AKADÉMIAI KIADÓ

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RESEARCH ARTICLE

Clonal spread of PER-1 and OXA-23 producing extensively drug resistant *Acinetobacter baumannii* during an outbreak in a burn intensive care unit in Tunisia

AYMEN MABROUK[1,2*], YOSRA CHEBBI[1,2], ANIS RADDAOUI[1,2], ASMA KRIR[4], AMEN ALLAH MESSADI[4], WAFA ACHOUR[1,2] and LAMIA THABET[2,3]

[1] Laboratory Ward, National Bone Marrow Transplant Center, 1006, Tunis, Tunisia

[2] Tunis El Manar University, Faculty of Medicine of Tunis, LR18ES39, 1006, Tunis, Tunisia

[3] Laboratory Ward, Traumatology and Great Burned Center, 2074, Ben Arous, Tunisia

[4] Burns Intensive Care Unit, Traumatology and Great Burned Center, 2074, Ben Arous, Tunisia

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ABSTRACT

Extensively drug resistant *Acinetobacter baumannii* (XDR-*Ab*), has emerged as an important pathogen in several outbreaks. The aim of our study was to investigate the eventual genetic relatedness of XDR-*Ab* strains recovered from burn patients and environment sites in the largest Tunisian Burn Intensive Care Unit (BICU) and to characterize β-lactamase encoding genes in these strains. Between March 04th, 2019 and April 22nd, 2019 an outbreak of XDR-*Ab* was suspected. Environmental screening was done. All isolates were screened by simplex PCR for β-lactamase genes. Genetic relatedness was determined by pulsed field gel electrophoresis (PFGE) of *Apa*I-digested total DNA. During the study period, 21 strains of *A. baumannii* were isolated in burn patients, mainly in blood culture ($n = 7$) and central vascular catheter ($n = 6$). All strains were susceptible to colistin but resistant to imipenem ($n = 23$), ciprofloxacin ($n = 23$), amikacin ($n = 22$), tigecyclin ($n = 5$) and rifampicin ($n = 4$). The $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA23,}}$ and bla_{ADC} genes were present in all strains. These resistance determinants were associated with $bla_{\text{PER-1}}$ in 10 strains. The IS*Aba1* was inserted upstream of $bla_{\text{OXA-23}}$ in all isolates. PFGE revealed two major clusters A ($n = 11$) and B ($n = 5$). This is the first description in Tunisia of clonally related PER-1 producing XDR-*Ab* in burn patients with probable environmental origin.

KEYWORDS

A. baumannii, Outbreak, Carbapenemase, extensively drug resistant, burn intensive care unit

INTRODUCTION

Acinetobcater baumannii is an important nosocomial pathogen that can cause a variety of infections. Its environmental tenacity and its wide variety of resistance determinants are responsible for large outbreaks [1]. Multidrug-resistant or even extensively-drug-resistant *A. baumannii* (XDR-*Ab*) strains are increasingly reported in these outbreaks, particularly in intensive care units and burn units [2].

Carbapenem resistance in *A. baumannii* is mainly mediated by class D carbapenemases (OXA-23, OXA-24/40, OXA-58, and OXA-143) [3]. Class B (SPM, GIM, IMP, VIM, and NDM) and class A (GES-14) carbapenemases are less frequently detected in this microorganism [4, 5].

On the other hand, resistance to third generation cephalosporins is mainly the result of over-expression of Ambler class C bla_{ADC} gene [6] or rarely due to the acquisition of

*Corresponding author. Laboratory Ward, National Bone Marrow Transplant Center, 1006, Tunis, Tunisia. Tel.: +216 54597383. E-mail: aymen.mabrouk10@yahoo.fr

extended spectrum β-lactamases (ESBL) (PER, TEM, VEB, CTX-M, SHV, GES) encoding genes [7].

Outbreaks caused by XDR-*Ab* are difficult to control and have substantial influence on recovery of burn wounds. In Tunisia, despite the scarcity of epidemiology and molecular studies of carbapenem resistant *A. baumannii* strains, new resistant determinants are continuously discovered, such as GES-14 [5] and NDM-1 [8].

Our study was conducted following the suspicion of an outbreak of XDR-*Ab* associated with several deaths in the Burn Intensive Care Unit (BICU) of The Trauma and Burn Centre of Ben Arous, to investigate the eventual genetic relatedness of XDR-*Ab* strains recovered from burn patients and environment sites and to characterize β-lactamase encoding genes in these strains.

MATERIAL AND METHODS

Hospital setting

TheTrauma and Burn Centre of Ben Arous is a 168 beds hospital. It includes seven floors, ten surgical rooms and several departments, such as plastic surgery, neurosurgery and orthopedic units. The BICU, which is the largest one in Tunisia, includes 20 beds, all in single rooms.

Outbreak description and environmental investigation

During the period between March 04th, 2019 and April 22nd, 2019, an unusual large number of XDR-*Ab* ($n = 21$) was isolated among burn patients in the BICU and it was associated with 15 deaths. Thus, an outbreak of XDR-*Ab* was suspected. Environmental screening was performed to identify the possible source of this eventual outbreak. It was carried out on April 22nd, 2019. Infection control measures were immediately reinforced: this included improved environmental cleaning, decontamination of materials and strict hand washing. The outbreak had been so stopped.

Microbiological identification and antimicrobial susceptibility testing

A. baumannii were identified by API 20 NE system (bioMerieux, Marcy l'Etoile, France) and PCR amplification of $bla_{\text{OXA-51}}$ [9].

Antimicrobial susceptibility testing was determined by disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2019) [10] and analyzed by the SIRscan system. Bacterial suspension at 0.5-McFarland standard turbidity was inoculated on a Mueller-Hinton agar plate (Merck, Frankfurt, Germany). For the quality control in susceptibility testing, *Escherichia coli CIP* 7624 (ATCC 25922) was used as reference strain for internal quality control [10], and external quality controls were conducted regularly by the Tunisian Health Ministry.

XDR-*Ab* was defined as resistant, in addition to carbapenems, to at least three classes of antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and fluroquinolones [11].

The minimum inhibitory concentrations (MIC) of imipenem was determined by E-test method (Biomérieux®) and the MIC of colistin was determined by broth dilution method (UMIC Biocenric®).

Phenotypic detection of carbapenemases and metallo-beta-lactamases

Carbapenemase screening was performed by Carbapenem Inactivation Method (CIM) as follows: A suspension was made by suspending a full 10 µL inoculation loop of culture in 400 µL water. Afterward, a 10 µg meropenem disk (Oxoid Ltd., Hampshire, United Kingdom) was immersed in the suspension and incubated for a minimum of 2 h at 35 °C. After incubation, the disk was removed and placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator strain (ATCC 29522) at a turbidity equivalent to a 0.5-McFarland standard and incubated at 35 °C. The uninhibited growth of *E. coli* was considered as a positive result [12].

Metallo-beta-lactamases (MBLs) screening was performed using the EDTA-disk test. Briefly, two 10 µg of imipenem disks were placed on the surface of an inoculated plate with bacterial suspension at a turbidity equivalent to 0.5 McFarland standards. A 10 µL of 0.5 M EDTA solution was added to one of the imipenem disks. After overnight incubation, the inhibition zone of imipenem and imipenem plus EDTA disks were compared, and the enhancement in the inhibition zone of imipenem plus EDTA disk (≥7 mm) was considered as positive for metallo-beta-lactamases (MBL) production [13].

Detection of resistance genes

The genomic DNA extraction was performed by boiling method [14]. In this end, about 4–7 fresh colonies were removed and dissolved in 1.5 mL micro tubes containing 350 µL of sterilized deionized water then placed in boiling water for 10 min. Lastly, centrifuge step was operated at 12,000 rpm at 4 °C for 10 min. The supernatant containing DNA was transferred to a sterile micro tube and stored at −20 °C. All isolates were screened by simplex PCR for the following β-lactamase genes: class A (bla_{TEM}, bla_{VEB}, bla_{PER}, bla_{SHV}, bla_{CTX-M}, bla_{KPC}, and bla_{GES}); class B (bla_{NDM}, bla_{GIM}, bla_{VIM}, bla_{SIM}, bla_{SPM}, and bla_{IMP}); class C (bla_{ADC}); and class D ($bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, and $bla_{OXA\text{-}51\text{-like}}$) (Table1). PCR mapping experiments using combinations of the *ISAba1* primers and OXA-23-like reverse primers were carried out as previously described (Table1). All PCR assays were performed using Taq polymerase (hot firePol) (0.02 U/µL) in a T100™ Thermal Cycler (BIO-RAD).

Typing by pulsed-field gel electrophoresis

Clonal relatedness of strains was assessed by examining the chromosomal DNA macro-restriction pattern. For these purpose, all isolates were typed by pulsed-field gel electrophoresis (PFGE), using *Apa*I enzyme, according to a method previously published by Tenover et al. [17]. Briefly, restriction fragments were separated by electrophoresis in 1% agarose (BIOLINE,

Table 1. Primers used for PCR reactions

Gene	Primer name	Sequence (5′-3′)	Size (pb)	References
$bla_{\text{OXA-51}}$	$bla_{\text{OXA-51-F}}$	TAAGCTTTGATCGGCCTTG	353	[9]
	$bla_{\text{OXA-51-R}}$	TGGATTGCACTTCATCTTGG		
$bla_{\text{OXA-23}}$	$bla_{\text{OXA-23-F}}$	GATCGGATTGGAGAACCAG	501	[9]
	$bla_{\text{OXA-23-R}}$	ATTTCTGACCGCATTTCCAT		
$bla_{\text{OXA-24}}$	$bla_{\text{OXA-24-F}}$	GGTTAGTTGGCCCCCTTAAA	246	[9]
	$bla_{\text{OXA-24-F}}$	AGTTGAGCGAAAAGGGGATT		
$bla_{\text{OXA-58}}$	$bla_{\text{OXA-58-F}}$	AAGTATTGGGGCTTGTGCTG	599	[9]
	$bla_{\text{OXA-58-F}}$	CCCCTCTGC GCTCTACATAC		
bla_{SHV}	$bla_{\text{SHV-F}}$	ATGCGTTATATTCGCCTGTG	753	[15]
	$bla_{\text{SHV-R}}$	TGCTTTGTTATTCGGGCCAA		
bla_{VEB}	$bla_{\text{VEB-F}}$	ATGAAAATCGTAAAAAGGATATT	780	[15]
	$bla_{\text{VEB-R}}$	TTATTTATTCAAATAGTAATTCC		
$bla_{\text{PER-1}}$	$bla_{\text{PER-1-F}}$	ATGAATGTCATTATAAAAG	927	[15]
	$bla_{\text{PER-1-R}}$	TTGGGCTTAGGGCAG		
bla_{GES}	$bla_{\text{GES-F}}$	ATGCGCTTCATTCACGCAC	863	[38]
	$bla_{\text{GES-R}}$	CTATTTGTCCGTGCTCAGGA		
bla_{KPC}	$bla_{\text{KPC-F}}$	ATGTCACTGTATCGCCGTCT	881	[38]
	$bla_{\text{KPC-R}}$	TTACTGCCCGTTGACGCCCA		
$bla_{\text{CTX-M}}$	$bla_{\text{CTX-M-F}}$	ATGATGACTCAGAGCATTCGCCGCT	876	[39]
	$bla_{\text{CTX-M-R}}$	ATGATGACTCAGAGCATTCGCCGCT		
bla_{TEM}	$bla_{\text{TEM-F}}$	GCACGAGTGGGTTACATCGA	310	[15]
	$bla_{\text{TEM-R}}$	GGTCCTCCGATCGTTGTCAG		
bla_{VIM}	$bla_{\text{VIM-F}}$	TTTGGTCGCATATCGCAACG	500	[15]
	$bla_{\text{VIM-R}}$	CCATTCAGCCAGATCGGCAT		
bla_{IMP}	$bla_{\text{IMP-F}}$	GTTTATGTTCATACATCG	432	[15]
	$bla_{\text{IMP-R}}$	GGTTTAAAAAACAACCAC		
bla_{GIM}	$bla_{\text{GIM-F}}$	ATATTACTTGTAGCGTTGCCAGC	729	[15]
	$bla_{\text{GIM-R}}$	TTAATCAGCCGACGCTTCAG		
bla_{SIM}	$bla_{\text{SIM-F}}$	ATGAGAACTTTATTGATTTT	741	[15]
	$bla_{\text{SIM-R}}$	TTAATTAATGAGCGGCGGTT		
bla_{SPM}	$bla_{\text{SPM-F}}$	GCGTTTTGTTTGTTGCTC	780	[15]
	$bla_{\text{SPM-R}}$	TTGGGGATGTGAGACTAC		
bla_{NDM}	$bla_{\text{NDM-F}}$	GCTTTGGCGATCTGGTTTTC	620	[40]
	$bla_{\text{NDM-R}}$	CGGAATGGCTCATCACGATC		
IsAba-1 F/OXA-23 R	ISaba1-23F	CACGAATGCAGAAGTTG	1456	[16]
	ISaba1-23-R	TTAAATAATATTCAGCTGT		

USA) at 6 V/cm for 20 h; the pulse time was increased from 1 to 30 son a CHEF DR II (Bio-Rad, California, USA). The gel was stained with 1 mg/ml ethidium bromide and visualized under ultraviolet illumination. The PFGE patterns were analyzed using the computer software Gelcompar II for Windows version 6.6 (Applied Math, Belgium). Obtained patterns were compared by using the Dice coefficient, according to the instructions of the Gelcompar manufacturer. A tolerance of 1% in the band position was applied during the comparison of PFGE fingerprinting patterns.

RESULTS

Microbiological results

During the study period, 21 strains of *A. baumannii* were isolated among burn patients: seven in blood culture, six in central vascular catheter, six in skin samples and two in respiratory samples. Two strains were isolated from environmental sites: one from a respirator and one from a working desk.

All strains were susceptible to colistin but resistant to ceftazidime (23), imipenem (n = 23), meropenem (n = 23), ciprofloxacin (n = 23), trimethoprime-sulfamid (n = 23), amikacin (n = 22), tigecyclin (n = 5) and rifampicin (n = 4).

MICs of imipenem were >32 μg/mL in all isolates.

The CIM test was positive for all strains. No strain has been producing MBLs according to the EDTA-disk test.

Molecular analysis

The $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA23,}}$ and bla_{ADC} genes were present in all strains. They were associated with $bla_{\text{PER-1}}$ in 10 strains. No isolates were positive for the remaining tested genes. The IS*Aba1* was inserted upstream of $bla_{\text{OXA-23}}$ in all isolates.

PFGE identified nine different clusters and revealed that most strains belonged to two major clusters A (n = 11; 48%) and B (n = 5; 22%) followed by clusters C, D, E, F, G, H, and I (one strain in each cluster). The two environmental strains belonged to the cluster A, which included all PER-1 strains (Fig. 1). In the cluster A, only the first strain isolated (March 09th, 2019) from an endotracheal tube, was PER-1 negative.

DISCUSSION

In our study, we reported an outbreak of OXA-23 and PER-1 XDR-*Ab* with probable environmental origin during a period not exceeding two months. *A. baumannii* remains one of the highest causes of outbreaks in BICU [18] and most of them have been traced to environmental sources [19]. The ability of *A. baumannii* to form biofilms on abiotic surfaces, its high degree of resistance to drying and disinfectants and its ability to develop resistance to commonly used antimicrobial agents, lead to its long term persistence in the environmental setting [20].

In our series, twenty one strains of *A. baumannii* were responsible for colonization and/or infection in burn

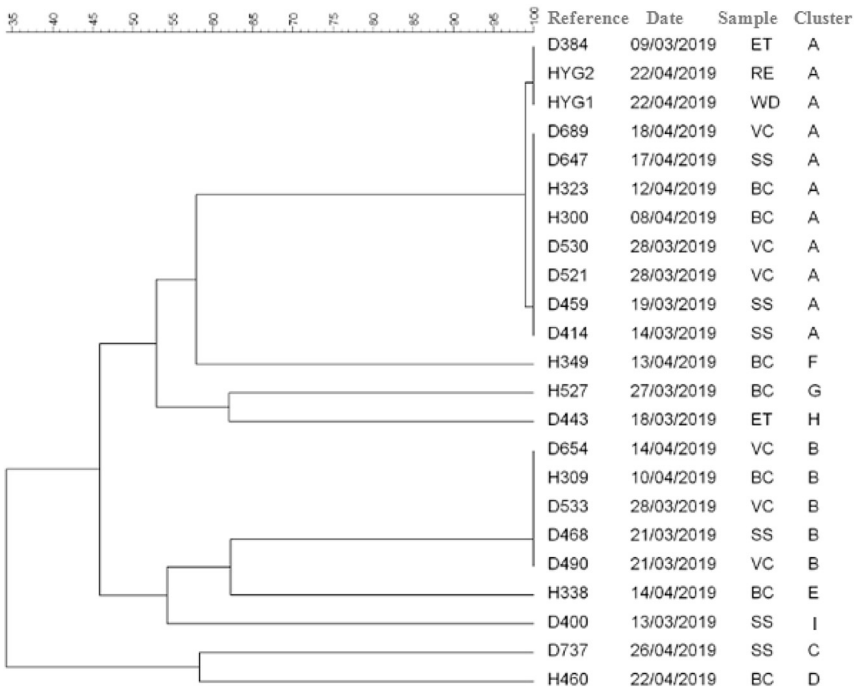

Fig. 1. A dendrogram of pulsed-field gel electrophoresis (PFGE) fingerprinting of 23 *Acinetobacter baumannii* isolates after digestion with *ApaI*. Dice: Optimization: 1% (Tolerance: 1%, Tolerance change: 1%). SS: Skin Samples, ET: Endotracheal tube, BC: Blood culture, VC: Vascular catheter, RE: respirator, WD; Working desk

patients: seven in blood culture, six in central vascular catheter, six in skin samples and two in respiratory samples. *A. baumannii* is frequently responsible for bacteremia and pneumonia [21]. The gateways of bacteremia are mainly intravascular and respiratory tract catheter. Surgical wounds, burns and the urinary tract are less encountered. Concerning *A. baumannii* isolated from the respiratory tracts, it is often difficult to differentiate between upper airway colonization from true pneumonia [21].

Two strains were isolated from environment sites: one from a respirator and one from a working desk. *Acinetobacter* spp. can endure dry conditions for long periods thus it is frequently isolated from reusable medical equipment such as ventilator tubing, arterial pressure monitoring devices, humidifiers, washbasins, plastic urinals and respirometers. It has also been isolated from the skin of healthcare personnel, mattresses, pillows and in all types of ventilator equipment and moist situations which allow it to diffuse easily [22, 23].

In our study, the outbreak was associated with 15 deaths. Rates of mortality due to *A. baumannii* in burn patients were high in many studies. The XDR character is considered by some authors as a major risk factor of mortality in these patients [24]. Besides, some others believe that mortality in burn victims is multifactorial [25].

In our study, *A. baumannii* strains had high rates of resistance to imipenem (n = 23), ciprofloxacin (n = 23), trimethoprime-sulfamid (n = 23) and amikacin (n = 22). This finding is similar to previous studies that report the high resistance rates of *A. baumannii* among their burn patients, primarily attributed to prolonged hospitalization [26]. It has been found that strains responsible for outbreaks were more resistant to antibiotics than those causing sporadic infections [23]. Our strains were susceptible to colistin and had a low rates of resistance to tigecyclin (n = 5). Recently, resistance to both agents (colistin and tigecyclin), considered till now like antibiotics of last resort for XDR-*Ab* infections, has been described with enhance emergence of colistin resistance in several parts of the world [27, 28].

In the present study, the $bla_{\text{OXA-51}}$, bla_{OXA23}, and bla_{ADC} genes were present in all strains. The $bla_{\text{OXA-51}}$ and bla_{ADC} are intrinsic to *A. baumannii* and are responsible for low level of resistance to β-lactams. OXA-type β-lactamases, especially OXA-23 are the most common mechanism of resistance to carbapenems in *A. baumannii* in Tunisia and worldwide [29]. OXA-23 producing *A. baumannii* outbreaks were reported all over the world. The first one described in Tunisia was in the University Hospital Sahloul in 2005 [30]. The OXA-23 dissemination was related mainly to the international clone I or II [31].

In our study, IS*Aba1* was inserted upstream of $bla_{\text{OXA-23}}$ in all isolates. IS*Aba1* has an important role in the overexpression of carbapenem resistance encoding genes, especially those encoding classe D carbapenemases [32]. It's one of the main mechanism by which *A. baumannii* acquires a high level of resistance to carbapenems [32].

PER-1 β-lactamase was detected in association with OXA-23 in 10 strains. PER enzymes have been reported in *Acinetobacter* isolates all over the world (Saudi Arabia, France, Belgium, Iran, South Korea, Argentina, India) [33–35] but never in Tunisia. To our knowledge, this is the first description of PER-1 in *A. baumannii* in Tunisia.

In order to investigate the clonality, and eventually determine the epidemic origin of this outbreak, PFGE was done. Analysis revealed that two major clusters (A) (n = 11; 48%) and (B) (n = 5; 22%) were circulating in the BICU. The two environmental strains belonged to the cluster A, which containing all PER-1 strains, and so considered the source of the cluster (A) outbreak. The first isolate of cluster (A) was $bla_{\text{PER-1}}$ deprived strain, suggesting that $bla_{PER\text{-}1}$ gene was acquired during the outbreak period [36].

Previous study had showed that sequential *A. baumannii* outbreak can occur in the same intensive care unit and were caused by different clones, one replacing the other in a well-defined temporal order [37].

The outbreak has been stopped after reinforcement of hygienic measures and material decontamination.

In conclusion, we confirmed in this study, by PFGE, a clonal diffusion of XDR-*Ab* co-harboring PER-1 and OXA-23 β-lactamases genes in burns with a probable multiple environmental origin stopped by reinforcing of infection control measures. It is the first description of PER-1 in *A. baumannii* in Tunisia.

ETHICAL APPROVAL

This study was performed with approval from the Local Medical Committee of Charles Nicolle Hospital, Tunis, Tunisia.

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CONFLICT OF INTEREST

None.

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