacter* isolates were identified by molecular and biochemical methods from patients. All of which were isolates of MDR *Acinetobacter*. The highest percentage of *Acinetobacter* isolates was reported for upper respiratory tract samples and the lowest for urinary tract. The highest percentage of infection was related to *Acinetobacter* co-infection with one bacterium in patients aged 45 to 87 years old. As the number of male patients with accident trauma was more than women, the percentage is higher in men. The percentage of patients with *Acinetobacter* infection in ICUs was higher than in other sections. These results show the evidence of necessity to examine the transmission ways and the increasing incidence of hospital infections.

1. Introduction

Nosocomial infections have been among the major problems of the past centuries and today, which cause staggering costs to the health systems, prolongation of hospitalization duration, and increased mortality and morbidity of patients (El Kettani *et al.,* 2017; Munoz-Price *et al.*,2008). *Acinetobacter* bacterium is one of the most important causes of nosocomial infections, and gram-negative coccobacillus is saprophytic and an opportunistic pathogen belonging to Neisseriaceae family, which does not ferment sugars, and can be isolated from many human and environmental sources. Its prevalence is more during summer than in other seasons. This bacterium is a negative oxidase, non-mobile, non-fermenting, forced aerobic bacterium which is typically found in the soil, water, and wastewater. *Acinetobacter* usually has a low virulence and causes infection through the assistant associated with the respiratory system and contaminated catheters. Infection with this bacterium especially in patients hospitalized in ICU wards of hospitals is very dangerous (Munoz-Price *et al.*,2008; Sinha *et al.*,2007).

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The food requirements of this bacterium are not complex, and they can easily grow on typical food environments. They even naturally exist on the skin of a healthy human and can remain in the hospital environment for a long time and are transmitted among the patients. This organism is known as the pathogen of tropical and humid regions (Adams *et al.*, 2011; Lee *et al.*, 2007; Peleg *et al.*, 2008). These bacteria are considered as one of the problematic pathogens in the intensive care units around the world. In this regard, due to its considerable clinical properties especially in recent years and its ability in acquiring drug resistance, it is regarded as one of the threatening microorganisms in relation to treatment with antimicrobial drugs (Fournier *et al.*, 2007; Wisplinghoff *et al.*, 2007; Mostofi *et al.*, 2011; Wroblewska *et al.*, 2007).

The strains of Acinetobacter have shown resistance to most of the antibiotics that have been reported so far. The reason behind the improvement of this resistance system is the abnormal intrinsic ability of *Acinetobacter baumannii* in long-term survival in all hospital environments, causing hospital development of this bacterium (Godoy *et al.*, 2017; Karah *et al.*, 2012). Currently, *Acinetobacter* is resistant to many drugs including quinolones, co-trimoxazole, doxycycline, imipenem, meropenem, and polymyxin B, which may be effective against nosocomial infections. The rapid resistance to quinolones has been reported worldwide. Sulbactams are effective in some cases of multidrug resistance (MDR). Tazobactam plus clavulanic acid is less effective. This issue is an important health problem in many countries especially for patients hospitalized in intensive care units as well as burn and surgery wards (Charalampous et al., 2019; Almasaudi, 2018; Prashanth *et al.*, 2004; Elham and Fawzia, 2109). Treating *Acinetobacter* infections mostly in cases where the resistant phenotypes are multidrug is difficult. Currently, carbapenem is used as the drug of choice for treating MDR Acinetobacter infections, though resistant to carbapenem is also increasing (Demoz *et al.*, 2018; Jamulitrat *et al.*, 2007; Ranjbar *et al.*, 2007; Coelho *et al.*, 2004). The aim of this research is to evaluate the frequency and pattern of antibiotic resistance of Acinetobacter isolates isolated from trauma patients in Shahid Rajaei hospital in Shiraz.

2. Materials and Methods

2.1. Clinical isolates

This study was conducted as cross-sectional-descriptive on 794 samples with upper respiratory tract, surgical position, blood, cerebrospinal fluid, and urine of trauma patients hospitalized in academic Shahid Rajaei hospital within the period of September 2014 to June 2015, by preparing a questionnaire and gaining permission from the patient and following ethical principles. Eventually, 248 Acinetobacter samples were isolated.

2.2. Acinetobacter isolation from clinical samples

In order to isolate the bacteria, first mucus, wound, and cerebrospinal fluid samples were transferred to Thioglycolate medium using a sterilized cotton swab. It was then incubated for 24 h at 37°C. Blood specimens were cultured using BACTEC. For primary isolation of the bacteria, Mac-Conkey Agar and Blood Agar medium containing 5% ovine blood was used. The samples were incubated at 37°C for 24 h and then, the isolates were kept at -80°C in nutrient broth containing 50% glycerol. Bacterial isolates were identified by Gram staining, cell and colony morphology and the following properties: motility, SIM, growth at 42°C, citrate utilization, catalase, oxidase and urease production and oxidative/fermentation (OF)-glucose test (Meumann et al., 2019; Avery et al., 2018; Sung et al., 2018).

2.3. Determining the antibiotic resistance patterns

Antibiotic resistance testing was done by the Kirby-Bauer disc diffusion method (CLSI, 2015). For disc diffusion assays, isolates were incubated in Mueller Hinton broth at 37°C overnight and the turbidity of the fresh culture was adjusted to 0.5 McFarland scale. A 100 µl of adjusted, fresh overnight culture was plated out onto Mueller Hinton agar. After drying the plates, antibiotic discs were applied onto the surface using a dispenser. The antibiotic discs used in this study included gentamicin (10 mcg), piperacillin (100 mcg), meropenem (10 mcg), colistin (10 mcg), trimethoprim (5 mcg), ciprofloxacin (5 mcg), imipenem (10 mcg),

ampicillin (10 mcg) and chloramphenicol (30 mcg). All plates were incubated at 35°C for 18 h as described in the CLSI (CLSI, 2015) guidelines. After incubation, the diameter of the inhibition zone was measured and the isolates were grouped into the categories susceptible, intermediate or resistant, based on the diameter of the inhibition zone for the respective antibiotic. The *Acinetobacter* isolates which showed resistance to three or more antibiotic classes were defined as the MDR strains. Based on this, the MDR *Acinetobacter* isolates were chosen (Avery et al., 2018; Sung et al., 2018). All antibiotic resistance determinations were done in duplicate.

2.4. Molecular detection of Acinetobacter

The aim of the Polymerase Chain Reaction (PCR) is synthesis of the new DNA strands according to the string pattern which is repeated chain. The PCR is widely used in molecular biology. Lorenz (8). DNA extraction was performed by using the high pure DNA isolation kit (Yekta Tajhiz Azma-Tehran-Iran) according to the manufacturer's instructions. 16S rRNA of specific primers forward and reverse *Acinetobacter* which was synthesized by Malaysia 1st BASE were used for smarter identification and amplification of fragment 353 bp gene region. Polymerase chain reaction was accomplished by a thermocycler (Bio Rad-USA). All components were purchased from Yekta Tajhiz Azma (Tehran, Iran Company). The reaction mixture contained: 25 µl Master Mix (including PCR buffer at concentration of 10 times, magnesium chloride, dNTP (10Mm), Taq DNA polymerase enzyme (5U/µl), 3 µl DNA (100ng/L), 2 µl forward primer (10pmol) (5'-TAA TGG TTT GAT CGG CCT TG-3'), 2 µl reverse Primer (10pmol) (5'-TGG ATT GCA CTT CAT CTT GG-3'-3') and 18 µl water. In this technique in order to start the polymerization process, thermal cycler machine was set at 94 ℃ for 1 minute followed by 35 cycles of PCR carried out at 94 ℃ for 1 minute, 58.8 ℃ for 30 seconds and at 72 ℃ for 1 minute. Eventually 4 minutes of elongation was done at 72 ℃. Finally, in order to ensure the amplification gene 16S rDNA, electrophoresis on 2% agarose gel containing TBE 1X buffer was performed for 60 minutes at 90v. Martin et al (9). The gel results were observed by using Ultraviolet (UV Transilluminator machine-USA) (Nepal *et al.*, 2018; Jafari *et al.*, 2014; Moazamian *et al.*, 2018; Bahmani *et al.*, 2019). Standard strain of *A. baumanni* [1855] was provided from the Persian Type Culture Collection (PTCC, Iran) which was tested at the same time.

2.5. Statistical analysis

For statistical analysis of the results, and to plot the diagrams, SPSS 21 plus EXCEL 2016 were used.

3. Results

3.1. The studied population

Out of 794 clinical samples isolated from trauma patients, 248 Acinetobacter isolates were identified, where 204 and 44 patients were male and female, respectively. The maximum frequency was related to upper respiratory tract, while the minimum frequency belonged to urine. The greatest involvement was related to October 2014, while the minimum involvement was associated with January 2015. The maximum extent of comorbid infection of Acinetobacter with another bacterium was related to 45-87-year-old age group. Further, the maximum number of Acinetobacter infections was related to ICUs.

3.2. Acinetobacter isolation from clinical samples

In this research, all isolates were observed as gram-negative coccobacillus after gram-staining, which also had a similar biochemical pattern. Their biochemical characteristics included the ability of using citrate as the only carbon source, positive catalase, negative oxidase, indole-negative, hydrogen sulfide negative, mobility and growth on the Mac-Conkey Agar medium at 42 ℃ negative, Urea production and OF glucose were variable.

3.3. Determining the antibiotic resistance pattern

The drug resistance pattern of the *Acinetobacter* isolates indicated that all of the studied *Acinetobacter* isolates showed resistance to all antibiotics in the ordinary diffusion disk

Agar method. This means that all of the Acinetobacter isolates were MDR.

3.4. Molecular identification of Acinetobacter

For molecular identification of *Acinetobacter,* polymerase chain reaction was used, and the results obtained by electrophoresis gel image can be seen in Figure 1.

3.5. Statistical analysis

Out of the 794 patients hospitalized in the ward, 248 of them had *Acinetobacter* infection, where 204 (82.26%) were male and 44 (17.74%) were female. After isolating and identification *Acinetobacter* from the clinical samples, the frequency of this bacterium was obtained as follows: upper respiratory tract in 154 samples (55.19%), surgical site in 75 samples (26.88%), blood in 18 samples (6.45%), cerebrospinal fluid in 18 samples (6.45%), and urine in 14 samples (5.01%). Overall, 248 *Acinetobacter* isolates were isolated, with the results provided in figure 2. The maximum frequency percentage of *Acinetobacter* isolates was related to the upper respiratory tract samples followed by surgical site (figure 2).

The maximum percentage of infection with *Acinetobacter* was observed in the third three months of 2014 (34.33%), while the lowest number of infection was associated with the fourth three months of 2014 (25.33%). The percentage of the *Acinetobacter* isolates across different months can be observed in Table 1.

The percentage of *Acinetobacter* infection and co-infection with one bacterium and several bacteria is shown in figure 3. The maximum number of infection with *Acinetobacter* is related to co-infection with one bacterium. The percentage of hospitalization of patients with *Acinetobacter* was 64.11%, 32.26%, and 3.63% in ICU, other hospitalization wards, and Post ICU, respectively. Based on this statistics, the percentage of patients hospitalized in ICU is higher than that of other wards (figure 4).

The minimum and maximum ages of patients with *Acinetobacter* were 14 and 87 years old, respectively, and the mean age of the patient was 50.5 years old. Since the number of male patients injured due to accident is higher than the number of women, thus the percentage of infection is higher among men. The maximum percentage of involvement in the patients was related to concurrent infection of *Acinetobacter* and another bacterium, and between the ages of 45 and 87 years. As p-value was calculated to be larger than 0.05, thus there is no significant relationship with regards to age.

Table 1. The percentage of infection with *Acinetobacter* across different months.

Sampling time	percentage
September 2014	38%
October 2014	40%
November 2014	34%
December 2014	29%
January 2015	23%
February 2015	28%
March 2015	25%
April 2015	37%
May 2015	31%
June 2015	27%

4. Discussion

Many dangerous and deadly human diseases have been increased due to surgical infections, weakness of immune system, hospital injuries, the probability of infection and secondary infections with opportunistic pathogenic bacteria.

A. bumannii is an opportunistic pathogen in hospital that causes severe infections in intensive care units. Due to drug resistance of this bacterium, there are major problems in patients' treatment, especially in surgical wounds, burns and ICUs (Katayama *et al.,* 2000; Garnacho-Montero *et al.,* 2019; Dora *et al.,* 2008). In addition to undesirable clinical effects, it has increased the cost of treatment.

The patients suffering from cystic fibrosis, compromised immune system, and healthy individuals are at risk of infection with Acinetobacter due to breakdown of defensive and protective barriers. *Acinetobacter* is an important cause of infection in weak patients, while aggregation of this bacterium as colonization imposes no risk to healthy individuals (El Salabi *et al.,* 2013).

In a study carried out in JIPMER, Pondicherry, India, 43 patients admitted to the hospital who developed *Acinetobacter* species infection or colonization were evaluated. Among these it was seen that respiratory tract infections accounted for maximum (48.8%) isolation followed by blood stream infection (16.27%),

followed by secondary meningitis (14%). Other infections included urinary tract infections, peritonitis, corneal infection, necrotizing fascitis and osteomylelitis. Of the *Acinetobacter* species from secondary meningitis, *Acinetobacter johnsonii* was isolated in one case and the rest were *A. baumannii* (Nageshwari *et al.*, 2012)

In their study Shakibaie *et al*., they found that many isolates of *Acinetobacter* species were resistant to almost all antibiotics routinely used in the ICUs of their hospital. There is limited data on β-lactamase producing *Acinetobacter* species from India (Shakibaie et al., 2012).

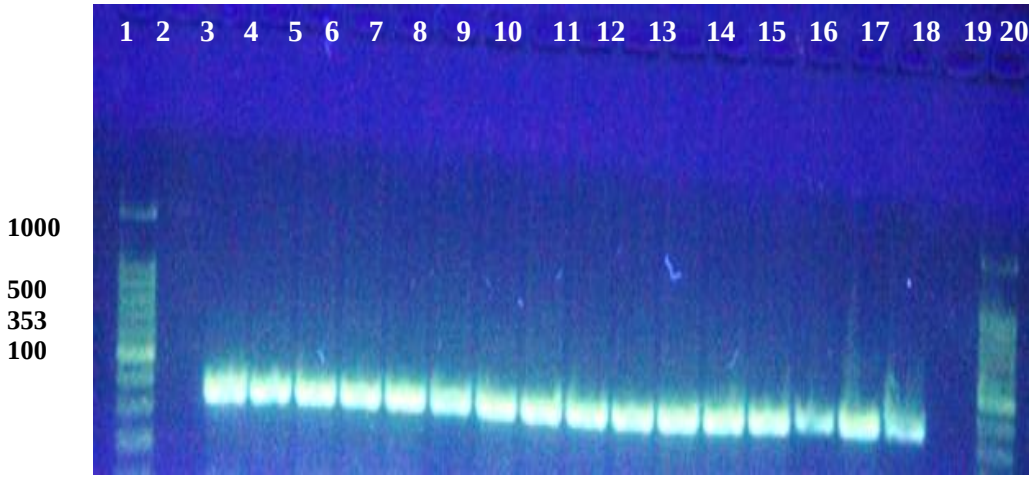

Fig. 1. The electrophoresis gel image of 14 *Acinetobacter* isolates: columns 1 and 20 are marker (100 bp SinaGen company), columns 2 and 19 are negative control, 3 and 18 are positive control, and 4-17 are the samples which have given a band at 353 bp.

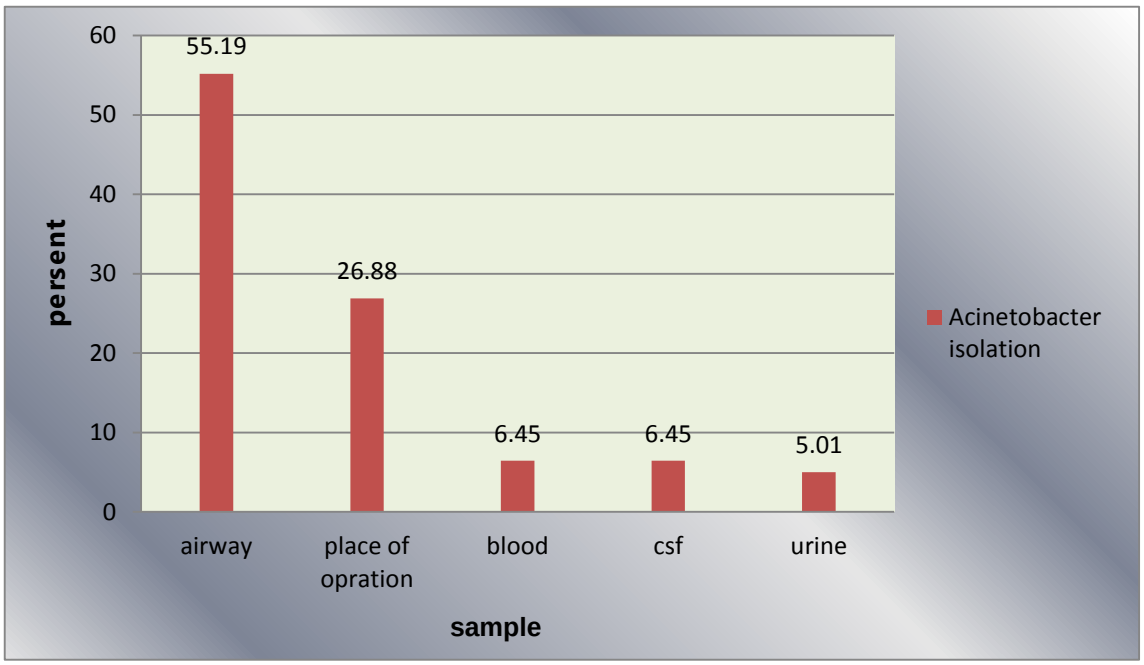

Fig. 2. The frequency of *Acinetobacter* in the clinical samples.

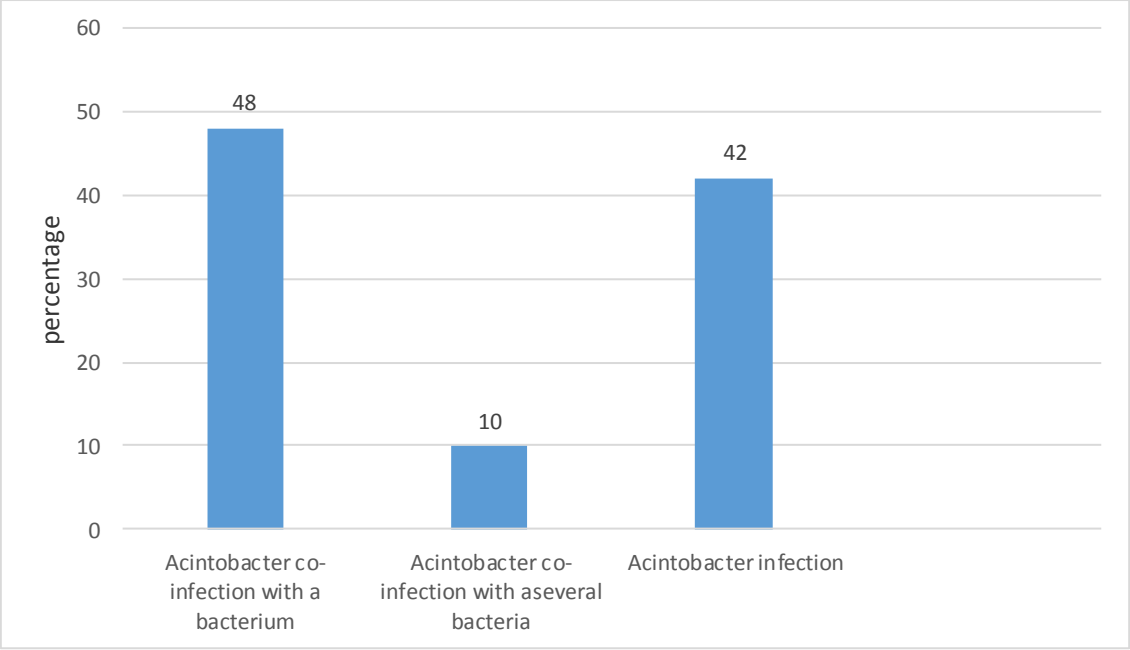

Fig. 3. The percentage of *Acinetobacter* infection and co-infection with one and several bacteria.

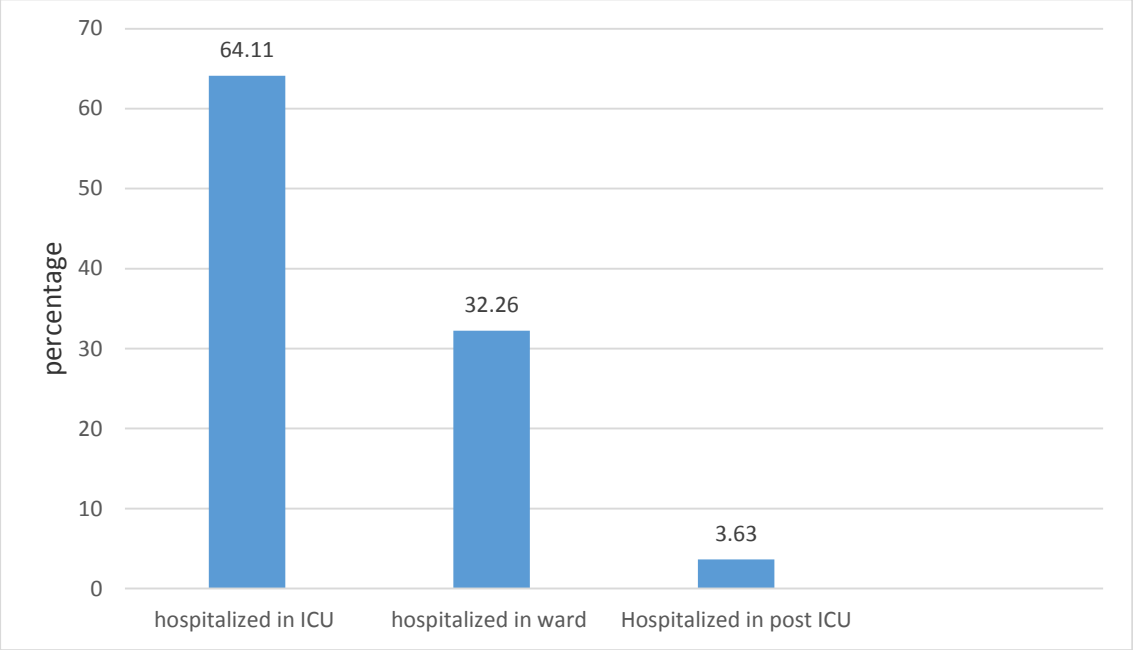

Fig 4. The percentage of hospitalization of patients in different wards.

In this study, the extent of isolation of *Acinetobacter* was the maximum in ICU ward, as compared with other wards. This issue is critical due to the different invasive interventions on the patient's, the long period of hospitalization, and weak or suppressed immune system. The maximum prevalence of nosocomial infection in the research was related to respiratory tract and pulmonary infection with a prevalence of 55.19%, which is in accordance with the findings obtained by Ganguly et al as 45.5% (Ganguly *et al.*, 1995) as well as Luzzati as 64% (Luzzati *et al.*, 2001). Furthermore, it is in complete congruence with a large number of studies that consider respiratory tract and pulmonary infections as the most common

nosocomial infection in ICU (Wieland et al., 2018; Bayuga et al., 2002).

In a study with Gupta et al, *Acinetobacter* accounted for 38% of total nonfermenters. Previously, published studies have accounted 12.9% (Lahiri et al., 2004) and 4.8% (Lone et al., 2009) of *Acinetobacter* isolates from total infected samples, respectively. In various countries, studies on *Acinetobacter* isolation have shown predominance in urine (21-27%) and tracheobronchial secretions (24.8-48.8%) (Lone et al., 2009) nevertheless there is an increase in occurrence of *Acinetobacter* in hemocultures in some hospital departments (Gupta et al., 2015). Wieland and coworkers reported that 131 of the 131 *A. baumannii* is isolated from patients in German hospital were 113 isolated MDRs (Wieland et al., 2018).

The findings of this study in line with the research by Bayuga et al (2002) along with Joshi (2003) indicated that antibiotic resistance is increasing seriously. In this research, 31.2% of the isolates were *Acinetobacter*, while 68.8% were other bacteria, where 100% of the studied *Acinetobacter* isolates had MDR phenotype. In their studies, they reported the extent of isolation of MDR *Acinetobacter baumannii* strains as about 45-75% (Bayuga et al., 2002).

Sileem et al. reported the most common respiratory tract infections in Egypt (79.5%), followed by urinary tract infection (14.1%) (Sileem et al., 2017). In this research, the nosocomial infection prevalence was reported as 31.2%. In the present study, no significant relationship was observed between the age and contracting the infection. However, in the studies by Ganguly et al and Lunzatti et al, a significant relationship was observed between age and development of nosocomial infection. The reason of this incongruence can be the high mean age of the patients hospitalized in the ICU ward of the studied hospital, complicating proper statistical comparison among the age groups of patients. The different antibiotic resistance patterns among the pathogenic hospital bacteria can considerably differ from country to country or region to region in a single country. Previous studies have shown that the first line therapy for infections resulting from *A. baumannii* includes amikacin, carbapenem (imipenem, meropenem, and doripenem), Ceftazidime, and quinolones (Ganguly et al., 1995; Luzzati et al., 2001; Prashanth et al., 2004).

In addition, most pathogenic bacteria have become almost completely resistant to some new antibiotics including broad-spectrum Cephalosporins (e.g. Cefotaxime and Ceftazidime). Previously, Imipenem was the most active drug against infections resulting from Acinetobacter worldwide. However recently, evidence of distribution of imipenem-resistant strains has been suggested (Demoz et al., 2018).

Among *Acinetobacters*, the greatest resistance to imipenem was observed in *A. baumannii* genus. Emergence of extensive resistance to Imipenem is a serious threat for treatment in the near future. Antibiotic resistance and sensitivity differs from country to country due to various environmental factors and application of antimicrobial agents (Jamulitrat et al., 2007). In this study, high resistance to Imipenem by Acinetobacter was also observed.

Conclusion

Since in our country very few studies have been conducted on epidemiological properties and drug resistance patterns of *Acinetobacter* isolates, thus paying attention to the role of this bacterium as a potentially dangerous cause of nosocomial infections and the increase in MDR Acinetobacter strains in clinical samples seems to be essential. It is suggested that periodic supervisions be performed on the resistance pattern. Based on the results obtained from this research and previous studies, which all suggest the progressive increase in antibiotic resistance in *Acinetobacter* strains, it can be concluded that the indiscriminate use of antibiotics, illogical prescription of medications, and not having a systematic and regular therapeutic policy in the country are among the main causes of development of high resistance in this bacterium. Thus, proper medications and new antimicrobial agents should be developed for controlling the infection.

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Refereces

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