



HAL
open science

Investigation of multidrug-resistant ST2 *Acinetobacter baumannii* isolated from Saint George hospital in Lebanon

Tania Nawfal Dagher, Charbel Al-Bayssari, Selma Chabou, Nadine Antar, Seydina Diene, Eid Azar, Jean-Marc Rolain

► To cite this version:

Tania Nawfal Dagher, Charbel Al-Bayssari, Selma Chabou, Nadine Antar, Seydina Diene, et al.. Investigation of multidrug-resistant ST2 *Acinetobacter baumannii* isolated from Saint George hospital in Lebanon. *BMC Microbiology*, 2019, 19 (1), 10.1186/s12866-019-1401-2 . hal-02202235

HAL Id: hal-02202235

<https://amu.hal.science/hal-02202235>

Submitted on 18 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

RESEARCH ARTICLE

Open Access



Investigation of multidrug-resistant ST2 *Acinetobacter baumannii* isolated from Saint George hospital in Lebanon

Tania Nawfal Dagher^{1,2}, Charbel Al-Bayssari³, Selma Chabou¹, Nadine Antar², Seydina M. Diene¹, Eid Azar² and Jean-Marc Rolain^{1*} 

Abstract

Background: *Acinetobacter baumannii* is an opportunistic pathogen causing various nosocomial infections. The spread of multidrug-resistant *A. baumannii* is a major public health problem. The aim of this study was to investigate the molecular epidemiology and the genetic support of multidrug-resistant *A. baumannii* isolates collected from Saint-Georges Hospital in Lebanon.

Methods: Between January and August 2016, 31 *A. baumannii* isolates were collected from sputum samples of patients infected with ventilator-associated pneumonia (VAP) and treated with colistin-carbapenem combination therapy. Antibiotic susceptibility testing was performed using the disk diffusion method. Carbapenemases, extended spectrum β -lactamases encoding genes and *mcr-1/2* genes were investigated by RT-PCR and standard PCR. The epidemiological relatedness of the strains was studied using MLST analysis.

Results: Most of the isolates exhibited multidrug-resistant phenotypes. All the isolates were carbapenem-resistant and among them, 30 carried the class D carbapenemase *bla*_{OXA-23} gene while one isolate carried *bla*_{OXA-72} gene. MLST results revealed three sequence types, namely ST2, ST699, and ST627. Isolates having ST2 were the most prevalent clone (29/31, 93.5%).

Conclusions: This study shows a nosocomial spread of multidrug-resistant *A. baumannii* ST2 having *bla*_{OXA-23} gene in Saint-George in Lebanon. Monitoring and control measures need to be adopted to avoid the spread of *A. baumannii* to patients.

Keywords: *Acinetobacter baumannii*, OXA-23, ST2 clone

Background

Acinetobacter baumannii is a glucose non-fermentative, gram-negative, opportunistic pathogen and is one of the leading causes of nosocomial and community infections [1, 2]. These features make *A. baumannii* capable of causing a wide variety of clinical complications such as pneumonia, particularly ventilator-associated pneumonia (VAP), bloodstream and urinary tract infections, meningitis, surgical site and wound infections especially in intensive care units [2, 3]. Carbapenems are the first

choice in the treatment of severe *A. baumannii* infections [4]. Carbapenem resistant *A. baumannii* nosocomial outbreaks have become a major concern worldwide since they lead to limited treatment options [5]. Several mechanisms contribute to carbapenem resistance in *A. baumannii* such as the expression of β -lactamases, alteration of cell membrane permeability, increased expression of efflux pumps, DNA gyrases, and topoisomerases [1]. In addition, the presence of three different types of β -lactamases in *A. baumannii* leads to β -lactame resistance such as: (1) *bla*_{GES-14}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{KPC} belonging to Ambler class A β -lactamases; (2) *bla*_{IMP-like}, *bla*_{VIM-like}, *bla*_{SIM-1}, and *bla*_{NDM-1} belonging to metallo- β -lactamases and (3) *bla*_{OXA-23-like}, *bla*_{OXA-24-like}, *bla*_{OXA-58-like}, *bla*_{OXA-143}, *bla*_{OXA-235-like}, and the intrinsic

* Correspondence: jean-marc.rolain@univ-amu.fr

¹Aix Marseille Univ, IRD, APHM, MEPHI, IHU-Méditerranée Infection, Faculté de Médecine et de Pharmacie, 19-21 boulevard Jean Moulin, 13385 Marseille, Cedex 05, France

Full list of author information is available at the end of the article



*bla*_{OXA-51-like}, which are found by nature on the chromosome of *A. baumannii* and belonging to oxacillinases [6–8]. Oxacillinases usually do not hydrolyse carbapenems, although some of them produce resistance to carbapenem and are known as “Carbapenem hydrolyzing class D beta-lactamases” or CHDLs. The most common of these enzymes were identified in *Acinetobacter* spp., notably in *A. baumannii*, and are responsible for carbapenem resistance [9].

The global dissemination of *A. baumannii* have been described in several countries in the world such as those of Europe, America, Asia, the Middle East, Australia, and South Africa [10]. A study done by Flamm et al demonstrated that the Mediterranean regions and Europe have the highest frequency of MDR *A. baumannii* isolates [11]. For example, in South and Southeast Asia, the carbapenem-resistant *A. baumannii* is a major challenge in public health, where these strains are predominant in nosocomial infections [12]. Regarding Europe, a lower rate of carbapenem resistance was shown in France, Germany and Sweden (10–20, 8 and 4%, respectively) whereas the rates increase up to 50–80% in Turkey, 85% in Greece, 60% in Italy and 45% in Spain [13]. It has been noticed that in many cases, one or two epidemic strains were perceived in a certain epidemiological setting. This is due to the transfer of colonized patients who transmit these strains between hospitals [14]. To date, in Lebanon, there has been a higher level of carbapenem-resistant *A. baumannii* (CRAB) strains [15]. While the *bla*_{OXA-24} [16] and *bla*_{OXA-58} [17] have been identified in our country, the *bla*_{OXA-23-like} remains the most common among the CRAB isolates. Furthermore, the international clone ST2 was found to be broadly spread among this country [18]. At the same institution where this present study was done, the outbreak of *A. baumannii* began in 2010 as an epidemic with a high level of resistance due to the high usage of carbapenem [19]. Several studies showed the dissemination of CRAB isolates harboring mainly the *bla*_{OXA-23-like} and belonging to the international clone ST2 [20, 21] at Saint-George Hospital. The aim of the current study was to investigate the molecular epidemiology and the genetic support of multidrug-resistant (MDR) *A. baumannii* isolates collected from patients treated with colistin-carbapenem combination therapy in Saint-George Hospital, Beirut, to investigate if there is a change in the pattern of isolates recovered from patients at the same institution.

Results

Antimicrobial susceptibility testing of isolated strains

A total of 31 strains isolated from Saint-George Hospital in Beirut were identified by MALDI-TOF as *A. baumannii*. These isolates were collected from the sputum of the respiratory tract (Table 1). Antibiotic susceptibility

testing results revealed high levels of resistance rates of all isolates to ticarcillin, ticarcillin clavulanic acid, piperacillin tazobactam, ceftazidime, cefepime, cefotaxime, imipenem, meropenem, ciprofloxacin, and levofloxacin. In addition, 3.2% of the isolates were resistant to gentamicin, tobramycin, and amikacin. E-tests showed high-level of resistance to imipenem, with MIC greater than 32 µg/ml for all the isolates. None of the isolates was resistant to colistin (MIC < 2 µg/ml).

Detection of beta lactamase genes

Results of PCR for carbapenemase-encoding genes showed that 30/31 of the isolates harbored the acquired OXA carbapenemase *bla*_{OXA-23-like} and one isolate expressed the *bla*_{OXA-24-like} gene.

In addition the β-lactamases gene *bla*_{TEM} was detected in all isolates. Using ARG-ANNOT, the analysis of the sequenced genes revealed that the *bla*_{OXA-23-like} encoded for the OXA-23 whereas the *bla*_{OXA-24-like} encoded for the OXA-72 variant. All the sequences of the *bla*_{TEM} gene were identified as *bla*_{TEM-1}. None of the isolates harbored *bla*_{NDM-1}, *bla*_{OXA-58}, *bla*_{VIM} gene, *bla*_{SHV}, *bla*_{CTX-M} and *mcr-1*, 2, 3, 4 and 5 genes.

MLST analysis

MLST analysis showed that 93.5% (29/31) of the *A. baumannii* isolates belonged to ST2 sequence type, whereas two isolates were assigned to ST699 and ST627, respectively. The most common clone (ST2), harboring the *bla*_{OXA-23} and *bla*_{TEM-1} genes, was found to be circulating in the hospital. The isolate belonging to ST627 was associated with the production of the *bla*_{OXA-72} and *bla*_{TEM-1} genes (Table 1).

Discussion

Acinetobacter baumannii has been identified as one of the most successful pathogens responsible for nosocomial infections especially for patients admitted to intensive care units (ICUs) [22]. *A. baumannii* is able to acquire resistance to broad types of antibiotics including carbapenems. Carbapenem-resistant *A. baumannii* has been reported worldwide and has become a significant health problem due to the limited options for antibiotic treatment [23, 24].

Between 1999 to 2009, carbapenem-resistant *A. baumannii* harboring the *bla*_{OXA-58} gene were predominant in the hospital flora of many Mediterranean countries such as Lebanon, Italy, Greece, and Turkey [25]. After 2009, a huge shift from OXA-58 *A. baumannii* to OXA-23 producing belonging to the international clonal I and II lineages has been observed globally [25].

An outbreak of MDR *A. baumannii* has been observed in Saint George Hospital in Beirut, Lebanon between November 2004 and October 2005 [17].

Table 1 Phenotypic and genotypic features of 31 *Acinetobacter baumannii* isolated from Saint Georges hospital in Lebanon

| Isolate | M / F | Collection date | Types of sputum | IPM | IMP MIC (µg/ml) | Carbapenemases | ESBL | MLST |
|---------|-------|-----------------|-------------------------|-----|-----------------|----------------|-------|-------|
| 1 | M | 10-06-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 2 | M | 06-07-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 3 | M | 08-06-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 4 | F | 23-07-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 5 | M | 10-06-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 6 | M | 08-08-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 7 | M | 20-06-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 8 | F | 01-07-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 9 | M | 15-06-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 10 | F | 07-07-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 11 | F | 06-02-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 12 | F | 30-03-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 13 | M | 16-03-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 14 | F | 15-02-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 15 | M | 11-03-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 16 | M | 02-05-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 17 | M | 14-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 18 | M | 28-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 19 | M | 06-02-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 20 | M | 08-02-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 21 | M | 24-03-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 22 | F | 28-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 23 | M | 31-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 24 | M | 08-03-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 25 | M | 08-03-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 26 | F | 27-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST699 |
| 27 | M | 31-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 28 | F | 30-01-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 29 | M | 16-02-16 | Upper Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 30 | M | 12-01-16 | Lower Respiratory Tract | R | > 32 | OXA-23 | TEM-1 | ST2 |
| 31 | M | 12-01-16 | Lower Respiratory Tract | R | > 32 | OXA-72 | TEM-1 | ST627 |

M male, F female, ESBL extended spectrum β-lactamases, MLST multilocus sequence typing, ST sequence type

In our study, the *bla*_{OXA-23} gene was found in 30 carbapenem-resistant *A. baumannii* isolates (96.7%) recovered from Saint George hospital in Beirut. In 2015, a study done in Lebanon showed the predominance of Imipenem-resistant *A. baumannii* *bla*_{OXA-23} and *bla*_{GES-11} gene -among the majority of *A. baumannii*. This dissemination of OXA-23 carbapenemase in Lebanon is consistent with the worldwide epidemiology of OXA-23 [16]. Also in 2016, Al Atrouni et al. showed the high dissemination of carbapenem-resistant *A. baumannii* harboring the *bla*_{OXA-23} gene and belonging to the international clone II lineage [26] (Table 2).

All the isolates in our study were resistant to imipenem. In Lebanon in 2012, it has been shown that 88%

of *A. baumannii* were imipenem-resistant. This number is closely related to the rates of 70% in Egypt, 24–72% in Turkey, 25–75% in Spain, and approximating 100% in Italy [16].

In addition to the *bla*_{OXA-23} gene, we identified the presence of the *bla*_{OXA-24-like} gene in one isolate. A study in Lebanon showed that there was a replacement of the predominant OXA-58 producing *A. baumannii* by OXA-23 producers and belonged to the International Clone II, using isolates recovered from patients from the same institution as those used in the present study, as well as there was the presence of *bla*_{OXA-24-like} gene in two isolates that harbored also the *bla*_{OXA-23-like} gene [20]. Our study revealed that the *bla*_{OXA-24-like} encoded for the OXA-72

Table 2 Study of carbapenemase *Acinetobacter baumannii* in Lebanon

| Origine | Carbapenemase genes | Other resistant genes | ST | Ref |
|-------------------|-------------------------------------|-----------------------|--|------|
| Clinical isolates | OXA-23, OXA-24, OXA-58, NDM-1 | | ST2, ST25, ST46, ST85, ST193, ST424, ST570, ST85, ST600, ST622, ST636, ST690, ST702, ST715, ST706, ST707, ST1, ST708, ST713, ST807, ST808, ST809, ST810, ST811 and ST812 | [26] |
| Clinical isolates | OXA-23, OXA-24 | | ST2, ST4, ST10 and ST14 | [20] |
| Clinical isolates | OXA-23, OXA-24, OXA-58 | | | [30] |
| Clinical isolates | OXA-23, OXA-51, OXA66, OXA69, NDM-1 | | ST2, ST1, ST460, ST85, ST6, ST25, ST103, ST154, ST3, ST158, ST146, ST459, ST284, ST150, ST108, ST461, ST462 | [31] |
| Clinical isolates | MBL, OXA | | | [46] |
| Clinical isolates | OXA-143 | | ST286 to ST296 and ST464 to ST476 | [47] |
| Clinical isolates | OXA-71 | | | [48] |
| Clinical isolates | OXA-23, OXA-24 | | ST2 | [21] |
| Clinical isolates | OXA-58 | GES-5 | | [49] |
| Clinical isolates | OXA-23, OXA-24 | GES-11 | | [15] |
| Clinical isolates | OXA-23, OXA-24 | GES-11 | | [50] |
| Livestock | OXA-23, OXA-58 | | ST491, ST492, ST493, ST2 and ST20 | [51] |

ST Sequence Type, Ref reference

variant, which was firstly reported in 2004 in an *A. baumannii* isolated from Thailand. After that the *Acinetobacter* spp. who carried this gene have been reported in different countries [27]. In Lebanon, the *bla*_{OXA-72} was firstly described in *A. calcoaceticus* isolated from purchased vegetables in Beirut [28]. It is also found in *A. pittii* isolated from patients admitted to Lebanese hospital in North of Lebanon, in 2015 [29]. Moreover, Rafei et al reported that among 31 carbapenem-resistant strains collected from different hospitals in Beirut and Northern Lebanon, 28 isolates carried the *bla*_{OXA-23} gene, 1 strain the *bla*_{OXA-24} gene and 2 strains the *bla*_{OXA-58} gene [30]. She also reported in 2015 the spread of the international clone II lineage with high incidence of *bla*_{OXA-23} carbapenemase, in addition to the presence of *bla*_{NDM-1}, *bla*_{OXA-51}, *bla*_{OXA-66} and *bla*_{OXA-69} in different hospitals in Tripoli, Lebanon [31].

Moreover, we found that the majority of our strains harbored the β -lactamases *bla*_{TEM-1} gene. A study in Egypt in 2017 showed that *bla*_{TEM} is the most frequent gene for ESBL [32]. In Saudi Arabia, Aly et al revealed that some of the isolates harbored the *bla*_{TEM} resistance genes as well as the *bla*_{PER-1} gene [33]. Also in Turkey, a study by Beris showed that *bla*_{TEM} was the most prevalent ESBL type amongst *A. baumannii* strains isolated from different regions [34].

In our study, the analysis of MLST showed that the strains belonged to three different clones, ST2, ST699, and ST627, where the ST2 was the most common clone (29/31). The ST2 and ST699 clones were associated with the production of OXA-23 carbapenemase, and the clone ST627 was associated with OXA-72. The β -lactamases

(ESBL) *bla*_{TEM-1} gene was found in all STs clones (Table 1). It has been shown that the international complexes CC1, CC2, and CC3 account for the most *A. baumannii* infections around the world and are frequently related with the production of OXA-23-like, OXA-24-like, or OXA-58-like enzymes [35]. Moreover, a study was done by the SENTRY Antimicrobial Surveillance Program in six countries of the Asia-Pacific region such as China, Hong Kong, India, Korea, Singapore and Thailand showed that there was a high distribution of *A. baumannii* having the *bla*_{OXA-23} carbapenemase genes [36]. In addition, in the United States, the OXA-23 has been described as the major mechanism responsible to the resistance of *A. baumannii*. These outbreaks are mostly associated to the worldwide spread of the international clones I and II [37]. The ST2 clone has also been reported in several Mediterranean countries. From 1999 to 2009, a study in four Mediterranean countries (Greece, Italy, Lebanon and Turkey) showed that *A. baumannii* outbreaks were caused by the spread of strains belonging in particular to ST2 and, to a lesser extent to ST1, ST25, ST78 and ST20. These clones harbored the *bla*_{OXA-58}, *bla*_{OXA-23} and *bla*_{OXA-72} genes [18]. In Greece, it has been observed that the ST2 was the most common clone circulating in Greek hospitals. These clones harbored the *bla*_{OXA-23} gene that was displacing the *bla*_{OXA-58} gene, which was the only carbapenemase found among carbapenem-resistant *A. baumannii* isolates until 2009 [38]. Moreover, the clone ST699, which belongs to the international clone CC2, was found for the first time in Lebanon but was reported in Beijing, China in a study that described the predominance of ST699 in

65 out of 97 clinical *A. baumannii* isolates collected from patients having nosocomial bloodstream infection [39]. In addition, the clone ST627, was also found for the first time in Lebanon, and was reported in Thailand in the database of Pasteur institute without a published paper.

Conclusion

In conclusion, this study describes the detection of *bla*_{OXA-23} and *bla*_{OXA-72} genes in clinical isolates of *A. baumannii* in Lebanon. MLST showed that there was a major circulating clone ST2. In addition, the ST699 and ST627 clones had not been previously detected in Lebanon. The resistant *A. baumannii* isolates found in Saint George hospital in Lebanon in the present study belonged to international clone II lineage and harbored the *bla*_{OXA-23}, which were firstly reported by Dahdouh et al and then again by Soudeihia et al in the same institution. Thus, it is clear that this clone has become endemic in the hospital and that an urgent strategy needs to be adopted to control the spread of such resistant microorganisms among patients as well as appropriate infection control measures and surveillance programs must be implemented. In addition, we believe that it is necessary to set up quality training for health professionals to promote a safe environment for staff, patients and visitors, in order that the risk of healthcare associated infections are kept to a minimum.

Methods

Bacterial isolates

Between January and August 2016, 31 *A. baumannii* were isolated from sputum of the respiratory tract of patients infected with ventilator-associated pneumonia (VAP) and receiving colistin-carbapenem combination therapy in Saint-George Hospital in Beirut. 29/31 samples were collected from the sputum of the upper respiratory tract and 2/31 from the sputum of the lower respiratory tract from hospitalized patients and kept at -80°C before being transported to the laboratory in Marseille. Once arrived, the isolates were cultivated for 24 h at 37°C on Trypticase Sodium Agar medium (TSA). Colonies growing on this medium were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex; Bruker Daltonics, Bremen, Germany) as previously described [40].

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar as recommended by the European Committee of Antimicrobial Susceptibility Testing (EUCAST) 2017. Fourteen different antibiotics were tested: ticarcillin, ticarcillin clavulanic

acid, piperacillin tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin, tobramycin, colistin, ciprofloxacin, levofloxacin, and cefotaxime. Interpretations of the results of antibiotic sensitivity testing were made according to EUCAST recommendations. In addition, E-test method (bioMérieux) was performed to determine the minimal inhibitory concentration (MIC) of imipenem as recommended by the 2017 European Committee of Antimicrobial Susceptibility Testing (EUCAST).

Moreover, the minimal inhibitory concentration (MIC) of colistin was determined using the broth microdilution method (Biocentric) according to EUCAST 2017.

DNA extraction

Bacterial DNA was extracted using the automatic robot EZ1 (Qiagen BioRobot EZ1-, Tokyo, Japan), with the extraction kit (EZ1 DNA, Qiagen, Hilden, Germany), following the manufacturer's instructions. The extracted DNA was eluted in 200 μL of elution buffer and was stored at -20°C .

Screening of samples by real-time PCR and molecular characterization of beta lactamase genes

Real-time PCR was performed to screen for the presence of carbapenemase-encoding genes using specific primers previously described for *bla*_{NDM-1}, *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{OXA-58}, *bla*_{VIM} and *bla*_{SHV}. All MDR bacteria were also screened for β -lactamases (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}) genes and for the *mcr*-1, 2, 3, 4 and 5 genes as described previously [41–44]. Negative and positive controls were used in each assay. The positive PCR products for any gene tested were sequenced using BigDye1 terminator chemistry on an automated ABI 3130 sequencer (PE Applied Biosystems, Foster City, CA). The sequences of the genes obtained were analyzed using the ARG-ANNOT database [45] (<http://backup.mediterranee-infection.com/article.php?lar-ef=282&titre=arg-annot>), and compared to other genes using the BlastN and BlastP of the National Center for Biotechnology Information (NCBI) database (<https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>).

Multilocus sequence typing

Molecular typing of the isolates was done to determine the genetic relationship among the clinical isolates by using the seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) as described on Institute Pasteur's MLST Web site (<https://pubmlst.org/abaumannii>). Each single locus has different allele and the allelic profile or sequence types (ST) of the seven loci were given a specific identification number.

Abbreviations

AST: Antibiotic susceptibility testing; ESBL: Extended spectrum beta lactamase; MALDI-TOF: Matrix-assisted laser desorption and ionization

time-of-flight mass spectrometry; MDR-AB: Multidrug-resistant *Acinetobacter baumannii*; MLST: Multilocus sequence typing; PCR: Polymerase chain reaction; RT-PCR: Real time polymerase chain reaction; ST: Sequence type

Acknowledgments

The authors thank CookieTrad for English language corrections.

Funding

This work was supported by the Lebanese Council for Research and the French Government under the « Investissements d'avenir » (Investments for the Future) program managed by the Agence Nationale de la Recherche (ANR, fr: National Agency for Research), (reference: Méditerranée Infection 10-IAHU-03. This work was supported by Région Provence Alpes Côte d'Azur and European funding FEDER PRIM1.

Availability of data and materials

Not applicable.

Authors' contributions

TND and SC wrote the manuscript, performed experiments, and analyzed the data. EA and NA provided the strains and helped draft the manuscript. CAB and SMD helped draft the manuscript. JMR conceived the study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable. No formal ethics approval was required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Aix Marseille Univ, IRD, APHM, MEPHI, IHU-Méditerranée Infection, Faculté de Médecine et de Pharmacie, 19-21 boulevard Jean Moulin, 13385 Marseille, Cedex 05, France. ²Saint George Hospital University Medical Center, Faculty of Medicine and Medical Sciences, University of Balamand, Beirut, Lebanon. ³Faculty of Sciences III, Lebanese University, Tripoli, Lebanon.

Received: 25 July 2018 Accepted: 24 January 2019

Published online: 02 February 2019

References

- Rolain J-M, Diene SM, Kempf M, Gimenez G, Robert C, Raoult D. Real-time sequencing to decipher the molecular mechanism of resistance of a clinical pan-drug-resistant *Acinetobacter baumannii* isolate from Marseille, France. *Antimicrob Agents Chemother.* 2013;57:592–6.
- Zarrilli R, Crispino M, Bagattini M, Barretta E, Di Popolo A, Triassi M, et al. Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance. *J Clin Microbiol.* 2004;42:946–53.
- Pogue JM, Mann T, Barber KE, Kaye KS. Carbapenem-resistant *Acinetobacter baumannii*: epidemiology, surveillance and management. *Expert Rev Anti-Infect Ther.* 2013;11:383–93.
- Ramoul A, Loucif L, Bakour S, Amiri S, Dekhil M, Rolain J-M. Co-occurrence of bla NDM-1 with bla OXA-23 or bla OXA-58 in clinical multidrug-resistant *Acinetobacter baumannii* isolates in Algeria. *J Glob Antimicrob Resist.* 2016;6:136–41.
- Bakour S, Olaitan AO, Ammari H, Touati A, Saoudi S, Saoudi K, et al. Emergence of colistin- and carbapenem-resistant *Acinetobacter baumannii* ST2 clinical isolate in Algeria: first case report. *Microb Drug Resist.* 2015;21:279–85.
- Biglari S, Hanafiah A, Mohd Puzi S, Ramli R, Rahman M, Lopes BS. Antimicrobial resistance mechanisms and genetic diversity of multidrug-resistant *Acinetobacter baumannii* isolated from a teaching hospital in Malaysia. *Microb Drug Resist.* 2017;23:545–55.
- Mathlouthi N, El Salabi AA, Ben Jomaa-Jemili M, Bakour S, Al-Bayssari C, Zorgani AA, et al. Early detection of metallo- β -lactamase NDM-1- and OXA-23 carbapenemase-producing *Acinetobacter baumannii* in Libyan hospitals. *Int J Antimicrob Agents.* 2016;48:46–50.
- Evans BA, Amyes SGB. OXA β -Lactamases. *Clin Microbiol Rev.* 2014;27:241–63.
- Antunes LCS, Visca P, Towner KJ. *Acinetobacter baumannii*: evolution of a global pathogen. *Pathog Dis.* 2014;71:292–301.
- Huber CA, Sartor AL, Mcodimba F, Shah R, Shivachi P, Sidjabat HE, et al. Outbreaks of multidrug-resistant *Acinetobacter baumannii* strains in a Kenyan teaching hospital. *Integr Med Res.* 2014;2:190–3.
- Flamm RK, Castanheira M, Streit JM, Jones RN. Minocycline activity tested against *Acinetobacter baumannii* complex, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia* species complex isolates from a global surveillance program (2013). *Diagn Microbiol Infect Dis.* 2016;85:352–5.
- Hsu L-Y, Apisarnthanarak A, Khan E, Suwantarant N, Ghafur A, Tambyah PA. Carbapenem-Resistant *Acinetobacter baumannii* and Enterobacteriaceae in South and Southeast Asia. *Clin Microbiol Rev.* 2017;30:1–22.
- Kempf M, Rolain J-M, Azza S, Diene S, Joly-Guillou M-L, Dubourg G, et al. Investigation of *Acinetobacter baumannii* resistance to carbapenems in Marseille hospitals, south of France: a transition from an epidemic to an endemic situation. *APMIS.* 2013;121:64–71.
- Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev.* 2008;21:538–82.
- Hammoudi Halat D, Moubareck CA, Sarkis DK. Heterogeneity of carbapenem resistance mechanisms among gram-negative pathogens in Lebanon: results of the first cross-sectional countrywide study. *Microb Drug Resist.* 2017;23:733–43.
- Hammoudi D, Moubareck CA, Hakime N, Houmani M, Barakat A, Najjar Z, et al. Spread of imipenem-resistant *Acinetobacter baumannii* co-expressing OXA-23 and GES-11 carbapenemases in Lebanon. *Int J Infect Dis.* 2015;36:56–61.
- Zarrilli R, Vitale D, Di Popolo A, Bagattini M, Daoud Z, Khan AU, et al. A plasmid-borne blaOXA-58 gene confers imipenem resistance to *Acinetobacter baumannii* isolates from a Lebanese hospital. *Antimicrob Agents Chemother.* 2008;52:4115–20.
- Di Popolo A, Giannouli M, Triassi M, Brisse S, Zarrilli R. Molecular epidemiological investigation of multidrug-resistant *Acinetobacter baumannii* strains in four Mediterranean countries with a multilocus sequence typing scheme. *Clin Microbiol Infect.* 2011;17:197–201.
- Ballouz Z, Aridi J, Afif C, Irani J, Lakis C, Nasreddine R, et al. Risk factors, clinical presentation, and outcome of *Acinetobacter baumannii* bacteremia. *Front Cell Infect Microbiol.* 2017;7:156.
- Dahdouh E, Hajjar M, Suarez M, Daoud Z. *Acinetobacter baumannii* isolated from Lebanese patients: phenotypes and genotypes of resistance, Clonality, and determinants of pathogenicity. *Front Cell Infect Microbiol.* 2016;6:163.
- Soudeihah MAH, Dahdouh EA, Azar E, Sarkis DK, Daoud Z. In vitro evaluation of the colistin-carbapenem combination in clinical isolates of *A. baumannii* using the checkerboard, Etest, and time-kill curve techniques. *Front Cell Infect Microbiol.* 2017;7:209.
- Blanco N, Harris AD, Rock C, Johnson JK, Pineles L, Bonomo RA, et al. Risk factors and outcomes associated with multidrug-resistant *Acinetobacter baumannii* upon intensive care unit admission. *Antimicrob Agents Chemother.* 2017;62:e01631–17.
- Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int J Antimicrob Agents.* 2013;41:11–9.
- Kempf M, Rolain J-M. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *Int J Antimicrob Agents.* 2012;39:105–14.
- Liakopoulos A, Miriagou V, Katsifas EA, Karagouni AD, Daikos GL, Tzouveleki LS, et al. Identification of OXA-23-producing *Acinetobacter baumannii* in Greece, 2010 to 2011. *Euro Surveill.* 2012;17:20117.
- Al Atrouni A, Hamze M, Jisr T, Lemarié C, Eveillard M, Joly-Guillou M-L, et al. Wide spread of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* belonging to clonal complex II in different hospitals in Lebanon. *Int J Infect Dis.* 2016;52:29–36.
- Montealegre MC, Maya JJ, Correa A, Espinal P, Mojica MF, Ruiz SJ, et al. First identification of OXA-72 carbapenemase from *Acinetobacter pittii* in Colombia. *Antimicrob Agents Chemother. Am Soc Microbiol (ASM).* 2012; 56:3996–8.

28. Al Atrouni A, Kempf M, Eveillard M, Rafei R, Hamze M, Joly-Guillou M-L. First report of Oxa-72-producing *Acinetobacter calcoaceticus* in Lebanon. *New Microbes New Infect.* 2016;9:11–2.
29. Al Atrouni A, Joly-Guillou M-L, Hamze M, Kempf M. Emergence of NDM-1 and OXA-72 producing *Acinetobacter pittii* clinical isolates in Lebanon. *New Microbes New Infect.* 2016;12:43–4.
30. Rafei R, Dabboussi F, Hamze M, Eveillard M, Lemarié C, Gaultier M-P, et al. Molecular analysis of *Acinetobacter baumannii* strains isolated in Lebanon using four different typing methods. *PLoS One.* 2014;9:e115969 Hakami RM, editor.
31. Rafei R, Pailhoriès H, Hamze M, Eveillard M, Mallat H, Dabboussi F, et al. Molecular epidemiology of *Acinetobacter baumannii* in different hospitals in Tripoli, Lebanon using bla(OXA-51-like) sequence based typing. *BMC Microbiol.* 2015;15:103.
32. Alkasaby NM, El Sayed Zaki M. Molecular study of *Acinetobacter baumannii* isolates for Metallo- β -lactamases and extended-spectrum- β -lactamases genes in intensive care unit, Mansoura University Hospital, Egypt. *Int J Microbiol.* 2017;2017:3925868.
33. Aly MM, Abu Alsoud NM, Elrohb MS, Al Johani SM, Balkhy HH. High prevalence of the PER-1 gene among carbapenem-resistant *Acinetobacter baumannii* in Riyadh, Saudi Arabia. *Eur J Clin Microbiol Infect Dis.* 2016;35:1759–66.
34. Beriş FŞ, Budak EE, Gülek D, Uzun A, Çizmeçi Z, Mengeloğlu FZ, et al. Investigation of the frequency and distribution of beta-lactamase genes in the clinical isolates of *Acinetobacter baumannii* collected from different regions of Turkey: a multicenter study. *Mikrobiyol Bul.* 2016;50: 511–21.
35. Levy-Blitchein S, Roca I, Plasencia-Rebata S, Vicente-Taboada W, Velásquez-Pomar J, Muñoz L, et al. Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* international clones II and III in Lima, Peru. *Emerg Microbes Infect.* 2018;7:119 Nature Publishing Group.
36. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, –24/40 and –58 carbapenemases among *Acinetobacter spp.* in Asia-Pacific nations: report from the SENTRY surveillance program. *J Antimicrob Chemother.* 2009;63:55–9.
37. Ganjo AR, Maghdid DM, Mansoor IY, Kok DJ, Severin JA, Verbrugh HA, et al. OXA-carbapenemases present in clinical *Acinetobacter baumannii-calcoaceticus* complex isolates from patients in Kurdistan region, Iraq. *Microb Drug Resist.* 2016;22:627–37.
38. Pournaras S, Dafopoulou K, Del Franco M, Zarkotou O, Dimitroulia E, Protonotariou E, et al. Predominance of international clone 2 OXA-23-producing *Acinetobacter baumannii* clinical isolates in Greece, 2015: results of a nationwide study. *Int J Antimicrob Agents.* 2017;49:749–53.
39. Guo L, Bao C, Liu J, Hao C, Cao J, Ye L, et al. Molecular Epidemiology of Bloodstream Infection-causing *Acinetobacter baumannii* from Five Tertiary Hospitals in Beijing, China. *Int J Clin Med Microbiol.* 2017;2:127.
40. Seng P, Rolain J-M, Fournier PE, La Scola B, Drancourt M, Raoult D. MALDI-TOF-mass spectrometry applications in clinical microbiology. *Future Microbiol.* 2010;5:1733–54.
41. Kruger T, Szabo D, Keddy KH, Deeley K, Marsh JW, Hujer AM, et al. Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrob Agents Chemother.* 2004;48:4263–70.
42. Roschanski N, Fischer J, Guerra B, Roesler U. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in Enterobacteriaceae. *PLoS One.* 2014;9:e100956 Bereswill S, editor. Public Library of Science.
43. Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y. A preliminary survey of extended-spectrum β -lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. *FEMS Microbiol Lett.* 2000;184:53–6 Oxford University Press.
44. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. *Eurosurveillance.* 2018;23. <https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672>.
45. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob Agents Chemother.* 2014;58:212–20.
46. Dahdouh E, Shoucair SH, Salem SE, Daoud Z. Mutant prevention concentrations of imipenem and meropenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Sci World J.* 2014;2014:1–7.
47. Rafei R, Hamze M, Pailhoriès H, Eveillard M, Marsollier L, Joly-Guillou M-L, et al. Extrahuman epidemiology of *Acinetobacter baumannii* in Lebanon. *Appl Environ Microbiol.* 2015;81:2359–67 Macfarlane GT, editor.
48. Pournaras S, Gogou V, Giannouli M, Dimitroulia E, Dafopoulou K, Tsakris A, et al. Single-locus-sequence-based typing of blaOXA-51-like genes for rapid assignment of *Acinetobacter baumannii* clinical isolates to international clonal lineages. *J Clin Microbiol.* 2014;52:1653–7.
49. Dahdouh E, Gómez-Gil R, Sanz S, González-Zorn B, Daoud Z, Mingorance J, et al. A novel mutation in pmrB mediates colistin resistance during therapy of *Acinetobacter baumannii*. *Int J Antimicrob Agents.* 2017;49:727–33.
50. Hammoudi D, Moubareck CA, Kanso A, Nordmann P, Sarkis DK. Surveillance of carbapenem non-susceptible gram negative strains and characterization of carbapenemases of classes A, B and D in a Lebanese hospital. *J Med Liban.* 2015;63:66–73.
51. Al Bayssari C, Dabboussi F, Hamze M, Rolain J-M. Emergence of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in livestock animals in Lebanon. *J Antimicrob Chemother.* 2015;70:950–1.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://www.biomedcentral.com/submissions)

