Folia Microbiol
DOI 10.1007/s12223-017-0507-x

CrossMark

Acquisition of Tn*6018*-3′ CS regions increases colistin MICs against *Acinetobacter baumannii* isolates harboring new variants of AbaRs

Mohammad Savari[1] · Alireza Ekrami[2] · Saeed Shoja[3] · Abbas Bahador[1,4,5]

Received: 3 November 2016 / Accepted: 14 February 2017
© Institute of Microbiology, Academy of Sciences of the Czech Republic, v.v.i. 2017

Abstract Colistin is the last hope to treat extensively drug resistance (XDR) *Acinetobacter baumannii* (*A. baumannii*) infections, but resistance to colistin is currently reported in clinical centers all over the world. Here, we studied two colistin-resistant *A. baumannii* isolates with a difference in minimum inhibitory concentrations (MICs) that were isolated from a single burn patient during treatment in the hospitalization period. The international clonal (IC) lineage, multilocus sequence typing (MLST), and multiple loci variable number tandem repeat (VNTR) analysis (MLVA) typing were used to characterize the relatedness of *A. baumannii* isolates. Lipopolysaccharides (LPS) and PmrAB system analysis by PCR sequencing, polyacrylamide gel electrophoresis (PAGE), and real-time PCR were performed to determine the intactness and probable modifications of the LPS as the main resistance mechanisms to colistin. A combination of PCR, sequencing, and restriction fragment length polymorphism (RFLP) was used for *A. baumannii* resistance islands (AbaR) mapping as resistance-determinant reservoirs. Two isolates were identical at all MLST and VNTR marker loci that indicated the isolates were the same strain. In comparison to colistin-heteroresistant *A. baumannii* strain TEH267 (MIC = 1.5 mg/L), colistin-resistant *A. baumannii* strain TEH273 (MIC ≥256 mg/L) acquired two genomic regions including Tn*6018-topA* sequence and *topA* sequence-3′ CS in its AbaR structure containing *ispA* and *cadA* genes which, it would appear, could be associated with eightfold increase in colistin MIC. Both isolates had new variants of AbaR-like structures which could be derivatives of the typical AbaR3. According to the results of this study, AbaRs could be associated with an increase in MIC to colistin.

✉ Abbas Bahador
abahador@sina.tums.ac.ir; ab.bahador@gmail.com

1 Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Keshavarz Blvd, 100 Poursina Ave, Tehran 1416753955, Iran

2 Health Research Institute, Infectious and Tropical Disease Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran

3 Infectious and Tropical Disease Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

4 Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

5 Laser Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Introduction

Since the first report in 1999 from a blood culture in a hospital in Czech Republic, colistin-resistant *Acinetobacter baumannii* (CoR-AB) has become a new challenge to the clinical centers all over the world and threatens the antibiotic era (Cai et al. 2012; Perez et al. 2007).

Resistance to colistin mainly derived from heteroresistance phenotypes. Colistin heteroresistance among *A. baumannii* clinical isolates first described in 2006 by Li et al. and attributed to therapy with inadequate dosage leads to emergence of resistance subpopulations and eventually complete resistance to colistin (Li et al. 2006; Cheah et al. 2016).

Colistin binds to the outer membrane of Gram-negative bacteria and disrupts the negative charge of it. There are currently two known mechanisms of the colistin resistance. The first is complete loss of lipopolysaccharides (LPS) as a result of inactivation of a lipid A biosynthesis gene *lpxA*, *lpxC*, or *lpxD* (Moffatt et al. 2010).

The second is reduction of net negative charge of the outer membrane through point or frameshift mutations in a two-

Published online: 25 February 2017

component regulatory system, PmrAB, that leads to LPS modifications, like addition of phosphorylethanolamine to hepta-acylated lipid A (Adams et al. 2009). Proteolytic cleavage of the antimicrobial compound and exclusion of peptides by a broad-spectrum efflux pump are other possible resistance mechanisms (Poirel et al. 2011).

The genome of XDR *A. baumannii* (including colistin-resistant strains) has a high ability to acquire mobile genetic elements that could lead to resistance to a broad spectrum of antimicrobials (Pagano et al. 2016; Gallagher et al. 2015; Stokes and Gillings 2011). AbaRs are large clusters of horizontally transferred genes integrated in the *comM* gene which encodes an ATPase domain and contains multiple antimicrobial resistance regions (MARR). These regions differ in different AbaR types. The MARR is surrounded by a backbone including a Tn*6019*-Tn*6018* region on both sides of the *ATPase* gene. The MARR contains a novel transposon, Tn*6020*, and segments of Tn*1696*, Tn*21*, Tn*1721*, and Tn*5393*, and all of these components are resistance gene reservoirs (Post et al. 2010; Krizova et al. 2011). Outbreaks are associated with clonal dissemination of *A. baumannii* strains, which could lead to a pervasive incidence of colistin resistance strains and these resistance gene reservoirs, and that would be a real threat (Karaha et al. 2012).

The aim of the present study was to illustrate the reasons for an increase in colistin MICs among two *A. baumannii* isolates isolated from the same burn patient hospitalized for a month-length period.

Materials and methods

Characterization of strains

Two *A. baumannii* strains were collected from January to February 2012 (Bahador et al. 2015) from the burn wound infection of a hospitalized patient in Shahid Motahari Hospital, a referral burn center in Tehran, Iran. The *A. baumannii* strain TEH273 was isolated 2 weeks after the isolation of *A. baumannii* strain TEH267. Isolates were initially characterized using routine biochemical tests, and then final identification was performed by multiplex PCR using *gyrB*-directed primers according to the study of Higgins et al. (2010).

Susceptibility testing

The Clinical and Laboratory Standards Institute (CLSI) guideline (2015) for MICs using the E-test was used to assess the susceptibility of two *A. baumannii* isolates to colistin (Ezy MIC TM strips, Himedia, India). MIC of ≤2 mg/L was considered as susceptible and MIC of ≥4 mg/L was considered as resistant for colistin (Li et al. 2006; Cheah et al. 2016). Heteroresistance of *A. baumannii* was defined as the emergence of resistance to colistin by a subpopulation from an otherwise susceptible (MIC ≤2 mg/L) population (Cai et al. 2012). The susceptibility of these two CoR-AB isolates to other antimicrobial agents such as amikacin, ampicillin-sulbactam, cefepime, ceftazidime, ciprofloxacin, gentamicin, levofloxacin, meropenem, minocycline, piperacillin, piperacillin-tazobactam, rifampicin, tetracycline, tigecycline, tobramycin, and trimethoprim- sulfamethoxazole was also assessed using the disk diffusion test (Himedia, India). *Escherichia coli* (*E. coli*) ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *E. coli* ATCC35218 were used as quality control organisms. The phenotype of *A. baumannii* isolates was defined as MDR, XDR, and pandrug resistance (PDR), according to the international expert proposal for interim standard guidelines (Magiorakos et al. 2012).

Characterization of genetic relatedness among isolates

To evaluate the genetic relatedness between the two isolates, we performed three molecular methods as follows:

Identification of international clone types by multiplex PCR

International clonal (IC) types were determined based on presence or absence of the alleles of the outer membrane protein A (*omp*A), chaperone-subunit usher E (*csu*E), and the intrinsic carbapenemase ($bla_{\text{OXA-51-like}}$)-encoding genes in two simultaneously multiplex PCRs, as previously described (Turton et al. 2007).

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed according to the Bartual method described previously (Bartual et al. 2005). Allele sequences, clonal complexes (CC), sequence types (STs), primer sequences, and other details are available from the MLST website at http://pubmlst.org.

Multiple loci VNTR analysis

Isolates were genotyped using the multiple loci variable number tandem repeat (VNTR) analysis (MLVA)-8 scheme method developed by Pourcel et al. (2011).

Analysis of LPS

Molecular analysis

PCR and sequencing on lipid A biosynthesis genes including *lpxA*, *lpxC*, and *lpxD* were performed using the primers described by Moffat et al. (Moffatt et al. 2010) to determine if the

increase in colistin MIC was referring to complete loss of LPS resulting from any mutation in LPS biosynthesis genes, or not.

PAGE analysis

LPS purification from overnight cultures of *A. baumannii* isolates, was performed, followed by polyacrylamide gel electrophoresis (PAGE) using the previously described methods by Moffat et al. (Moffatt et al. 2010) to determine if the LPS was intact or not.

Analysis of PmrAB system

Analysis of PmrAB systems was carried out using sequencing and real-time PCR methods as previously described (Adams et al. 2009; Park et al. 2011; Beceiro et al. 2011). Briefly, DNA sequence of *pmrA* and *pmrB* genes between two isolates was compared, and then the expression of these genes was assessed using the real-time PCR method.

AbaR mapping and AbaR-associated genes detection

Genomic DNA used as a template for PCR amplifications was extracted using the High Pure PCR Template Preparation Kit (Roche Inc., Mannheim, Germany), following the manufacturer's instructions. For the purpose of AbaR mapping and detection of AbaR-associated genes, a combination of PCR, restriction fragment length polymorphism (RFLP), and sequencing methods was used using primers and conditions described previously by Krizova et al. (2011). Up to 40 PCR reactions were performed for each isolate. Long PCR amplicons were amplified by AccuPower ProFi Taq PCR PreMix (Bioneer, South Korea). PCR products were purified for sequencing after separation on agarose gel using the GF-1 PCR Clean up Kit (Vivantis Inc., Malaysia), following the manufacturer's instructions. Direct sequencing was determined, assembled, compared to sequences in the GenBank database, and annotated. Sequencing was performed in an ABI Prism 377 sequencer using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit V2 (PE Applied Biosystems, Foster City, CA), according to the manufacturer's recommendations.

A. baumannii strain NIPH321, dedicated kindly by Alexander Nemec, was used as AbaR3 positive control.

GenBank accession number

The partial nucleotide sequences of the AbaR structures have been deposited in the GenBank database under accession numbers KU880771 (AbaR from *A. baumannii* strain TEH267), KU880772 (AbaR from *A. baumannii* strain TEH273), and KU508671 (IS*26*).

Results

The isolates were identical at all MLST (1-3-3-2-2-11-3) and VNTR marker loci (12-7-5-5-9-17-40-57), which indicated that the isolates were the same strain. The two isolates belonged to CC-92 (ST75) and the international clone II (IC II). Both isolates were resistant to imipenem, meropenem, ceftazidime, cefepime, ceftriaxone, piperacillin, piperacillin-tazobactam, gentamicin, tobramycin, amikacin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, rifampin, and aztreonam and were susceptible to tigecycline and ampicillin-sulbactam, and both isolates revealed the XDR phenotypes. According to the definition (Cai et al. 2012), *A. baumannii* strain TEH267 (MIC = 1.5 mg/L) was considered as colistin heteroresistance while *A. baumannii* strain TEH273 (MIC ≥256 mg/L) was considered as colistin resistant (Table 1). By molecular and PAGE analysis of LPS, *A. baumannii* strain TEH267 and *A. baumannii* strain TEH273 isolates both did not have any mutation in their lipid A biosynthesis genes and had an intact LPS layer in their outer membranes. By sequencing of *pmrA* and *pmrB* genes in these two isolates, there was no difference in both isolates, and by real-time PCR, there was not a significant difference in expression of these genes in both isolates. The reasons for eightfold increase in colistin MIC were revealed by

Table 1 Characteristics of the *A. baumannii* strains used in the present study

Strain name	City, country, year of isolation	IC (international clone)	Gene cassettes of AbaR-associated class 1 integron	AbaR type	MIC (μg/mL)			
					CST	TGC	MEM	IMP
NIPH321	Tábor, CZ, 1994	IC II	*aacC1-(orfP)2-orfQ-aadA1*	AbaR3	0.5	0.25	0.5	1
TEH267	Tehran, Iran, 2012	IC II	None	AbaR TEH267	1.5	1.5	12	32
TEH273	Tehran, Iran, 2012	IC II	None	AbaR TEH273	>256	1.5	12	32

CST colistin, *TGC* tigecycline, *IMP* imipenem, *MEM* meropenem

structural analysis of AbaRs. AbaR mapping of these two isolates indicated a large of region deletions and a major difference with the typic AbaR3 structure. In comparison to the AbaR from *A. baumannii* strain TEH267 (AbaRTEH267), the AbaR from *A. baumannii* strain TEH273 (AbaRTEH273) acquired two genomic regions in the hospitalization period. These regions were Tn*6018*-*topA* sequence and *topA* sequence-3′ CS (Fig. 1). These two regions contained six genes at least, including *cadA* (cadmium-translocating ATPase), *ispA* (geranyltranstransferase), *tpnA* (transposase IS*26*), *topA* (topoisomerase I), *sul1* (sulphonamide resistance gene), and *orf5* (acetyltransferase) (Fig. 2). Up to 30 AbaR-associated genes (mostly antimicrobial resistance genes) were assessed in our isolates. *A. baumannii* strain TEH267 and *A. baumannii* strain TEH273 showed complete resemblance except for six additional genes which were present in strain TEH273, but not in strain TEH267. These genes can be associated with the notable increase in colistin MICs. Among AbaR-associated genes, IS*26*, a well-known transposase, was present in both isolates and considered to have a major role in construction of AbaR new variants. Of these two colistin resistance isolates, both were positive for $bla_{\text{OXA-23-like}}$ carbapenemase gene. Additionally, IS*Aba1* was also present upstream of $bla_{\text{OXA-23-like}}$ gene in both isolates. This explains the high degree of resistance to carbapenems among these isolates. Other investigated genes such as $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, bla_{VIM}, bla_{IMP}, bla_{SPM}, and bla_{NDM} were negative.

Discussion

Primary exposure to colistin and therefore the natural selection process contribution during the therapy period could lead to selection of the resistant subpopulations with lost or modified LPS and eventually emergence of CoR-AB clones. MICs ranged from 4 to >256 mg/L among CoR-AB clinical isolates, showing that multifactors probably involved in colistin resistance and other factors should be considered (Qureshi et al. 2015).

Since we isolated both *A. baumannii* isolates from a single burn patient during the antimicrobial therapy in the hospitalization period, we considered the possibility of selection pressure to be a major factor in emergence of an isolate with colistin MIC of ≥256 mg/L. LPS and PmrAB system analysis were performed to examine the intactness and modifications of LPS in these isolates, but the results rejected these possibilities. Both isolates had an intact LPS in their outer membranes and this rejected the possibility of complete loss of LPS as the result of mutations in lipid A synthesizing genes (*lpxA*, *C*, *D*). PmrAB system analysis by sequencing and real-time PCR showed no significant difference in sequences and expression of these two-component system genes in both isolates, and therefore, the possibility of PmrAB system involvement was rejected too. In continuation of the present study, we focused on AbaR maps of these two *A. baumannii* isolates as the resistance gene reservoirs of the genus. We showed that *A. baumannii* strain TEH273 acquired two genomic regions in hospitalization period and these regions had at least six additional genes.

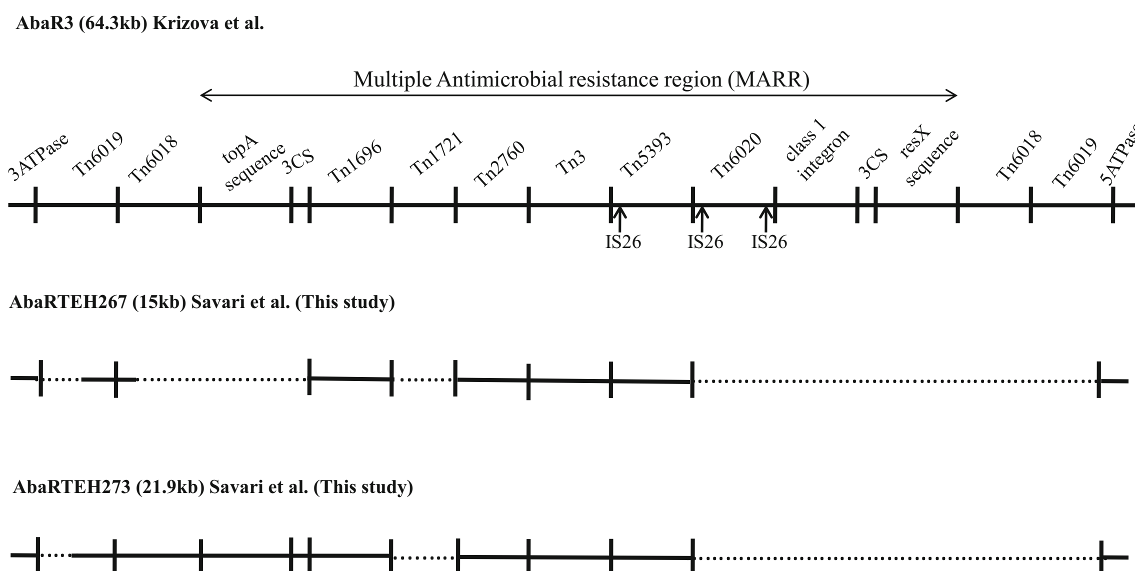

Fig. 1 Schematic overview of AbaR variants in our clinical isolates. An AbaR3 map is shown at the *top*. Our local AbaR variants are lined up *below* AbaR3 according to the presence of the regions homologous to those of AbaR3. *Unbroken and dashed horizontal lines* indicate, respectively, the AbaR3 regions present or absent in each of the AbaRs. Partial deletion of an AbaR3 component is indicated by the *absence of a vertical line*. The sequences of AbaR variants among colistin resistance *A. baumannii* isolates TEH267 (AbaRTEH267) and TEH273 (AbaRTEH273) are available in the GenBank database under accession numbers KU880771 and KU880772, respectively

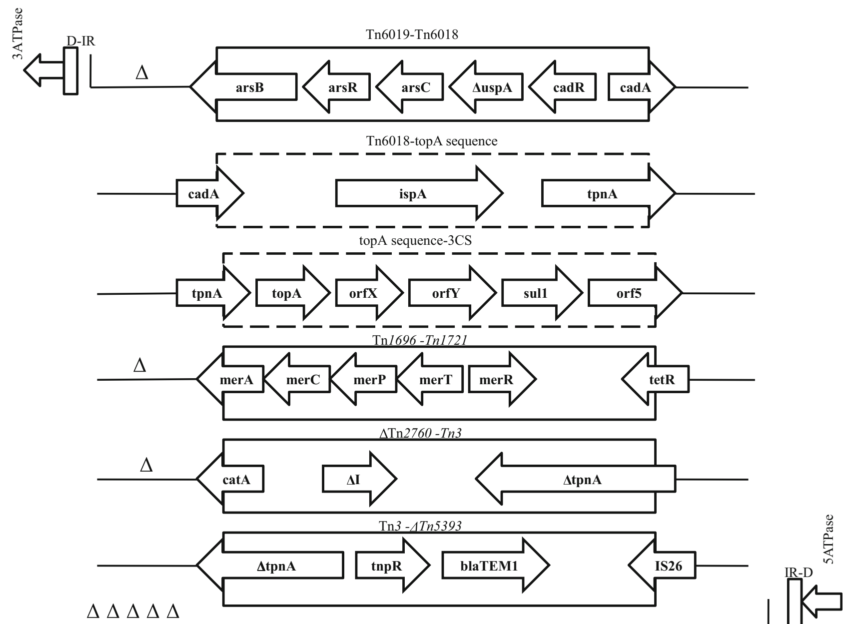

Fig. 2 AbaR mapping of *Acinetobacter baumannii TEH273* isolate according to PCR-RFLP and sequencing analysis. *IR-D* represents 5 bp direct repeats (D) and 26 bp imperfect terminal inverted repeats (IR). *RectanglesPDR with dashed lines* represent the regions missing in *TEH267* but present in *TEH273*. *Triangles* represents the numbers of regions missing in TEH267 and TEH273 strains but present in typical AbaR3

Among these genes, existence of *ispA* and *cadA* genes might be associated with the eightfold increase in colistin MICs. The *ispA* encodes for a geranyltranstransferase. This enzyme has a role in synthesis of ubiquinone, isoprenoids, and some other components. Ubiquinone is a key electron carrier in the electron transport chain of cellular respiration, and isoprenoids serve as biosynthetic precursors for lipids, the key component of the outer membrane LPS. Geranyltranstransferase probably could transfer some components to lipid A of *A. baumannii* LPS and leads directly to colistin resistance (Mac Síomóin et al. 1996). Recently, a set of studies in China, Europe, and the USA reported a plasmid-borne colistin resistance gene; *mcr-1* encodes for a transferase enzyme. These studies assigned the phosphorylethanolamine transfer to lipid A and eventually colistin resistance, to this gene (Liu et al. 2016). Existence of the *cadA* gene on the other hand acts as cadmium-translocating ATPase, effluxing ambivalence cations such as cadmium, and leads to decrease the negative charge around lipid A which might lead to colistin resistance. *CadA* is also involved in environmental sensing of pH and ion concentration, the same role of the PmrAB system, and could affect the expression of some superficial component-related genes. Cadmium also can act as a pollutant stress that leads to transient repression of the synthesis of the outer membrane protein *OmpF* and also to a transient decrease in the rate of synthesis of other proteins. It is known that the decreased expression of *OmpF* causes resistance to multiple antibiotics. These changes also might lead to a decrease in uptake of colistin (Kishii and Takei 2009; Faber et al. 1993). It is obvious that *A. baumannii* strains harboring these genes are not necessarily colistin resistant, because of the different gene expression patterns in different organisms and environments. On the other hand, constructing *ispA* and *cadA* mutants could be very confirmative in proving the role of these genes in colistin resistance.

In the present study, we reported new variants of AbaRs in our clinical isolates. Deletions in the right hand of *ATPase* gene caused by IS*26*-mediated deletions were prevalent and led to omission of Tn*6020* and other regions coming after this transposon. Not completely like AbaR6 and AbaR7, the left hand of *ATPase* gene in our clinical isolates had partial deletions, but with PCR sequencing on 3′ CS-*resX* sequence region, AbaR10 determinant, amplification was performed. So, these were new variants of AbaR-like structures (Fig. 1). These structures derive from the complete AbaR3-type islands through deletion events mediated by IS*26*. The importance of IS*26* is its ability to induce mutations with a high rate through deletion and acquisition of genomic regions. According to the fact that IS*26*-mediated deletions are nonspecific, additional AbaR variants can be expected in clinical populations (Krizova et al. 2011).

The heteroresistance rate of *A. baumannii* to colistin is generally higher than the resistance rate which suggests that

the prescription of sublethal colistin dosage could lead to emergence of resistant subpopulations. Mutation preventing concentration (MPC) experiments showed that 90% of the *A. baumannii* isolates need at least >128 mg/L of antibiotic concentration to prevent mutation which is higher than the colistin plasma concentration, achieved by the routine dosage strategy which has used in clinical centers (Cai et al. 2012).

CoR-AB is considered as XDR phenotype and it is difficult to omit its reservoirs in hospital environments (Weber et al. 2010). There are some reports about outbreaks of colistin-resistant *A. baumannii* infections in the ICU wards, showing that all isolates belong to a single clone (Valencia et al. 2003).

Colistin is the last-line therapy for infections caused by carbapenem resistance *A. baumannii*, and therefore, emergence of colistin resistance must be considers as a source of anxiety. Nowadays, emergence of simultaneous resistances to colistin and carbapenem, and thus PDR strains have been reported in many studies and have proved that colistin resistance emerged from a single progenitor colistin-susceptible isolate (Viehman et al. 2014; Lesho et al. 2013). Here, we reported coincidental resistance to colistin and to the carbapenems and also showed that the colistin-heteroresistant and colistin-resistant *A. baumannii* were derived from a single clone. Both isolates included in this study belonged to the same international clone (IC II) and were identical at all MLST and VNTR marker loci and therefore emerged from a single ancestor colistin-susceptible isolate. Recently in Iran, cases with resistance to colistin among local *A. baumannii* clinical isolates have been reported (Bahador et al. 2013; Zahedi Bialvaei and Samadi Kafil 2015; Sepahvand et al. 2015; Moradi et al. 2015). Thus, colistin susceptibility testing should be considered in the purpose of effective therapy, especially in treating patients with prior exposure to this antimicrobial agent. According to these data, focusing on colistin resistance and screening for such a phenomenon should be unstoppable in health systems. The clonal dissemination of *A. baumannii* infections is another problem and should be considered as an important issue.

According to the results of this study, AbaRs and AbaR-associated genes, including *cadA* and *ispA*, could be associated with an increase in MIC to colistin. This is the first report to show an association between differences in AbaR structures and colistin resistance in *A. baumannii* clinical isolates.

Acknowledgements We thank Lenka Krizova, Lenie Dijkshoorn, and Alexandr Nemec for providing *Acinetobacter baumannii* strain NIPH321 that we used as positive control for AbaR3.

Compliance with ethical standards

Funding This research has been supported by Tehran University of Medical Sciences & Health Services, Grant No. 93-04-103-27532.

Transparency declarations We have none to declare.

References

Adams MD, Nickel GC, Bajaksouzian S, Lavender H, Murthy AR, Jacobs MR, Bonomo RA (2009) Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the pmrAB two component system. Antimicrob Agents Chemother 53:3628–3634

Bahador A, Taheri M, Pourakbari B, Hashemizadeh Z, Rostami H, Mansoori N, Raoofian R (2013) Emergence of rifampicin, tigecycline, and colistin-resistant *Acinetobacter baumannii* in Iran; spreading of MDR strains of novel international clone variants. Microb Drug Resist 19:397–406

Bahador A, Raoo An R, Farshadzadeh Z, Beitollahi L, Khaledi A, Rahimi S, Mokhtaran M, Mehrabi Tavana A, Esmaeili D (2015) The prevalence of ISAba 1 and ISAba 4 in *Acinetobacter baumannii* species of different international clone lineages among patients with burning in Tehran, Iran. Jundishapur J Microbiol 8:e17167

Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodríguez Valera F (2005) Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii. J Clin Microbiol 43:4382–4390

Beceiro A, Llobet E, Aranda J, Bengoechea JA, Doumith M, Hornsey M, Dhanji H, Chart H, Bou G, Livermore DM, Woodford N (2011) Phosphoethanolamine modification of lipid A in colistin-resistant variants of *Acinetobacter baumannii* mediated by the pmrAB two-component regulatory system. Antimicrob Agents Chemother 55: 3370–3379

Cai Y, Chai D, Wang R, Liang B, Bai N (2012) Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 67:1607–1615

Cheah SE, Li J, Tsuji BT, Forrest A, Bulitta JB, Nation RL (2016) Colistin and polymyxin B dosage regimens against Acinetobacter baumannii: differences in activity and the emergence of resistance. Antimicrob Agents Chemother 60(7):3921–3933

Faber F, Egli T, Harder W (1993) Transient repression of the synthesis of OmpF and aspartate transcarbamoylase in *Escherichia coli* K12 as a response to pollutant stress. FEMS Microbiol Lett 111:189–195

Gallagher LA, Ramage E, Weiss EJ, Radey M, Hayden HS, Held KG, Huse HK, Zurawski DV, Brittnacher MJ, Manoil C (2015) Resources for genetic and genomic analysis of emerging pathogen Acinetobacter baumannii. J Bacteriol 197:2027–2035

Higgins PG, Lehmann M, Wisplinghoff H, Seifert H (2010) gyrB multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3. J Clin Microbiol 48:4592–4594

Karaha N, Sundsfjord A, Townerc K, Samuelsena Ø (2012) Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. Drug Resist Updat 15:237–247

Kishii R, Takei M (2009) Relationship between the expression of ompF and quinolone resistance in *Escherichia coli*. J Infect Chemother 15: 361–366

Krizova L, Dijkshoorn L, Nemec A (2011) Diversity and evolution of AbaR genomic resistance islands in *Acinetobacter baumannii* strains of European clone I. Antimicrob Agents Chemother 55: 3201–3206

Lesho E, Yoon EJ, McGann P, Snesrud E, Kwak Y, Milillo M, Onmus-Leone F, Preston L, St Clair K, Nikolich M, Viscount H, Wortmann G, Zapor M, Grillot-Courvalin C, Courvalin P, Clifford R, Waterman PE (2013) Emergence of colistin-resistance in extremely drug-resistant Acinetobacter baumannii containing a novel pmrCAB operon during colistin therapy of wound infections. J Infect Dis 208: 1142–1151

Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, Liolios L (2006) Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 50(9): 2946–2950

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16:161–168

Mac Síomóin RA, Nakata N, Murai T, Yoshikawa M, Tsuji H, Sasakawa C (1996) Identification and characterization of ispA, a Shigella flexneri chromosomal gene essential for normal in vivo cell division and intracellular spreading. Mol Microbiol 19:599–609

Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas MF, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug resistant, extensively drug-resistant and pandrug- resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:268–281

Moffatt JH, Harper M, Harrison P, Hale JDF, Vinogradov E, Seemann T, Henry R, Crane B, Michael F, Cox AD, Ben Adler B, Nation RL, Li J, Boyce JD (2010) Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 54:4971–4977

Moradi J, Hashemi BF, Bahador A (2015) Antibiotic resistance of *Acinetobacter baumannii* in Iran: a systemic review of the published literature. Osong Public Health Res Perspect 6:79–86

Pagano M, Martins AF, Barth AL (2016) Mobile genetic elements related to carbapenem resistance in *Acinetobacter baumannii*. Braz J Microbiol. doi:10.1016/j.bjm.2016.06.005

Park YK, Choi JY, Shin D, Ko KS (2011) Correlation between overexpression and amino acid substitution of the PmrAB locus and colistin resistance in Acinetobacter baumannii. Int J Antimicrob Agents 37:525–530

Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA (2007) Global challenge of multidrug resistant Acinetobacter baumannii. Antimicrob Agents Chemother 51:3471–3484

Poirel L, Bonnin RA, Nordmann P (2011) Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. IUBMB Life 63: 1061–1067

Post V, White PA, Hall RM (2010) Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 65:1162–1170

Pourcel C, Minandri F, Hauck Y, D'Arezzo S, Imperi F, Vergnaud G, Visca P (2011) Identification of variable-number tandem-repeat (VNTR) sequences in *Acinetobacter baumannii* and interlaboratory validation of an optimized multiple-locus VNTR analysis typing scheme. J Clin Microbiol 49:539–548

Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, Pasculle AW, Ernst RK, Doi Y (2015) Colistin-resistant Acinetobacter baumannii: beyond carbapenem resistance. Clin Infect Dis 60:1295–1303

Sepahvand V, Davarpanah MA, Hejazi SH (2015) Epidemiology of colistin-resistant *Acinetobacter baumannii* in Shiraz, Iran. J Appl Environ Biol Sci 5:45–48

Stokes HW, Gillings MR (2011) Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiol Rev 35:790–819

Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL (2007) Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. Clin Microbiol Infect 13:807–815

Valencia R, Arroyo LA, Conde M, Aldana JM, Torres MJ, Fernández-Cuenca F, Garnacho-Montero J, Cisneros JM, Ortíz C, Pachón J, Aznar J (2003) Nosocomial outbreak of infection with pan-drug-resistant *Acinetobacter baumannii* in a tertiary care university hospital. Infect Control Hosp Epidemiol 30:257–263

Viehman JA, Nguyen MH, Doi Y (2014) Treatment options for carbapenem-resistant and extensively drug-resistant *Acinetobacter baumannii infections*. Drugs 74:1315–1333

Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E (2010) Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control 38:S25–S33

Zahedi Bialvaei A, Samadi Kafil H (2015) Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin 31:707–721