Journal of Global Antimicrobial Resistance 19 (2019) 210–215

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Tracing the dissemination of the international clones of multidrug-resistant *Acinetobacter baumannii* among cancer patients in Egypt using the PCR-based open reading frame typing (POT) method

Miran Y. El Far[a], Hadir A. El-Mahallawy[b], Ahmed S. Attia[c,*]

[a] Department of Microbiology and Immunology, Faculty of Pharmacy, Ahram Canadian University, Giza, Egypt
[b] Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt
[c] Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

ARTICLE INFO

Article history:
Received 26 February 2019
Received in revised form 23 July 2019
Accepted 25 July 2019
Available online 2 August 2019

Keywords:
POT type
Acinetobacter baumannii
Clonal diversity
Low cost, rapid typing
Cancer
Egypt

ABSTRACT

Objectives: The aim of this study was to perform an epidemiological surveillance of multidrug-resistant (MDR) *Acinetobacter baumannii* genetic lineages among cancer patients in Egypt using the PCR-based open reading frame typing (POT) method.
Methods: A total of 160 MDR *A. baumannii* isolates were collected between January 2015 and December 2017 at a tertiary-care centre in Egypt. VITEK® 2 system was used for preliminary species identification and antimicrobial susceptibility testing. The POT method was applied for confirmation of species identification and molecular epidemiological typing of the isolates.
Results: MDR *A. baumannii* isolates were classified into 15 POT types, including POT 122 ($n = 69$), POT 69 ($n = 22$) and other miscellaneous POT types (MPOTs) including POT 0, 8, 10, 12, 14, 40, 44, 52, 56, 93, 104, 106 and 108 ($n = 69$). POT 122 isolates infected or colonised 61% of patients hospitalised in surgical wards and 54% of patients diagnosed with solid tumours and 51% were adults; whereas MPOT isolates infected or colonised 51% of patients hospitalised in paediatric wards and 49% of patients diagnosed with haematological malignancies and 51% were paediatric patients ($P = 0.007$, 0.001 and 0.004, respectively). MPOT isolates were recovered from 46% of the collected blood specimens, whilst POT 122 isolates were recovered from 61% of the collected respiratory specimens ($P = 0.05$).
Conclusion: The current study demonstrates that the easy, low-cost POT method is convenient for rapid delineation of *A. baumannii* clonal diversity in a tertiary-care hospital in Egypt.

1. Introduction

There is an increasing incidence of multidrug-resistant (MDR) pathogens causing infections in high-risk patients, among which *Acinetobacter baumannii* is one of the most threatening. *Acinetobacter baumannii* is responsible for 2–10% of all Gram-negative hospital-acquired infections [1]. With regard to high-risk patients, MDR *A. baumannii* are widely spreading in intensive care units (ICUs) all over the world and are of particular interest in developing countries such as Egypt [2–4]. Moreover, among Egyptian cancer patients *A. baumannii* accounted for 10–13% of infections caused by the so-called 'ESKAPE' organisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) [5]. The morbidity and mortality rates associated with these bacterial infections are significant [5].

It is not yet clear which molecular typing method is more suitable to determine or confirm institutional outbreaks as well as to identify epidemiologically related isolates from different geographical areas [6]. The emergence of *A. baumannii* as an important nosocomial pathogen responsible for outbreaks has resulted in the need for deploying expeditious epidemiological typing methods. The newly described PCR-based open reading frame (ORF) typing (POT) method [7] has been shown to be capable of rapidly distinguishing *A. baumannii* sequence types (STs) corresponding to the international clones (IC1 and IC2) from other STs and *Acinetobacter* spp. in ordinary clinical laboratories without nucleotide sequencing analysis [7–10].

In this method, the distribution pattern of several ORFs that showed good correlation with the ST results generated by multilocus sequence typing (MLST) are selected to represent

* Corresponding author. Present address: Department of Microbiology and Immunology, Room #D404, Faculty of Pharmacy, Cairo University, Kasr El-Ainy Street, Cairo 11562, Egypt.
E-mail address: ahmed.s.attia@staff.cu.edu.eg (A.S. Attia).

http://dx.doi.org/10.1016/j.jgar.2019.07.025

specific *A. baumannii* clonal lineages [international clone 1 (POT 69) and international clone 2 (POT 122)] or *Acinetobacter* spp. by comparing their genome data [7,11]. Distribution patterns of the selected ORFs are visualised by agarose gel electrophoresis after multiplex PCR amplification and are digitised as numeric data with plus (expressed as 1) or minus (expressed as 0) depending on the presence or absence of the band of the amplicon DNA [7].

In the present study, the POT method proved to be of great help to public-health professionals towards more effective and affordable outbreak detection and prevention approaches in limited-resources countries such as Egypt. This method was successfully applied for daily surveillance and detection of outbreaks in their very early stages regarding MDR *A. baumannii* clinical isolates obtained over a 3-year period in a tertiary-care cancer institute in Cairo, Egypt.

2. Materials and methods

2.1. Study design

This was a laboratory-based surveillance study where MDR *A. baumannii* isolates were collected from clinical specimens during the period extending from January 2015 to December 2017. The study was carried out at the National Cancer Institute, Cairo University, which is a tertiary-care centre receiving cancer cases from all over Egypt. Isolates were recovered from different types of specimens including blood, respiratory secretions and surgical wound exudates. Study approval was obtained from the Research Ethics Committee of the Faculty of Pharmacy of Cairo University. Written informed consent was not obtained as the isolates were collected from the routine work of the clinical microbiology laboratory and no additional clinical specimens were collected for the purpose of the research.

2.2. Patient data

Data collected from cancer patients included age, sex, cancer diagnosis, length of stay in the ICU, duration of the infection episode, site of infection, outcome at the end of hospital stay, date of hospital admission, date of specimen collection and the respective hospital ward.

2.3. Bacterial isolates

Isolates were identified phenotypically as *A. baumannii* by VITEK®2 (bioMérieux, Marcy l'Étoile, France). Among these, 160 isolates were confirmed genotypically as *A. baumannii* by the presence of the *A. baumannii*-specific bla_{OXA-51} gene [12] and were used for the current study. Pure bacterial isolates were stored at −80 °C in 15% v/v glycerol in Mueller–Hinton broth.

2.4. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using a VITEK®2 system with the AST-GN73 antimicrobial susceptibility test card (bioMérieux) according to the manufacturer's instructions. Isolates were categorised as susceptible, intermediate-susceptible or resistant based upon the interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) [13].

2.5. PCR-based open reading frame typing (POT) genotyping

Epidemiological typing of all of the *A. baumannii* isolates was done by the POT method as described previously [7]. Briefly, seven ORFs are used to maximise the discriminatory power and reliability of the identification of the international clones IC1 and IC2 of *A. baumannii*. The ORFs with their respective primer pairs as well as one *A. baumannii*-specific ORF (bla_{OXA-51}) [12] are listed in Table 1. PCR was performed using standard procedures [14]. The seven ORFs were scored in the order of their amplicon size using a binary code with either '1' = present or '0' = absent. These scores were then converted to decimal numbers, where the results of each binary in the code were multiplied by 2^n (n = 6–0) and added.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v.22 (IBM Corp., Armonk, NY, USA). Numerical data were expressed as

Table 1
Primers used in the current study.

Target ORF	Primer direction	Sequence (5′→3′)[a]	Amplicon size (bp)	Final concentration (μM)
bla_{OXA-51}	Forward	GCTTCGACCTTCAAAATGCT	465	0.4
	Reverse	TCCAGTTAACCAGCCTACTTGT		0.4
AB57_2484	Forward	TATGTACAAAGCCAACCGGA	271	0.4
	Reverse	GAATTTGAGCdGAAGCCATTA		0.4
ACICU_02042	Forward1	CCGCGTCTTTCATAATAAGCAA	234	0.2
	Forward2	CCACGTCTCTCATAATAAGCAA		0.2
	Reverse1	TGGAGAAATAGATTCTTCAAAAGTTGT		0.2
	Reverse2	TGCAGAAATAGATTCTTCAmAAATTGT		0.2
ACICU_02966	Forward	ACCGTAyCCCTTTTTAATAAGTTCA	189	0.4
	Reverse	GGGCAAACTTATCATAGTTATATCGAC		0.4
ACICU_01870	Forward	GCTGCAACCCAACCAATwA	151	0.4
	Reverse	AATTGGCTTCGhTGGATATTTATG		0.4
AB57_3308	Forward	GCAACAGTTTCAAAATTAAATGG	122	0.4
	Reverse1	ACTGTTTGTATGGGTATTGCAG		0.2
	Reverse2	ACTGTTTGTATAGGCATTGCAG		0.2
ACICU_03137	Forward	CCyGCACTGCTCTACGATAATG	102	0.4
	Reverse	TTGyTCATAATGAAAAGCCGCA		0.4
AB57_0815	Forward	CTTTAGAmGAGGCACGTTGGTTTG	81	0.4
	Reverse	TTTCACAyGGCTCACCGT		0.4

ORF, open-reading frame.
The table shows eight sets of oligonucleotide primers used as mentioned above for small genomic islet (SGI) ORFs adopted for multiplex PCR amplification; bla_{OXA-51} for *A. baumannii* identification; four ORFs for distinguishing international clones (I and II), comprising ORFs specific to international clone I (AB57_0815 and AB57_3308) and those specific to international clone II (ACICU_02966 and ACICU_03137); and three ORFs (AB57_2484, ACICU_02042 and ACICU_01870) to improve discrimination between international clones IC1 and IC2 and other strains.
[a] Mixed nucleotide residues were described according to standard code (d, A/G/T; m, A/C; y, C/T; w, A/T; and h, A/C/T).

the mean ± standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. The χ^2 test or Fisher's exact test was used to examine the relationship between qualitative variables. For quantitative data, comparison between three groups was done using the Kruskal–Wallis test (non-parametric ANOVA) and then post-hoc test was used for pairwise comparison based on Kruskal–Wallis distribution. All tests were two-tailed. A P-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Clinical isolates and patients

The numbers of *A. baumannii* infection or colonisation recorded each month throughout the duration of the study are presented in Fig. 1A, with the highest number of infections or colonisation detected in June 2015. On the other hand, no *A. baumannii* infections or colonisation were detected in the period August–October 2017. The infected or colonised cancer patients had a median age of 35 years (range 1–81 years). The characteristics of the patients were as follows: 62% were ≥18 years old; 58% were male; 54% had haematological malignancy; 63% were admitted to the ICU for >15 days; 53% had an infection or colonisation episode duration of <7 days; and 64% died at the end of their hospital stay (Table 2). Blood specimens represented 56% of the collected specimens. All cancer patients in the study were hospitalised.

3.2. Antimicrobial susceptibility profiles

Of the 160 *A. baumannii* clinical isolates, 43% were resistant to multiple classes of antibiotics. Specifically, 89% (n = 142) of the isolates showed a high resistance rate to meropenem. Regarding fluoroquinolones and aminoglycosides, 89% (n = 143) were resistant to ciprofloxacin, 73% (n = 116) were resistant to levofloxacin and 71% (n = 114) were resistant to gentamicin, whilst only 43% (n = 69) were resistant to tobramycin.

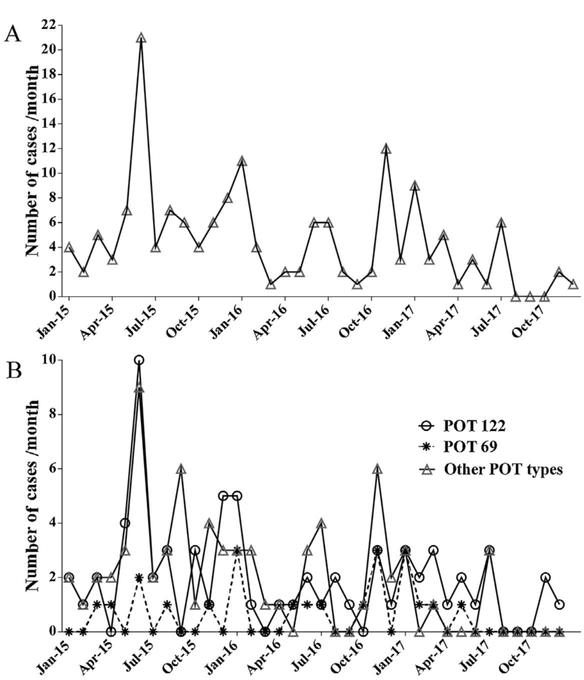

Fig. 1. Frequency of isolation of *Acinetobacter baumannii* during the course of the study: (A) total number of *A. baumannii* cases per month; and (B) frequency of each of the main *A. baumannii* clonal lineages, including POT 122, POT 69 and other POT types. The charts were generated using GraphPad Prism v.5.0 (GraphPad Software, Inc., La Jolla, CA, USA). POT, PCR-based open reading frame typing.

3.3. PCR-based open reading frame typing (POT) genotyping

Multiplex PCR resulted in fragments of the expected sizes (81, 102, 122, 151, 189, 234 and 271 bp). The *A. baumannii* isolates were classified into 15 POT types. The POT numbers were represented by numerical labels as follows: POT 69 (n = 22); POT 122 (n = 69); and miscellaneous POTs types (MPOTs) (n = 69), including POT numbers 0 (n = 16), 8 (n = 9), 10 (n = 3), 12 (n = 6), 14 (n = 4), 40 (n = 4), 44 (n = 2), 52 (n = 1), 56 (n = 5), 93 (n = 2), 104 (n = 3), 106 (n = 6) and 108 (n = 8). International clones 1 and 2 were represented by POT 69 and POT 122, respectively, as previously reported [7,11]. They were distinguished from other POT types by the presence of more than two differences in the bands of POT ladder patterns as shown in Fig. 2 and Table 3.

Table 3 shows the POT ladder used with small genomic islet (SGI) ORFs adopted for multiplex PCR amplification of *A. baumannii* IC1 and IC2 and $bla_{\text{OXA-51}}$ for *A. baumannii* identification. Four ORFs were used for distinguishing IC1 and IC2, i.e. ORFs specific to the IC1 strains (AB57_0815 and AB57_3308) and those specific to IC2 strains (ACICU_02966 and ACICU_03137). Three ORFs specific for strains (AB57_2484, ACICU_02042 and ACICU_01870) were selected to improve the discrimination between the international clones and other lineages relative to the *A. baumannii* reference strain ATCC 17978.

The relationship between different POT types and various clinical epidemiological data of the cases is shown in Table 2. POT 122 isolates were significantly more frequently recovered from respiratory specimens of adult patients diagnosed with solid tumours; in contrast, MPOT isolates were significantly more frequently recovered from blood specimens of paediatric patients diagnosed with haematological malignancies. There was no correlation between different POT types and either length of ICU stay or the duration of the infection episode. However, there was a trend towards a higher percentage of clonal POT 122 isolates in male patients. It was found that 62% (43/69) of POT 122 isolates infected or colonised male patients.

The POT method showed the remarkable changes in the frequency and number of different *A. baumannii* clonal lineages per month in the hospital over the study period, as demonstrated in Fig. 1B. POT 122 and MPOT isolates were responsible for the majority of *A. baumannii* cases in the different wards of the tertiary-care centre, with persistence of POT 122 isolates but with MPOT isolates declining by the end of the study period. MPOT isolates were more susceptible to antimicrobials active against *Acinetobacter* relative to POT 122 isolates.

4. Discussion

The World Health Organization (WHO) has recently categorised carbapenem-resistant *A. baumannii* as a critical priority for the research and development of new antibiotics [15]. In the present study, 89% of the *A. baumannii* clinical isolates were resistant to carbapenems. Meanwhile, the POT method was capable of identifying and epidemiologically tracing the *A. baumannii* isolates collected in a tertiary cancer centre in Egypt over a period of 3 years. *Acinetobacter baumannii* clinical isolates were classified into 15 POT types, with international clones POT 122 and POT 69 representing 57% (91/160) of the collected isolates. Of the 15 POT types, 13 MPOTs represented *A. baumannii* isolates other than the international clones POT 122 (IC2) and POT 69 (IC1). This is in contrast to Japan where *A. baumannii* clinical isolates were classified into 17 POT types, with international clones POT 122 and POT 69 representing only 37% (30/81) of isolates through 11

Table 2
Statistical relationship between different PCR-based open reading frame typing (POT) types with various clinical and epidemiological data.

Clinical data	Variable	No. of isolates	% of isolates				*P*-value
			POT 0	POT 69	POT 122	MPOTs	
Age	<18 years	61	10	16	23	51	0.004*
	≥18 years	99	10	12	51	27	
Sex	Male	92	12	11	47	30	0.085
	Female	68	7	18	31	44	
Diagnosis	HM	86	7	16	28	49	0.001*
	SOT	74	14	11	54	22	
Specimen	Blood	90	9	13	32	46	0.050*
	Respiratory	33	6	15	61	18	
	Surgical	37	16	14	41	30	
Ward	Medical	68	9	12	46	34	0.007*
	Paediatrics	61	10	16	23	51	
	Surgical	31	13	13	61	13	
ICU LoS	≤15 days	60	10	13	50	27	0.184
	>15 days	100	10	14	34	42	
Episode duration	<7 days	85	9	17	33	41	0.211
	≥7 days	75	11	11	48	31	

HM, haematological malignancy; SOT, solid tumour; ICU. intensive care unit; LoS, length of stay.
* $P \leq 0.05$ was considered statistically significant.

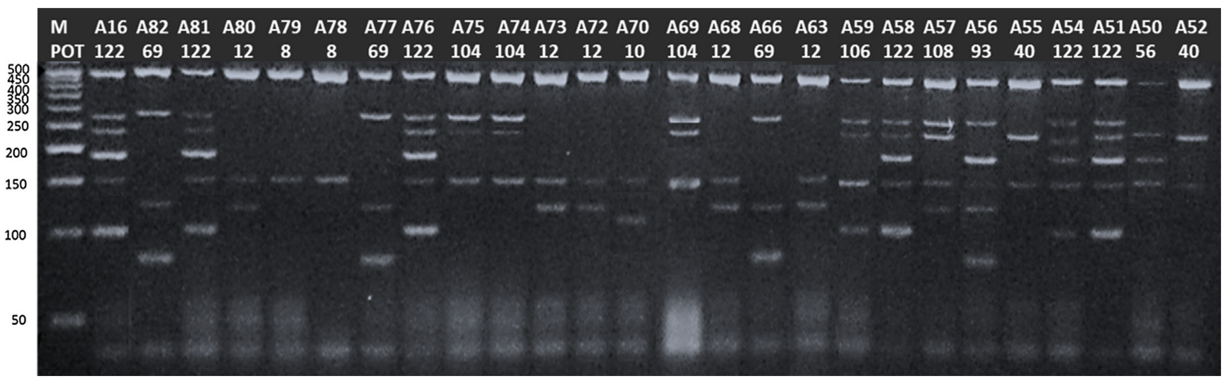

Fig. 2. Multiplex PCR-based open reading frame typing (POT) for identification and epidemiological typing of *Acinetobacter baumannii* clinical isolates. A photograph of an agarose gel (2%) showing patterns of the 8-plex PCR. The first lane contained a 50-bp ladder marker (M); the other lanes show representative isolates with different POT numbers; codes of the shown isolates were A50–A52, A54–59, A63, A66, A68–A70, A72–A82 and A16. The international clones are POT 122 (IC2) and POT 69 (IC1), while other strain types are miscellaneous POT types (MPOTs) (8, 10, 12, 40, 56, 93, 104, 106 and 108).

years of collection [7]. The current study showed 9 of 13 MPOT types similar to other POT types revealed by the previous study in Japan, namely POT 0, 8, 10, 40, 44, 56, 104, 106 and 108. However, four MPOTs (12, 14, 52 and 93) were not previously reported.

Adams et al. reported that *A. baumannii* isolates belonging to different clonal groups (European clones 1 and 2) had related resistance islands [16]. They explained this finding by an island acquired by a common *A. baumannii* ancestor before its divergence into two different clonal lineages. Indeed, previous studies on *A. baumannii* isolates recovered from cancer patients in Egypt showed a large diversity of *A. baumannii* isolated through short periods of time from a few centres in Egypt using different genotyping methods [4,17].

POT 122 (IC2) isolates showed persistence, whilst MPOT isolates showed rises and declines in different wards towards the end of the study (Fig. 1B). Hua et al. demonstrated that IC2 was the terminal destination for the bacteria after within-host evolution and that during this process most of the alteration was replacement of non-IC2 strains by IC2 strains [18]. Moreover, the current study showed that all of the detected POT 122 isolates were extensively resistant to the used antibiotics from different classes. This might indicate that isolates from IC2 harboured more resistance genes and mobile elements than non-IC2 isolates [18]. The contribution of POT 122 in the present study correlates with other studies in developed countries using other genotyping methods, especially MLST [19–22]. However, Wright et al. suggested that much of the evolution detected was in fact under selection [23]. The success of *A. baumannii* as a human nosocomial pathogen seemed to be intimately linked with the acquisition of antibiotic resistance genes, which might in turn modulate bacterial fitness and virulence potential [24–26].

Cancer patients used to be infected mainly with Escherichia coli and Klebsiella spp. [27,28]. These infections are usually acquired endogenously via the gastrointestinal tract after the initiation of chemotherapy. Recently, there has been an increase in the proportion of bloodstream infections caused by *P. aeruginosa*, *A. baumannii* and other non-fermenting bacilli along with the evolution of MDR phenotypes among cancer patients in some countries of the Mediterranean area and Europe [5,27–29]. *Acinetobacter baumannii* primarily causes pneumonia and bloodstream infection [30]. *Acinetobacter baumannii* infection often originates in the respiratory tract and bacteria then migrate to the bloodstream causing bacteraemia [30].

As shown in Fig. 1B, both POT 122 and MPOT isolates were responsible for most of the *A. baumannii* cases. As shown in Table 2, *A. baumannii* POT 122 isolates infected or colonised adult patients diagnosed with solid tumours and were recovered from respiratory specimens, whilst *A. baumannii* MPOT isolates infected paediatric patients diagnosed with haematological malignancies and were recovered from blood specimens. This could be explained

Table 3
PCR-based open reading frame (ORF) typing (POT) ladder patterns of the seven ORF amplicons and its correlation with previously characterised *Acinetobacter baumannii* strains.

Isolate	*bla*OXA-51 (465 bp)	POT code							POT no.	Previously characterised strains [a]
		***AB57_2484 (271 bp)	***ACICU_02042(234 bp)	*ACICU_02966 (189 bp)	***ACICU_01870 (151 bp)	**AB57_3308 (122 bp)	*ACICU_03137 (102 bp)	**AB57_0815 (81 bp)		
A16	+	1	1	1	1	0	1	0	122	ACICU ST2
A66	+	1	0	0	0	1	0	1	69	AB0057 ST1
A17	+	0	0	0	0	0	0	0	0	
A78	+	0	0	0	1	0	0	0	8	
A70	+	0	0	0	1	0	1	0	10	
A63	+	0	0	0	1	1	0	0	12	
A43	+	0	0	0	1	1	1	0	14	
A55	+	0	1	0	1	0	0	0	40	
A152	+	0	1	0	1	1	0	0	44	
A37	+	0	1	1	0	1	0	0	52	
A50	+	0	1	1	1	0	0	0	56	ATCC 17978 ST77S/V
A56	+	1	0	1	1	1	0	1	93	
A69	+	1	1	0	1	0	0	0	104	
A59	+	1	1	0	1	0	1	0	106	
A38	+	1	1	0	1	1	0	0	108	

IC, international clone.
*ORFs specific to IC2.
**ORFs specific to IC1.
***Discriminatory ORFs between IC1 and IC2 and other genetic lineages; their distribution patterns in the international clones were divergent in at least two alleles compared with other lineages.
[a]Three strains used for selecting ORFs with the same distribution patterns among reference clonal strains and clinical isolates used in the *A. baumannii* POT method.

by the fact that nosocomial infections are the result of a complex interplay of several dynamic factors, including: (i) pathogenicity and drug resistance of the infecting bacteria [25,26]; (ii) the host's immune status and microbiota composition; and (iii) environmental persistence (cross-colonisation and resistance acquisition) [24]. When all of these factors interact, the bacteria responsible for nosocomial infections constantly evolve, with an adverse outcome of the rapid and widespread increase of MDR organisms [31].

In summary, the POT method represents an easy, inexpensive tool for rapid tracing of international clonal lineages IC1 and IC2 and daily epidemiological surveillance of *A. baumannii* clinical isolates [8,9]. The method is additionally advantageous at genotyping clinical isolates owing to its simplicity in expressing the result outputs since the POT value only ranges from 0 corresponding to the binary code (0000000) to 127 with binary code (1111111). Moreover, different STs belonging to clonal complex (CC) 109 and 92 are represented by only POT 69 and 122, respectively, which are easy to quickly compare around the world. Further studies will be required to optimise the POT method for other well-described *A. baumannii* clonal lineages. This might be useful for monitoring the dissemination of MDR *A. baumannii*.

A major limitation of this study is that only POT types 122 and 69 were shown to identify isolates belonging to IC1 and IC2, whilst other POT types were not correlated with other international clonal *A. baumannii* lineages. Another limitation was that the POT method could only give a glance at the genetic relatedness among isolates with no comparative genome sequence analysis. In other words, MLST and other next-generation typing methods might be preferred in meticulous research with more vertical genomic data.

5. Conclusion

In Egypt; the POT method has proved to be advantageous for depicting horizontal epidemiological surveillance data on a daily basis. It indicated that there is a challenging presence of international clones IC1 and IC2 in addition to some miscellaneous POT types among the *A. baumannii* clinical isolates in a single tertiary-care cancer hospital in Egypt. This illustrates the increased spread of the two international clones, which are known for their tendency for epidemic spread and resistance to most common antimicrobials. More importantly, this fact requires immediate infection control measures to contain this emerging threat.

Funding

None.

Competing interests

None declared.

Ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Pharmacy, Cairo University (Cairo, Egypt) [approval no. MI(1205)].

References

[1] Geisinger E, Mortman NJ, Vargas-Cuebas G, Tai AK, Isberg RR. A global regulatory system links virulence and antibiotic resistance to envelope homeostasis in *Acinetobacter baumannii*. PLoS Pathog 2018;14:e1007030, doi: http://dx.doi.org/10.1371/journal.ppat.1007030.

[2] Fouad M, Attia AS, Tawakkol WM, Hashem AM. Emergence of carbapenem-resistant *Acinetobacter baumannii* harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. Int J Infect Dis 2013;17:e1252–4, doi: http://dx.doi.org/10.1016/j.ijid.2013.07.012.

[3] El-Sayed-Ahmed MA, Amin MA, Tawakol WM, Loucif L, Bakour S, Rolain JM. High prevalence of bla_{NDM-1} carbapenemase-encoding gene and 16S rRNA *armA* methyltransferase gene among *Acinetobacter baumannii* clinical isolates in Egypt. Antimicrob Agents Chemother 2015;59:3602–5, doi:http://dx.doi.org/10.1128/AAC.04412-14.

[4] Ghaith DM, Zafer MM, Al-Agamy MH, Alyamani EJ, Booq RY, Almoazzamy O. The emergence of a novel sequence type of MDR *Acinetobacter baumannii* from the intensive care unit of an Egyptian tertiary care hospital. Ann Clin Microbiol Antimicrob 2017;16:34, doi:http://dx.doi.org/10.1186/s12941-017-0208-y.

[5] El-Mahallawy HA, Hassan SS, El-Wakil M, Moneer MM. Bacteremia due to ESKAPE pathogens: an emerging problem in cancer patients. J Egypt Natl Canc Inst 2016;28:157–62, doi:http://dx.doi.org/10.1016/j.jnci.2016.05.002.

[6] Johnson JK, Robinson GL, Zhao L, Harris AD, Stine OC, Thom KA. Comparison of molecular typing methods for the analyses of *Acinetobacter baumannii* from ICU patients. Diagn Microbiol Infect Dis 2016;86:345–50, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.024.

[7] Suzuki M, Hosoba E, Matsui M, Arakawa Y. New PCR-based open reading frame typing method for easy, rapid, and reliable identification of *Acinetobacter baumannii* international epidemic clones without performing multilocus sequence typing. J Clin Microbiol 2014;52:2925–32, doi:http://dx.doi.org/10.1128/JCM.01064-14.

[8] Fujikura Y, Yuki A, Hamamoto T, Ichimura S, Kawana A, Ohkusu K, et al. Evaluation and validity of a polymerase chain reaction-based open reading frame typing method to dissect the molecular epidemiology for *Acinetobacter baumannii* in an epidemiologic study of a hospital outbreak. Am J Infect Control 2016;44:e275–8, doi:http://dx.doi.org/10.1016/j.ajic.2016.03.059.

[9] Nakaie K, Yamada K, Park K, Nakamura Y, Okada Y, Fujita A, et al. Effectiveness of weekly polymerase chain reaction-based open reading frame typing analysis of all newly isolated methicillin-resistant *Staphylococcus aureus* strains for controlling nosocomial infections. J Infect Chemother 2016;22:733–7, doi:http://dx.doi.org/10.1016/j.jiac.2016.07.007.

[10] Suzuki M, Yamada K, Aoki M, Hosoba E, Matsumoto M, Baba H, et al. Applying a PCR-based open-reading frame typing method for easy genotyping and molecular epidemiological analysis of *Pseudomonas aeruginosa*. J Appl Microbiol 2016;120:487–97, doi:http://dx.doi.org/10.1111/jam.13016.

[11] Yamada Y, Endo K, Sawase K, Anetai M, Narita K, Hatakeyama Y, et al. Rapid species identification and epidemiological analysis of carbapenem-resistant *Acinetobacter* spp. by a PCR-based open reading frame typing method. J Med Microbiol 2016;65:923–7, doi:http://dx.doi.org/10.1099/jmm.0.000314.

[12] Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. Clin Microbiol Infect 2007;13:807–15, doi:http://dx.doi.org/10.1111/j.1469-0691.2007.01759.x.

[13] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 26th ed. Wayne, PA: CLSI; 2016 CLSI supplement M100S.

[14] Sambrook J, Russell DW. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2001.

[15] World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva, Switzerland: WHO; 2017.

[16] Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H, Jamison JJ, et al. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J Bacteriol 2008;190:8053–64, doi:http://dx.doi.org/10.1128/JB.00834-08.

[17] Al-Hassan L, El Mehallawy H, Amyes SG. Diversity in *Acinetobacter baumannii* isolates from paediatric cancer patients in Egypt. Clin Microbiol Infect 2013;19:1082–8, doi:http://dx.doi.org/10.1111/1469-0691.12143.

[18] Hua X, Zhou Z, Yang Q, Shi Q, Xu Q, Wang J, et al. Evolution of *Acinetobacter baumannii* in vivo: international clone II, more resistance to ceftazidime, mutation in *ptk*. Front Microbiol 2017;8:1256, doi:http://dx.doi.org/10.3389/fmicb.2017.01256.

[19] Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 2010;5:e10034, doi:http://dx.doi.org/10.1371/journal.pone.0010034.

[20] Antunes LC, Visca P, Towner KJ. *Acinetobacter baumannii*: evolution of a global pathogen. Pathog Dis 2014;71:292–301, doi:http://dx.doi.org/10.1111/2049-632X.12125.

[21] Liu F, Zhu Y, Yi Y, Lu N, Zhu B, Hu Y. Comparative genomic analysis of *Acinetobacter baumannii* clinical isolates reveals extensive genomic variation and diverse antibiotic resistance determinants. BMC Genomics 2014;15:1163, doi:http://dx.doi.org/10.1186/1471-2164-15-1163.

[22] Matsui M, Suzuki M, Suzuki M, Yatsuyanagi J, Watahiki M, Hiraki Y, et al. Distribution and molecular characterization of *Acinetobacter baumannii* international clone II lineage in Japan. Antimicrob Agents Chemother 2018;62:, doi:http://dx.doi.org/10.1128/AAC.02190-17 pii: e02190-17.

[23] Wright MS, Iovleva A, Jacobs MR, Bonomo RA, Adams MD. Genome dynamics of multidrug-resistant *Acinetobacter baumannii* during infection and treatment. Genome Med 2016;8:26, doi:http://dx.doi.org/10.1186/s13073-016-0279-y.

[24] Geisinger E, Isberg RR. Interplay between antibiotic resistance and virulence during disease promoted by multidrug-resistant bacteria. J Infect Dis 2017;215 (Suppl 1):S9–S17, doi:http://dx.doi.org/10.1093/infdis/jiw402.

[25] Lean SS, Yeo CC. Small, enigmatic plasmids of the nosocomial pathogen, *Acinetobacter baumannii*: good, bad, who knows? Front Microbiol 2017;8:1547, doi:http://dx.doi.org/10.3389/fmicb.2017.01547.

[26] Lopez M, Rueda A, Florido JP, Blasco L, Fernandez-Garcia L, Trastoy R, et al. Evolution of the quorum network and the mobilome (plasmids and bacteriophages) in clinical strains of *Acinetobacter baumannii* during a decade. Sci Rep 2018;8:2523, doi:http://dx.doi.org/10.1038/s41598-018-20847-7.

[27] Averbuch D, Avaky C, Harit M, Stepensky P, Fried I, Ben-Ami T, et al. Non-fermentative Gram-negative rods bacteremia in children with cancer: a 14-year single-center experience. Infection 2017;45:327–34, doi:http://dx.doi.org/10.1007/s15010-017-0988-1.

[28] Islas-Munoz B, Volkow-Fernandez P, Ibanes-Gutierrez C, Villamar-Ramirez A, Vilar-Compte D, Cornejo-Juarez P. Bloodstream infections in cancer patients. Risk factors associated with mortality. Int J Infect Dis 2018;71:59–64, doi: http://dx.doi.org/10.1016/j.ijid.2018.03.022.

[29] Kamel NA, El-Tayeb WN, El-Ansary MR, Mansour MT, Aboshanab KM. Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt. PLoS One 2018;13:e0202119, doi:http://dx.doi.org/10.1371/journal.pone.0202119.

[30] Tilley D, Law R, Warren S, Samis JA, Kumar A. CpaA a novel protease from *Acinetobacter baumannii* clinical isolates deregulates blood coagulation. FEMS Microbiol Lett 2014;356:53–61, doi:http://dx.doi.org/10.1111/1574-6968.12496.

[31] Zhang T, Wang M, Xie Y, Li X, Dong Z, Liu Y, et al. Active efflux pump *adeB* is involved in multidrug resistance of *Acinetobacter baumannii* induced by antibacterial agents. Exp Ther Med 2017;13:1538–46, doi:http://dx.doi.org/10.3892/etm.2017.4141.