Investigation of a possible outbreak of carbapenem-resistant Acinetobacter baumannii in Odense, Denmark using PFGE, MLST and whole-genome-based SNPs

Hammerum, Anette M.; Hansen, Frank; Skov, Marianne N.; Stegger, Marc; Andersen, Paal Skytt; Holm, Anette; Jakobsen, Lotte; Justesen, Ulrik Stenz

Published in:
Journal of Antimicrobial Chemotherapy

DOI:
10.1093/jac/dkv072

Publication date:
2015

Document version
Publisher's PDF, also known as Version of record

J Antimicrob Chemother 2015; **70**: 1965–1968
doi:10.1093/jac/dkv072 Advance Access publication 19 March 2015

Journal of Antimicrobial Chemotherapy

Investigation of a possible outbreak of carbapenem-resistant *Acinetobacter baumannii* in Odense, Denmark using PFGE, MLST and whole-genome-based SNPs

Anette M. Hammerum[1]*, Frank Hansen[1], Marianne N. Skov[2], Marc Stegger[1,3], Paal S. Andersen[1,3,4], Anette Holm[2], Lotte Jakobsen[1] and Ulrik S. Justesen[2]

[1]*Statens Serum Institut, Copenhagen S, Denmark;* [2]*Department of Clinical Microbiology, Odense University Hospital, Odense C, Denmark;* [3]*Pathogen Genomics Division, Translational Genomics Research Institute, Flagstaff, AZ, USA;* [4]*Veterinary Disease Biology, University of Copenhagen, Copenhagen, Denmark*

*Corresponding author. Department of Microbiology and Infection Control, Statens Serum Institut, Artillerivej 5 (47/219), DK-2300 Copenhagen S, Denmark. Tel: +45-3268-3399; Fax: +45-3268-3231; E-mail: ama@ssi.dk

Received 16 December 2014; returned 1 February 2015; revised 16 February 2015; accepted 25 February 2015

Objectives: The objectives were to study a possible outbreak of carbapenem-resistant *Acinetobacter baumannii* by comparing three different typing methods (PFGE, MLST and whole-genome SNPs) and to compare the resistance gene profiles of the isolates.

Methods: From December 2012 to October 2013, eight carbapenem-resistant *A. baumannii* were detected at Odense University Hospital, Odense, Denmark. These isolates were typed by PFGE, with ApaI and SmaI, respectively, and subjected to WGS. The WGS data were used for *in silico* extraction of MLST types using two different schemes, resistance genes and SNPs, to which 31 publicly available *A. baumannii* genomes were added.

Results: Using ApaI, the eight isolates had four different PFGE profiles, which were further differentiated using SmaI, separating one of the profiles into two distinct PFGE types. Five ST2 (Pasteur MLST) OXA-23-producing isolates, two ST1 OXA-72-producing isolates and one ST158 OXA-23-producing isolate were detected. The five ST2 isolates were subdivided into ST195, ST208 and ST218 using the Oxford MLST scheme. The phylogenetic analysis based on the core genome showed that six of the eight Danish *A. baumannii* isolates were located in three distinct clusters. The two remaining isolates did not cluster with other Danish or international isolates included in the study. Isolates that clustered using PFGE, Oxford MLST and phylogenetic analysis also shared similar resistance gene profiles.

Conclusions: The SNP profile, Oxford MLST, PFGE and resistance gene profiles clearly indicated spread of three different *A. baumannii* strains.

Keywords: antimicrobial resistance, antimicrobial resistance epidemiology, carbapenems

Introduction

Carbapenem resistance in *Acinetobacter baumannii* is most often encoded by oxacillinases (OXAs) and less frequently by metallo-β-lactamases.[1] Four main OXA groups, which can be further subgrouped, have been associated with carbapenem resistance in *A. baumannii*: the chromosomally located intrinsic OXA-51-like group and the three acquired OXA groups—OXA-23-like, OXA-40-like and OXA-58-like.[2,3] Furthermore, two novel OXA enzymes, OXA-143 and OXA-235, were detected in *A. baumannii* isolates in 2009 and 2013, respectively.[4,5]

Spread of *A. baumannii* in hospitals has been investigated by several typing methods. The most commonly used method for investigation of clonality is PFGE using ApaI as the restriction enzyme.[2] For investigation of population structure and global bacterial epidemiology, MLST has been the golden standard.[2] There are two MLST schemes for *A. baumannii*: the Oxford MLST scheme and the Pasteur MLST scheme (http://pubmlst.org/abaumannii). Furthermore, WGS can be used for comparison of *A. baumannii* isolates.

In recent years, Danish departments of clinical microbiology have, on a voluntary basis, submitted carbapenem-resistant *A. baumannii* isolates for verification and genotyping at the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

To our knowledge, spread of OXA-23-producing *A. baumannii* has previously only been detected at one hospital in the Capital

Region in Denmark, whereas single cases of OXA-23-like- or OXA-40-like-producing *A. baumannii*, most often related to prior travel, have been reported in other parts of Denmark too.[6,7]

The aim of this study was: (i) to study possible outbreak(s) of carbapenem-resistant *A. baumannii* detected at Odense University Hospital using three different typing methods [PFGE, MLST (two different schemes) and SNP analysis]; (ii) to compare these typing methods; and (iii) to compare the resistance gene profiles detected from the whole-genome sequences.

Methods

Isolates

In October 2013, carbapenem-resistant *A. baumannii* were detected in four patients hospitalized at Odense University Hospital, a tertiary referral hospital with approximately 1100 beds in the Region of Southern Denmark (1.2 million inhabitants).

To investigate a possible outbreak, the isolates were characterized using different typing methods. Their profiles were compared with four other carbapenem-resistant *A. baumannii* isolates previously detected at the hospital (December 2012 to September 2013) (Table 1). The eight isolates were the only carbapenem-resistant *A. baumannii* isolates detected during December 2012 to October 2013 at the Department of Clinical Microbiology at Odense University Hospital. Isolates were identified to species level by MALDI-TOF MS.

Susceptibility testing

The eight isolates were tested using Sensititre Trek panels (Thermo Scientific, Waltham, MA, USA; ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefoperazone, ceftriaxone, cefotaxime, cefepime, aztreonam, imipenem, meropenem, lomefloxacin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, gentamicin, tobramycin, amikacin, tetracycline and colistin) as per the manufacturer's instructions. The microbroth dilution method was performed in accordance with the CLSI.[8]

PCR

The bla_{OXA} genogroups in the eight carbapenem-resistant *A. baumannii* isolates were identified by multiplex PCR as previously described.[9,10]

PFGE

Bacterial DNA for PFGE was prepared using the *Salmonella* standard protocol of CDC PulseNet (http://www.cdc.gov/pulsenet/). Agarose plugs containing DNA were digested with 25 U of the restriction enzyme ApaI or with 30 U of the restriction enzyme SmaI for 4 h. Electrophoresis (1% gel) was performed at 6 V/cm and with the following run parameters for the two different enzymes: ApaI, 5–20 s for 18.5 h; and SmaI, 5–20 s for 18.5 h followed by 5–10 s for 4 h. Addition of thiourea (100 μL of 1 M) in the running buffer was not able to stop the degradation of the DNA from two of the strains when digested with SmaI (isolate AMA 517 and isolate AMA 525). The CDC standard H9812 isolate was digested with 50 U of XbaI and used as the molecular size marker. All visible bands more than 55 kb in size were included in the interpretation of PFGE patterns. An isolate was considered to be closely related to the outbreak strain if its PFGE pattern differed from the outbreak pattern by changes consistent with a single genetic event, i.e. a point mutation or any major insertions or deletions. Such changes typically result in two or three band differences. Patterns that were closely or possibly related to the outbreak pattern were considered subtypes of A and were designated type A1, type A2 etc.[11]

WGS and assembly

Genomic DNA was extracted (DNeasy Blood and Tissue Kit, Qiagen, Copenhagen, Denmark) and fragment libraries constructed using the Nextera Kit (Illumina, Little Chesterford, UK) followed by 251 bp paired-end sequencing (MiSeq, Illumina) according to the manufacturer's instructions.

In addition to the eight genomes of the Danish *A. baumannii* isolates (NCBI BioProject ID PRJNA266271), the chromosomes of the 31 publicly available complete and scaffold *A. baumannii* genomes, representing 31 different genome groups according to GenBank, were included in this study. The sequence data were aligned against the chromosome of the *A. baumannii* BJAB0715 reference genome (GenBank accession ID CP003847) using the Burrows–Wheeler Aligner (BWA). Identification of SNP variants was performed using the GATK Unified Genotyper with filtering using NASP (http://tgennorth.github.io/NASP/) to remove positions with less than 10× coverage and <90% unambiguous variant calls, or within duplicated regions of the reference using NUCmer. Phylogenetic analyses of the identified SNPs was performed using MEGA 6.0.6.[12] The paired-end Illumina data were assembled using CLCBio's Genomic Workbench 7.5 (Qiagen, Aarhus, Denmark).

MLST

MLST was performed from the whole-genome sequences of the eight isolates using the MLST web server (www.genomicepidemiology.org).[13] Two different MLST schemes were used: the Oxford MLST scheme and the Pasteur MLST scheme (http://pubmlst.org/abaumannii).

Identification of resistance genes

The ResFinder web server (www.genomicepdemiology.org) was used to identify acquired antimicrobial resistance genes in the assembled WGS data, using a threshold of 100% identity for the genes encoding β-lactamases and 98.00% identity for all other genes. ResFinder detects the presence of resistance genes, but not functional integrity and expression or resistance due to acquired variation in housekeeping genes.[14]

Results and discussion

Screening for bla_{OXA} genes with multiplex PCR detected six isolates with $bla_{OXA\text{-}23\text{-}like}$ genes and two isolates with $bla_{OXA\text{-}40\text{-}like}$ genes (data not shown). ResFinder analysis of the WGS data identified $bla_{OXA\text{-}23}$ in the six isolates with $bla_{OXA\text{-}23\text{-}like}$ genes and $bla_{OXA\text{-}72}$ genes in the two $bla_{OXA\text{-}40\text{-}like}$ group isolates. Furthermore, $bla_{OXA\text{-}65}$ ($n=1$), $bla_{OXA\text{-}66}$ ($n=5$) and $bla_{OXA\text{-}92}$ ($n=2$), all belonging to the intrinsic $bla_{OXA\text{-}51}$ group, were identified (Table 1). To our knowledge, $bla_{OXA\text{-}92}$ is rare and has only previously been reported from a clinical isolate from a patient in Greece.[15]

The eight isolates had four different PFGE profiles using ApaI as restriction enzyme; four of the isolates had related types (B1, B2) (Table 1 and Figure S1, available as Supplementary data at *JAC* Online). PFGE using SmaI as restriction enzyme revealed that these four isolates comprised two unrelated types (Figure S2).

Using the Pasteur MLST scheme, five of the eight isolates belonged to ST2 (CC2; where CC stands for clonal complex), two isolates to ST1 (CC1) and one isolate to ST158 (Table 1). The five isolates belonging to ST2 (using the Pasteur scheme) were further divided into ST195, ST208 and ST218 using the Oxford MLST scheme. Furthermore, ST499 and the new ST812 (double-locus variant of ST321) were identified for three isolates using the Oxford MLST scheme (Table 1). The Oxford MLST scheme had a higher discriminatory power than the Pasteur MLST scheme.

Table 1. Description of eight *A. baumannii* isolates from Odense University Hospital

Isolate no.	Origin	Sample date	PFGE ApaI	PFGE SmaI	Pasteur MLST	Oxford MLST	SNP profile	Background	Patient's link to other countries	Resistance profile[a]	β-Lactamase	Genes encoding non-β-lactam resistance
AMA 341	respiratory secretions	16-12-2012	D	E	158	499			travel to Egypt	1	bla_{OXA-23}, bla_{OXA-65}, bla_{GES-11}	*aph(3′)-VI-a*, *aac(6′)-Ib-cr*, *aadB*, *mph*(E), *mrs*(E), *sul1*, *dfrA7*
AMA 474	drain tip	18-08-2013	C	F	2	218			hospitalized in Slovakia	1	bla_{OXA-23}, bla_{OXA-66}, bla_{ADC-25}	*aadA1*, *aac(6′)Ib-cr*, *aph(3′)-VI-a*, *aph(3′)Ic*, *armA*, *strB*, *strA*, *mph*(E), *mrs*(E), *catB8*, *sul1*, *tet*(B)
AMA 441	culture from lower leg	09-06-2013	B2	G	2	208			hospitalized in Greece	2	bla_{OXA-23}, bla_{OXA-66}, bla_{ADC-25}, bla_{TEM-1D}	*aph(3′)-Ic*, *aph(3′)-Ic*, *armA*, *strA*, *strB*, *mrs*(E), *mph*(E), *tet*(B)
AMA 495	urinary catheter	14-09-2013	B2	G	2	208	same SNP profile as AMA 441	stayed in the same nursing home as patient with AMA 441	none	2	bla_{OXA-23}, bla_{OXA-66}, bla_{ADC-25}, bla_{TEM-1D}	*aph(3′)-Ic*, *aph(3′)-Ic*, *armA*, *strA*, *strB*, *mrs*(E), *mph*(E), *tet*(B)
AMA 517	respiratory secretions	04-10-2013	A	NT	1	812			born in Serbia	1	$bla_{OXA-72,}$ bla_{OXA-92}, bla_{TEM-1D}	*aadA1*, *aac(6′)Ib-cr*, *aph(3′)-VI-a*, *aph(3′)Ic*, *catA1*, *sul1*, *tet*(A)
AMA 525	respiratory secretions	14-10-2013	A	NT	1	812	same SNP profile as AMA 517	this patient stayed in the same hospital room as the patient with AMA 517	none	1	bla_{OXA-72}, bla_{OXA-92}, bla_{TEM-1D}	*aadA1*, *aac(6′)Ib-cr*, *aph(3′)-VI-a*, *aph(3′)Ic*, *catA1*, *sul1*, *tet*(A)
AMA 520	sputum	11-10-2013	B1	I	2	195			born in Pakistan	1	bla_{OXA-23}, bla_{OXA-66}, bla_{ADC-25}	*armA*, *strA*, *strB*, *mph*(E), *mrs*(E), *sul2*, *tet*(B)
AMA 524	sputum	14-10-2013	B1	I	2	195	same SNP profile as AMA 520	this patient stayed in the same hospital ward as the patient with AMA 520	none	1	bla_{OXA-23}, bla_{OXA-66}, bla_{ADC-25}	*armA*, *strA*, *strB*, *mph*(E), *mrs*(E), *sul2*, *tet*(B)

NT, non-typeable.

[a]Resistance profiles: profile 1, resistant to ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftriaxone, cefotaxime, ceftazidime, cefoperazone, cefepime, aztreonam, imipenem, meropenem, lomefloxacin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, gentamicin, tobramycin, amikacin and tetracycline; and profile 2, as profile 1, except susceptible to trimethoprim/sulfamethoxazole.

The phylogenetic analyses showed that six of the eight Danish *A. baumannii* isolates clustered into three clusters each consisting of two isolates (Table 1). AMA 474 and AMA 341 did not cluster with other Danish or international isolates included in the study.

The PFGE, MLST and SNP profiles discarded the suspicion of an outbreak consisting of all four isolates detected in October 2013, but strongly suggested a spread of carbapenem-resistant *A. baumannii* in three instances: between two patients (with AMA 517 and AMA 525) staying in the same room during hospitalization; between two patients hospitalized in the same ward (with AMA 520 and AMA 524); and between two patients living in the same nursing home (with AMA 441 and AMA 495) (Table 1). In addition to the bla_{OXA} genes, 8–13 other resistance genes were identified from the assembled genomes. *In silico* analyses showed isolates sharing SNP profiles and Oxford MLST profiles had identical resistance gene profiles (Table 1). The isolates were resistant to almost all tested antimicrobial agents and patterns were consistent between the three sets of related isolates (Table 1).

MLST can be useful for investigation of global epidemiology. In the present study, three of the patients had been travelling abroad prior to OXA-carbapenemase detection and two persons were of foreign ethnicity living in Denmark. The ST158 (Pasteur MLST) isolate was detected in a patient who had been to Egypt prior to detection (Table 1). To our knowledge, ST158 is rare and has only been reported from Iowa, USA, but ST615 and ST618, which are single-locus variants of ST158, have been reported from Egypt (Pasteur MLST).

The patient with *A. baumannii* ST218 (Oxford MLST) had been to Slovakia prior to detection of the isolate; however, according to the Oxford MLST database, ST218 has only been reported from Japan.

One of the two patients with ST208 (Oxford MLST) had been to Greece before detection, but, according to the Oxford database, ST208 has only been reported from Egypt, the USA and China.

One of the two patients with *A. baumannii* ST195 (Oxford MLST) was a Pakistani male living in Denmark. *A. baumannii* ST195 has previously been reported from several Asian countries.[16,17] Furthermore, Karah *et al*.[18] found an ST195 OXA-23-like-producing *A. baumannii* in Norway from a patient travelling to Thailand.

In conclusion, the SNP profile, Oxford MLST and the resistance gene profiles obtained from the WGS data were useful for typing of the eight carbapenem-resistant *A. baumannii*. WGS analysis, including SNP calling, MLST and resistance gene profiles, adds significant information for comparison and potential tracing of international-spreading clones and should replace PFGE typing.

Acknowledgements

Karin Sixhøj Pedersen at Statens Serum Institut and Elisa Knudsen at Odense University Hospital are thanked for their excellent technical assistance.

Funding

This work was supported by the Danish Ministry of Health and Prevention as part of The Integrated Surveillance of ESBL/AmpC Producing *E. coli* and Carbapenemase Producing Bacteria.

Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 2006; **12**: 826–36.

2 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.

3 Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β-lactamases. *Antimicrob Agents Chemother* 2010; **54**: 24–38.

4 Higgins PG, Poirel L, Lehmann M *et al*. OXA-143, a novel carbapenem-hydrolyzing class D β-lactamase in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; **53**: 5035–8.

5 Higgins PG, Pérez-Llarena FJ, Zander E *et al*. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2013; **57**: 2121–6.

6 DANMAP 2012. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria From Food Animals, Food and Humans in Denmark*. http://www.danmap.org/~/media/Projekt%20sites/Danmap/DANMAP%20reports/DANMAP%202012/Danmap_2012.ashx.

7 DANMAP 2011. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria From Food Animals, Food and Humans in Denmark*. http://www.danmap.org/~/media/Projekt%20sites/Danmap/DANMAP%20reports/Danmap_2011.ashx.

8 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Tenth Edition: Approved Standard M07-A10*. CLSI, Wayne, PA, USA, 2015.

9 Higgins PG, Lehmann M, Seifert H. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2010; **35**: 305.

10 Woodford N, Ellington MJ, Coelho JM *et al*. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. *Int J Antimicrob Agents* 2006; **27**: 351–3.

11 Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel-electrophoresis—criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.

12 Tamura K, Stecher G, Peterson D *et al*. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013; **30**: 2725–9.

13 Larsen MV, Cosentino S, Rasmussen S *et al*. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 2012; **50**: 1355–61.

14 Zankari E, Hasman H, Cosentino S *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; **67**: 2640–4.

15 Tsakris A, Ikonomidis A, Spanakis N *et al*. Identification of a novel bla_{OXA-51} variant, bla_{OXA-92}, from a clinical isolate of *Acinetobacter baumannii*. *Clin Microbiol Infect* 2007; **13**: 348–9.

16 Endo S, Yano H, Hirakata Y *et al*. Molecular epidemiology of carbapenem-non-susceptible *Acinetobacter baumannii* in Japan. *J Antimicrob Chemother* 2012; **67**: 1623–6.

17 Tada T, Miyoshi-Akiyama T, Kato Y *et al*. Emergence of 16S rRNA methylase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in hospitals in Vietnam. *BMC Infect Dis* 2013; **13**: 251.

18 Karah N, Haldorsen B, Hermansen NO *et al*. Emergence of OXA-carbapenemase- and 16S rRNA methylase-producing international clones of *Acinetobacter baumannii* in Norway. *J Med Microbiol* 2011; **60**: 515–21.