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Expression of MFS efflux pumps among multidrug resistant *Acinetobacter baumannii* clinical isolates

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ABSTRACT

Multidrug resistance (MDR) strains of Acinetobacter baumannii have been declared as a significant threat worldwide by World Health Organization (WHO). Researchers have shown that the prevalence of MDR strains of A. baumannii has reached an alarming rate all over the world. Among various assumed antibiotic resistance mechanisms of this bacterium, efflux pumps are regarded as one of the most important types. In this study, 100 clinical isolates of A.baumannii isolated from patients hospitalized in Intensive Care Unit (ICU); after biochemical and microbiological tests, the antibiotic sensitivity test was performed and determined the Minimum Inhibitory Concentration (MIC),then genes expression of selected Major facilitator superfamily (MFS) transporters were evaluated in presence of antibiotics. Results showed that the abaye genes of (002647, 1603) there were 100% in Acinetobacter baumannii isolates, percentage of abaye genes of (2281468, 1582001) were 88%, and percentage of abaye genes of (3671432, 3641859) were 0. The expression rate of abaye genes (3671432, 3641859, and 1603) in presence of all antibioticswere0.The expression rate of abaye2281468 in presence of ciprofloxacin antibiotic was 75%. This study revealed that MFS transporter of abaye2281468 unlike other MFS transporter of A.baumannii has an expression in presence of ciprofloxacin comparison with the fact that such phenomenon did not happen without presence of this antibiotic which can indicate the role of this MFS transporter in resistance to ciprofloxacin. Of course to evaluate the expression of these genes other molecular methods such as cloning and Real Time PCR should be used.

Keywords:, *Acinetobacter baumannii*, Expression, Efflux pump**,** MFS transporter

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INTRODUCTION

Acinetobacterbaumannii is increasingly introduces itself as a pathogen which can cause severely infection with high mortality rate, especially in patients hospitalized in ICU, immunocompromised patients, and patients treated with broad-spectrum antibiotics[1]. At present drug resistance is emerging in often pathogens and most medical efforts

have been unsuccessful in their attempt to its control [2]. In recent decades, infections resulted from *Acinetobacter baumannii* could be treated with available conventional antibiotics[3]; but nowadays, it has become resistant to most antibiotic classes and selecting an empirical treatment for infections resulted from this bacterium has become difficult [4, 5]. MDR strains of *A. baumannii* are strains that are able to resist fatal antibiotic doses which are enough to eradicate non-MDR strains [6]. MDR has been considered as a significant threat for human health by WHO. Researchers have revealed that the prevalence of MDR strains is high in Iran like other regions of the world and there is a growing concern in relation to life-threatening infections caused by this organism[7]. Among different mechanisms of antibiotic resistance to this bacterium, the efflux pumps are among the most significant antibiotic resistance mechanisms [8]. Efflux pumps almost are found in all bacteria species while the coding genes of these proteins have found on chromosomes or plasmids[9, 10]. Genomic sequence analysis shows that efflux pumps composed 10% of bacterial transporters averagely; moreover, they can also transport a wide range of compounds [11]. The presence of efflux pumps that are able to increase antibiotic resistance would lead to reduction in Drug accumulation as well as an increased MIC. Most antibiotics are pumped outside bacterial cell are including; macrolides, quinolones, and tetracycline [12]. Multiple Efflux Pumps (MEPs) are divided into following antibiotic classes[13]:

1. ATP-binding cassette (ABC)
2. Major facilitator superfamily (MFS)
3. Resistance nodulation cell division (RND)
4. Multidrug and toxic compound extrusion (MATE)
5. Small multidrug resistance (SMR) family
6. Drug/metabolite transporter (DMT) superfamily

In *A. baumannii*, resistance resulted from efflux pumps are often caused by RND and MFS families [12]. Over 300 proteins of MFS superfamily have been recognized [14]. 5 clusters of MFS proteins are also detected which have a role in a) drug resistance, b) sugar uptake, c) uptake of Krebs cycle intermediates, d) phosphate ester/phosphate antiport, and e) oligosaccharide uptake[15]. The most important genes involved in antibiotic resistance from MFS superfamily are *abaye* genes. Therefore, considering the relative lack of research regarding these genes in Iran, this study has been conducted to evaluate the expression of MFS efflux pumps and their genes in abiotic resistance from clinical isolates of *A.baumannii* isolates.

MATERIALS AND METHODS

Statistical Population

In this cross-sectional study, 100 clinical isolates were detected using microbial and biochemical tests [16]. All clinical isolates (n=100) were positive for *blaOXA-51-like*gene [17] and were collected as *A.baumannii* from patients hospitalized in ICU of Motahari Hospital of Gonbad during a one year period (2011-2012); the samples entered the study to evaluating the effect of studied antibiotics on expression of 6 selected MFS transporter genes. As noted above, the role of MFS efflux pumps in antibiotic resistance were determined indifferent bacteria and regarding that the efflux pumps of various families play an important rolein *A. baumannii* antibiotic resistance and because the role of mentioned family in drug resistance in *A.baumannii* has not been investigated, for this in present study this multiplex(6 plex)will be discussed.

Bacterial strains and antimicrobial susceptibility testing

In order to growof bacterial strains, TSB (Toy Soy Broth) was used in 37 °C; antimicrobial susceptibility testing was performed through Kirby-Bauer disk diffusion (KB) method using polymxyin B, cefepime, erythromycin, piperacillin-tazobactam, ampicillin-sulbactam, ticarcillin-clavulanic acid, minocycline, doxycycline, tigecycline, rifampicin, netilmicin, kanamaycin, kanamaycin, colistin, ceftazidime clavulanic acid, doripenem, tobramycin, ceftriaxone, ceftazidime, amikacin, tetracycline, tetracycline, ciprofloxacin, gentamicin, imipenem, and sulfamethoxasole antibiotics[18].

MIC determination of *Acinetobacterbaumannii* isolates

Based on CLSI guidelines, *A.baumannii* isolates were placed alongside selected antibiotics (rifampin, imipenem, tetracycline, ticarcillinclavulanic acid, piperacillin, gentamicin, ciprofloxacin, trimethoprim sulfamethoxazole, and ceftriaxone) and their MIC were determined using micro broth dilution method (Table 1) [19]. The controls were standard reference strains of *Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa ATCC 27853*.

Table 1. MIC of selected antibiotics for isolates of *A. baumannii* based on CLSI

Antibiotics types	MIC (µg /ml)		
	S	I	R
Piperacillin	8-16	32-64	≥128
Ticarcillin-clavulanic acid	8-16	32-64	≥128
Gentamicin	≤4	≤8	16-32
Ciprofloxacin	≤1	2≤	4-8
Trimethoprim-Sulfamethoxasole	≤2	≤4	≥8
Ceftriaxone	≤ 8	16-32	64-128
Tetracycline	≤4	≤ 8	16-32
Imipenem	≤2	4-8	≥16
Rifampin	≤2	4-8	≥16

Note: MIC; minimum inhibitory concentration, S: susceptibility, I: intermediate, R: resistant

DNA Extraction

DNA extraction was done using method previously described[20].

Evaluating the presence of selected MFS genes using multiplex PCR method

In order to assessing the presence of selected MSF genes, Multiplex PCR method was used. PCR reaction's initial setup was as Uniplex which then changed to Multiplex. Required primers for multiplex PCRare listed in Table 2. Required values for each reaction are listed as;10 µl of Master Mix (Ampliqon, Denmark), 1.5 µl of each primer (Takapouzist, Iran) and 2µl of 100 pg of genomic DNA in 25 µl PCR reaction and 7 µl distilled water, primers for *fum* and *abaye* genes were used at 2.5 picomol concentration. The condition for PCR cycle was as following: initial denaturation was done in 94 °C for 3 minutes, 30 cycles in 94 °C for 45 seconds, 47 °C for 30 seconds and 72 °C for 30 seconds and afinalelongationstepat72° C for5minutes; finally, electrophoresis of products was carried out on 2% agarose gel /and painting was done through safety dye.

Table 2. The necessary primers for *abaye* genes

***abaye* genes**	**Primers sequences (5' to 3')**	**Amplicons size (bp)**
3671432	TCACCAATCTAAGCTCTATCG GAATGGTCTGCGTAGTATTG	140
2281468	CTAACCTACATATTGCCGATAC TAGCTGAATGACTGTCGTAA	212
002647	GGCCTGATTTCGTTTGTATATC CTAATAATGGACCAATGGCAG	316
3641859	GTCTTCTTCATCATGCAACTCGTTCCACGTTGTTATAGAAG	451
1603	CACTCCCAAATACTCTACCTAT GAGAGTAGTACACCTGTCAAT'	538
1582001	CTTATTCCTGCCACTTTATCC AATACCTAGGCTCGATACTCTA	660

Evaluation of *abaye* genes expression using reverse transcriptase - PCR (RT-PCR)

For evaluating the expression of *abaye* genes, RT-PCR technique was used [21]. In brief, after incubation with or without antimicrobial agents, the expressions of desired genes were evaluated. RNA of isolates was extracted using Qiagen kit (Qiagen, Valencia, C) . After eliminating the genomic DNA through RNase-free DNase (Qiagen) treatment, reverse transcription was done using Hexamerss random (Qiagen). Lastly, the data were analyzed using SPSS software (version 19.0.0). Significant differences between variables were analyzed through x^2 test.

RESULTS

Tracing of *abaye* genes from mfs superfamily through multiplex RT-PCR

After performing antibiogramwith mentioned antibiotics for 100 isolates of *A.baumannii* (data not shown), MIC determination revealed that ciprofloxacin's MIC washigh and≥32 (Table 3). PCR reaction was initially applied as uniplex and later changed to multiplex (Figure 1).Results showed that the *abaye* genes of (002647, 1603) there were 100% in *Acinetobacterbaumannii* isolates, percentage of *abaye* genes of (2281468, 1582001) were 88%, and percentage of *abaye* genes of (3671432, 3641859) were 0. The expression rate of *abaye* genes (3671432, 3641859, and 1603) in presence of all antibiotics were 0 while the expression rate of *abaye*2281468, 002647, and 1582001were 27.7%, 10.5% and 12.5%, respectively. The expression rate of *abaye*2281468 in presence of ciprofloxacin antibiotic was 75% (Table 4). According statically analysis as shown in table 4 no significant

relationship was found between MFS gene expression and the presence and lack of mentioned antibiotics (P>0.05). The only exception in this regard, significant expression of*abaye*2281468in presence of ciprofloxacin (P=0.001).

Table 3. Determining the MIC of selected antibiotics for100*Acinetobacter baumannii* isolates

Antibiotics types	MIC (µg/ml)		
	S	I	R
Piperacillin	35	25	40
Ticarcillin-clavulanic acid	40	30	30
Gentamicin	20	35	45
Ciprofloxacin	30	30	40
Trimethoprim-Sulfamethoxasole	40	25	35
Ceftriaxone	35	35	40
Tetracycline	40	30	30
Imipenem	50	25	25
Rifampin	40	30	30

Note: MIC; minimum inhibitory concentration, S: susceptibility, I: intermediate, R: resistant

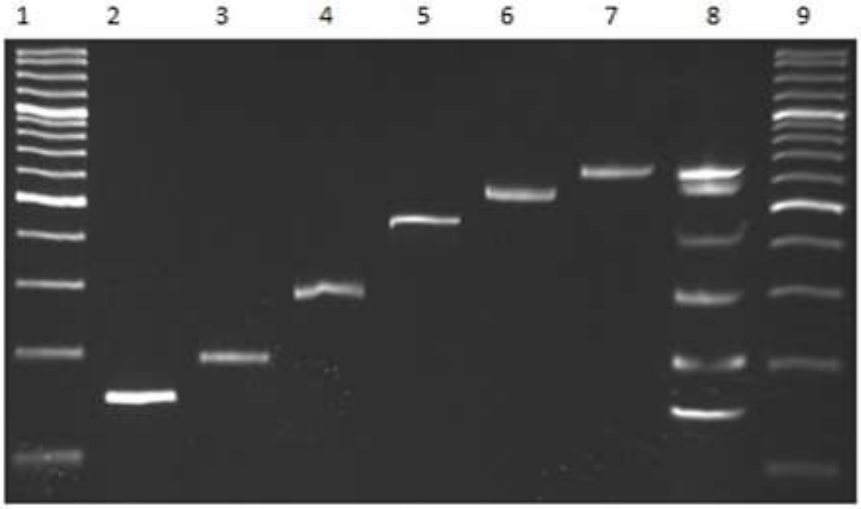

Figure1. Multiplex PCR setup for presence of *abaye* genes from MFS superfamily

Lanes 2-7. Uniplex PCR product of selected genes; lane 1 100 bp DNA Ladder, lane2 band 140 bp from abaye 3671432 gene, lane 3 band 212 bp corresponds to the abaye 2281468 gene; lane 4 band 316 bp related to product of abaye 002647 gene, lane 5 Uniplex PCR product of 451bp for abaye 3641859 gene, lane 6 band 538 bp of of abaye 1603 gene, lane 7 band 660 bp corresponds to the abaye 1582001 gene, lane 8 Multiplex PCR product (6Plex PCR) of selected genes and lane 9 100 bp *DNA Ladder*

Table 4. The expression rate of *abaye* genes in the presence of selected antibiotics and without them using multiplex RT- PCR

In presence of Antibiotics	*Expression rate of abaye genes* (%)					
	abaye 3671432	*abaye* 2281468	*abaye* 002647	*abaye* 3641859	*abaye* 1603	*abaye* 1582001
Piperacillin	0	10	20	0	0	25
Absence	0	0	15	0	0	25
Ticarcillin-clavulanic acid	0	10	10	0	0	15
Absence	0	0	15	0	0	25
Gentamicin	0	10	10	0	0	10
Absence	0	0	15	0	0	25
Ciprofoxacin	0	75	15	0	0	25
Absence	0	0	15	0	0	25
Sulfamethoxasole	0	15	5	0	0	5
Absence	0	0	15	0	0	25
Ceftriaxone	0	10	10	0	0	15
Absence	0	0	15	0	0	25
Tetracycline	0	15	10	0	0	10
Absence	0	0	15	0	0	25
Imipenem	0	15	5	0	0	15
Absence	0	0	15	0	0	25
Rifampin	0	20	10	0	0	2
Absence	0	0	15	0	0	25
Total	0	27.7	10.5	0	0	12.4

DISCUSSION

As we know, bacterial resistance toward antibiotics are partly through efflux pumps in gram-negative organisms; but, resistance resulted from over-expression of efflux pumps cannot lead to high-level clinically significant resistance alone and other mechanisms involved in antibiotic resistance certainly[22]. One of these classes is efflux pump of mfs superfamily which has a notable role in antibiotic resistance [14]. Several efflux pumps such as Tet(A) and Tet(B) of MFS superfamily have been identified in *A.baumannii* which have a role in creating resistance to tetracycline and minocycline [23, 24]. This research also revealed that several studied isolates had a high MIC (≥32) for ciprofloxacin which was higher than conventional MIC declared by CLSI (MIC=4-8),which represents the resistance of the isolates to ciprofloxacin. Moreover, *abaye*2281468 had a 75% expression in presence of ciprofloxacin which shows the role of this gene in increasing the MIC of ciprofloxacin antibiotic for *A. baumannii* isolates. Until now, no MFS family gene has been found that would have a role in ciprofloxacin resistance; but, researches have shown that in *E. coli*, MdfA protein of MFS family has a role in resistance towards ciprofloxacin, chloramphenicol and several other antibiotics [25]. Similar our study, AbeM protein of MATE family has a role in resistance to ciprofloxacin and norfloxacin as well as other antibiotics in *A.baumannii* [13]. Although it should be noted in addition to efflux pumps, resistance against fluoroquinolones can also be through mutations in *gyrA* and *parC* genes and therefore antibiotic resistance of this family is in fact through a mix of different mechanisms [26]. In a study, the role of NorM and YdhE effluxes of the same family in formation of the resistance to ciprofloxacin and norfloxacin has been confirmed [27]. Other studied genes of *abaye*002647 and *abaye*1582001due to their expression with and without the presence of antibiotic as well as the low amount of their expression cannot be expected to play a role in resistance to selected antibiotics and possibly other resistance mechanisms play a role in this case**.**

CONCLUSION

This study revealed that MFS transporter of *abaye*2281468 unlike other MFS transporter has an expression in the presence of ciprofloxacin comparison with the fact that such phenomenon did not happen without presence of this antibiotic which can indicate the role of this MFS transporter in resistance to ciprofloxacin. Of course to evaluate the expression of these genes, other molecular methods such as cloning and Real Time PCR should be used.

REFERENCES

[1] MI Borges-Walmsley, AR Walmsley. *Trends Microbiol.***2001**;9(2):71-9.
[2] SB Levy. *Springer*; **1995**. p. 1-13.
[3] B Liu, Y Liu, X Di, X Zhang, RWang, Y Bai, et al. *Rev Soc Bras Med Trop*. **2014**;47(4):3641859-6.
[4] YJOh, SH Song, SH Baik, HH Lee, IM Han, DHOh. *Korean JIntern Med*. **2013**;28(4):486-90.
[5] ABahador, A Bazargani, M Taheri, Z Hashemizadeh, A Khaledi, H Rostami, et al. *J Pure Appl Micribiol*. **2013**;7:1559-66.
[6] PKiratisin, AApisarnthanarak, SKaewdaeng. *Int J Antimicro Ag*. **2010**;36(3):243-6.
[7] J Moradi, FB Hashemi, A Bahador. *Osong Public HealthRes Perspect*.**2015**;6(2):79-86.
[8] J Sun, Z Deng, A Yan. *Res. Commun*. **2014**;453(2:(254-67.
[9] K Poole. *Ann Med*. **2007**;39(3):162-76.
[10]LJPiddock. *Nat Rev Microbiol.***2006**;4(8):629-36.
[11] ITPaulsen. *CurrOpin Microbiol*. **2003**;6(5):446-51.
[12] KF Barker. *Br JClinPharmacol.***1999**;48(2):109-24.
[13] J Vila, S Martí,J Sánchez-Céspedes. *J AntimicrobChemother*.**2007**;59(6):1210-5.
[14] IT Paulsen, MH Brown, RA Skurray. *Microbiol Rev*. **1996**;60(4):575-608.
[15] IT Paulsen, RA Skurray. *Gene*. **1993**;124(1):1-11.
[16] B-CJeon, SHJeong, IKBae, SBKwon, KLee, DYoung, et al. *J Clin Microbiol*. **2005**;43(5):2241-5.
[17] JFTurton, NWoodford, J Glover ,SYarde, ME Kaufmann, TLPitt. *J Clin Microbiol.***2006**;44(8):2974-6.
[18] M Hombach, GV Bloemberg, EC Böttger. *J AntimicrobChemother*.**2011**:dkr524.
[19] H Seifert, R Baginski, A Schulze, G. Pulverer. *Antimicrob Agents Chemother*. **1993**;37(4):750-3.
[20] MR Green, J Sambrook. *Cold Spring Harbor Laboratory Press New York*; **2012**.
[21] HW Lee, YKoh , J Kim, JC Lee, YC Lee, SY Seol, et al. *Clin Microbiol Infect*. **2008**;14(1):49-54.
[22] M Webber, L Piddock. *J Antimicrob Chemother*. **2003**;51(1):9-11.
[23] A Ribera, I Roca, J Ruiz, I Gibert, J Vila. *J Antimicrob Chemother*. **2003**;52(3):477-80.

[24] S Marti, F Fernandez-Cuenca, A Pascual, A Ribera, J Rodriguez-Bano, G Bou, et al. *EnfermInfecc Microbiol Clin*. **2006**;24(2):77.
[25] R Edgar, E Bibi. *J bacteriol*. **1997**;179(7):2274-80.
[26] JS Wolfson, DCHooper. *Rev Infect Dis*. **1989**;11(Supplement 5):S960-S8.
[27] Y Morita, K Kodama, S Shiota, T Mine, A Kataoka, T Mizushima, et al. *Antimicrob Agent Chemother*. **1998**;42(7):1778-82.