Journal of Global Antimicrobial Resistance 5 (2016) 47–50

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

GES-11-producing *Acinetobacter baumannii* clinical isolates from Tunisian hospitals: Long-term dissemination of GES-type carbapenemases in North Africa

H. Chihi [a,e], R.A. Bonnin [a,c,d,g], A. Bourouis [e], S. Mahrouki [e], S. Besbes [f], M. Ben Moussa [b], O. Belhadj [e], T. Naas [a,c,d,g,*]

[a] *Research Unit EA7361, LabEx LERMIT, Faculty of Medicine, Université Paris-Sud, Le Kremlin-Bicêtre, France*
[b] *Biochemistry and Technobiology, Faculty of Sciences of Tunis, Tunis, Tunisia*
[c] *French National Reference Center for Antibiotic Resistance, Le Kremlin-Bicêtre, France*
[d] *Joint Research Unit EERA 'Evolution and Ecology of Resistance to Antibiotics', Institut Pasteur-APHP-Université Paris-Sud, Paris, France*
[e] *Microbiology Unit, Hôpital Militaire de Tunis, Tunis, Tunisia*
[f] *Microbiology Unit, Institut Orthopédique Mohamed El Kassab, Tunis, Tunisia*
[g] *Bacteriology-Hygiene Unit, Hôpital Bicêtre, APHP, Le Kremlin-Bicêtre, France*

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 5 March 2016
Accepted 22 March 2016
Available online 2 May 2016

Keywords:
Acinetobacter baumannii
Carbapenemase
GES
Tunisia
Case report

ABSTRACT

Acinetobacter baumannii is an emerging threat in healthcare facilities owing to its ability to be multidrug-resistant (MDR) and to be involved in outbreaks. GES-type extended-spectrum β-lactamases (ESBLs) have been increasingly identified in *A. baumannii*. In this study, clinical *A. baumannii* isolates were characterised using standard biochemical methods and antibiotic susceptibility testing. Antibiotic resistance genes were sought by PCR and sequencing. Genetic support was characterised using S1 nuclease pulsed-field gel electrophoresis (PFGE) mapping, conjugation and electroporation assays. The genetic environment was investigated by PCR, and genetic relatedness was investigated by PFGE. Two MDR *A. baumannii* clinical isolates susceptible only to colistin and rifampicin were isolated from a tracheal aspirate of a 49-year-old woman hospitalised in 2006 at the Military Hospital of Tunis, Tunisia, and from a tracheal aspirate of a 53-year-old man hospitalised in 2010 at the Institut Orthopédique Mohamed El Kassab of Tunis, Tunisia. PCR revealed that the two isolates harboured the acquired carbapenemase bla_{OXA-23} and ESBL bla_{GES-11} genes along with chromosomally-encoded bla_{OXA-51} and $bla_{ADC-like}$ genes. PFGE revealed that these *A. baumannii* isolates were unrelated; nevertheless, plasmid analysis revealed a similar sized plasmid following electrophoresis of the isolates. In addition, *A. baumannii* CIP70.10 transformants displayed similar resistance patterns. bla_{GES-11} was integron-borne and the IS*AbaI* element was identified upstream of bla_{OXA-23} and $bla_{ADC-like}$. Here we described two unrelated clinical *A. baumannii* isolates producing GES-11 ESBL and OXA-23 carbapenemase from two Tunisian hospitals. This work further illustrates the emergence of GES-type β-lactamases in *A. baumannii* in North Africa as early as 2006.

1. Introduction

Acinetobacter baumannii is an important opportunistic pathogen that has the potential to spread among hospitalised patients and to persist in the hospital environment [1]. Public health is facing a rapid and global emergence of *A. baumannii* strains resistant to almost all antibiotic families including β-lactams, aminoglycosides, quinolones, tetracyclines–glycylcyclines, polymyxins and trimethoprim/sulfamethoxazole [2]. Carbapenems are often considered as an antibiotic of last resort but the increasing carbapenem resistance menace now compromises the uses of these compounds [1–3]. The main mechanism of carbapenem resistance in *A. baumannii* is the production of carbapenem-hydrolysing Ambler class D β-lactamases (CHDLs) [3]. Very recently, GES-type carbapenemases belonging to Ambler class A

* Corresponding author at: Service de Bactériologie-Hygiene, Hôpital Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France.
Tel.: +33 1 45 21 29 86; fax: +33 1 45 21 63 40.
E-mail address: thierry.naas@bct.aphp.fr (T. Naas).

http://dx.doi.org/10.1016/j.jgar.2016.03.005
2213-7165/

have been reported in *A. baumannii* [4–8]. Extended-spectrum β-lactamases (ESBLs) of the GES type (for Guiana extended-spectrum β-lactamase) were first identified in a clinical isolate of *Klebsiella pneumoniae* in 2000 and then in other Gram-negative rods including Enterobacteriaceae, *Pseudomonas aeruginosa* and, recently, in *A. baumannii* [4–7,9]. Several GES-type β-lactamases have been identified in *A. baumannii*: the bla_{GES-11} gene in France, Kuwait, Turkey, Egypt, the Palestinian territories and, recently, Tunisia; the bla_{GES-12} gene in Egypt, France and Belgium; and the bla_{GES-14} gene in France, Turkey and Kuwait [3–6,8,10,11]. GES-11 and GES-1 differ by a single amino acid substitution at position 243 (Gly243Ala in GES-11), a position known to be involved in extension of the hydrolysis spectrum towards aztreonam but also with reduced activity against carbapenems [5,9].

Here we report the earliest cases of bla_{GES-11}-positive *A. baumannii* isolated from patients in countries of North Africa.

2. Materials and methods

2.1. Strains, identification and susceptibility testing

Strain identification was performed using an automated VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) and an API 20NE system (bioMérieux). *A. baumannii* CIP70.10 was used in transformation experiments. All bacterial strains were routinely cultured at 37 °C on Mueller–Hinton agar medium (Bio-Rad, Marnes-la-Coquette, France) or in trypticase soy broth (Bio-Rad). Disc diffusion antibiograms were performed and were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org). Minimum inhibitory concentrations (MICs) of antimicrobial agents for these strains were determined by the dilution method on Mueller–Hinton agar and were interpreted using EUCAST guidelines.

2.2. Screening for β-lactamases

Total DNA of clinical isolates and the obtained transformants was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Courtaboeuf, France) and was screened by PCR for the presence of $bla_{VEB-like}$, $bla_{PER-like}$, $bla_{GES-like}$, bla_{OXA-23}, $bla_{OXA-24/-40}$, bla_{OXA-58}, $bla_{ADC-like}$ and $bla_{OXA-51-like}$ genes using specific primers as previously described [3]. Presence of the insertion sequence IS*AbaI* and integron structures was investigated by PCR as previously described [3,6]. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN) and were sequenced on both strands using an ABI 3100 Automated Sequencer (Applied Biosystems, Foster City, CA). The nucleotide and deduced amino acid sequences were analysed with software available on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/).

2.3. Genetic support and genetic environment of the bla_{GES-11} gene

Determination of the genetic support was performed using S1 nuclease pulsed-field gel electrophoresis (PFGE) mapping. Briefly, bacterial suspensions at an optical density at 600 nm (OD_{600}) of 0.8–1 were re-suspended in low-melting-point agarose (Bio-Rad) according to the manufacturer's recommendations. Agarose plugs were then digested with S1 nuclease (Thermo Fisher Scientific, Illkirch, France) according to the manufacturer's instructions. Electrophoresis was performed on 1% agarose 0.5× TBE [Tris–borate–ethylene diamine tetra-acetic acid (EDTA)] using a CHEF-DR II system (Bio-Rad). Following electrophoresis, the gel was first transferred to a nylon membrane and was hybridised with a specific probe for bla_{GES} as described previously [5,11].

Conjugation with rifampicin-resistant *A. baumannii* BM4547 (MIC > 256 μg/mL) was attempted as previously reported [6]. Extraction of plasmid DNA from *A. baumannii* isolates was attempted using the Kieser extraction method [6,12]. Plasmid extracts were analysed by electrophoresis on a 0.7% agarose gel. Extracted plasmids were electroporated into *A. baumannii* CIP70.10 using a Gene Pulser® II Electroporator (Bio-Rad) as previously described [6]. Electroporants were selected on trypticase soy agar plates (bioMérieux) containing 100 μg/mL ticarcillin (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Characterisation of the plasmid type was performed using *A. baumannii* PCR-based replicon typing (AB-PBRT) as described previously [13].

2.4. Clonal relationship of the clinical isolates

The clonal relationship of the two isolates was investigated using PFGE as described previously [6]. Multilocus sequence typing (MLST) using the Institut Pasteur scheme was performed as described previously [14].

2.5. GenBank accession no.

The nucleotide sequence data reported in this work have been deposited in the GenBank nucleotide database under accession no. JQ764759.

3. Results and discussion

3.1. Case report and clinical data

The first case corresponded to *A. baumannii* 4037 that was isolated from a tracheal aspiration of a 49-year-old woman hospitalised in 2006 at the Military Hospital of Tunis, Tunisia, who had been admitted for lymphoblastic leukaemia type B in the internal medicine unit. Ten days after hospitalisation, a urinary tract infection due to *Escherichia coli* was treated with cefotaxime and ciprofloxacin for 5 days. Subsequent to severe dyspnoea syndrome, the patient was transferred to the intensive care unit. She was intubated and placed on mechanical ventilation and she suffered from acute renal failure and septic shock. An *Aspergillus* spp. was isolated and was treated with caspofungin acetate for 10 days. The patient remained febrile and received empirical treatment composed of piperacillin/tazobactam (4 g × 3 per day for 16 days), amikacin for 4 days and teicoplanin (400 mg × 2 per day for 15 days). Following treatment, a carbapenem-non-susceptible *A. baumannii* was recovered from a tracheal aspiration. Despite treatment, the patient continued to deteriorate and died 24 h later.

The second case corresponded to *A. baumannii* 2735 that was isolated from a tracheal aspirate in January 2010 at the Institut Orthopédique Mohamed El Kassab of Tunis, Tunisia. Both patients had no international travel history. These two cases were identified 4 years apart and in two separate hospitals with no epidemiological link between them.

3.2. Susceptibility testing

Both isolates were resistant to penicillins and cephalosporins and exhibited reduced susceptibility to imipenem (MIC = 32 μg/mL). The isolates were also resistant to aminoglycosides, quinolones and chloramphenicol but remained susceptible to colistin and rifampicin. The ESBL-producing transformants were resistant to ceftazidime and cefotaxime and exhibited lower MICs to imipenem (MIC = 0.75 μg/mL) compared with the parental isolates (Table 1). The ESBL-producing transformants displayed additional resistances to kanamycin, gentamicin, netilmicin and trimethoprim.