Profile of Eight Prophage Sequences Present in the Genomes of Different *Acinetobacter baumannii* Strains

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ABSTRACT

Background and Objective: Prophage sequences are major contributors to interstrain variations within the same bacterial species. *Acinetobacter baumannii* is a gram-negative bacterium that causes a wide range of nosocomial infections, especially in intensive care unit inpatients. Prophage sequences constitute a considerable proportion of several sequenced complete genomes of *A. baumannii*. The aim of this study was to investigate the presence of prophage sequences in *A. baumannii* strains isolated from burn patients, and compare the results with other studies.

Methods: Presence of eight prophage sequences was investigated in the genome of ten multi-drug resistant *A. baumannii* isolates obtained from burn sites of 10 burn patients in a hospital in Isfahan, Iran. PCR and sequencing were performed to detect the prophage sequences. The presence of the eight prophage sequences in the genome of *A. baumannii* strains from other studies was investigated by BLAST analysis of whole nucleotide sequence of prophage sequences.

Results: The isolates in the present study had different prophage sequence profiles. Two isolates did not contain any of the sequences, while two isolates contained three and two of the prophage sequences. Other isolates contained only one sequence. The prophage sequence profiles observed in this study were not found in *A. baumannii* isolates from other studies.

Conclusion: The results of this study indicate that the prophage sequences profile can be useful for studying the epidemiology of *A. baumannii* strains.

Keywords: *Acinetobacter baumannii*, genome, prophage sequences.

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INTRODUCTION

Horizontal gene transfer is the main cause of genetic variation (evolutionary change) in bacteria. Temperate bacteriophages (prophages) are recognized as important agents in horizontal gene transfer. Phages play an important role in the emergence of new bacterial pathogens or epidemic clones by introducing new fitness factors, virulence factors and antibiotic resistance genes to bacteria (1-4). Prophage sequences have been identified in genomes of thousands of sequenced bacteria. Prophage sequences constitute up to 20% of the bacterial genome, and are major contributors to interstrain variations within a same bacterial species (2, 5-7). Thus, investigation of the presence of the prophage sequences in the bacterial genome could be useful for rapid identification of the epidemic strains of pathogens.

Acinetobacter baumannii is one of the important pathogens that contained the prophage sequences in its genome. This gram-negative bacterium causes a wide range of nosocomial infections, especially among patients in intensive care unit (8-11). Several complete genome sequences have been registered for *A. baumannii* in NCBI database. A number of prophage sequences have been identified in each of these complete genome sequences, which constitute a considerable proportion of the genome, and are usually different among the sequenced genomes. However, identical prophage sequences may be present in different strains. *A. baumannii* AYE and *A. baumannii* SDF have six and eight putative prophage regions (5.1% and 6.7% of the genome) with no similarity within and between two genomes, respectively (12). In study of Adams et al., a disproportionate number of likely cryptic prophage regions were identified in genomes of different strains of *A. baumannii* (*A. baumannii* AB0057, AB307-0294, AB900, AYE, ACICU, ATCC 17978 and SDF), and one prophage was identified in different genomic locations of two *A. baumannii* strains (*A. baumannii* AB0057 and *A. baumannii* ACICU) (13). By comparing the whole genome sequences of *A. baumannii* ACICU (an epidemic, multidrug-resistant *A. baumannii* strain) and *A. baumannii* ATCC 17978, Iacono et al. (2008) identified four prophage regions in the ACICU genome that were absent in the ATCC 17978 The "prophage LambdaSo, transcriptional genome (14). Iacono et al. (2008) also identified a phage sequence in *A. baumannii* ACICU plasmid pACICU1 (14). Golemboski and Eardly identified two prophages with sequences identical to that of *Acinetobacter* phage Bphi-B1251 and *Psychrobacter* phage Psymv2 in genomes of two *A. baumannii* isolates obtained from two different body sites of the same patient (*A. baumannii* BU310 from a urinary catheter and *A. baumannii* BR097 from a sputum specimen). They also detected another prophage (enterobacterial phage phiX174) in strain BR097. Overall, they identified 134 and 143 phage genes in the BU310 and BR097 strains, respectively (15). Huang et al. detected many phage-like elements in the *A. baumannii* MDR-TJ plasmid pABTJ2. Nine of these phage-like genes encoded proteins related to DNA packaging/morphogenesis and host-cell lysis, while seven of the genes were similar to those of the *Salmonella* phage SSU5 (16).

Due to the importance of prophage sequences in interstrain diversity of *A. baumannii*, this study aimed to detect already identified prophage sequences in 10 *A. baumannii* strains isolated from burn sites of 10 burn patients.

MATERIAL AND METHODS

Presence of prophage sequences was investigated in genome of 10 multi-drug resistant *A. baumannii* isolates (*A. baumannii* ImdAb04, ImdAb06, ImdAb10, ImdAb12, ImdAb35, ImdAb47, ImdAb48, ImdAb68, ImdAb72 and ImdAb74) obtained from burn sites of 10 burn patients in ICU of a hospital in Isfahan, Iran (17). Among the prophage sequences investigated, eight have been identified in the whole genome sequences of different *A. baumannii* strains from different parts of the world (Table 1), three of which were related to *A. baumannii* SDF isolated from human body louse.

Identification of the prophage sequences in the *A. baumannii* isolates was done by PCR using primers (designed by Primer-Blast) specific for each of the eight prophage sequences (Table 1). PCR was carried out using a 25 µl reaction mixture containing 3 µl of extracted bacterial DNA (by boiling method), one unit of Smar *Taq* DNA Polymerase (Cinnagen, Iran), 0.4 µM of each primer (forward and

reverse), 0.2 mM of each dNTP, 20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$ and sterile double-distilled water. Thermal conditions of the amplification process for all reactions were as follows: initial denaturation at 95 ˚C for 7 min, 35 cycles of denaturation at 95 ˚C for 30 sec, annealing at 55 ˚C for 30 sec, extension at 72 ˚C for 30 sec, and final extension at 72 ˚C for 5 min. The prophage sequences were identified in PCR products extracted from agarose gel using GeneJET Gel Extraction Kit (Fermentas, Germany) according to the manufacturer's protocol. The extracted products were sent to Macrogen Company (Republic of Korea) for sequencing. Results of sequencing were analyzed using Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Presence of the eight prophage sequences in the genome of *A. baumannii* strains form other studies was assessed by BLAST analysis of whole nucleotide sequence of prophage sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Nucleotide sequences of the four prophages identified in this study are available on the GenBank nucleotide database under accession numbers KP677275, KP677276, KP677277 and KP677278.

RESULTS

All three putative prophage integrase sequences and peptidase U35 phage prohead HK97 sequence were not found in any of the 10 *A. baumannii* isolates tested using the 17978i, SDF2, SDF3 and HK97 primers.

The "bacteriophage protein; prophage terminase large subunit" sequence was detected in *A. baumannii* ImdAb68 using SDF1 primers (Table 2). Sequencing and BLAST analysis of the 851 bp of obtained sequence revealed 96% nucleotide sequence identity with the sequence of *A. baumannii* SDF, and 93% identity with the sequences of *A. baumannii* MDR-TJ and *A. baumannii* MDR-ZJ06.

The "prophage LambdaSo, transcriptional regulator, Cro/CI family" sequence was detected in *A. baumannii* ImdAb35, ImdAb47, ImdAb48 and ImdAb68 isolates using AB57C primers (Table 2). The 418 bp obtained sequence had 99% nucleotide sequence identity with that of *A. baumannii* AB0057, *A. baumannii* ACICU and *A. baumannii* TCDC-AB0715, and 86% identity with that of *A. oleivorans* DR1. BLAST analysis also revealed that 80% of this 418 bp is present in the genome of *A. baumannii* 1656-2 with 75% nucleotide sequence identity.

The sequence for "prophage LambdaBa04, DnaD replication protein" was detected in the genomes of *A. baumannii* ImdAb04, ImdAb06, ImdAb10, ImdAb68 and ImdAb72 isolates using AB57D primers (Table 2). The 595 bp sequence obtained had 100% nucleotide sequence identity with the sequences of *A. baumannii* AB0057 and *A. baumannii* BJAB0868, and 99% identity with the sequence of *A. baumannii* AB031. BLAST analysis also revealed that 22% of this 595 bp sequence is present in *A. baumannii* lytic bacteriophage IME-AB2 (a member of the *Myoviridae* family) (18) and *Acinetobacter* phage YMC-13-01-C62 (belonging to *Myoviridae*) (http://www.ncbi.nlm.nih.gov) with 70% identity.

The "prophage antirepressor" sequence was detected in *A. baumannii* ImdAb04 (Table 2) using TCDC primers. The 370 bp sequence obtained had 97% nucleotide sequence identity with the sequence of *A. baumannii* TCDC-AB0715, and 96% identity with the sequence of *A. baumannii* SDF. BLAST analysis also revealed that part of the sequence for the "prophage antirepressor" investigated in this study is present in the genomes of *A. baumannii* ACICU and *A. baumannii* AB030. PCR products with a size close to the size of expected products were observed for *A. baumannii* ImdAb06, ImdAb10 and ImdAb12 using TCDC primers. Sequencing of these products revealed that they were not the expected "prophage antirepressor" sequence.

Table 1- Primers designed for detection of prophage sequences in *A. baumannii* isolates from burn wounds.

Name	Sequence (5' to 3')	Target region	*A. baumannii* strain (Reference)	Product length (bp)
17978iF	CGCTTAGACTTGGGCACCTA	Phage integrase[a]	*A. baumannii* ATCC 17978 (24)	836
17978iR	TGTCGTCTTAGGCGTTCCAT			
HK97F	TCACATGGCGACATCATCCG	Peptidase U35 phage prohead HK97	*A. baumannii* ATCC 17978 (24)	510
HK97R	TTCACCCTCTGACTCGCTCA			
SDF1F	GAGGGCATGAGCTACGTTGT	Bacteriophage protein; prophage terminase large subunit	*A. baumannii* SDF (12)	1270
SDF1R	TCACGCTATCTGCTCGGTTC			
SDF2F	CTCTAGCAGAAGCCCGCATA	Putative prophage integrase[a]	*A. baumannii* SDF (12)	1030
SDF2R	GAGTGGCCAATCACAGCTTC			
SDF3F	ACCCGTTACCCGTATGCTTT	Putative prophage integrase[a]	*A. baumannii* SDF (12)	983
SDF3R	TCATGCAGTCGCGTTTCTGA			
AB57CF	GCTAAATGGGCAGGCGTAAC	Prophage LambdaSo, transcriptional regulator, Cro/CI family	*A. baumannii* AB0057 (13)	476
AB57CR	GCCTTGAAGCCACCTTTACC			
TCDCF	GTGGCTATATTTCAGGTCAG	Prophage antirepressor	*A. baumannii* strain TCDC-AB0715 (25)	418
TCDCR	ATTGCAAGCCAAGCATCTCG			
AB57DF	TCGTCCGACTATTGTCCGTG	Prophage LambdaBa04, DnaD replication protein	*A. baumannii* AB0057 (13)	651
AB57DR	GCTGGCTTCCAGTTGTAACG			

Table 2- Comparison of different *A. baumannii* strains (present study and previous studies) on the presence of the eight prophage sequences investigated in this study.

A. baumannii strain	Country, year of isolation (reference)	Prophage sequences							
		Seq. 1	Seq. 2	Seq. 3	Seq. 4	Seq. 5	Seq. 6	Seq. 7	Seq. 8
ATCC 17978	France, 1951 (26)	+	+	-	-	-	-	-	-
AB030	Canada, 2006-2009 (27)	+	-	-	+*	-	-	-	+*
D1279779	Australia, 2009 (28)	+	-	-	-	-	-	-	-
IOMTU 433	Japan (http://www.ncbi.nlm.nih.gov)	+	-	-	+	-	-	-	-
AB0057	USA, 2004 (13)	+	-	-	-	-	+	+	-
NCGM 237 DNA	Japan, 2012 (29)	-	+	-	+*	-	-	-	-
BJAB0868	China, 2008 (22)	-	+	-	-	-	-	+	-
BJAB07104	China, 2007 (22)	-	+	-	-	-	-	-	-
TYTH-1	Taiwan, 2008 (23)	-	+	-	-	-	-	-	-
MDR-TJ	China (30)	-	+	-	-	+	-	-	-
SDF	France, 1997 (31)	-	-	+	+	+	-	-	+
AbH12O-A2	Spain, 2006-2008 (32)	-	-	-	+*	-	-	-	-
TCDC-AB0715	Taiwan, 2007-2009 (25)	-	-	-	+*	-	+	-	+
AB031	Canada, 2006-2009 (27)	-	-	-	+*	-	-	+	-
1656-2	South Korea, 2004-2005 (33-35)	-	-	-	+*	-	+*	-	-
MDR-ZJ06	China, 2006 (36)	-	-	-	-	+	-	-	-
ACICU	Italy, 2005 (14)	-	-	-	-	-	+	-	+*
ImdAb04	Iran, 2014 (17)	-	-	-	-	-	-	+	+
ImdAb06	Iran, 2014 (17)	-	-	-	-	-	-	+	-
ImdAb10	Iran, 2014 (17)	-	-	-	-	-	-	+	-
ImdAb12	Iran, 2014 (17)	-	-	-	-	-	-	-	-
ImdAb35	Iran, 2013 (17)	-	-	-	-	-	+	-	-
ImdAb47	Iran, 2013 (17)	-	-	-	-	-	+	-	-
ImdAb48	Iran, 2013 (17)	-	-	-	-	-	+	-	-
ImdAb68	Iran, 2013 (17)	-	-	-	-	+	+	+	-
ImdAb72	Iran, 2013 (17)	-	-	-	-	-	-	+	-
ImdAb74	Iran, 2013 (17)	-	-	-	-	-	-	-	-
Other species of *Acinetobacter*									
A. oleivorans DR1	South Korea, isolated from soil (37)	-	-	-	-	-	+	-	-
A. guillouiae NBRC 110550	Japan, isolated from soil (38)	-	-	+*	-	-	-	-	-

Seq. 1- Phage integrase, Seq.2- Peptidase U35 phage prohead HK97, Seq.3: Putative prophage integrase, Seq.4: Putative prophage integrase, Seq.5: Bacteriophage protein; prophage terminase large subunit, Seq.6: Prophage LambdaSo, transcriptional regulator, Cro/CI family, Seq.7: Prophage LambdaBa04, DnaD replication protein, Seq.8: Prophage antirepressor

***Partial prophage sequence: using BLAST search, partial sequence of the complete expected prophage sequence was detected in bacterial genome**

DISCUSSION

The partial presence of phage sequences in the genome of the bacteria may be due to imprecise or mistaken excision of prophage, leaving part of the prophage in the bacterium (19-21). It could be also due to decay and inactivation of prophage to a defective one that lacks the excision and lysis ability (2, 4, 7). Sequences from one or multiple phages may be present in a bacterial genome. These integrations may confer phenotypic changes and increase survival fitness of the host, antibiotic resistance, pathogenicity, production of virulence factors such as toxins, and the metabolic potential of the bacterium (2, 5, 7). Furthermore, phage integration may cause gene disruption that may lead to loss of some bacterial functions or attaining new properties (2).

Profile of the eight tested prophage sequences was different in the tested bacteria. None of the sequences was identified in two bacteria (*A. baumannii* ImdAb12 and *A. baumannii* ImdAb74), while *A. baumannii* ImdAb68 and *A. baumannii* ImdAb04 contained three and two of the phage sequences, respectively. The rest of the isolates contained only one sequence. As shown in Table 2, different profiles of the eight sequences studied are present in the *A. baumannii* isolates from different parts of the world during different periods (containing 1 to 4 prophage sequences). In addition, these bacteria had different antibiotic resistance patterns. The prophage sequences profiles identical to those observed in this study were not present in the isolates from other parts of the world (Table 2). It has been suggested that prophage sequences are major contributors to diversification of strains within the same bacterial species (2, 3). This is further confirmed by our results and the difference in prophage sequences in *A. baumannii* strains isolated from different countries over different periods (13, 14, 22, 23, 25-36), indicating that the prophage sequences profile can be useful for studying the epidemiology of this bacterium.

A. baumannii ImdAb35, *A. baumannii* ImdAb47 and *A. baumannii* ImdAb48 that were isolated from patients hospitalized at the same time and place, had identical prophage sequences profiles. Moreover, *A. baumannii* ImdAb06, *A. baumannii* ImdAb10 and *A. baumannii* ImdAb72 that were isolated from patients hospitalized at different times but in the same place, had the same prophage sequences profile. These contradictory results may show that bacterial isolates from a hospital may have similar or different origins. According to previous studies, *A. baumannii* BJAB07104 isolate in China (22) and *A. baumannii* TYTH-1 isolate in Taiwan (23) have the same prophage sequences profile (due to close geographical location), while other isolates have different prophage sequences profiles (Table 2).

Since prophage sequences could constitute up to 20% of a bacterial genome (5-7), different strains of the same species that contain different prophage sequences may have different properties (e.g. different antibiotic resistance patterns). For example, *A. baumannii* AYE and *A. baumannii* SDF that contain different prophage sequences have different antibiotic resistance patterns (12). In this study, isolates ImdAb48 and ImdAb68 as well as isolates ImdAb72 and ImdAb74 had the same antibiotic resistance patterns while having different prophage sequences profiles. On the other hand, isolates ImdAb06, ImdAb10 and ImdAb72 with different antibiotic resistance patterns had the same prophage sequences profile. Isolates ImdAb35 and ImdAb47 from patients hospitalized at the same time had similar prophage sequences profile and antibiotic resistance pattern. Further studies with larger study populations and prophage sequences are required to explain and interpret the exact correlation between antibiotic resistance patterns and prophage sequence profiles. Two prophage sequences detected in this study were present in two other species of *Acinetobacter* (*A. oleivorans* DR1 and *A. guillouiae* NBRC 110550) (Table 2). This indicates that these species either have the same origin or have been infected by the same phage. In this study, isolates ImdAb35, ImdAb47 and ImdAb48 had prophage sequence profiles identical to that of *A. oleivorans* DR1, which is isolated from the soil of rice paddies (Table 2). This may indicate that different species of a genus can have identical prophage sequence profiles. It may also indicate occurrence of horizontal prophage sequence transfer among different species of a genus, or the same origin of these

isolates. However, these results need further testing using larger sample sizes.

The BLAST search revealed the partial presence of the "prophage LambdaBa04, DnaD replication protein" sequence in bacteriophages IME-AB2 (18) and YMC-13-01-C62 (http://www.ncbi.nlm.nih.gov) as well as in the genomes of five *A. baumannii* isolates in this study, and in the genome of three other isolates in other studies (Table 2). This could indicate bacterial infection with these bacteriophages. Considering the 100% identity of this sequence between the two bacteriophages, it can be concluded that different phages may be the origin of the same prophage sequences.

CONCLUSION

The results of this study indicate that the prophage sequences profile could be useful for studying the epidemiology and discrimination of epidemic strains of *A. baumannii*.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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