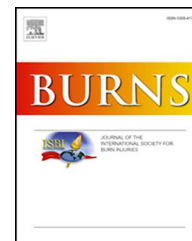


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Infection control measures to stop the spread of sequence type 15 OXA-23-producing *Acinetobacter baumannii* in a Swedish Burn Center

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ABSTRACT

Objective: To describe the course of the outbreak and infection control measures to stop the spread of sequence type 15 OXA-23-producing *Acinetobacter baumannii* in the Burn Center of Uppsala University Hospital, between November 2014 and the end of April 2015.

Methods: Compliance with hand hygiene, dress code, and cleaning routines were reviewed, the ward's environment was systematically investigated to identify potential environmental sources. Sampling routines for *A. baumannii*, from patients and environment, were established, and the epidemiological relationship was analysed for all carbapenem-resistant *A. baumannii* isolates using arbitrarily primed polymerase chain reaction (AP-PCR) and pulsed-field gel electrophoresis (PFGE).

Results: A total of 54 patients were treated at the burn intensive care unit during the studied, approximately five months period, and an OXA-23-producing *A. baumannii* was isolated from nine patients (9/54, 17%), whereof two died (2/9, 22.2%). All isolates shared identical PFGE-genotype patterns and belonged to sequence type 15; AP-PCR was eligible for prompt epidemiological investigations.

Conclusions: Higher awareness and increased compliance with hand hygiene and dress code as well as intensified cleaning protocols of the environment and equipment were successfully established and likely to have led to stop the spread of sequence type 15 OXA-23-producing *Acinetobacter baumannii*.

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1. Introduction

Over the past decades, infections caused by multidrug-resistant *Acinetobacter baumannii*-species have been noted [1–4],

Table 1 – Direct observations of seven hygiene practice dimensions.

1) Hands and forearms disinfected immediately before clean procedures and contact with a patient**	2) Hands and forearms disinfected after contact with patient and after dirty procedures	3) Gloves should be used in contact with bodily fluids (e.g. wounds, blood, secretion, urine, etc.) and surface disinfectants	4) Disposable apron or protective coat should be used in patient care, direct contact with the patient's bed, as well as handling of contaminated items	5) Scrubs should have short sleeves and be changed daily and/or when they get wet or visibly contaminated	6) Hands and forearms should be free of rings, watches, bracelets, etc	7) Hair set up or cut short
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*Direct observations of seven hygiene practice dimensions continuously monitored monthly: * **Hands and forearms disinfected (LIV Handdesinfection IPA 60 by Clemondo AB, Helsingborg, Sweden).

especially in units like intensive care units (ICU) and burn intensive care units (BICU) [5–9].

Previous documentation on the epidemiology of outbreaks caused by multidrug-resistant *A. baumannii* in Scandinavia is sparse and in 2021 no international emerging clone sequence type ST15 (Pasteur's multi-locus sequence typing (MLST) scheme) and ST236 (Oxford's MLST scheme) had, to the best of our knowledge, been documented [10,11]. In contrast, pan-resistant *A. baumannii* belonging to ST15 (Pasteur) was widespread in Brazil between 2008 and 2011 and is endemic in South America [12,13]. Reports on multidrug-resistant *A. baumannii* belonging to ST15 (Pasteur) are available from an isolate collection from Kuwait between 2011 and 2012; and ST236 (Oxford) was described from an isolate collection that originated from Saudi Arabia and Egypt between 2013 and 2014 [14,15].

A. baumannii infections mainly affect vulnerable patients, like burns with extensive wounds, and intensive care patients. Nevertheless, in the context of healthcare associated infections, *A. baumannii* infections are dreaded, as they contribute to considerable morbidity and mortality [16–18]. Furthermore, the pathogen is known to be persistent in hospital settings due to its resistance to antibiotics, disinfectants, and desiccation. In particular, resistance to fluoroquinolones and carbapenems is regarded a significant factor for epidemic spread and hospital outbreaks [19,20]. As far as the literature is concerned no published Scandinavian studies are extant addressing measures to control *A. baumannii* outbreaks in burn centers.

The present study aims to describe the course of the outbreak and infection control measures taken to stop the outbreak of ST15 OXA-23-producing *A. baumannii* in the Burn Center of Uppsala University Hospital, from November 2014 and to the end of April 2015.

2. Methods

The Swedish Ethical Review Authority approved a waiver of requirement (2020–06285) for informed consent for this study.

2.1. Case definition

A patient was defined as a case when it was treated at the burn center at Uppsala University Hospital, with a positive culture with the outbreak strain of *A. baumannii*, isolated from clinical or screening specimens in the period between November 2014, and the end of April 2015. Cases were identified by reviewing the clinical microbiology laboratory registers and patient medical records, and through establishing the screening culture routines during the epidemic period.

2.2. Infection control measures before the outbreak

2.2.1. Education, hand hygiene, dress code, surface disinfection, and patient-based surveillance

Education about microbiology, hygiene, and burns was standard in the introduction program when healthcare staff was employed. Occasionally it was highlighted in special

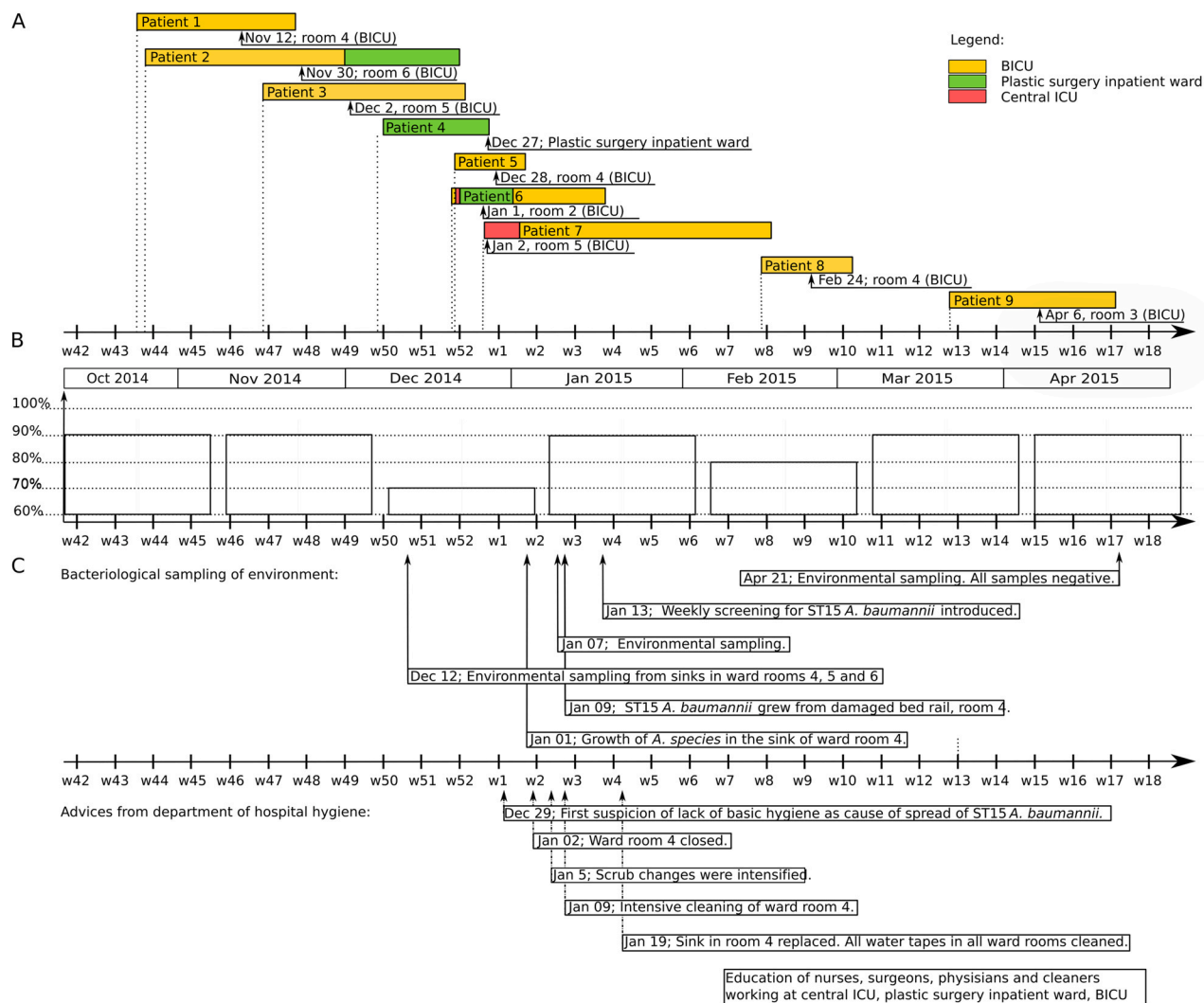


Fig. 1 – Timeline of the outbreak. 2 A) Colours indicate hospitalization time and location (ward). Arrow = first positive sample detected; yellow boxes - BICU; green boxes - Plastic surgery inpatient ward; orange boxes - Central ICU. 2 B) Timeline of hand hygiene and dress code monthly measurements. 2 C). Measurement timeline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

educational training. Direct observations of seven hygiene practice dimensions were continuously monitored (Table 1). Ten co-workers of the staff category (physician, nurse, assistant nurse, and paramedics) were monitored per month; a co-worker is assessed as failure of compliance when at least one error to the rules is made and counted as 10% error per category (Fig. 1B) [21].

All surfaces close to patients including medical equipment were cleaned once a day by healthcare professionals with isopropanol (IPA) disinfectants containing tensides (Liv DES +45 by Clemondo AB, Helsingborg, Sweden). Surfaces in the wardroom, sanitary facility, and decontamination areas were cleaned once a day by cleaning staff. Cleaning staff wiped surfaces with IPA disinfectants containing tensides (Liv DES +45) and floors were cleaned with mops and water. After the patient was discharged, the wardroom and items (which do not tolerate heat) were cleaned with IPA disinfectants containing tensides. Items tolerating heat

underwent a disinfection in a washer disinfectant. The floor and walls were cleaned with detergents (Allotol natur by Nilfisk AB, Mölndal, Sweden). All cleaning equipment was room-bound and disposable cloths and mops were used. From all patients, specimens were taken from wounds, airways, and blood stream based on clinical indications using established standards.

2.3. Infection control measures implemented during the outbreak

2.3.1. Education, hand hygiene, dress code, surface disinfection, environmental investigations, and patient-based surveillance

An extended education program was implemented and all categories of healthcare professionals including cleaning staff participated in the lectures about *A. baumannii*. Education and motivational discussions were held for each



Fig. 2 – Map of the burn intensive care unit section, (cropped image of Burn Center) Uppsala University Hospital. Patient room 3, 4, 5, 6, OR and hydrotherapy room are highlighted, where patients infected with ST15 bla_{OXA-23} *A. baumannii* were treated.

staff category at least once a week and included hand hygiene, dress code, cleaning protocols, and feedback on monitoring results at least once a week by the hospital infection control team, and nurse specialists in BICU.

Supplementary cleaning to the pre outbreak strategy with 1% sodium hypochlorite was performed with an effect time of at least 10 min, on touched surfaces. All surfaces that did not tolerate sodium hypochlorite were disinfected ones more with IPA disinfectants containing tensides [22]. Surfaces treated with sodium hypochlorite were bedside tables, bed panels, lamps, and patient beds, including all handles and buttons that the patient could have been in contact with. In the hygiene room sodium hypochlorite was used to clean toilet seat, washbasin, flush button, door handle, taps, and toilet paper holders. Finally, the cleaning equipment was disinfected after each use. After a patient was discharged from the ward, the above-described cleaning procedure was carried out in these steps. Since March 2015, an ultraviolet-C light decontaminator (Tru-D™ SmartUVC, Lumalier Corporation, Memphis, TN, USA) was used after completed cleaning.

In order to identify possible environmental sources, and to prove the wards cleaning strategy, environmental sampling was performed on three occasions, during calendar week 50, 2014, week 2, 2015 and week 16, 2015. Specimens were taken using sterile saline-soaked swabs from a variety of surfaces. In week 50, 2014 sinks in rooms 4–6, BICU, were sampled ($n = 3$). In week 2, 2015, specimens were taken in BICU from room 4 ($n = 16$), the operating room ($n = 21$), and mobile phones and pagers belonging to healthcare professionals, mainly physicians ($n = 12$). In week 16, 2015 environmental specimens were taken once again in room 4, BICU ($n = 31$, Fig. 1C).

A patient-based surveillance for carbapenem resistance *A. baumannii* was implemented in order to find cases that needed to receive adapted treatment in time and as an effect measurement of the interventions. All patients treated in the BICU were sampled from perineum/feces, throat, and three wounds using swabs once a week until 31 August 2015. Additionally, all inpatients at the Central-ICU (calendar week 2 and 7, 2015) and plastic surgery ward were tested (calendar week 2, 2015) (Fig. 1A).

2.4. Microbiology

2.4.1. Species identification

Swabs were transported in Stuart medium containing charcoal (Copan Diagnostics, Murrieta, CA, USA) to the laboratory within 24 h. The swabs were plated onto ChromID CPS agar (bioMérieux SA, Marcy l'Etoile France) and Cystine–lactose–electrolyte-deficient agar (CLED; Oxoid Limited, Hampshire, United Kingdom), and incubated for 48 h at 35 °C in room atmosphere. After inoculating the plates, each swab was put into 4.5 mL Luria-Bertani broth (LB; Oxoid Limited, Hampshire, United Kingdom) with an antimicrobial susceptibility disc containing 10 mg ertapenem. The broth was incubated overnight at 35 °C, and 10 mL were inoculated on MacConkey agar plates with a meropenem disc (10 mg), followed by incubation in 35 °C in room atmosphere overnight. Suspicious *A. baumannii* isolates were identified with standard laboratory procedures and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) using MALDI Biotyper (Bruker Corporation, Billerica, MA, USA) according to the manufacturer's recommendations with a score > 2.0. Species identification of *A. baumannii* was verified using 16S ribosomal RNA gene sequencing according to Kommedal et al. [23]. Fig. 2.

2.4.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on all isolates according to NordiCAST's (<http://www.nordicast.org/>) recommendations, using broth microdilution as implemented in the Sensititre System (Thermo Fisher Scientific, USA), and using the BMD-EUCAST reading guide for determination of minimal inhibition concentrations. All isolates were tested for the carbapenems meropenem and imipenem; trimethoprim/sulfamethoxazole; ciprofloxacin; tigecycline, the aminoglycosides gentamicin, tobramycin, amikacin; and colistin. Isolates with reduced susceptibility to one of the carbapenems were analysed for the presence of beta-lactamases in a Check-MDR CT103XL (Check-Points Health B.V, Wageningen, The Netherlands) according to the manufacturer's recommendations.

2.4.3. Epidemiological analysis

All carbapenem-resistant *A. baumannii*, were analysed for their relatedness using PFGE performed at the National Public Health Institute, Stockholm, Sweden, by using restriction enzyme Apa1 (Fig. 3). Species identification with 16S ribosomal RNA and analyses with PFGE caused a significant timely delay, therefore an AP-PCR was always performed at the Department of clinical microbiology, Uppsala University Hospital, according to Grundmann et al. using primers ERIC1R 5'-ATG TAA GCT CCT GGG GAT TCA C-3' or ERIC2 5'-AAG TAA GTG ACT GGG GTG AGC G-3' [24].

2.4.4. Whole genome sequence analysis

The isolates from patients (n = 9) were analyzed by whole-genome sequencing (WGS) as described previously [25]. Library preparation and sequencing was performed at the National Genomics Infrastructure in Stockholm, Sweden, using the Illumina NovaSeq 6000 SP platform (Stockholm, Sweden), generating 150bp paired end reads. Paired-end reads were

assembled using SPAdes assembler (v3.11.1, <http://cab.spbu.ru/software/spades/>), multi-locus sequence typing was performed according to Pasteur and Oxford scheme, and antimicrobial resistance genes were analysed using the MobileElementFinder (March 2021) (Table 2) [26,27]. Nucleotide sequences generated within this study are publicly available through European Nucleotide Archives (ENA) under project accession number PRJEB44847.

3. Results

3.1. Outbreak description

On the fourth of November 2014, a carbapenem resistant *A. baumannii* was isolated from patient 1 (index patient). During the outbreak period from November 2014 to the end of April 2015 a total of nine patients had samples with a growth of a carbapenem-resistant *A. baumannii*, that were subsequently found to belong to the outbreak strain. Patients that were assessed cases had a median age of 54 (range, 28–73), with a Total Body Surface Area burned with median 28.5%, and mean 26.4% (range, 9 – 42%). In the outbreak 67% males and 33% females were included. The patients' median length of stay was 22 days (range, 7–31days). Of the nine patients positive for the *A. baumannii* outbreak strain 2014/2015 two (22.2%) died. Fatal outcome for patient 3 was considered likely due to a ventilator-associated pneumonia and for patient 5 fatality was caused likely by bacteremia caused by the outbreak strain. The median number of antibiotics used to treat suspected infections due to the outbreak strain were four (range, 0–8): carbapenems (meropenem or imipenem) were used in five patients (5/9, 56%), and piperacillin/tazobactam in six patients (6/9, 67%). The outbreak strain was susceptible to tobramycin in four patients (4/9; 44%; patients 1, 2, 7, and 9), and colistin in three patients (3/9, 33%; patients 1, 3, and 5).

When patient 2 and 3 were found to be infected with a carbapenem resistant *A. baumannii*, the hospital infection control team initiated a first investigation and environmental sampling were performed from sinks in ward rooms 4, 5, and 6 (Nov 2014) where patients 1, 2, and 3 were cared for, respectively. Patient 4 was not previously treated in the BICU; however, this patient was nursed simultaneously at the plastic surgery ward with patient 2. Patient 7 was first admitted to the Central ICU and was infected with the outbreak strain before admission to BICU. No additional cases were found in the Central ICU; therefore, the transmission was considered as a lack of compliance with hand hygiene and dress code by healthcare professionals from the BICU consulting Central ICU or other consulting healthcare staff. All other patients infected with the outbreak strain were treated at the BICU and were the most likely site of infection.

The majority of the environmental specimens were negative to the outbreak strain. In January 2015, environmental sampling from room 4 at BICU showed growth of the outbreak strain. The specimen was taken from a ragged bed rail after thorough cleaning and disinfection procedure. Patients 1, 5, and 8 were treated in this room (Fig. 2). When patient 5 was discharged the room was cleaned according to the

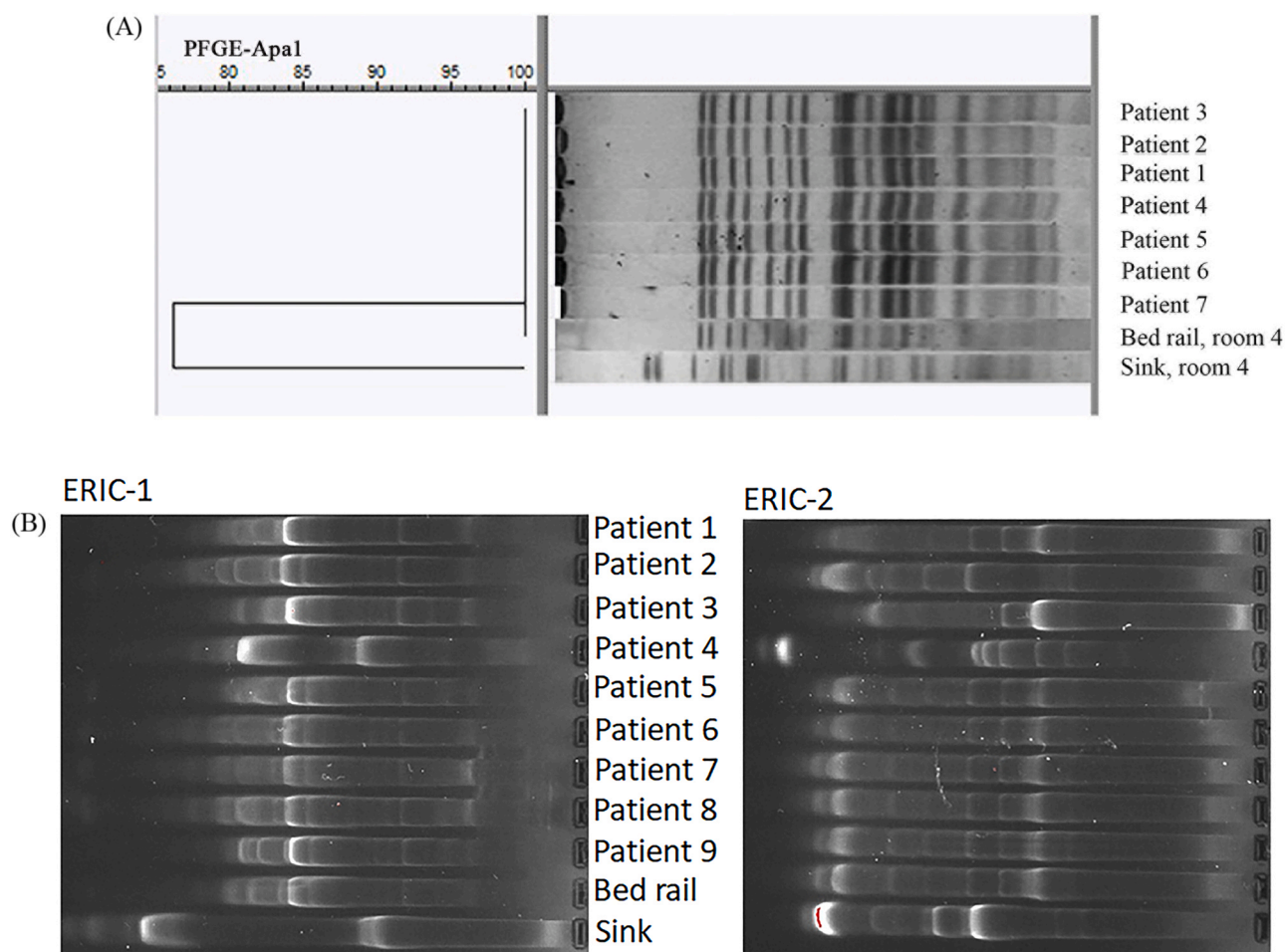


Fig. 3 – Images of gel electrophoresis analysis after PFGE (3 A) and AP-PCR (3B) analysis for a representative selection of *A. baumannii* isolates. The first *A. baumannii* isolate identified per affected patient was chosen. 3 A: PFGE patterns for isolates from patients 1–7, and bed frame are illustrated. A *A. guillouiae* isolated from a sink in room 4 was used as root. 3B: AP-PCR patterns for isolates from patients 1–9, sink and bed frame are illustrated; primer ERIC-1 on the left and primer ERIC-2 on the right.

above-described protocol and were closed for six weeks (3 January - 15 February). On 15 February, patient 8 was admitted to room 4, and nine days later wound cultures were positive for the outbreak-strain. The last patient, 9, was treated in room 3 three weeks after patient 8 had left the ward. Weekly surveillance sampling for carbapenem resistant *A. baumannii* continued until August 2015, and the clinical microbiologists have continued to read clinical samples from BICU with high alertness for carbapenem resistant *A. baumannii* until today. No more spread of the outbreak strain was found during the following five years after the last patient (Fig. 2).

3.2. Infection control measures

3.2.1. Education

All intended education, involving 13 lectures and weekly discussions at workplace meetings were performed during March 2014 until the end of May 2015 for all healthcare professionals. The weekly feedback worked as continuous

reminders on cleaning routines and to keep hand hygiene, and dress code, on a level above 80% (Figs. 1B and 1C).

3.2.2. Hand hygiene and dress code

The monitoring of the compliance with hand hygiene and dress code were maximum 90% during the time of observation and ranged from 70% to 90%. Compliance with hand hygiene, especially hands and forearms disinfection before patient contact, accounted for the most frequent errors: seven failures during the period compared to dress code failure which was only found in three cases. During December 2014, compliance with hand hygiene dropped to 70%, and during this period the frequency of new cases was high (Fig. 1B).

3.2.3. Surface disinfection and environmental investigations

Intensified cleaning protocols were implemented. Broken items were discarded and replaced by new ones and equipment not suitable for cleaning protocols were exchanged, items replaced were: chairs and computer keyboards inside

Table 2 – Overview over the antibiotic resistance genes and antibiotic resistance per representative patient isolate. Table header: MEM (meropenem), IMP (imipenem), CN (gentamicin), TOB (tobramycin), AK (amikacin), TGC (tigecycline), STX (ciprofloxacin), CIP (ciprofloxacin), CT (colistin); numbers in parenthesis relate to clinical breakpoints according to NORDCAST (version v11.0, 2021) per substance with first number indication the breakpoint for susceptible (\leq) and the second for resistant ($>$). All MIC values are specified in mg/L.

Patient	Antibiotic resistance genes	MEM (2/8)	IMP (2/4)	CN (4/4)	TOB 4/4	AK (8/8)	TGC (0.5/0.5)*	STX (2/4)	CIP (0.001/1)	CT (2/2)
1	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (4)	S (1)	R (> 32)	S (0.5)	R (8)	R (> 2)	S (1)
2	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (4)	S (1)	R (> 32)	S (0.5)	R (8)	R (> 2)	S (1)
3	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (4)	S (1)	R (> 32)	S (0.5)	R (> 8)	R (> 2)	S (1)
4	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (4)	S (1)	R (> 32)	S (0.5)	R (8)	R (> 2)	S (1)
5	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	R (> 8)	S (4)	R (16)	R (2)	R (> 8)	R (> 2)	S (2)
6	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (2)	S (1)	R (> 32)	S (0.5)	R (8)	R (> 2)	S (1)
7	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (4)	S (1)	R (> 32)	S (0.5)	R (8)	R (> 2)	S (1)
8	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	R (> 8)	S (4)	R (16)	S (0.5)	R (8)	R (> 2)	S (1)
9	<i>aph(3')-Via</i> , <i>bla_{OXA-23}</i> (IS6), <i>bla_{OXA-51}</i> , <i>bla_{ADC-25}</i>	R (> 16)	R (> 16)	S (2)	S (1)	R (> 32)	S (0.5)	R (8)	R (> 2)	S (1)

the patient-rooms and in nurses' offices; textile gowns to single-use disposable aprons; cabinets to shelves in the patients' toilets; textile sofa in the patients' family room to a wipeable one; sink (week 3, 2015), ragged bed rail (week 3, 2015), and the decontaminator, all in room 4, BICU; broken protective cover for X-ray plate; water locks in all patient-rooms, OR, and hydrotherapy room (week 3, 2015).

Furthermore, all HEPA-filters in patient-rooms were cleaned and the number of cleaning staff was increased from 1 to 1.5 (and is still the same) employees and a consistent group of cleaners worked at the BICU after specific training. During the environmental sampling in calendar week 2, 2015 (n = 49), the outbreak strain was isolated from a broken bed rail in room 4. N.B. The sampling was performed after the discharge of patient 5 and intense cleaning procedures. During the investigation, no unique common source of dissemination in the environment could be identified (Fig. 1).

3.2.4. Patient-based surveillance

A total of 368 microbiology specimens were taken from the affected nine patients during the outbreak and carbapenem resistant *A. baumannii* was found in 64 samples (17.4%). Out of the 64 *A. baumannii* positive samples, 41 (64%) specimens were wound swabs, blood stream isolates (n = 6), tracheal secretions (n = 3), central line catheter (n = 3), throat (n = 2), feces (n = 2), sputum (n = 2), nostrils (n = 2), urine (n = 1), perineum (n = 1), and pharynx (n = 1).

3.3. Microbiology

3.3.1. Antimicrobial susceptibility

All *A. baumannii* isolates related to the outbreak strain were reported resistant to meropenem, imipenem, ciprofloxacin, and co-trimoxazol; and susceptible to colistin and tobramycin. The isolates showed varying susceptibility to gentamycin and amikacin, the same was observed for isolates derived from the same patient. Most of the isolates carried the carbapenemase gene *bla_{OXA-23}* that was associated to an insertion sequence of IS6 family. However, for the blood stream isolate of patient 5 no *bla_{OXA-23}* could be found. The chromosomal beta-lactamases *bla_{OXA-51}* and *bla_{ADC-25}* were present in all nine isolates. All isolates carried genes for the dihydropteroate synthase *sul2* that was associated to insertion sequence IS91 and the aminoglycoside 3'-phosphotransferase *aph(3')-Via* was present in 6/9 isolates (Table 2).

3.3.2. Epidemiological analysis

The outbreak strain showed the same band pattern after PFGE analysis for all representative isolates from nine patients and the bed rail. The isolate *Acinetobacter guillouiae*, from a sink in wardroom 4, was thus not interpreted to be part of the outbreak. AP-PCR showed a consistent pattern for all isolates but from patient 4 using primer ERIC-1, and for all isolates but from patient 3 and 4 for ERIC-2. The outbreak strain gave robust results with AP-PCR while the outbreak progressed, and as AP-PCR was accessible at the hospital and could be run the same day, AP-PCR was used as first line epidemiological tool. Still, all new cases were confirmed with PFGE. MLST analysis on whole genome sequences resulted in

ST15 according to Pasteur's MLST scheme and ST236 according to Oxford's MLST scheme.

4. Discussion

Here, we describe the investigation of an outbreak caused by an ST15 OXA-23-producing *A. baumannii* in a burn center at Uppsala University Hospital in Sweden with focus on the infectious control measures undertaken to impede the spread. Hard focus on obedience to correct hand hygiene and dress code by all categories of health care professionals and intensified cleaning protocols led to control of further spread of the outbreak strain and prevented new outbreaks for the following five years.

Since 2007, the Swedish National Board of Health and Welfare has governed hand hygiene and dress code in the regulation SOSFS 2015:10, and all health care providers must monitor compliance with these rules. The regulation gives no advice to what extent the compliance with the rules should be, and in 2014/15, at the time of the here described outbreak the hospital's goal for compliance was set to 90% for dress code and 80% for hand hygiene compliance. Nevertheless, in December 2014 the staff's compliance with hand hygiene and dress code was as low as 70% in the BICU. During that period, the BICU employed more staff than usual, and the staff was working with high demands due to Christmas holidays, leading to a markedly high workload situation that was further aggravated by the exceptionally high number of severely burned patients compared to normal. The impact of workload on hand hygiene compliance has been highlighted previously, and Chang et al. showed that hand hygiene compliance dropped significantly when the workload reached 30 opportunities for proper hand hygiene per hour [28]. In a literature review by Erasmus et al. [29] compliance with hand hygiene ranged between 4% and 100% with a median compliance of 40%, which is a low value compared to the BICU at Uppsala University Hospital. Nevertheless, lack of hand hygiene compliance was assumed to be the only possibility for infection of patients 4 and 7 with the outbreak strain, as none of them were nursed at the BICU, and several consultants oversaw patients both at the BICU, the plastic surgery ward, and the Central ICU where patients 4 and 7 were treated, respectively.

The outbreak and the infection control measures generated a pre-understanding over time, by the staff, about *A. baumannii* and its impact on patients, likewise as Cheng et al. [30] describes in their review. Learning via lectures took place in parallel with multimodal environmental measures taken and feedback and reminders from the management continuously every week during the outbreak.

A. baumannii is a species known to be well adapted to hospital settings due to its tolerance to biocides and desiccation, effective cleaning strategies are a challenge. Another aspect is that broken items and equipment can be a bacterial hotbed and must be reviewed regularly. Disinfectants used during the outbreak were Liv DES 60% IPA for hand disinfection, Liv DES +45 IPA and 1% sodium hypochlorite for surface disinfection, in the late phase of the outbreak an UVC decontaminator was introduced. Ethanol

and isopropanol have been shown to effectively reduce the bacterial load of *A. baumannii* when used for hand hygiene, however, an efficient number of bacteria may still survive the disinfection procedure and be causative for patient-to-patient transfer by health care workers [31,32]. Although transient carriage of bacteria on the skin is regarded as one of the most important factors for spread, especially outbreaks have frequently been associated with environmental sources. *A. baumannii* strains related to outbreaks have been shown to survive on inanimate dry surfaces for up to four months [33]. Therefore, effective environmental cleaning protocols of dry surfaces are regarded with special significance. The importance of concentration and contact time for effective disinfections has been highlighted previously. The staff of the BICU applied 1% sodium hypochlorite with a contact time of 10 min on inanimate surfaces, which is expected to be sufficient when considering the results presented by Liu et al. reporting 0.5% sodium hypochlorite with a contact time of 30 s as efficient to eradicate an imipenem-resistant *A. baumannii* outbreak strain [34]. Furthermore, bactericidal concentrations for sodium hypochlorite to *A. baumannii* were previously measured between 4 and 160 mg/L, which is 100-fold below the concentration of 1% sodium hypochlorite [35]. Nevertheless, the outbreak strain was isolated from a ragged bed rail after intense cleaning procedures with Liv DES +45% and 1% sodium hypochlorite and might illustrate the discrepancy of laboratory test settings where plain surfaces are used and where human failure, that could miss proper cleaning at locations difficult to access, has significant influence on the results. However, the environmental sampling during calendar week 2, 2015, led to only one sample where the outbreak strain could be found (1/49.2%), a fact that could also be interpreted as proof of efficacy of the cleaning protocol.

A. baumannii isolates belonging to clonal complex ST15 have been described from Brazil, Kuwait, The Netherlands, Czech Republic, and Argentina and isolates producing OXA-23 were common [15,36–38]. In concordance to previous studies, OXA-23-producing and OXA-23-non-producing ST-15 isolates were reported highly resistant to meropenem and imipenem [15]. The carbapenemase *bla*_{OXA-51-like} has been shown to be intrinsic to *A. baumannii* and is overexpressed when the insertion sequence ISAb9 providing promotor sequences is present upstream of *bla*_{OXA-51} [39,40]. In all sequenced isolates from the here reported outbreak, both *bla*_{OXA-51} and ISAb9 were present, however, not assembled on the same contig. All isolates were susceptible to colistin and tobramycin, and these substances were mainly used when infection with *A. baumannii* was judged to need treatment.

5. Conclusions

Our recommendation is to have close collaboration with the hospital hygiene department and the microbiology laboratory. Utmost attention to suspected outbreaks by everyone in the staff and the management. Limiting the number of staff that nurse the patient and avoiding non-necessary relocations to other units/ hospitals is necessary. Increased

education, compliance with hand hygiene and dress code, intensified cleaning protocols for the environment and equipment were successfully established in this outbreak.

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