Original Article

Prevalence of resistance to colistin, tigecycline and minocycline in Acinetobacter baumannii isolated from clinical samples in 2014

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Abstract

Background: Colonization rate of Acinetobacter baumannii is increasing in hospitalized patients especially in long term hospitalized one and / or who were treat with extended spectrum antibiotics or anticancer. Antibiotic resistance in A. baumannii is considerable because more prevalence of them cause nosocomial infections and can impose high cost to health systems and patients. The aim of this study was determination of tigecycline, minocycline and colistin resistance A. baumannii in selected center in Tehran, Iran. **Materials and Methods:** This study was descriptive and functional foundation. In this study A. baumannii were collected from Milad, Mofid, Taleghani, Motahari and Loghman hospital, Tehran and transferred to laboratory of pediatric infections research center. Collected bacteria were identified by conventional microbiology tests. Antibiotic susceptibility testing was determined according to CLSI guide line. Tigecycline, minocycline and colistin resistance strains were isolated. **Results:** In this study, 105 A. baumannii were collected from five selected hospitals: 48 (46%) from Milad, 33 (31%) from Motahari, 17 (16%) from Loghman, 4 (4%) from Mofid and 3 (3%) from Taleghani hospital. The highest resistance was observed against cefepime and high frequency of carbapenem and minocycline was observed. On the other hand, observed resistance to aminoglycosides was 93% at least. Tigecycline is the most effective antibiotic after colistin. Colistin resistant confirmed just in one isolate by E. test. **Conclusion:** The results of this study indicated that high rate of antibiotic resistance in A. baumannii even resistant to third and fourth generation of cephalosporin and carbapenem antibiotics. The treatment of MDR strains of A. baumannii become more complicated if the spread of them were not been controlled.

Keywords: Acinetobacter baumannii, Antibiotic resistance, Tigecycline, Minocycline

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Introduction

Colonization rate of Acinetobacter baumannii is increasing in hospitalized patient especially in patients who were hospitalized for a long time or who were treated with extended spectrum antibiotics or anti cancers [1, 2]. A. baumannii usually can involve in respiratory tract infection in hospitals. Also, A. baumannii can cause nosocomial urinary tract infections and wound infections, and even the infection can develop into septicemia [3]. Antibiotic resistance in A. baumannii is considerable that can be

intrinsic or it can be acquired through transferable genetic elements. Most strains of A. baumannii are resistant to ampicillin, amoxicillin-clavulanic acid, extended spectrum cephalosporins, tetracycline, macrolides and chloramphenicol. A. baumannii has presented various mechanisms for resistant to extended spectrum antibiotic. One of intrinsic resistance mechanism is mainly due to the low permeability of outer membrane and also structural expression of specific efflux pumps. A. baumannii can simply acquire genetic elements such as plasmids, transposons, and integrons [3- 8]. MDR isolates are being reported increasingly on the basis of so many studies that were done in developing and developed countries all over the world. Resistance to carbapenems, that are one of the last chances of antibiotic therapy in some cases, is also being seen [4, 7]. In some of the studies, the effect of combination therapy with tigecycline, colistin and minocycline in resistant cases is being discussed [9, 11]. Because of prevalence of antibiotic resistant strains of A. baumannii in nosocomial infections and high costs of detection and especially treatment of this bacteria, more research and study about this are needed. Therefore, the aim of this study is determination of the prevalence of tigecycline, minocycline and colistin resistance A. baumannii in selected Medical centers in Tehran, Iran.

Methods

Bacterial strains. A. *baumannii* was collected from different clinical specimens (blood, urine, wound, sputum, etc.) of hospitalized patients in Taleghani, Motahari and Loghman hospitals in 2014. Identification of isolated A. *baumannii* strains by conventional microbiology and biochemistry methods such as oxidase, TSI, SIM, etc.

Antibiotic Susceptibility Testing.Determination of Antibiotic resistance pattern was prepared by disk diffusion method according to the CLSI protocol; minocycline (30µg), co-trimoxazole (1.25/23.75 µg), ceftazidime (30 µg), cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin(5 µg), amikacin (30 µg), imipenem (10 µg), cefepime(30 µg), ceftriaxone (30 µg), meropenem (10 µg), piperacillin- tazobactam (100/10 µg), tigecycline (15 µg). Determination of colistin resistant was determined MIC by E-Test.

Phenotypic ESBL detection. Combination disc method by clavulanic acid was used for the detection of ESBL producing strains according to CLSI guideline. In this method, cefotaxime and cefotaxime + clavulanic acid and also ceftazidime and ceftazidime + clavulanic acid discs were used.

Strains that have increase at least 5mm around the combined cephalosporin disc compare to cephalosporin alone were considered as an ESBL producing strains.

Data analysis. In this study data analysis was done by SPSS 24.

Results

In this study, 105 A. baumannii strains were confirmed from five selected hospitals include; 48 (46%) strains from Milad, 33 (31%) strains are from Motahari, 17 (16%) strains from Loghman hospital, 4 (4%) strains from Mofid hospital and 3 (3%) strains from Taleghani hospital. Percentage of collected strains from different hospitals is demonstrated in the Figure 1.

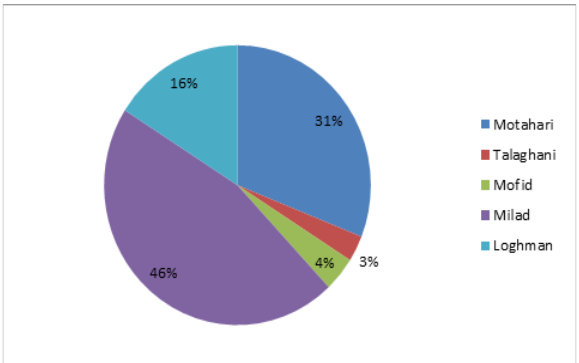

Figure 1. Collected Acinetobacter strains from different hospitals

Antibiotic resistant percentage of A. baumannii strains are demonstrated in table 1. The rate of resistance and sensitivity of these bacteria to studied antibiotics is demonstrating that the highest rate of resistance belongs to cefotaxime with 100% rate and the highest rate of sensitivity belongs to tigecycline with 43.8% rate.

According to table 1, the highest rate of resistance belongs to cefotaxime and the lowest rate of resistance belongs to imipenem.

Table1. Antibiotic resistant frequency of collected A. baumannii

Antibiotics	Resistant (%)
Minocycline	98 (93.33)
Trimethoprim/Sulfamethoxazole	97 (92.38)
Piperacillin	103 (98.09)
Ceftazidime	101 (96.19)
Cefotaxime	105 (100)
Gentamicin	101 (96.19)
Ciprofloxacin	104 (99.04)
Amikacin	98 (93.33)
Tigecycline	59 (56.19)
Imipenem	100 (95.23)
Cefepime	104 (99.04)
Ceftriaxone	104 (99.04)
Meropenem	103 (98.09)
Piperacillin/tazobactam	101 (96.19)

MN: minocycline ،SXT: co-trimoxazole ،CAZ: ceftazidime ، CTX: cefotaxime ،GM: gentamicin, CIP: ciprofloxacin ،AK: amikacin ،IMI: imipenem ،FEP: cefepime ،CTR: ceftriaxone ، MRP: meropenem ،PTZ:piperacillin- tazobactam

Detection of ESBL. Among the studied strains, 50 (48%) strains were confirmed as an ESBL producing strains by phenotypic method used in this study (figure 2).

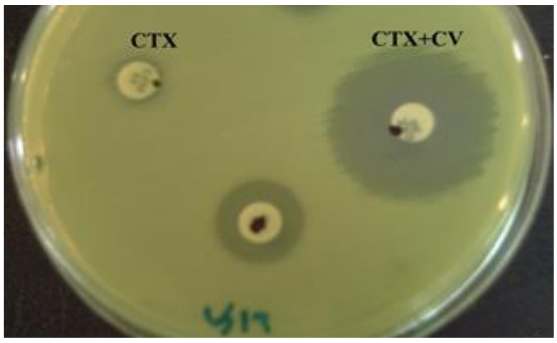

Figure 2. ESBL positive strain

Study of colistin sensitivity by E- Test. In all strains studied in this research, MIC of colistin was determined by E-test antibiotic strips (figure 3). Except one strain that belongs to Motahari hospital, the rest are sensitive to this antibiotic. The MIC of resistant strain was 4mg concentration.

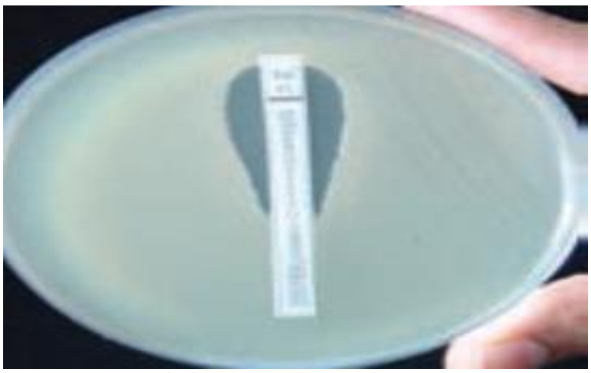

Figure 3. E-Test of colistin resistant strain

Discussion

Gram-negative bacteria are always considered as one of the most important cause of nosocomial infections in health system. *A. baumannii* is one of the common non-fermenting gram-negative bacteria that is an agent of nosocomial infection especially in burned patients .Nowadays, Antibiotic resistance has become an important challenge in treatment of infections caused by gram-negative bacteria such as *A. baumannii* [12- 19]. Over use and in some cases miss use of antibiotics in treatment of infections caused by this bacterial species can cause MDR strains to appear [12-15, 20, 21]. Prolonged hospitalization time of patients in hospital and imposing more costs on patients and also health system can be consequence results of high prevalence of MDR bacteria in hospitals [15, 21-23]. As results of this study 99% of studied strains have shown resistance to cefepime. Cefepime is a fourth generation cephalosporin and observation of such a high percentage of resistance to it can be considerable. On the other hand, the minimum observed resistance to tested aminoglycosides in this study is 93% and this resistance is very high and important. Thus, the reason and source of this observation must be follow ups. Also, resistance to minocycline and carbapenem of collected strains were more than 90%. Carbapenem and minocycline are the important therapeutic choices in serious infection caused by *A.baumannii* [21, 22]. Thus, identification of carbapenem resistant bacteria and diagnosis of resistance mechanism can be an important step for prevention of carbapenem and

minocycline resistant bacteria spreading in the health centers.

Owlia et al. studied on *A. baumannii* collected from burns in 2012. According to this study, 92% of strains were reported resistant to all tested beta lactam antibiotics [14].

In 2011, Peymani et al. studied on 100 clinical samples of *A. baumannii* collected from different clinical samples of patients admitted in hospital in Tabriz. In this study, 92% resistance to ceftazidime, 88% resistance to cefepime and 54% resistance to imipenem were reported [23] These rates are lower than the results of the current study. The reason for observed differences can be related to different therapeutic strategies in different hospitals or source of specimens.

In the current study, results of resistance to beta-lactams and aminoglycosides which many of them are part of therapeutic choice of patients are also high and almost similar to results of some other studies in Iran [14, 15, 20, 21].

Reason of this similarity can be related to similarity of strategy of antibiotic therapy in different parts of Iran. High rate of prescription of extended spectrum antibiotics for treating infection or in some cases incorrect use of extended spectrum antibiotics for prophylaxis in Iran may be possible cause of observed high frequency of antibiotic resistant bacteria in Iran. Hence, the use of extended spectrum antibiotics resulted in the selection of MDR bacteria and physician face to serious problematic antibiotic therapy.

Results of a study in 2015, showed that in most cases, antibiotic prescription is without considering results of determination of antibiotic sensitivity testing of microbiology laboratory in hospital [24].

Iran is among world's top 20 countries in drug use and ranks second after China in Asia. The most studies show 30 to 60 percent of prescribed drugs have been incorrect. Physicians, distributors or doing self-therapy may be involved in this health problem [25, 26].

Also in a study in Iran, in 2010, Dr. Gholami et al. studied on in emergency ward of educational hospital in Tehran [27].

More than half of samples collected in recent study are from Motahari hospital and burn samples. Bacteria collected from burn patients showed high frequency of antibiotic resistance that can because of high rate prescription of extended spectrum antibiotics in this group of patients.

Generally it should be noted that carbapenems are the last antibiotic choice in treatment of bacteria producing ESBL enzymes and in case of observation of carbapenem resistant bacteria which should be considered [23]. Another notable point in results of the current study is observation of a colistin resistant A. baumannii strain that according to MIC result. Colistin is the last antibiotic choice antibiotic in cases of carbapenem resistant and MDR or XDR A. baumannii infections. So, resistance to colistin is very serious and urgent problem in health system, especially in *A. baumannii* strains as one of the important nosocomial pathogens. On the other hand, molecular study on colistin resistant mechanisms is essential in this strain, because in 2016, plasmid born colistin resistance gene is reported from china [28]. Plasmid born colistin resistance can transfer horizontally to other bacteria and make unbelievable problem to antibiotic therapy. According to the results of the current study, more than half of collected *A. baumannii* were resistant to tigecycline. Tigecycline also can be used to antibiotic therapy if MDR *A. baumannii* was isolated [29- 32] and observation of high resistant rate to this antibiotic can be consider in hospitalized patients. In different studies, therapeutic effect of tigecycline as monotherapy or combination with colistin or rifampin has been studied that in some cases, resistance to tigecycline is being reported [33]. Results of the current study showed that there is a high percentage of antibiotic resistance in studied *A. baumannii* strains. Even resistance to third and fourth generation cephalosporins and carbapenems is high and in case of not controlling these MDR strains in studied health centers, treatment of patients with *A. baumannii* infection will be very complicated and problematic.

Conclusion

Results of these studies show that collected *A. baumannii* from burn patients are not only resistant to

cephalosporins but also are highly resistant to imipenem. Observation of this high frequency of antibiotic resistance *A. baumannii* can be related to high use of carbapenem in treatment and sometimes use in prophylaxis with extended spectrum beta lactam antibiotics in these patients.

Conflicts of Interest

There is no conflict of interest among authors.

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