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Long-term intensive care unit outbreak of carbapenamase-producing organisms associated with contaminated sink drains

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SUMMARY

Background: Between 2018 and 2022, a Belgian tertiary care hospital faced a growing issue with acquiring carbapenemase-producing organisms (CPO), mainly VIM-producing *P. aeruginosa* (PA-VIM) and NDM-producing Enterobacterales (CPE-NDM) among hospitalized patients in the adult intensive care unit (ICU).

Aim: To investigate this ICU long-term CPO outbreak involving multiple species and a persistent environmental reservoir.

Methods: Active case finding, environmental sampling, whole-genome sequencing (WGS) analysis of patient and environmental strains, and implemented control strategies were described in this study.

Findings: From 2018 to 2022, 37 patients became colonized or infected with PA-VIM and/or CPE-NDM during their ICU stay. WGS confirmed the epidemiological link between clinical and environmental strains collected from the sink drains with clonal strain dissemination and horizontal gene transfer mediated by plasmid conjugation and/or transposon jumps. Environmental disinfection by quaternary ammonium-based disinfectant and replacement of contaminated equipment failed to eradicate environmental sources. Interestingly, efflux pump genes conferring resistance to quaternary ammonium compounds were widespread in the isolates. As removing sinks was not feasible, a combination of a foaming product degrading the biofilm and foaming disinfectant based on peracetic acid and hydrogen peroxide has been evaluated and has so far prevented recolonization of the proximal sink drain by CPO.

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Conclusion: The persistence in the hospital environment of antibiotic- and disinfectant-resistant bacteria with the ability to transfer mobile genetic elements poses a serious threat to ICU patients with a risk of shifting towards an endemicity scenario. Innovative strategies are needed to address persistent environmental reservoirs and prevent CPO transmission.

Introduction

The prevalence of clinically relevant carbapenemase-producing organisms (CPO), such as *Pseudomonas aeruginosa* and Enterobacterales, has increased worldwide [1,2]. Genes encoding for carbapenemases, such as the Verona integron-encoded metallo-β-lactamases (VIM) and the New Delhi metallo-β-lactamases (NDM), coexist with many other resistance determinants and are often transmitted between organisms by mobile genetic elements, such as transposons and/or plasmids, contributing to their spread [3]. Healthcare-associated infections caused by CPO are particularly worrying since they are associated with an increased financial burden, prolonged hospital stays, and increased mortality [4—8]. In this context, the prevention of the acquisition and spread of these strains is a priority. Current infection prevention and control interventions include screening, hand hygiene promotion, barrier precautions, enhanced surface disinfection, waste management, and contaminated source identification and elimination [9,10].

Between 2018 and 2022, our healthcare facility was confronted with a rising number of CPO acquisitions, mainly VIM-producing *P. aeruginosa* (PA-VIM) and NDM-producing Enterobacterales (CPE-NDM) among hospitalized patients in the adult intensive care unit (ICU). We report the investigation of a CPO long-term outbreak in ICU and the identification of an environmental aquatic source with genomic analysis confirming the horizontal transfer of mobile genetic elements. The aim was to provide insight into the complexity of outbreak management in this specific type of outbreak involving a persistent reservoir and multiple species, which may be encountered in ICU settings worldwide, and to demonstrate the need for combined measures over time.

Methods

Setting

Cliniques universitaires Saint-Luc is a tertiary care hospital in Belgium, with approximately 1000 beds. The adult ICU includes 14 single-bed rooms. Each room contains a sink and a bedpan washer (Figure 1A and B).

Case definitions

Cases were defined as ICU colonized or infected patients identified with acquired CPO between January 2018 and December 2022. Colonized patients were defined as patients in whom CPO was identified only on screening samples (endotracheal aspirate, rectum and urine samples were collected upon admission and twice weekly). Infected patients were defined as patients with at least one clinical sample CPO positive. The acquisition was defined when CPO were identified in the patient ≥48 h after hospital admission.

CPO microbiological investigations

Routine rectal swabs were recovered with Copan ESwab® (Brescia, Italy) and inoculated on ChromID ESBL (bioMérieux, Marcy l'Etoile, France) medium.

Bacterial isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-Biotyper; Bruker Daltonics, Bremen, Germany). When CPO was suspected on rectal swabs or clinical samples based on antimicrobial susceptibility testing, the identification of carbapenemase type was confirmed by an in-house multiplex polymerase chain reaction or by immunochromatographic assay (K-SeT; Coris BioConcept, Gembloux, Belgium) [11].

Infection control measures

Specific detection of CPO acquisition prompted enhanced infection control procedures, maintained daily until patient's death or discharge and including alert in the electronic health record, contact precautions and environmental chlorine dioxide cleaning. Complete room disinfection was performed with Tristel Fuse® (Tristel, Anvers, Belgium) and misting with hydrogen peroxide, upon discharge of each CPO-positive patient. In February 2019, additional preventive interventions were implemented to mitigate contamination of sink drains and reduce CPO transmission. The ICU siphons were replaced by the HygieneSiphon® (Aquafree, Hamburg, Germany), consisting of a permanent drain valve with a replaceable inlet. The inlet was replaced once every three months and upon discharge of each CPO patient. Starting from September 2021, the inlet was replaced monthly, combined with daily cleaning with 1 L of 0.5% Incidin Pro® (2-phenoxyethanol, *N*,*N*-bis-(3-aminopropyl) dodecylamine, benzalkoniumchloride; Ecolab, Groot Bijgaarden, Belgium). The sink drains of the 14 rooms (from the drain valve to the wall including bottle trap (P1) and the pipe (P2) (Figure 3A)) were changed in November 2019, February 2021 and January 2022.

Environmental sampling

Between December 2018 and January 2023, intermittent environmental sampling of sink drains (inlet and/or drain valve) was performed. Environmental samples were recovered with ESwab, inoculated on ChromID Carba Smart (bioMérieux), and incubated for 48 h. Since January 2022, the remaining suspension of each sink drain swab was incubated in a Letheen broth at 37 °C for seven days before plating on ChromID Carba Smart to detect low concentrations of CPO.

Figure 1. (A) Map of the adult intensive care unit, which includes 14 single-bed rooms. (B) Each room contains a sink and a bedpan washer. Sinks in patient rooms were located <5 feet (<2 m) from patient beds. The sink is systematically on the side of the patient's feet. The pipes of the different rooms are connected to a horizontal drainage system within the unit.

To evaluate the colonization rate of the sink drain, samples were collected from the inlet or the drain valve of four ICU rooms before the inlet replacement and twice a week during four weeks after replacement. ESwabs were serially diluted and plated on Columbia blood agar (Becton Dickinson, Cockeysville, MD, USA) and ChromID CARBA to quantify the total number of bacteria and carbapenem-resistant bacteria, respectively. In parallel, a pre-enrichment in a Letheen broth of each swab was incubated at 37 °C for seven days before plating on ChromID Carba. Bacterial and CPO identification were assessed as described above.

Foam cleaning protocol evaluation

A new protocol combining enziSurf™ (OneLife, Louvain-la-Neuve, Belgium) and Phago'Spore® (Christeyns, Gent, Belgium) was evaluated on four contaminated ICU sink drains (without inlet) in November 2022. The enziSurf protocol is composed of two foaming products: enziSurf Descale (descaling agent containing phosphoric acid, lactic acid and anonic and non-ionic surfactants; applied 5 min) and enziSurf Drain (solution containing five enzymes known to degrade biofilm matrix (including protease, lipase, amylase, and DNase), applied for 15 min. The Phago'Spore is a foam non-quaternary ammonium-based detergent/disinfectant composed of peracetic acid 0.034% and hydrogen peroxide 3.26% applied 15 min after the enziSurf protocol. The water was run for a few seconds between each product, until complete flushing of foam. Two treatments were applied successively: a curative protocol (enziSurf and Phago'Spore every day for four days) followed by a preventive protocol (enziSurf and Phago'Spore twice weekly for four weeks). Prior to each application and twice a week, samples were collected from the proximal sink drain to a depth of 10 cm and were assessed as described above to estimate the cfu/mL of carbapenem-resistant bacteria and evaluate the presence of CPO.

In February 2023, the 14 ICU HygieneSiphons were replaced by standard chrome-plated brass sink drains and cleaned twice weekly with the preventive protocol combining enziSurf and Phago'Spore. Samples were collected from the proximal sink drain once per month and assessed as described above.

Whole-genome sequencing

Isolates were analysed by whole-genome sequencing (WGS). Libraries were constructed with Illumina DNA prep kit (Illumina, San Diego, CA, USA) and were sequenced on the Illumina MiSeq or NextSeq1000 platform according to the manufacturer's protocol. Sequence reads, whole-genome multi-locus sequence typing (wgMLST) and single-nucleotide polymorphism (SNP) were analysed using BioNumerics (version 8.0; Applied-Maths, Sint-Martens-Latem, Belgium). wgMLST was analysed with a scheme containing 15,143 loci for *P. aeruginosa*. SNP analysis was performed using as reference the contig harbouring

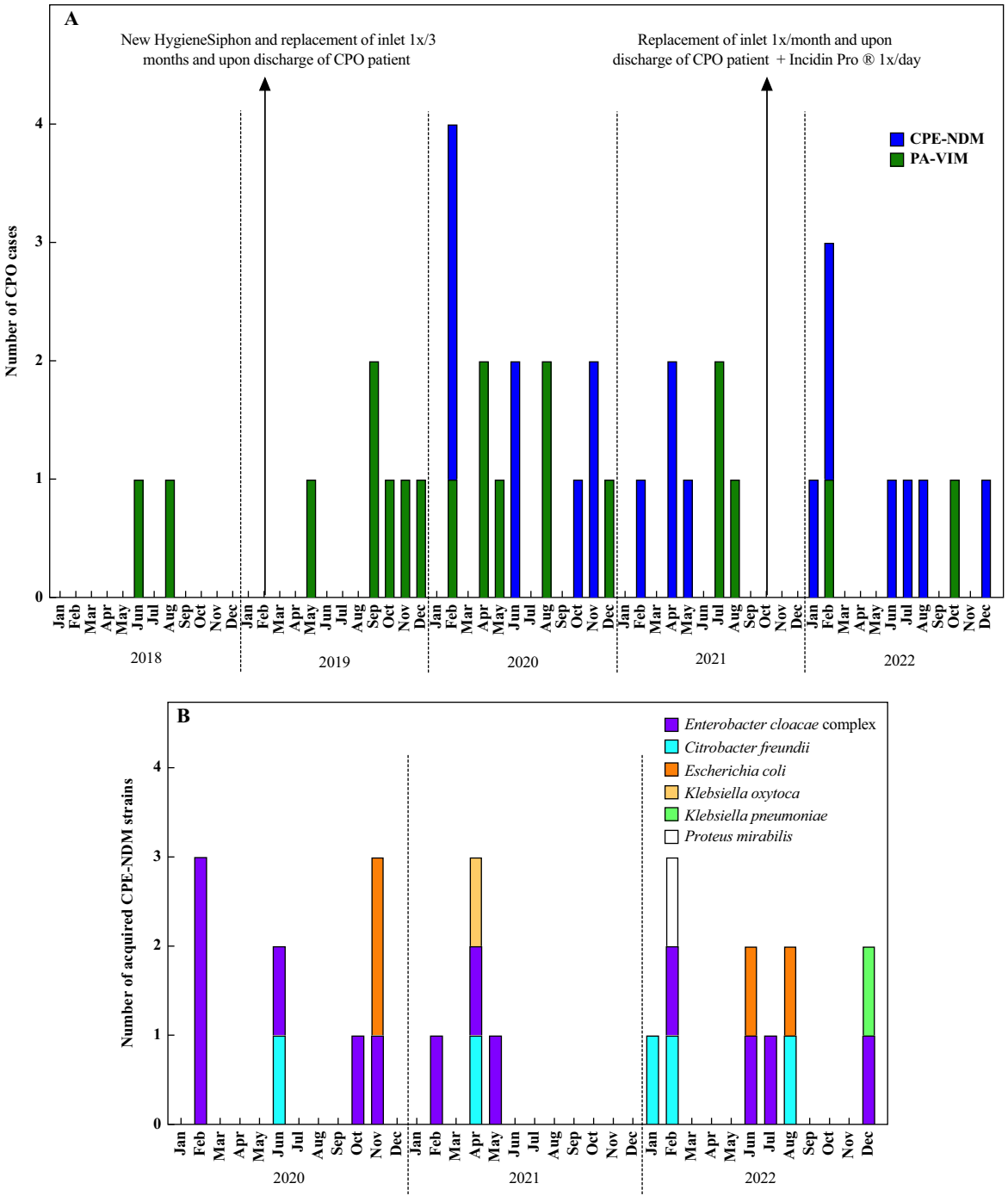

Figure 2. (A) Epidemic curve of VIM-producing *Pseudomonas aeruginosa* (PA-VIM) and/or NDM-producing Enterobacterales (CPE-NDM) patient acquisitions in the intensive care unit (ICU). (B) Epidemic curve of CPE-NDM strains acquisition in ICU.

bla_{NDM-1} isolated from the first CPE-NDM strain of the outbreak CPE275 (ST395; 136,152 pb; October 2019) for Enterobacterales and PA1936 the first PA-VIM strain of the outbreak (ST111; 7,001,756 pb; June 2018) for PA-VIM ST111.

Results

Outbreak description

CPO acquisition incidence per 1000 hospitalization-days increased in ICU from 2019. Notably, PA-VIM increased from 0.49 in 2018 to 1.72 in 2020 and CPE-NDM increased sharply from 0 in 2018 to 1.97 in 2020. The combined attack rate of PA-VIM and CPE-NDM increased from 0.17% in 2018 to 0.49% in 2019, 1.89% in 2020, 0.72% in 2021, and to 0.87% in 2022. From 2018 to 2022, 37 ICU patients were newly colonized or infected with acquired CPE-NDM and/or PA-VIM. The median ICU length of stay was 43 days, and 51% of patients died. Prior to CPO detection, the median ICU length of stay was 19 days, and all patients received anti-Gram-negative antibiotics. A total of 19 PA-VIM and 25 CPE-NDM were detected, including 13 *Enterobacter cloacae* complex, five *Citrobacter freundii*, four

Escherichia coli, one *Klebsiella oxytoca*, one *Proteus mirabilis*, and one *K. pneumoniae* (Figure 2A and B). One patient acquired both PA-VIM and two CPE-NDM isolates, and five patients harboured two different CPE-NDM isolates (Supplementary Table A1). Patients with acquired CPO were not related to one room, suggesting several persistent environmental reservoirs of PA-VIM and CPE-NDM during the five years.

Environmental investigations/sink colonization

The sink drain of ICU rooms (Figure 3A) has been suspected to be an environmental reservoir since 2019. Indeed, we investigated several environmental sources (sink drains, sink, faucet jetbreaker, water and bedpan washer) and no CPO was found in any of these samples except those from the sink drains. Between June 2018 and January 2023, intermittent sampling of the 14 sink drains (210 environmental samples from the inlets and 70 from the drain valves) confirmed their colonization with PA-VIM and/or CPE-NDM (Figure 3B). In October 2019, the sink drain of the rooms M4 and M10 were positive with CPE-NDM whereas no CPE-NDM-positive patients had previously been hospitalized in these rooms. Likewise the sink drain of room M2 was repeatedly positive for PA-VIM without any known PA-VIM-infected/colonized patients in this room. These observations suggested a contamination route of some sink drains independent of CPO-positive patients. The rate of colonization upon inlet replacement and despite daily cleaning was rapid. After one month, the four inlets were colonized by 10^3–10^4 cfu/mL of carbapenem-resistant bacteria, including PA-VIM, *E. cloacae* complex NDM, and *C. freundii* NDM. The permanent drain valves were colonized with 10^7–10^8 cfu/mL of carbapenem-resistant bacteria (Figure 4A). In January 2023, sampling of different siphon parts revealed a colonization of the whole siphon with CPO (Figure 3).

Sequencing results

One hundred and twenty-six isolates were sequenced, 40 (18 PA-VIM and 22 CPE-NDM) from patients and 86 (28 PA-VIM and 58 CPE-NDM) from environmental sampling.

VIM-producing P. aeruginosa

Polyclonality was observed among PA-VIM, including ST111 (N=32) as the predominant clone, ST179 (N=7), ST175 (N=2), ST245 (N=2), and ST235, ST253, and ST395 with one isolate of each (Supplementary Figure A1). Nevertheless, all isolates (except ST235) harboured bla_{VIM-2} and shared a large array of associated resistance genes (Supplementary Figure A2). In 93.3% (42/45) of isolates, the bla_{VIM-2} gene was found within a class I integron, inserted in a Tn21-like transposon. No plasmid was detected in PA-VIM isolates. The genetic environment of bla_{VIM-2} of ST175 and ST395 clinical isolates differs from the other strains and may thus not be linked to the same environmental reservoir. The wgMLST analysis showed the genetic proximity between the isolates within each MLST. Focusing on PA-VIM ST111, SNP analysis confirmed that most (30/32) clinical and environmental isolates originated from a common reservoir (<10 SNPs). However, isolates from room M2 differed genetically in ~37 SNPs, suggesting different origins (Supplementary Figure A3).

NDM-producing Enterobacterales

The 80 CPE-NDM isolates included 46 *E. cloacae* complex, 24 *C. freundii*, four *E. coli*, four *K. oxytoca*, one *K. pneumoniae*, and one *P. mirabilis*. Polyclonality was observed among *E. cloacae* complex, *C. freundii*, *E. coli*, and *K. oxytoca* with a clonal spread of ST595 *E. cloacae* complex (34/46) (Supplementary Figure A1). The plasmid belonging to the incompatibility (Inc)C (~140 kb) harbouring bla_{CMY-6} and bla_{NDM-1}, *sul1*, *qacEdelta1*, and *aac(6′)-Ib3* located within a class 1 integron was present in all Enterobacterales species from the outbreak, including patient and environmental strains (Supplementary Figure A4). SNP analysis confirmed that the CPE-NDM outbreak isolates differed by less than two SNPs in this genomic region regardless of species (Supplementary Figure A5). The same plasmid was found in a community-associated *E. coli* strain (CPE399), suggesting the spread of these genes within the community. The three ST544 NDM-1-*E. cloacae* complex isolates (from patients and sink drain) harboured *mcr-9* (mobilized colistin-resistance) genes carried by a different plasmid (Inc HI2/HI2A).

Interestingly, 116 isolates (72 CPE-NDM and 44 PA-VIM) from patients and sink drains harboured the gene *qacEΔ1* located in the 3′-CS of class 1 integron. The IncC plasmid also carried the *sugE* gene. These resistance genes encode for efflux pumps (small multidrug resistance (SMR) family), conferring resistance to quaternary ammonium compounds (QACs).

Mitigation strategies

Due to the detection of nine PA-VIM and CPE-NDM acquisitions in 2022 with the evidence for a water reservoir, and the ineffectiveness of measures, a new protocol combining enziSurf and Phago'Spore was evaluated on four ICU sink drains without inlet.

The efficacy of this protocol was compared during one month with the routine protocol (Figure 4B). After the four-day curative protocol, no carbapenem-resistant bacteria were detected in the drain valve. After four weeks of preventive protocol, two drain valves were colonized by 10^2–10^3 cfu/mL of carbapenem-resistant bacteria (*Pseudomonas monteilii*) but not by CPO, unlike the routine protocol where CPO quickly recolonized the four new inlets.

These preliminary results led to replacing all HygieneSiphons by standard chrome-plated brass sink drains and cleaning twice per week with the new preventive protocol combining enziSurf and Phago'Spore by the cleaning staff. The monthly control of the proximal drain showed aquatic (ex: *Pseudomonas oleovorans*) and skin bacteria but no CPO after seven months of prospective analysis, even when the distal parts (pipe connected to the wall) were colonized by CPO (Figure 3). Only one NDM-*E. cloacae* complex acquisition was detected in May 2023 in room M01 non-related with sink drain. The cleaning staff reported no fumes nor odour during the use of these products, but the application took longer and was less straightforward than the previous Incidin Pro protocol.

Training of healthcare workers on the correct use of the sinks in ICU patient rooms was performed in parallel. They focused on the appropriate use of the sinks for hand hygiene, the non-use of the sinks to pour intravenous bags and dialysis fluid down the drain, and the separation of non-contaminated and contaminated areas and tasks.

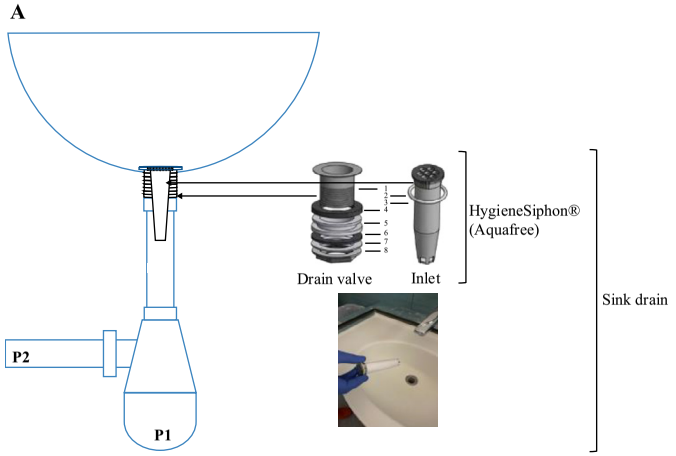

Figure 3. (A) Siphon scheme. HygienSiphon® (Aquafree) is composed of a permanent drain valve and a replaceable inlet to reduce aerosols from the drain traps during water running off. P1 indicates the bottle trap and P2 the pipe connected to the wall. (B) Acquisition of NDM-producing Enterobacterales (CPE-NDM) and/or VIM-producing *Pseudomonas aeruginosa* (PA-VIM) and environmental sampling of the different parts of the siphon (inlet, drain valve, P1 (bottle trap) and P2 (pipe connected to the wall)) according to the intensive care unit (ICU) between June 2018 and August 2023. Since February 2023, the HygieneSiphons of the 14 ICU rooms (M01 to M14) were replaced by standard chrome-plated brass sink drains and cleaned twice a week with the new preventive protocol (enziSurf™ and Phago'Spore®) by the ICU cleaning staff.

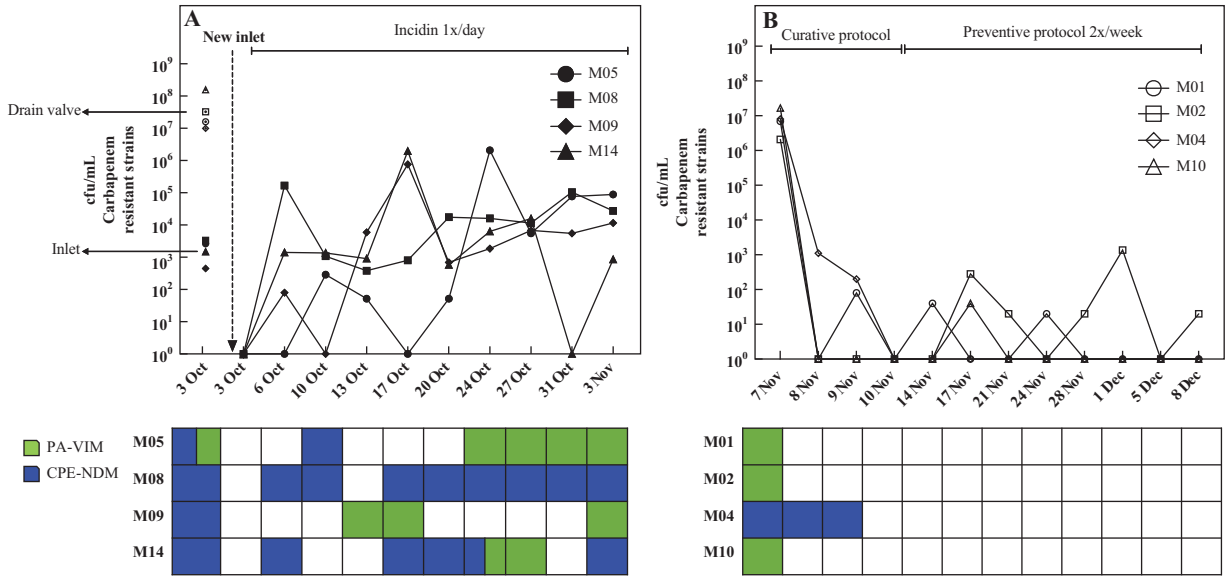

Figure 4. Colonization rate of sink drains by carbapenem-resistant Gram-negative bacteria. The routine protocol (daily cleaning with 1 L of 0.5% Incidin Pro® with new inlet replacement) (A) was compared to a new protocol combining enziSurf™ and Phago'Spore® (B) in four sinks. The number of carbapenemase-resistant bacteria was expressed in cfu/mL.

Discussion

To our knowledge, this investigation represents the first described long-term outbreak of CPO involving a diverse set of bacterial species with a common environmental reservoir.

Reported healthcare-associated CPO outbreaks are generally caused by a single, clonal strain. In this study, the WGS analysis revealed both a polyclonality among CPO strains with a clonal spread of *E. cloacae* complex ST595 and *P. aerginosa* ST111, and highly transmissible mobile genetic elements carrying a plethora of resistance. The VIM-2-producing *P. aeruginosa* ST111 is a high-risk, epidemic MDR/XDR lineage, globally widespread including Belgium and associated with high morbidity and mortality [12—15]. Unlike the latter, *E. cloacae* complex ST595 has only been previously described in two American studies carrying class A β-lactamase *Klebsiella pneumoniae* carbapenemase [16,17]. Multiple genetic mechanisms were involved in NDM or VIM transmission, including clonal spread and horizontal gene transfer mediated by plasmid and/or transposon jump. Both bla_{NDM-1} and bla_{VIM-2} genes were located within a class I integron, found in Tn*21*-like transposons integrated either in the IncC plasmid for Enterobacterales or in the chromosome for *P. aeruginosa* [18]. Some clones, such as *P. aeruginosa* ST245, *E. cloacae* complex ST513, and *C. freundii* NT, were mainly found in one room. We also observed within the ST111 group a low genetic distance between the clinical and/or environmental isolates collected within the same room. These observations may suggest a local ecological (e.g. pathogen introduction by the hospitalized patient) and evolutionary pressure within each ICU room.

Phylogenomic analysis confirmed the epidemiological link between clinical and environmental strains. Hospital sinks are well-known reservoirs for the transmission of Gram-negative pathogens in general, and CPO in particular [19—23]. Interestingly, some sink drains were contaminated by CPO without any known infected/colonized patients previously hospitalized in these rooms. The introduction of pathogens into the sink trap is multifactorial, such as the use of sinks for handwashing and disposal of waste and the transmission from neighbouring rooms via the horizontal drainage system [24]. During faucet operation, contaminated aerosols and drain contents are then dispersed to surrounding areas from the sink and bacteria may be transferred to healthcare workers and the patient [19,22,25,26]. Sub-optimal room and sink designs can put patients at risk [25—27]. In addition, it has been observed that sterile materials and devices intended for patient insertion were regularly misplaced in the very near surroundings of the sinks in the ICU [28].

A range of interventions to eradicate these reservoirs has been published, emphasizing disinfection, biofilm disruption, replacement of sink drain/plumbing, and complete removal of the reservoir [19,29]. Infection control strategies are often bundled together during outbreaks.

Disinfection alone fails to control the CPO reservoir, leading to hospital-acquired infection [19,29—31]. Biofilms may limit the penetration of disinfectants such as chlorhexidine and QACs (benzalkonium chloride) [32]. Several QAC efflux systems have been discovered in Gram-negative bacteria (*sugE*, *emrE*, *qacE*, and *qacEΔ1*), conferring resistance to QACs and multiple antimicrobials [33—36]. The *sugE* and *qacEΔ1* genes of antiseptic resistance were broadly identified in the clinical and environmental isolates of our outbreak. Although the exact role of *qacEΔ1* is still controversial, the daily use of quaternary ammonium-based disinfectant in our sink drains may have created selective pressure on CPO [37]. According to the review by Collet *et al.*, using hydrogen peroxide or peracetic acid constitutes an improbable risk for developing resistance to antimicrobials [34].

For the control of the CPO reservoir, it is therefore helpful to reduce the biofilm's density before applying the biocide. Most of the interventions described in the literature, such as pressurized steam [38,39], self-disinfecting traps with electromechanical vibration, bundled with heat or ultraviolet

radiation [21,40,41], and replacement of sinks and/or sink drains [29], showed only temporary reductions in transmission and sink colonization, as observed in our study. Removal of bacterial reservoirs with the implementation of waterless patient care was the most successful intervention in CPO outbreaks, showing an effect in all studies [25,42,43].

In our case, the combined actions of QAC daily, the design of the HygieneSiphon (inlet replaced monthly) and the sink drain replacement every year were insufficient to prevent sink drain recolonization and CPO acquisitions. Recolonization may occur after exposure to contaminated materials or retrograde growth from P-shaped traps or the water drainage network. Indeed, we observed that CPO colonized the whole siphon to the entrance in the wall. The removal of sinks and a change in the architecture of the rooms in our setting were unfortunately not feasible. We therefore looked for an efficient and inexpensive solution to limit CPO acquisition and sink drain colonization. The repeated combination of a foaming product able to degrade the biofilm and a foaming disinfectant based on peracetic acid and hydrogen peroxide, with a longer contact time, might be a promising solution based on literature review [34,44]. Additional risk mitigation strategies (enzymatic, probiotic, or phage-based approaches) to address persistent bacterial environmental reservoirs are under investigation but still need to be adequately tested in clinical environments [44]. Enzymes, such as proteases, DNAses, and polysaccharide depolymerases, may enhance the biocidal effect of chemical disinfectants or antibiotics by disrupting the biofilm matrix [45—49]. Here we evaluated the effect of an enzymatic cocktail on multi-species colonized sink drains for the first time. The new cleaning protocol allowed a 10,000-fold reduction in carbapenem-resistant bacteria and no CPO colonization was observed after seven months in the proximal sink drain of ICU rooms, although an extended observation period is required. In addition, the enzymatic cocktails may have a less negative impact on the non-targeted organisms and the environment than biocides routinely used: the enzymatic cocktail is composed of enzymes found in the environment and humans and is biodegradable (≥99%), unlike chemical disinfectants such as Incidin Pro, which is known to be highly toxic to aquatic life with long-lasting effects.

There are several limitations in our study. First, our evaluation focused on CPO acquisition. However, other carbapenem-resistant bacteria were present in the sink drain (*Stenotrophomonas maltophila*, non-carbapenemase-producing *Pseudomonas* sp. along with CPO). These bacteria might have a role in the biofilm persistence and the exchange of genetic material. Second, environmental sampling has not been done systematically, and the inoculation method has also been optimized over the years (addition of an enrichment medium), so we cannot assess whether there has been an increase in sink drain colonization. Third, because we directly evaluated the efficacy of the enzymatic cocktail combined with a peracetic acid and hydrogen peroxide disinfectant, the effect of the products separately should be investigated. Finally, we should have performed audits to confirm the correct application of hygiene instructions by the nursing team. The disposal by healthcare workers of leftover intravenous fluids or food supplements into the sink has been demonstrated to favour the durable establishment of pathogens in the latter [22,38,50].

In conclusion, hospital sinks provide a permissive environment for biofilm formation and microbial colonization and are the source of hospital outbreaks. The persistence of bacteria resistant to antibiotics and disinfectants with the ability to transfer mobile genetic elements makes the outbreak investigation and control complex. Emphasis should be placed not only on optimizing sink design and placement but also on innovative approaches to address persistent environmental reservoirs and prevent transmission of potentially dangerous pathogens from sinks.

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Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2023.10.010.

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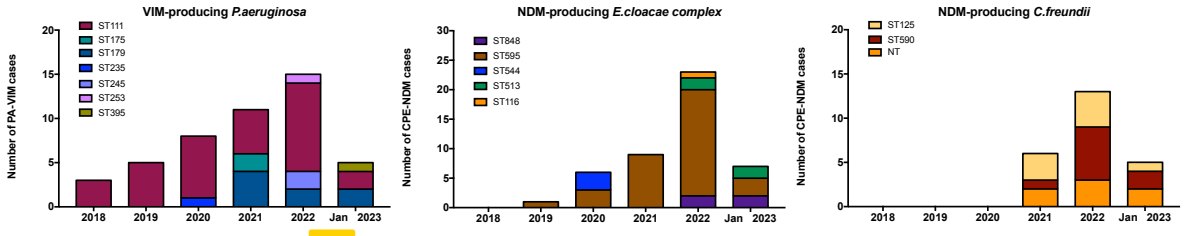
Supplementary material

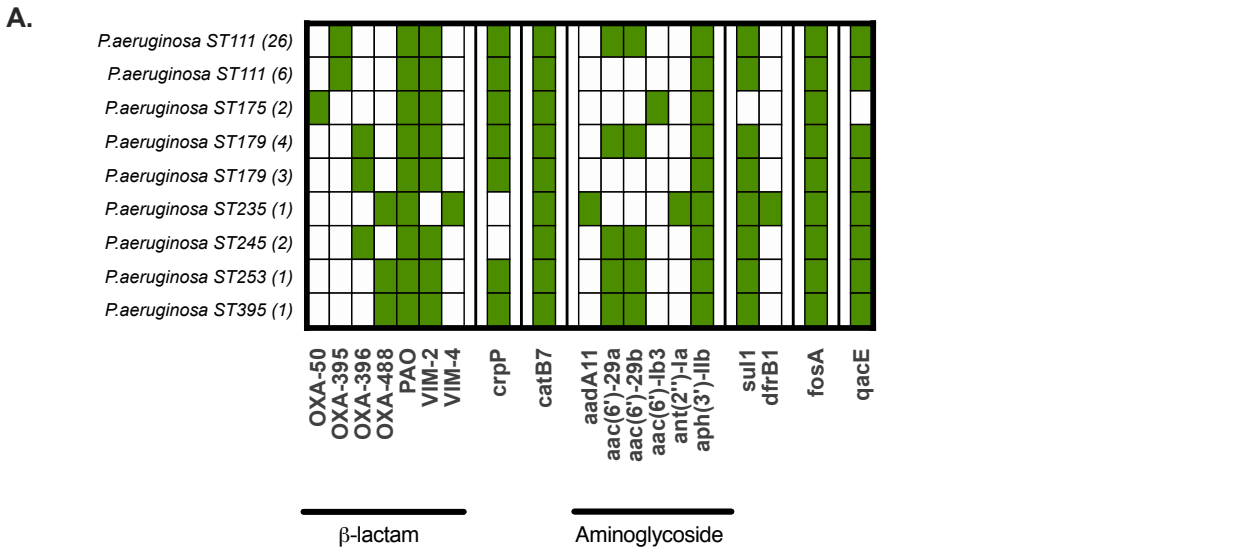
Patient	Age (y)	Species	MLST	Specimen	Sample date	ICU LOS (day)	ICU LOS prior to detection (day)	Antibiotic treatment prior to detection	Mechanical ventilation prior to detection	Reasons of ICU admission	ICU outcome	ICU room
P1	78	*P. aeruginosa*	ST111	Endotracheal aspirate	Jun-2018	39	20	CXM; CIP; CZD	YES	Bacterial pneumonia	discharge	M12
P2	82	*P. aeruginosa*	ST111	Rectal screening	Aug-2018	14	13	PTZ	NO	Pancreatic fistula	discharge	M05
P3	61	*P. aeruginosa*	ST111	Blood	May-2019	10	9	FEP; CXM	NO	Acute renal failure and duodenal perforation	death	M12
P4	50	*P. aeruginosa*	NR	Endotracheal aspirate	Sep-2019	44	15	PTZ; CXM; CZD; TEM; CIP	NO	Fulminant hepatic failure	discharge	M04
P5	41	*P. aeruginosa*	ST111	Urine	Sep-2019	95	64	CXM; AMC; AMK; CIP	YES	Multiple complication post oesophagectomy	discharge	M07/M09
P6	61	*P. aeruginosa*	ST111	Blood	Oct-2019	17	17	GEM	NO	Fulminant hepatitis secondary to bartonellosis	death	M08
P7	67	*P. aeruginosa*	ST111	Endotracheal aspirate	Nov-2019	40	36	CXM; CIP; MEM	YES	Multiple complication post mesocaval shunting	death	M04
P8	62	*E .cloacae* complex	ST544	Endotracheal aspirate	Feb-2020	74	30	MEM	YES	Sepsis and respiratory failure in bone marrow transplant recipient	death	M03
P9	59	*E. cloacae* complex	NR	Bronchoalveolar Lavage	Feb-2020	44	7	CRO; PTZ; AMK	YES	ARDS (Influenza)	discharge	M11
P10	47	*P. aeruginosa*	ST111	Urine	Feb-2020	46	42	PTZ; MEM; CIP	YES	Cardiac arrest and septic shock	death	M01/M14
P11	63	*E. cloacae* complex	ST544	Bronchoalveolar Lavage	Feb-2020	21	11	CRO; TEM	NO	Duodenal perforation and macrophagic activation syndrome	death	M05/M13
P12	54	*P. aeruginosa*	ST111	Endotracheal aspirate	Apr-2020	22	21	CXM	YES	ARDS (COVID-19)	discharge	M10
P13	64	*P. aeruginosa*	NR	Urine	Apr-2020	74	35	CXM; CIP; TEM; CZD	YES	ARDS (COVID-19)	discharge	M09
P14	77	*P. aeruginosa*	ST111	Rectal screening	May-2020	38	14	CXM; CIP; CZD	YES	ARDS (COVID-19)	death	M08
P15	61	*C. freundii*	NR	Rectal screening	Jun-2020	64	57	CZD; TEM; AMC	YES	ARDS (COVID-19)	discharge	M03
P16	59	*E. cloacae* complex	NR	Rectal screening	Jun-2020	12	6	CXM; CZD	NO	Fulminant hepatic failure	death	M10
P17	35	*P. aeruginosa*	ST111	Rectal screening	Aug-2020	13	13	AMC; PTZ	YES	Septic shock secondary to peritonitis	death	M08
P18	51	*P. aeruginosa*	ST111	Wound	Aug-2020	73	60	TEM; PTZ	TES	Variceal rupture (cirrhosis)	discharge	M09
P19	67	*E. cloacae* complex	ST595	Endotracheal aspirate	Oct-2020	80	25	CXM; CZD	YES	ARDS (COVID-19)	discharge	M10
P20	58	*E. cloacae* complex *E. coli*	ST595 ST69	Blood Rectal screening	Nov-2020	58	17	CXM	YES	ARDS (COVID-19)	death	M11
P21	65	*E. coli*	ST69	Urine	Nov-2020	135	31	CXM; CZD; MEM	YES	ARDS (COVID-19)	discharge	M09
P22	29	*P. aeruginosa*	ST111	Peritoneal fluid	Dec-2020	258	71	CZD; CIP; MEM	YES	ARDS (COVID-19)	death	M08
P23	56	*E. cloacae* complex	ST595	Urine	Feb-2021	262	46	CXM; CZD; PTZ; MEM; CIP	YES	Multiple complications post oesophagectomy	discharge	M03/M04
P24	30	*C. freundii* *K. oxytoca*	ST125 NT	Rectal screening Rectal screening	Apr-2021	258	156	CZD; CIP; MEM	YES	ARDS (COVID-19)	death	M08
P25	68	*E. cloacae* complex	ST595	Endotracheal aspirate	Apr-2021	29	19	CZD	YES	ARDS (COVID-19)	death	M12
P26	74	*E. cloacae* complex	ST595	Rectal screening	May-2021	68	8	NO	YES	ARDS (COVID-19)	death	M04
P27	42	*P. aeruginosa*	ST175	Urine	Jul-2021	22	9	PTZ; CXM; CIP; AMK	NO	Severe pancreatitis with necrosis surinfection	discharge	M06
P28	65	*P. aeruginosa*	ST175	Urine	Jul-2021	29	15	AZM; CIP	YES	ARDS (COVID-19)	discharge	M01
P29	50	*P. aeruginosa*	ST111	Endotracheal aspirate	Aug-2021	32	32	MXF	YES	Bacterial pneumonia	discharge	M12
P30	82	*C. freundii*	NT	Peritoneal fluid	Jan-2022	21	13	CXM; PTZ	YES	Septic shock secondary to peritonitis	death	M12
P31	53	*E. cloacae* complex *P. mirabilis* *P. aeruginosa*	ST595 NT ST111	Rectal screening Rectal screening Rectal screening	Feb-2022 Feb-2022 Feb-2022	63	23	CRO	YES	ARDS (COVID-19)	death	M04
P32	57	*C. freundii*	ST590	Rectal screening	Feb-2022	10	10	CRO; CZD; MEM	YES	ARDS (COVID-19)	death	M06
P33	58	*E. cloacae* complex *E. coli*	ST513 ST162	Endotracheal aspirate Rectal screening	Jun-2022	24	8	AMC	YES	Septic shock secondary to angiocholitis	death	M14
P34	72	*E. cloacae* complex	ST595	Rectal screening	Jul-2022	14	14	CRO	YES	ARDS (COVID-19)	death	M12
P35	47	*E. coli* *C. freundii*	ST5451 NT	Rectal screening Rectal screening	Aug-2022	88	24	CRO	YES	haematoma airway compression	discharge	M12
P36	65	*P. aeruginosa*	ST395	Rectal screening	OCt-2022	43	11	PTZ; MEM	NO	Septic shock secondary to spondylodiscitis	discharge	M01/M013
P37	34	*E. cloacae* complex *K. pneumoniae*	ST848 ST1081	Rectal screening Rectal screening	Dec-2022	89	50	PTZ; CRO; CZD; CIP	YES	Acute liver failure and liver transplantation	discharge	M09

Supplementary Table A1: Clinical characteristics of CPO cases

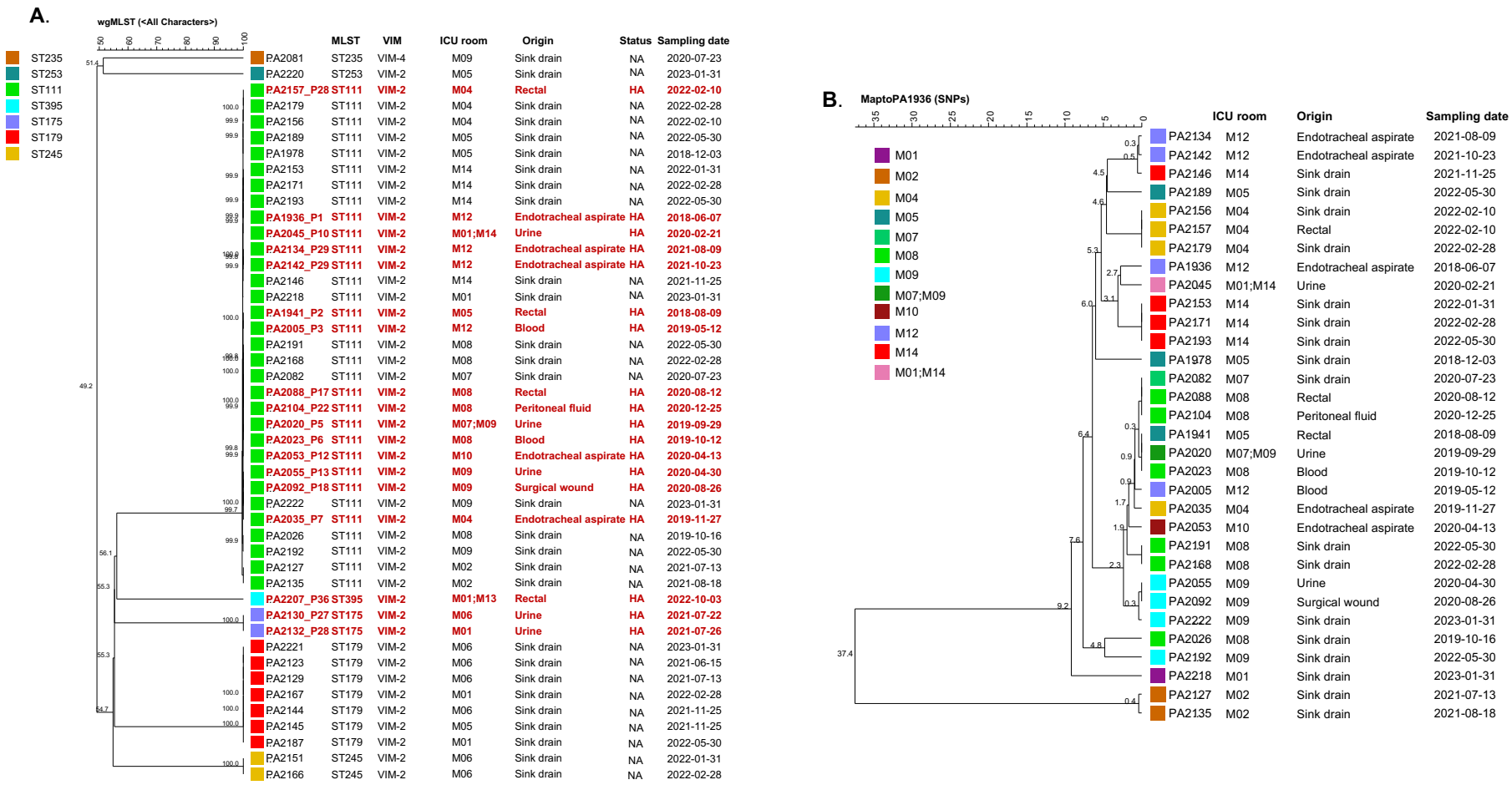
MLST: Multi-Locus Sequence Typing; ST: Sequence Type; NR: Sequencing not performed; NT: Sequence Type unknown; LOS: Length of stay; ICU: Intensive Care Unit; AMC: Amoxillin-clavulanate; AMK: Amikacin ; CIP: Ciprofloxacin; CRO: Ceftriaxone; CXM: Cefuroxim; CZD: Ceftazidim; FEP: Cefepim; MEM: Meropenem; MXF: Moxifloxacin; PTZ: Piperacillin-tazobactam; TEM: Temocillin



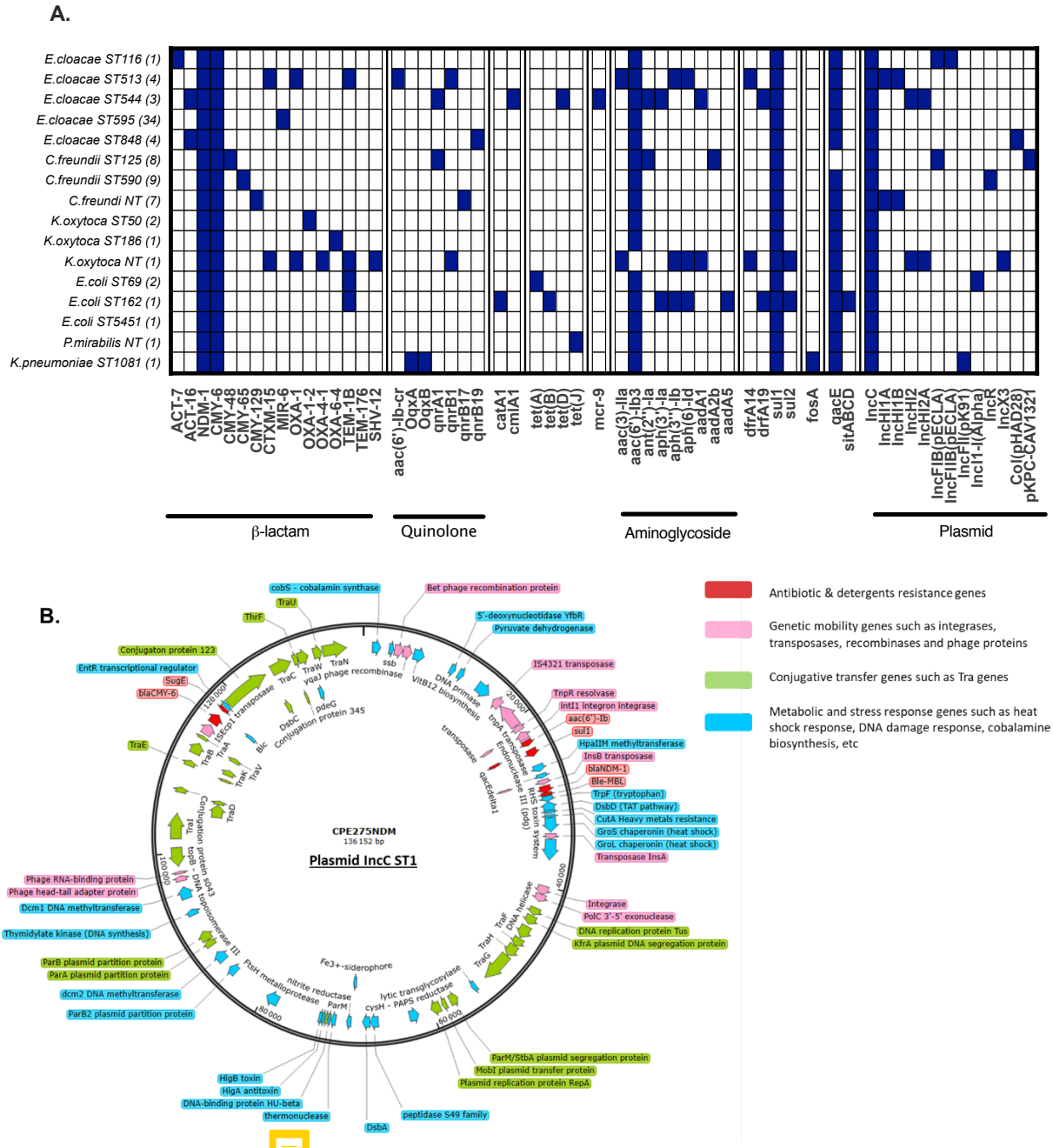
Supplementary figure A1 ST distribution of VIM-producing *P. aeruginosa* (n=46), NDM-producing *E. cloacae* complex (n=46) and NDM-producing *C. freundii* (n=24) isolates collected from patients and sink drains between June 2018 to January 2023 in ICU.



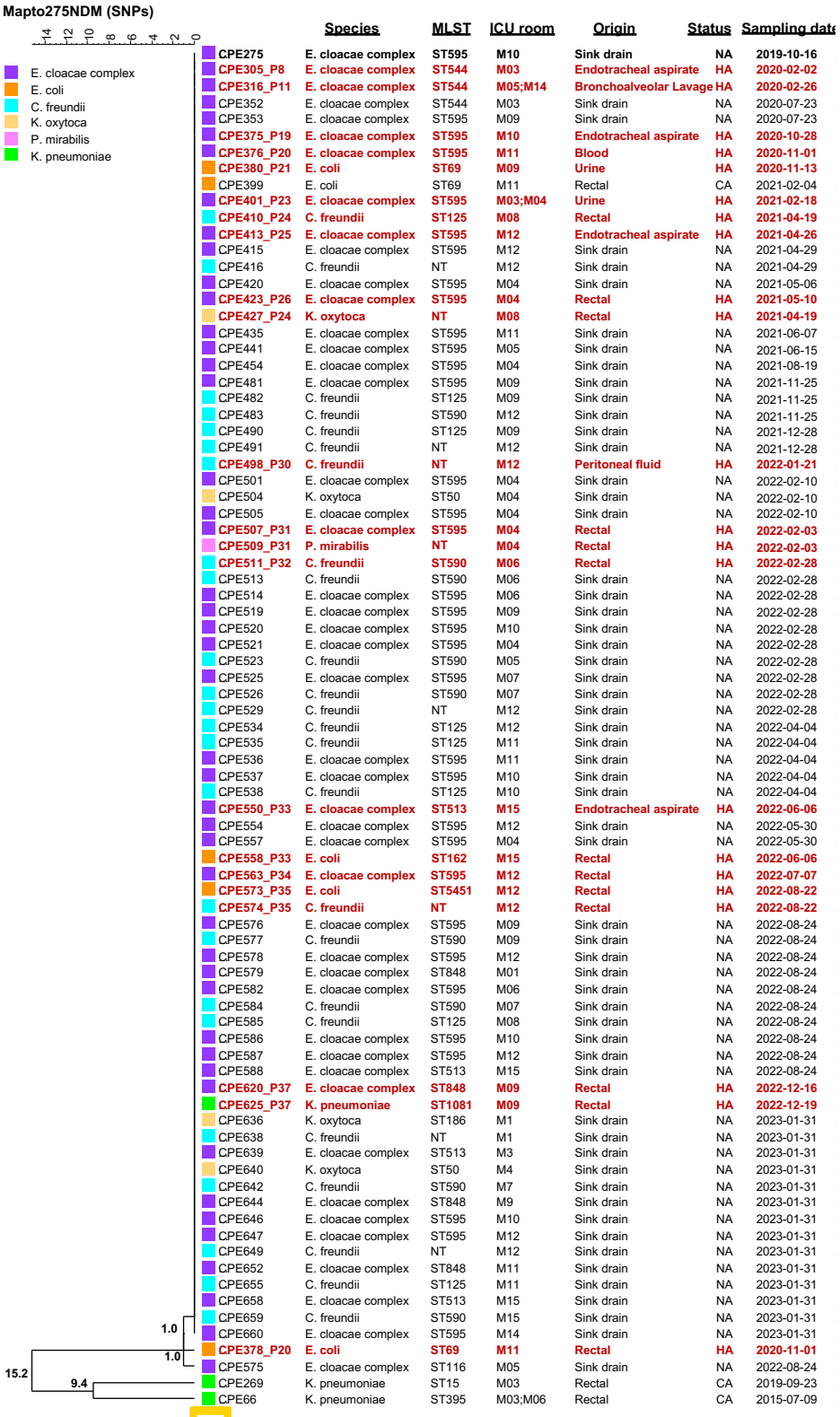
Supplementary figure A2 VIM-producing *P. aeruginosa* (n=46) isolates resistome (**A**) and annotation of the contig harbouring *bla*$_{VIM-2}$ (15967 pb; PA1936 ST111; June 2018, endotracheal aspirate; room M12) (**B**). Genes that confer resistance to β-lactams, quinolones, phenicol, aminoglycosides, trimethoprim, sulphonamide, fosfomycin and disinfectants were determined by the Resfinder database. Green cells and white cells indicate the gene's presence or absence respectively. The brackets refer to the number of isolates with the same resistome



Supplementary figure A: Clonal relationship based on the whole-genome multilocus between VIM-producing *P. aeruginosa* clinical and environment isolates collected during the outbreak **(A)** and based on the SNP analysis between PA-VIM ST111 isolates (**B**). The wgMLST phylogenic tree was generated with Bionumerics 8.0 using categorical values with a scaling factor of 100 (100*number of the loci in common/total number of loci in the complete comparison). SNP analysis was performed using as reference PA1936, the first PA-VIM strain of the outbreak (ST111; 7 001 756 pb; June 2018; Endotracheal aspirate). ST: sequence type; HA: Hospital-acquired PA-VIM; NA: Not applicable (environmental strains). No community-associated PA-VIM (CA PA-VIM) were included in the analysis because no CA PA-VIM was detected among ICU patients over the outbreak period.



Supplementary figure A4. Resistome of NDM-producing *Enterobacterales* (n=80) isolates (**A**) and annotation of the contig harbouring *bla*$_{NDM-1}$ (136 152 pb; CPE275 ST395; October 2019, sink drain of the room M10) (**B**). Genes that confer resistance to β-lactams, quinolones, phenicol, tetracycline, colistin, aminoglycosides, trimethoprim, sulfonamide and disinfectants were determined by the Resfinder database and plasmid sequences were determined by the PlasmidFinder database. Blue cells and white cells indicate the presence or absence of the gene respectively. The brackets refer to the number of isolates with the same resistome.



	Species	MLST	ICU room	Origin	Status	Sampling date
CPE275	**E. cloacae complex**	**ST595**	**M10**	**Sink drain**	**NA**	**2019-10-16**
CPE305_P8	**E. cloacae complex**	**ST544**	**M03**	**Endotracheal aspirate**	**HA**	**2020-02-02**
CPE316_P11	**E. cloacae complex**	**ST544**	**M05;M14**	**Bronchoalveolar Lavage**	**HA**	**2020-02-26**
CPE352	E. cloacae complex	ST544	M03	Sink drain	NA	2020-07-23
CPE353	E. cloacae complex	ST595	M09	Sink drain	NA	2020-07-23
CPE375_P19	**E. cloacae complex**	**ST595**	**M10**	**Endotracheal aspirate**	**HA**	**2020-10-28**
CPE376_P20	**E. cloacae complex**	**ST595**	**M11**	**Blood**	**HA**	**2020-11-01**
CPE380_P21	**E. coli**	**ST69**	**M09**	**Urine**	**HA**	**2020-11-13**
CPE399	E. coli	ST69	M11	Rectal	CA	2021-02-04
CPE401_P23	**E. cloacae complex**	**ST595**	**M03;M04**	**Urine**	**HA**	**2021-02-18**
CPE410_P24	**C. freundii**	**ST125**	**M08**	**Rectal**	**HA**	**2021-04-19**
CPE413_P25	**E. cloacae complex**	**ST595**	**M12**	**Endotracheal aspirate**	**HA**	**2021-04-26**
CPE415	E. cloacae complex	ST595	M12	Sink drain	NA	2021-04-29
CPE416	C. freundii	NT	M12	Sink drain	NA	2021-04-29
CPE420	E. cloacae complex	ST595	M04	Sink drain	NA	2021-05-06
CPE423_P26	**E. cloacae complex**	**ST595**	**M04**	**Rectal**	**HA**	**2021-05-10**
CPE427_P24	**K. oxytoca**	**NT**	**M08**	**Rectal**	**HA**	**2021-04-19**
CPE435	E. cloacae complex	ST595	M11	Sink drain	NA	2021-06-07
CPE441	E. cloacae complex	ST595	M05	Sink drain	NA	2021-06-15
CPE454	E. cloacae complex	ST595	M04	Sink drain	NA	2021-08-19
CPE481	E. cloacae complex	ST595	M09	Sink drain	NA	2021-11-25
CPE482	C. freundii	ST125	M09	Sink drain	NA	2021-11-25
CPE483	C. freundii	ST590	M12	Sink drain	NA	2021-11-25
CPE490	C. freundii	ST125	M09	Sink drain	NA	2021-12-28
CPE491	C. freundii	NT	M12	Sink drain	NA	2021-12-28
CPE498_P30	**C. freundii**	**NT**	**M12**	**Peritoneal fluid**	**HA**	**2022-01-21**
CPE501	E. cloacae complex	ST595	M04	Sink drain	NA	2022-02-10
CPE504	K. oxytoca	ST50	M04	Sink drain	NA	2022-02-10
CPE505	E. cloacae complex	ST595	M04	Sink drain	NA	2022-02-10
CPE507_P31	**E. cloacae complex**	**ST595**	**M04**	**Rectal**	**HA**	**2022-02-03**
CPE509_P31	**P. mirabilis**	**NT**	**M04**	**Rectal**	**HA**	**2022-02-03**
CPE511_P32	**C. freundii**	**ST590**	**M06**	**Rectal**	**HA**	**2022-02-28**
CPE513	C. freundii	ST590	M06	Sink drain	NA	2022-02-28
CPE514	E. cloacae complex	ST595	M06	Sink drain	NA	2022-02-28
CPE519	E. cloacae complex	ST595	M09	Sink drain	NA	2022-02-28
CPE520	E. cloacae complex	ST595	M10	Sink drain	NA	2022-02-28
CPE521	E. cloacae complex	ST595	M04	Sink drain	NA	2022-02-28
CPE523	C. freundii	ST590	M05	Sink drain	NA	2022-02-28
CPE525	E. cloacae complex	ST595	M07	Sink drain	NA	2022-02-28
CPE526	C. freundii	ST590	M07	Sink drain	NA	2022-02-28
CPE529	C. freundii	NT	M12	Sink drain	NA	2022-02-28
CPE534	C. freundii	ST125	M12	Sink drain	NA	2022-04-04
CPE535	C. freundii	ST125	M11	Sink drain	NA	2022-04-04
CPE536	E. cloacae complex	ST595	M11	Sink drain	NA	2022-04-04
CPE537	E. cloacae complex	ST595	M10	Sink drain	NA	2022-04-04
CPE538	C. freundii	ST125	M10	Sink drain	NA	2022-04-04
CPE550_P33	**E. cloacae complex**	**ST513**	**M15**	**Endotracheal aspirate**	**HA**	**2022-06-06**
CPE554	E. cloacae complex	ST595	M12	Sink drain	NA	2022-05-30
CPE557	E. cloacae complex	ST595	M04	Sink drain	NA	2022-05-30
CPE558_P33	**E. coli**	**ST162**	**M15**	**Rectal**	**HA**	**2022-06-06**
CPE563_P34	**E. cloacae complex**	**ST595**	**M12**	**Rectal**	**HA**	**2022-07-07**
CPE573_P35	**E. coli**	**ST5451**	**M12**	**Rectal**	**HA**	**2022-08-22**
CPE574_P35	**C. freundii**	**NT**	**M12**	**Rectal**	**HA**	**2022-08-22**
CPE576	E. cloacae complex	ST595	M09	Sink drain	NA	2022-08-24
CPE577	C. freundii	ST590	M09	Sink drain	NA	2022-08-24
CPE578	E. cloacae complex	ST595	M12	Sink drain	NA	2022-08-24
CPE579	E. cloacae complex	ST848	M01	Sink drain	NA	2022-08-24
CPE582	E. cloacae complex	ST595	M06	Sink drain	NA	2022-08-24
CPE584	C. freundii	ST590	M07	Sink drain	NA	2022-08-24
CPE585	C. freundii	ST125	M08	Sink drain	NA	2022-08-24
CPE586	E. cloacae complex	ST595	M10	Sink drain	NA	2022-08-24
CPE587	E. cloacae complex	ST595	M12	Sink drain	NA	2022-08-24
CPE588	E. cloacae complex	ST513	M15	Sink drain	NA	2022-08-24
CPE620_P37	**E. cloacae complex**	**ST848**	**M09**	**Rectal**	**HA**	**2022-12-16**
CPE625_P37	**K. pneumoniae**	**ST1081**	**M09**	**Rectal**	**HA**	**2022-12-19**
CPE636	K. oxytoca	ST186	M1	Sink drain	NA	2023-01-31
CPE638	C. freundii	NT	M1	Sink drain	NA	2023-01-31
CPE639	E. cloacae complex	ST513	M3	Sink drain	NA	2023-01-31
CPE640	K. oxytoca	ST50	M4	Sink drain	NA	2023-01-31
CPE642	C. freundii	ST590	M7	Sink drain	NA	2023-01-31
CPE644	E. cloacae complex	ST848	M9	Sink drain	NA	2023-01-31
CPE646	E. cloacae complex	ST595	M10	Sink drain	NA	2023-01-31
CPE647	E. cloacae complex	ST595	M12	Sink drain	NA	2023-01-31
CPE649	C. freundii	NT	M12	Sink drain	NA	2023-01-31
CPE652	E. cloacae complex	ST848	M11	Sink drain	NA	2023-01-31
CPE655	C. freundii	ST125	M11	Sink drain	NA	2023-01-31
CPE658	E. cloacae complex	ST513	M15	Sink drain	NA	2023-01-31
CPE659	C. freundii	ST590	M15	Sink drain	NA	2023-01-31
CPE660	E. cloacae complex	ST595	M14	Sink drain	NA	2023-01-31
CPE378_P20	**E. coli**	**ST69**	**M11**	**Rectal**	**HA**	**2020-11-01**
CPE575	E. cloacae complex	ST116	M05	Sink drain	NA	2022-08-24
CPE269	K. pneumoniae	ST15	M03	Rectal	CA	2019-09-23
CPE66	K. pneumoniae	ST395	M03;M06	Rectal	CA	2015-07-09

Supplementary figure A5. Single-nucleotide polymorphism phylogenetic analysis of NDM-producing Enterobacterales. SNP analysis was performed on BioNumerics using the contig harbouring *bla*NDM-1 isolated from the first CPE-NDM strain of the outbreak CPE275 (ST395; 136 152 pb; October 2019, sink drain of the room M10). CPE-NDM isolates from patients with an ICU linked acquisition were indicated in bold red. Three community-associated CPE NDM-1 detected among ICU-patients over the outbreak period were included in the analysis. The scale bar on the tree indicates the number of SNPs differences. SNP=single-nucleotide polymorphisms. ST: sequence type; HA: Hospital-acquired CPE-NDM; CA: community-associated CPE-NDM; NA: Not applicable (environmental strains).