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Published in:
International Journal of Antimicrobial Agents

DOI:
10.1016/j.ijantimicag.2023.106866

Publication date:
2023

Document version:
Final published version

International Journal of Antimicrobial Agents 62 (2023) 106866

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

Short Communication

Characterisation of Carbapenemase-producing *Acinetobacter baumannii* Isolates from Danish Patients 2014–2021: Detection of a New International Clone – IC11

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ARTICLE INFO

Article history:
Received 17 March 2023
Accepted 21 May 2023

Editor: H. Sader

Keywords:
OXA-23
IC2
cgMLST
MLST
WGS

ABSTRACT

Objectives: This study aimed to characterise carbapenemase-producing *Acinetobacter baumannii* (*A. baumannii*) isolates from Danish patients using whole genome sequencing (WGS). It also compared typing and epidemiological data for further investigation of the spread and origin of the carbapenemase-producing *A. baumannii* isolates.

Methods: From 1 January 2014 to 30 September 2021, 141 carbapenemase-producing *A. baumannii* isolates, received at the national reference laboratory at Statens Serum Institut, were investigated using WGS. Multilocus sequence typing (MLST) and cgMLST data, obtained by SeqSphere+ software, were linked to data related to source of isolation, patient age and sex, hospital admission and travel history.

Results: Most of the carbapenemase-producing *A. baumannii* isolates were from males (n = 100, 71%). Most patients (n = 88, 63%) had travelled outside Scandinavia before admission to a Danish hospital. The most prevalent carbapenemase gene was $bla_{\text{OXA-23}}$ (n = 124). Isolates belonging to the dominating international clone IC2 accounted for 78% of all isolates. A new international ST164/OXA-91 clone, proposed to be named IC11, was recognised and described. The cgMLST analysis revealed 17 clusters, reflecting both sporadic travel to similar geographical areas and confirmed outbreaks in Danish hospitals.

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https://doi.org/10.1016/j.ijantimicag.2023.106866

Conclusions: The occurrence of carbapenemase-producing *A. baumannii* in Denmark was still low; however, isolates belonging to major international clones with a high potential to spread within hospitals, mainly IC2, dominated. OXA-23 was by far the most prevalent carbapenemase detected. Sporadic and travel-related introductions to Danish hospitals, also intra-hospital transmission, could be confirmed, emphasising the need for continuing vigilance.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is an opportunistic, nosocomial pathogen belonging to the group of microorganisms called ESKAPE (an acronym for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.), associated with multidrug resistance, increased mortality and burden on healthcare systems globally [1]. *A. baumannii* can cause serious infections such as ventilator-associated pneumonia, burns and bloodstream infections [2]. Carbapenems are first-line beta-lactams for treatment of multidrug-resistant *A. baumannii* infections. Carbapenem-resistant *A. baumannii* (CRAB) is listed by the Center of Disease Control as an 'urgent threat' [3] and is the highest ranked 'critical-priority' pathogen on the WHO priority 2018 list of antibiotic-resistant bacteria [4]. There is a high risk of early mortality in patients with CRAB bacteraemia [5]. Relevant healthcare authorities and reference laboratories should therefore closely monitor the occurrence of this difficult-to-treat organism.

Acinetobacter baumannii intrinsically carries the chromosomal beta-lactamase gene $bla_{\text{OXA-51-like}}$. However, carbapenem resistance in *A. baumannii* is most often encoded by acquired class D beta-lactamases, especially $bla_{\text{OXA-23-like}}$ [6]. Other class D beta-lactamases have been detected: $bla_{\text{OXA-40-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-143-like}}$ and $bla_{\text{OXA-235-like.}}$ Class B metallo-beta-lactamases, IMP, VIM, SIM and NDM are less frequently reported from CRAB isolates [7].

Several typing methods such as Rep-PCR, pulsed-field gel electrophoresis (PFGE) and two multilocus sequence typing (MLST) schemes (Pasteur and Oxford) have been used for strain typing of *A. baumannii*. The introduction of whole genome sequencing (WGS) has made it possible to compare whole genomes for detecting outbreaks and international spread. In 2017, an *A. baumannii* core genome MLST (cgMLST) scheme based on 2390 genes was published by Higgins et al., with good correlation to PFGE [8].

Molecular epidemiological studies have assigned ten major International Clones (IC1-10) (Paul Higgins, personal communication) 6,7; the most common is IC2, which very often harbours the acquired carbapenemase OXA-23 6,9.

The Danish National Patient Registry (DNPR) became nationwide in 1978 and covers all hospitals in Denmark. Reporting was made compulsory for private hospitals and private outpatient specialty clinics in 2013. Since 1 January 2014, all Danish Departments of Clinical Microbiology (DCMs) have been submitting meropenem non-susceptible *A. baumannii* isolates to the Reference Laboratory of Antimicrobial Resistance at Statens Serum Institut (SSI) Copenhagen, Denmark, for confirmation of carbapenemase production and molecular characterisation. The samples originate both from hospitalised patients and persons sampled at general practitioners. Isolates were originally submitted on a voluntarily basis, but submission of these isolates (both from clinical and screening samples) became mandatory by the Danish health authorities as of 5 September 2018.

This study aimed to characterise the carbapenemase-producing *A. baumannii* isolates obtained from patients at Danish hospitals and combine genomic relatedness with epidemiological information based on recent travel history and hospital contacts.

2. Materials and Methods

2.1. Isolates

All carbapenemase-producing *A. baumannii* isolates referred to SSI from DCMs located in the five regions of Denmark from 1 January 2014 to 30 September 2021 were included in the study. Only one isolate per patient per 12 months was included.

2.2. International sequences for comparison for the International Clone 11

Sixteen sequences were used for comparison of the International Clone 11: seven were derived from Danish patients; six were shared by Paul Higgins: Ab-S-863 and Ab-S-1052 from China isolated in 2016 (shared by Paul Higgins on behalf of Nai-Kei Wong and Qing Pan), N00157-21 and UO17238-21 isolated in Germany in 2021, and K1569-22 and U343-22 also isolated in Germany 2022; PEG-16-83-61 isolated in Germany in 2016 was added (shared by Paul Higgins on behalf of Esther Wohlfarth); and two publicly available genomes from recent studies by Zafer et al. describing an NDM-1 producing ST164$^{\text{Pasteur}}$ *A. baumannii* from Cairo, Egypt [10], and Tada et al. describing ST164$^{\text{Pasteur}}$ /$_{bla\text{OXA-91}}$ CRAB from Myanmar (2016-2017) [11] were included.

2.3. Travel information

Available data on travel history abroad, 6 months prior to detection of the carbapenemase-producing *A. baumannii* isolate, were reported by the DCM.

2.4. Genotypic characterisation

DNA was extracted (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany), with subsequent library preparation (Nextera XT Kit, Illumina, San Diego, USA) and finally subjected to WGS (Nextseq, llumina) according to the manufacturer's instructions, to obtain paired-end reads of 2 × 150 bp in length.

Quality control, genome assembly (SKESA v. 2.2), detection of resistance genes and species identification were carried out using the Bifrost pipeline (https://github.com/ssi-dk/bifrost).

Assembled draft genomes were uploaded to SeqSphere+ version 8.5.1 (Ridom© GmbH, Münster, Germany) for phylogenetic analysis. The isolates were defined as belonging to an outbreak cluster if the distance to a minimum of one isolate was nine or fewer alleles [8]. The $bla_{\text{OXA-51-like}}$ variant combined with the Pasteur MLST allelic profile and cgMLST based cluster analysis using SeqSphere+ were used to assign the isolates to International Clones 7,8. Sequencing data from the study are available in GenBank PRJEB60981.

Table 1
Number of carbapenemase-producing *Acinetobacter baumannii* isolates associated with international clonal (IC) lineages and carbapenemases.

International Clone	NDM-1	OXA-23	OXA-23, NDM-1	OXA-23, OXA-58	OXA-239	OXA-23. OXA-72	OXA-58	OXA-72	Total
IC1	–	3	1	–	–	–	–	2	6
IC2	–	99	5	–	–	1	3	2	110
IC5	–	–	–	–	1	–	–	–	1
IC6	–	–	–	–	–	–	–	2	2
IC7	–	1	–	–	–	–	–	1	2
IC9	2	1	–	–	–	–	–	–	3
IC10	–	1	–	–	–	–	–	–	1
IC11	–	6	–	1	–	–	–	–	7
No IC	1	5	–	–	–	–	–	3	9
Total	3	116	6	1	1	1	3	10	141

2.5. The Danish National Patient Registry

Information reported to the DNPR includes contact data, diagnoses, examinations and treatment procedures. Contact data include: hospital and department identifiers, and dates of admission and discharge. For investigation of possible nosocomial transmission, hospitalisation data were retrieved from the DNPR for patients with similar isolates in relation to cgMLST cluster types and carbapenemase genes.

All hospitalisation contact data during the period from January 2014 to September 2021 were obtained for each patient and anonymised before analysis. Data were analysed in the program EpiLinx to detect whether the patients had been sharing the same ward or undergoing the same medical treatment at the same hospital (under development; Statens Serum Institut, Copenhagen, Denmark) [12].

3. Results and Discussion

A total of 141 carbapenemase-producing *A. baumannii* isolates were collected from DCMs from 1 January 2014 to 30 September 2021. Of 141 isolates, 101 were cultured from 100 male patients and 40 isolates from 40 female patients (Table 1). One male patient had two different carbapenemase-producing *A. baumannii* isolates (one $ST2^{Pasteur}$/$ST349^{Oxford}$ OXA-23/NDM-1 producer and one $ST2^{Pasteur}$/$ST1933^{Oxford}$ OXA-23 producer) isolated 2 years apart. The median age of the patients was 64 years (range 17–89 years).

The most common sources of cultured carbapenemase-producing *A. baumannii* were the lower respiratory tract, accounting for 37% of all isolates, followed by skin and soft tissue (25%) and the lower gastrointestinal tract (17%) (Supplementary Table 1). The predominance of isolates from male patients in this study was noticeable. A large study from Kunming, China, including 1600 patients with nosocomial *A. baumannii* infections found a male-to-female ratio (2.8:1) similar to this study (2.5:1). Focusing on the site of isolation, the largest male-to-female ratios were found in lower gastrointestinal tract (7:1) and lower respiratory tract (3.7:1). The review by Falagas et al. found that men more frequently develop respiratory tract infections than women in general [13], especially infections in the lower respiratory tract. Possible reasons included anatomical, lifestyle, behavioural and socioeconomic differences. Sex hormonal regulation of the immune system were also hypothesised to play a part.

Of the 140 patients, 88 had been travelling outside Scandinavia within 6 months before sampling and identification of CRAB at the DCMs; these travel destinations were known for 85 patients. Travel information was missing for 17 patients, and 35 patients did not travel before hospital admission. Reflecting common travel patterns for Danes (www.statbank.dk), travelling to Southern Europe was the most frequently noted region of travel destination reported (n = 29), followed by Western Asia (n = 15) (Supplementary Table 2).

The majority of the isolates produced OXA-23 (n = 116) (Table 1), in accordance with data reported by previous studies [6]. Other detected carbapenemases included OXA-72 (n = 10), OXA-23+NDM-1 (n = 6), OXA-58 (n = 3), NDM-1 (n = 3), OXA-23+OXA-58 (n = 1), OXA-23+OXA-72 (n = 1) and OXA-239 (n = 1) (Table 1).

To date, 10 international clones have been described. These clones are characterised as being highly genetically related (belonging to the same $ST^{Pasteur}$), producing the same intrinsic bla_{OXA-51} variant and being disseminated beyond country borders.

In the current study, 110 of the isolates belonged to IC2, among which bla_{OXA-23} (n = 99) was most frequently detected (Table 1), in accordance with previous studies 6,9. Five isolates carrying both bla_{OXA-23} and bla_{NDM-1} clustered with IC2 and one isolate clustered with IC1. Three of the isolates belonged to IC9. The IC9 lineage has recently been discovered and harbours the intrinsic OXA-51 variant bla_{OXA-94} [9]. *Acinetobacter baumannii* isolates from Belgium, Germany, Egypt, Italy and Pakistan have been assigned to IC9. Two of the IC9 *A. baumannii* isolates in the current study were NDM-1 producers, and the third isolate was an OXA-23 producer. NDM-1 producing *A. baumannii* isolates are rare, but Wohlfarth et al. have described an NDM-1 producing IC9 *A. baumannii* from Germany [14]. One of the patients with the IC9 NDM-1 producing *A. baumannii* reported travel to Morocco prior to detection.

The isolate connected to IC5 was recovered from a patient travelling to Mexico. It carried the bla_{OXA-23} variant $bla_{OXA-239}$ and belonged to $ST758^{Pasteur}$. Noteworthy, the IC5 lineage has been associated with the emergence of OXA-239 from isolates detected in Mexico [15]; it is believed that this is the first detection of OXA-239 outside Mexico.

One isolate belonged to the new (as yet unpublished) IC10 (Paul Higgins, personal communication). The features of the IC10 isolates are $ST158^{Pasteur}$ and OXA-65 as their intrinsic oxacillinase (however, it is encoded by a DNA sequence containing synonymous nucleotide substitions compared with the sequence of bla_{OXA-65} found in OXA-65 carrying IC5 isolates). Isolates belonging to IC10 has been detected from Egypt, Syria, and Ireland, and they commonly have GES-11 or GES-12 in addition to OXA-23 (Paul Higgins, personal communication). The Danish isolate carried bla_{GES-11} in addition to bla_{OXA-23} and was related to international travel with an unknown destination. Based on cgMLST a distinct cluster of seven Danish isolates differing from each other by 17–63 alleles could be recognised; these seven isolates all belonged to $ST164^{Pasteur}$ and carried the bla_{OXA-51} variant bla_{OXA-91}. They were associated with travel to Thailand (n = 5), Iran (n = 1) and Mauritius (n = 1), and therefore seemed to constitute a new international clone. The isolates were detected between 2015 and 2021 (data not shown). A recent study by Zafer et al. described an NDM-1 producing ST164 (Pasteur scheme) *A. baumannii* from Cairo, Egypt [10], also carrying the bla_{OXA-51} variant bla_{OXA-91}. *A. bauman-*

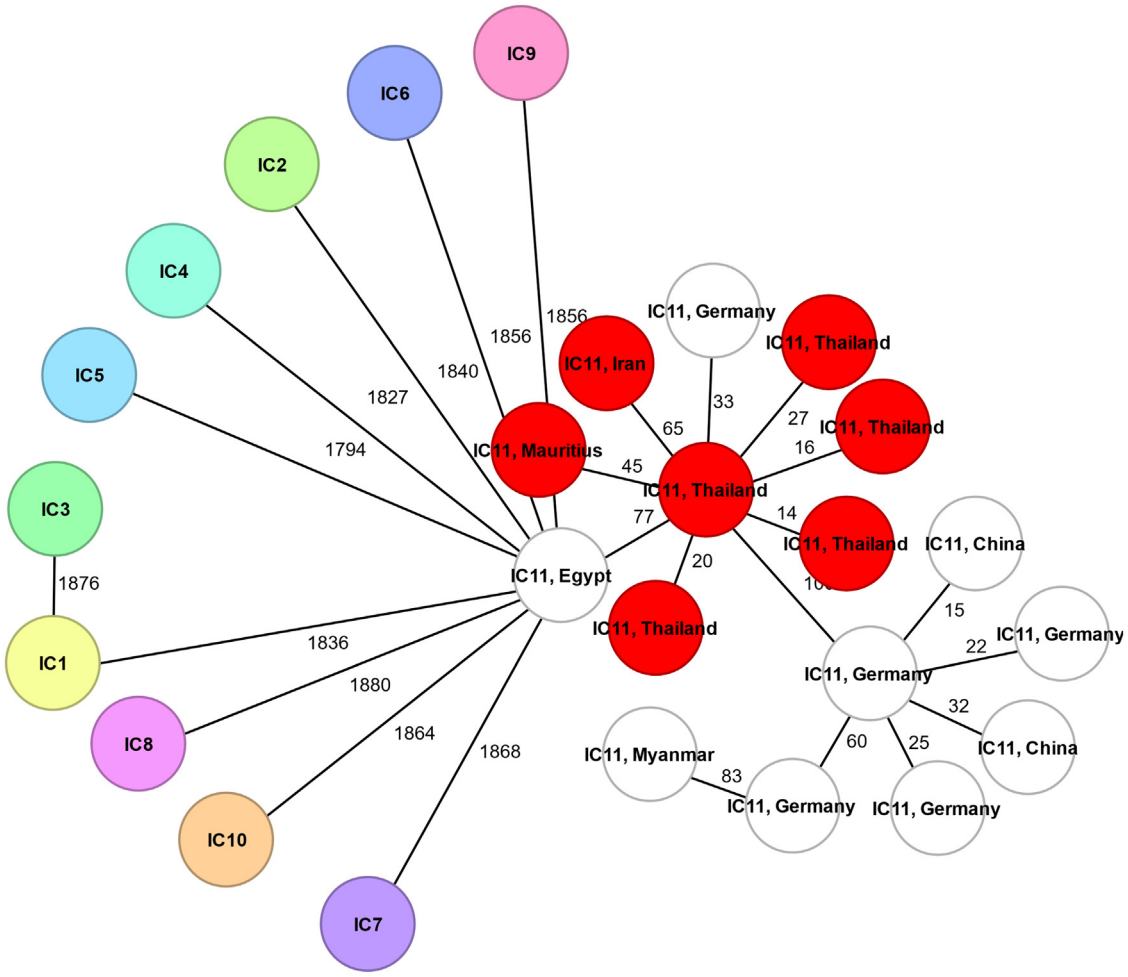

Fig. 1. The new IC11 clone. The red isolates are from Danes travelling to Thailand, Iran and Mauritius, whereas the white isolates are from patients from Myanmar, Germany, China and Egypt. Allele distances to representative isolates from IC1-IC10 are proportionally shortened for better visualisation.

nii isolates of this particular type have also been described from Myanmar (2016–2017) [11], Germany (2016, 2021 and 2022) and China (2016) (Paul Higgins and Esther Wohlfarth, personal communication) [14]. Their genome sequences (n = 9) were added to the seven Danish sequences in SeqSphere+, forming a cluster distinct from the other ICs (Fig. 1). A nationwide study from Thailand described the spread of multidrug-resistant *A. baumannii* belonging to ST164[Pasteur] [16] and this has also been described from Brazil [17]. Given these observations, it is suggested that this clone should be recognised as an international clone and the name IC11 is proposed, chronologically following the description of IC9 [8] and IC10 (Paul Higgins, personal communication).

With the exception of IC3, IC4 and IC8, all previously described international clones were represented in the study collection. Nine isolates did not cluster within known international clones.

The cgMLST analysis revealed a total of 17 possible outbreak clusters (Table 2), which comprised between two to 13 isolates. All clusters except one belonged to IC2, and had the carbapenemase gene $bla_{\text{OXA-23}}$. The non-IC2 cluster belonged to IC6 and was positive for $bla_{\text{OXA-72}}$. Using data from the DNPR and reported travel history, the molecular clusters were investigated for possible epidemiological links, as previously described [12]. It was observed that three clusters represented verified outbreaks in Danish hospitals without any patients with travel history. In four of the clusters, the index patient had travelled abroad, and the strain further transmitted in Danish hospitals. For two clusters, one patient travelled abroad, but the transmission to the other patients was not confirmed, while the remaining eight clusters reflected sporadic occurrences of very similar isolates rather than spread in Danish hospitals (Table 2). The largest cluster (Cluster 15) comprising 13 verified cases represented an outbreak of ST195[Pasteur] OXA-23 producing *A. baumannii* confined to a plastic surgery and burn wound treatment ward. The presumed index patient for this outbreak did not travel outside of Scandinavia before initial admission. This was also the case for the index patients with the isolates belonging to Cluster 1 and Cluster 17.

The remaining four verified outbreaks (Clusters 6, 9, 14 and 16) had suspected index patients reporting travel before hospital admission: Croatia, Serbia, Morocco and Bosnia, respectively.

Eight clusters were determined to derive from isolates related from travel to various geographical areas, rather than transmission events at Danish hospitals. The patients with isolates in Cluster 2 had travelled to Thailand and Sri Lanka. Patients with isolates in Cluster 3 had travelled to Spain. Likewise, Cluster 5 and Cluster 7 isolates were from patients who had travelled to Turkey. Patients with isolates belonging to Cluster 8 and Clusters 10–13 had travelled to Thailand, Indonesia, Greece, Turkey and Thailand, respectively. These countries are all known to have a high occurrence of CRAB [18–20]. Information about travel history, including contacts to healthcare systems abroad, is important information in relation to starting screening regimes to prevent transmission of carbapenemase-producing *A. baumannii* and other resistant bacteria in Danish hospitals.

The occurrence of carbapenemase-producing *A. baumannii* in Danish patients was a relatively rare event between 2014–2021, with an average of fewer than 20 cases per year. The rapid de-

Table 2
Descriptions of the 17 carbapenemase-producing *Acinetobacter baumannii* clusters in relation to possible origin.

Cluster	No. of patients	Isolation period	MLST Pasteur	MLST Oxford	cgMLST	International Clone	Carbapenemase	Travel destination (numbers)	Epidemiological summery
1	8	August 2014–November 2016	ST2	ST208	CT1451	IC2	OXA-23	None ($n = 8$)	Spread in Danish hospitals
2	3	December 2014–April 2016	ST2	ST195	CT607/CT1045	IC2	OXA-23	Thailand ($n = 1$), Sri Lanka ($n = 2$)	Travel related
3	2	December 2014–March 2016	ST2	ST208	CT1728	IC2	OXA-58	Spain ($n = 2$)	Travel related
4	2	March 2015	ST2	ST436	CT1462	IC2	OXA-23	Thailand ($n = 1$), unknown ($n = 1$)	Travel related and unknown origin
5	2	March 2015–March 2016	ST2	ST195	CT15	IC2	OXA-23	Turkey ($n = 2$)	Travel related
6	3	April 2015–January 2016	ST2	ST195	CT1414	IC2	OXA-23	Croatia ($n = 1$), unknown ($n = 1$), none ($n = 1$)	Import + spread in Danish hospitals
7	3	June 2015–October 2015	ST2	ST1114	CT1363	IC2	OXA-23	Turkey ($n = 3$)	Travel related
8	3	August 2015–March 2019	ST2	ST349	CT1466	IC2	NDM-1, OXA-23	Thailand ($n = 2$) unknown ($n = 1$)	Travel related
9	4	March 2016–April 2016	ST2	ST436	CT1454	IC2	OXA-23	Serbia ($n = 1$), Croatia ($n = 1$), unknown ($n = 1$), none ($n = 1$)	Import + spread in Danish hospitals
10	2	September 2016–November 2016	ST2	ST195	CT2136	IC2	OXA-23	Indonesia ($n = 2$)	Travel related
11	4	October 2017–October 2020	ST2	ST195/ST1816[1]	CT1626/CT2735/ CT2964	IC2	OXA-23	Greece ($n = 4$)	Travel related
12	3	October 2017–January 2018	ST2	ST556	CT1552	IC2	OXA-23	Turkey ($n = 1$), none ($n = 2$)	Travel related and unknown origin
13	2	August 2018–June 2019	ST2	ST208/ST1808[1]	CT2040	IC2	OXA-23	Thailand ($n = 1$) none ($n = 1$)	Travel related
14	2	August 2019–October 2020	ST2	ST1050/ST2058[1]	CT2729	IC2	OXA-23	Morocco ($n = 1$), none ($n = 1$)	Import + spread in Danish hospitals
15	13	July 2020–March 2021	ST2	ST195/ST1816[1]	CT6/CT715/ CT1044/ CT2896	IC2	OXA-23	none (n=13)	Spread in Danish hospitals
16	4	December 2020–March 2021	ST2	ST195/ST1816[1]	CT2045	IC2	OXA-23	Bosnia ($n = 1$), none ($n = 3$)	Import + spread in Danish hospitals
17	2	May 2021–June 2021	ST78	ST1757	CT3078	IC6	OXA-72	Unknown ($n = 2$)	Spread in Danish hospitals

[1] Oxford scheme STs after the / are derived from the presence of the *gdhB*2 paralog.

tection of CRAB in the Danish healthcare system (most importantly DCMs performing meropenem susceptibility testing of isolates from relevant patients) combined with the implementation of effective and appropriate infection control measures (e.g. screening protocols, isolation regimes) probably plays a major role in keeping the occurrence at this low level. As isolates of the most major international clones are frequently detected in Danish patients, it is important that the ongoing surveillance remains unimpaired.

In conclusion, this study showed that IC2 OXA-23 producing *A. baumannii* was the most common carbapenemase-producing *A. baumannii* among Danish patients. Furthermore, a new International Clonal lineage, IC11, was detected. Most of the carbapenemase-producing *A. baumannii* were associated with patients travelling abroad, with few outbreaks detected in the Danish hospitals during 2014–2021.

Acknowledgements

Karin Sixhøj Pedersen and Pia Thurø Hansen are thanked for excellent technical assistance. Esther Wohlfarth is thanked for sharing WGS data from one of the German IC11 isolates. The data were partly presented in the DANMAP reports DANMAP 2015 - DANMAP 2021.

Declarations

Funding: Part of this work was supported by the Danish Ministry of Health.

Competing interests: None declared.

Ethical Approval: Not required.

Sequence Information: In progress.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2023.106866.

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