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Carbapenemase-producing bacteria in the sink traps of a hospital

Bactéries productrices de carbapénémases dans les siphons sanitaires

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Abstract

This study focuses on carbapenemase-producing bacteria and investigates their potential presence in sink traps at the Centre Hospitalier Universitaire Vaudois (CHUV). Antibiotic-resistant bacteria represent a growing global health concern, as they render standard antibiotic therapies ineffective, complicating the treatment of bacterial infections. Preventing the spread of such organisms is a key priority in healthcare settings. In 2019, an outbreak of carbapenemase-producing *Enterobacteriaceae* (CPE) occurred in the Intensive Care Unit (ICU) of the CHUV. These bacteria produce carbapenemase enzymes, which confer resistance to carbapenems- a last resort class of antibiotics. A link was established between the outbreak and sink traps colonised by CPEs within the ICU. The objective of this study was to determine whether CPEs had spread beyond the ICU and could be detected in other parts of the hospital. A total of 44 sink traps located across six floors of the hospital were sampled. Sampling focused on rooms previously occupied by patients who had tested positive for CPE carriage. Samples were analysed using MALDI-TOF mass spectrometry and two complementary antibiotic resistance tests. No carbapenemase-producing organisms were detected in any of the sink traps analysed. These findings suggest that the CPE outbreak remained confined to the ICU.

Keywords: Carbapenemase, Enterobacteriaceae, sink trap, MALDI-TOF, CPE, antibiotic resistance, hospital outbreak.

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Résumé

Cette étude porte sur les bactéries productrices de carbapénémases et vise à évaluer leur éventuelle présence dans les siphons d'évier du Centre hospitalier universitaire vaudois (CHUV). Les bactéries résistantes aux antibiotiques représentent une menace croissante pour la santé publique mondiale, car elles rendent inefficaces les traitements antibiotiques standards et compliquent la prise en charge des infections bactériennes. La prévention de la propagation de ces agents pathogènes constitue donc une priorité dans les établissements de soins. En 2019, une épidémie d'Entérobactéries productrices de carbapénémases (CPE) a été détectée dans l'unité de soins intensifs (USI) du CHUV. Ces bactéries produisent des enzymes appelées carbapénémases, qui leur confèrent une résistance aux carbapénèmes, une classe d'antibiotiques de dernier recours. Un lien a été établi entre cette épidémie et des siphons d'évier colonisés par les CPE au sein de l'USI.

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L'objectif de cette étude était de déterminer si les CPE s'étaient propagées au-delà de l'USI et pouvaient être détectées dans d'autres zones de l'hôpital. Au total, 44 siphons d'évier répartis sur six étages du bâtiment hospitalier ont été échantillonnés. Les prélèvements ont été ciblés dans des chambres précédemment occupées par des patients porteurs de CPE. Les échantillons ont été analysés par spectrométrie de masse MALDI-TOF ainsi que par deux tests complémentaires de résistance aux antibiotiques. Aucun organisme producteur de carbapénémase n'a été détecté dans les siphons analysés. Ces résultats suggèrent que l'épidémie de CPE est restée confinée à l'unité de soins intensifs.

Mots-clés: carbapénémase, entérobactéries, siphon d'évier, MALDI-TOF, CPE, résistance aux antibiotiques, épidémie hospitalière.

Introduction

Background

In 2021 and 2022, the Centre Hospitalier Universitaire Vaudois (CHUV) experienced two outbreaks of Carbapenemase-producing Enterobacteriaceae (CPE) in its intensive care units (ICUs). Following these events, the Infection Prevention and Control team initiated an investigation to identify potential sources of transmission. Their findings pointed to contaminated sink traps in the affected ICUs as a plausible environmental reservoir. This hypothesis is consistent with the increasing proportion of hospital-acquired infections, which suggests that transmission is not exclusively of external origin.

Resistant bacteria were shown to colonize sink drains, particularly through the formation of biofilms on internal surfaces. These biofilms not only harbour persistent bacterial populations but also facilitate the horizontal transfer of resistance genes – most notably plasmid-encoded carbapenemases- to other bacteria. Several types of such carbapenemases were identified during the investigation, offering insight into the spread of resistance within the hospital environment, primarily through plasmids and other mobile genetic elements (MGEs) (Mauffrey *et al.* 2024).

Sink drains can thus act as persistent sources of infection, with bidirectional transmission occurring between colonised sinks and patients (Mauffrey *et al.* 2024). Transmission events often originate from activities conducted near contaminated drains, such as handwashing or emptying containers (Warren *et al.* 2024). In such cases, backflow or splashing of contaminated water may allow pathogens to come into contact with surrounding surfaces or individuals (Kotsanas *et al.* 2013).

The ESKAPE Pathogens

The acronym ESKAPE refers to six bacterial species known to be responsible for the majority of nosocomial infections (Mulani *et al.* 2019):

- *Enterococcus faecium,*
- *Staphylococcus aureus,*
- *Klebsiella pneumoniae,*
- *Acinetobacter baumannii,*
- *Pseudomonas aeruginosa,*
- *Enterobacter spp.*

Among these, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.* are of particular relevance to this present study, as they are frequently associated with carbapenem resistance conferred by plasmid-encoded carbapenemase genes (Mauffrey *et al.* 2024). All of these species are Gram-negative bacteria (Lin & Lan 2014; Oliveira & Reygaert 2023; Ryan *et al.* 2004).

Gram-negative bacteria are characterised by a complex cell envelope consisting of an inner cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane containing lipopolysaccharides. The peptidoglycan layer provides structural integrity and resistance to osmotic pressure. Importantly, it is this layer that is targeted by carbapenem antibiotics (Silhavy *et al.* 2010).

Mechanism of Action of Carbapenems

Carbapenems are broad-spectrum β-lactam antibiotics with a bacteriostatic effect, meaning they prevent their ability to reproduce. Their activity depends on their ability to penetrate the bacterial outer membrane through porins – channel-forming proteins that facilitate the diffusion of small molecules. Once inside the periplasmic space, carbapenems inhibit the function of transpeptidases, also known as penicillin-binding proteins (PBPs), which are essential for the synthesis and maintenance of the peptidoglycan layer.

By inhibiting PBPs, carbapenems disrupt the integrity of the cell wall, leading to the formation of defects through which water can enter the cell. Given the hyperosmotic nature of the bacterial cytoplasm, this influx of water eventually leads to cell lysis. A major advantage of carbapenems is their ability to bind to multiple PBPs, thereby enhancing their efficacy against a wide range of bacterial pathogens (Papp-Wallace *et al.* 2011).

Methods

Study Design and Sampling Strategy

This investigation follows the hospital's routine control of the procedure established by the CHUV (CHUV Laboratoire de diagnostic IMU-DAM, 2023; CHUV Laboratoires de diagnostic IMU-DAM, 2021). The aim was to assess the potential spread of carbapenemase-producing Enterobacteriaceae (CPE) beyond the ICU, by testing sink traps in rooms previously occupied by patients who had screened positive for CPE carriage.

Between January and May 2024, 44 sink drains located across six different floors of the hospital were selected for analysis. Inclusion criteria required that the selected rooms had been frequented by patients identified as CPE carriers through routine clinical screening. The sampling focused specifically on the drain traps of washbasins, which are known to serve as potential environmental reservoirs for resistant bacteria. Each sink drain was clearly marked prior to sampling.

Sample collection and preprocessing

Samples were collected using eSwabs, designed for microbial sampling and transport. Each swab was inserted into the trap of the sink drain and rotated thoroughly to collect biofilm and water residues. The swabs were then transferred onto CHROMagar™mSuperCarba agar petri dishes, a selective and differential medium specifically designed for the growth of carbapenem-resistant organisms.

The medium inhibits the growth of non-resistant bacteria while promoting the development of colonies with potential carbapenem resistance. It also enables preliminary differentiation based on colony morphology and pigmentation. After plating, the dishes were incubated at 35-37 °C for 18 to 24 hours to allow optimal growth of *Enterobacteriaceae*, which are adapted to the human body (Moreira de Gouveia *et al.* 2024).

In cases where overlapping or mixed colonies prevented precise sampling, an isolation step was conducted. Bacterial isolates were streaked onto two types of non-selective media, BBL™CHROMagar™Orientation (ORI) petri dish and BD™Columbia Agar petri dish supplemented with 5% sheep blood. The ORI medium permits orientation-based

identification of enteric species, while Columbia agar, being non-selective and non-inhibitory, supports stress-free bacterial growth for subsequent analysis.

Bacterial identification using MALDI-TOF Mass spectrometry

Pure bacterial colonies were subjected to protein fingerprinting using Matrix-Assisted Laser Desorption/Ionisation -Time-of-Flight Mass Spectrometry (MALDI-TOF-MS), a method increasingly used for rapid microbial identification (Li *et al.* 2022). A small quantity of bacterial biomass was deposited onto a steel target plate, overlaid with formic acid to break down bacteria to extract proteins and a matrix solution, then allowed to dry before analysis.

The MALDI-TOF process involves four main steps: desorption, ionisation, acceleration and detection. Laser pulses excite the matrix molecules, causing desorption of bacterial proteins, followed by ionisation. The resulting ions are accelerated through a flight tube under vacuum, and their time-of-flight (TOF) is measured. As heavier ions travel more slowly, the instrument calculates the mass-to-charge ratio (m/z) based on TOF. The output is a mass spectrum- a unique protein «fingerprint» representing the ribosomal protein composition of each bacterial species (Mortier *et al.* 2021).

These spectra were compared against a reference database to determine the identity of the isolates. Only isolates corresponding to members of the ESKAPE group species or other clinically relevant species were retained for further resistance testing.

Carbapenemase Resistance Testing

Bacterial isolates of interest – i.e., all ESKAPE group species as well as other known carbapenemase producers – underwent targeted resistance testing using two rapid diagnostic tests: the NG CARBA-5 and OXA-23 lateral flow tests.

The NG CARBA-5 rapid test detects the five major carbapenemase families that can be produced by ESKAPE species:

- KPC (Klebsiella pneumoniae carbapenemase) (class A).
- Three metallo β-lactamases or MBLs: IMP (imipenemases), VIM (Verona integron-encoded MBL) and NDM (New Dehli MBL) (class B).
- OXA-48 or oxacillin-48 (class D) (Queenan & Bush, 2007).

This test was applied to all isolates from the ESKAPE group and to any additional species known to potentially harbour carbapenemases.

The OXA-23 rapid which targets a specific oxacillinase (oxacillinase 23) prevalent in *Acinetobacter spp.*, was performed exclusively on isolates of this genus, as it offers a complementary diagnostic approach for these species (Coris BioConcept, 2023).

Isolates testing positive for any of these enzymes were classified as carbapenemase-producing organisms and flagged for epidemiological monitoring.

Results

Observation of bacterial growth on selective media

Among the 44 sink trap samples collected, six showed no bacterial growth after incubation on CHROMagar™ mSuperCarba medium, indicating the likely absence of carbapenem-resistant bacteria in those drains. The remaining 38 samples exhibited varying levels of bacterial growth. Morphological characteristics such as colony shape, size, colour, and distribution were recorded. In general, one to three distinct colony types were observed per Petri dish (see figure 1). In some cases, overgrowth prevented direct analysis and required prior isolation on additional media for species identification.

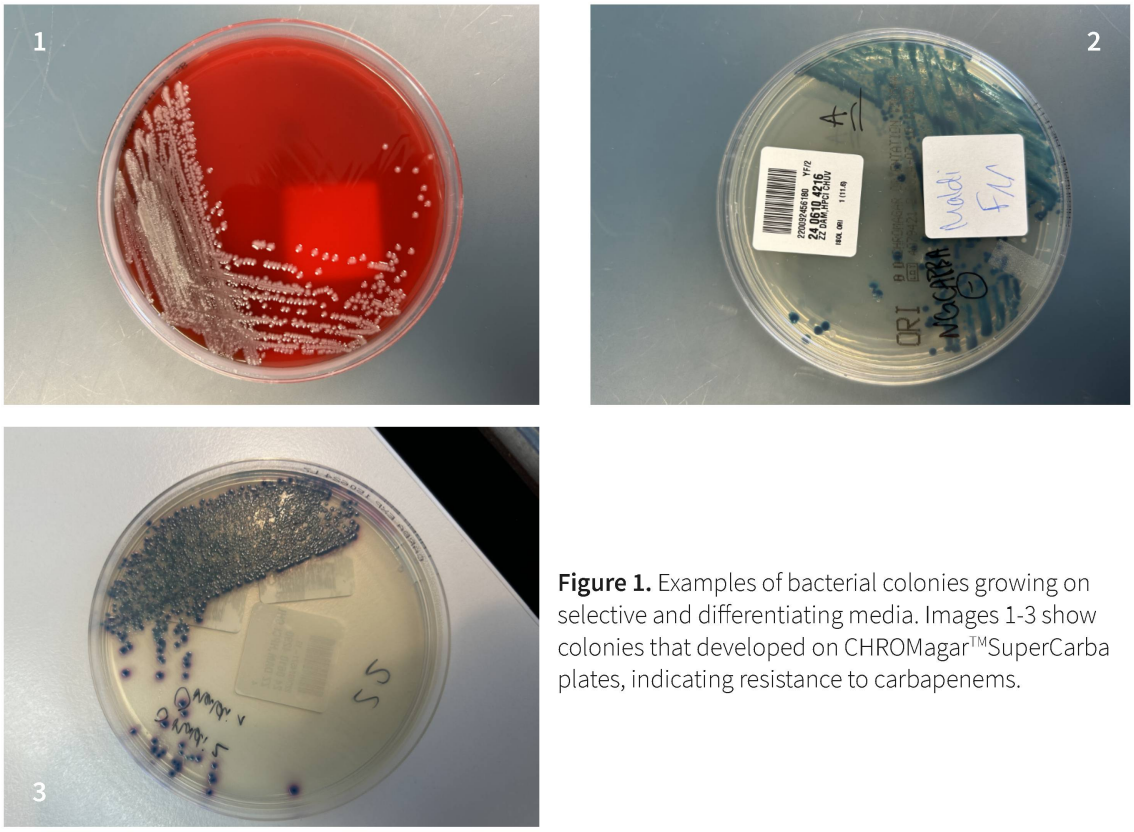

Figure 1. Examples of bacterial colonies growing on selective and differentiating media. Images 1-3 show colonies that developed on CHROMagar™SuperCarba plates, indicating resistance to carbapenems.

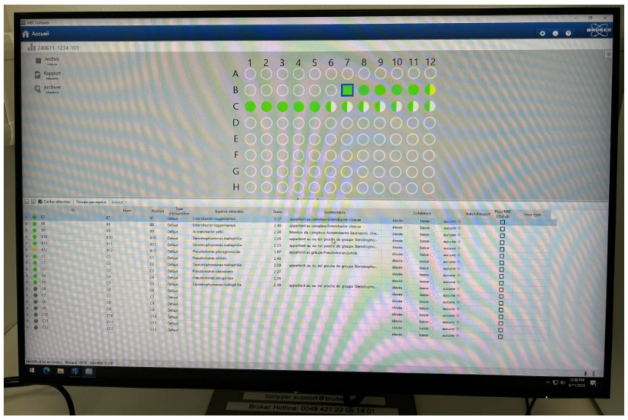

Figure 2. MALDI-TOF mass spectrometry interface used to analyse bacterial isolates. Green circles represent successfully analysed colonies; semi-green circles indicate ongoing analysis. The identified species are listed below the visual output.

Identification by MALDI-TOF

All morphologically distinct colonies were analysed using MALDI-TOF mass spectrometry (figure 2) and their identification is presented in table 1. The spectra allowed for precise taxonomic identification. A number of bacterial species known to potentially harbour carbapenemase genes – particularly members of the ESKAPE group – were identified and retained for further analysis (see table 2). These included *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.

Resistance testing

A total of 18 isolates were tested using the NG CARBA-5 rapid test and, when applicable (i.e., for *Acinetobacter spp.*), the OXA-23 rapid test. All resistance tests yielded negative results, indicating the absence of carbapenemase production among the tested strains. No carbapenemase-producing organisms (CPOs) were selected in the analysed samples.

Table 1. Bacterial species identified by the MALDI-TOF mass spectrometer from the 44 sink trap samples. Abbreviations used in subsequent sections are indicated below.

Sample	Species 1	Species 2	Species 3	Of interest
1	Acineto. pittii	Steno. malto.		Yes, species 1
2	Entero. rog.	Entero. rog.	Acineto. pittii	Yes, all 3
3	Steno. malto.			-
4	Steno. malto.			-
5	Serratia marcescens			Yes
6	Steno. malto.	Pseudo. mont.		-
7	Pseudo mont.			-
8	Steno. malto.			-
9	Pseudo. mont.	Steno. malto.		-
10	sterile			-
11	Entero. rog.	Pseudo. otitidis		Yes, species 1
12	Pseudo. mont			
13	Acineto. pittii			Yes
14	Pseudo. putida			-
15	Citro. Freundii	Steno. malto.		Yes, species 1
16	Acineto. pittii	Pseudo. nitroreducens		Yes, species 1
17	Sterile			-
18	Pseudo. aeruginosa			Yes
19	Steno. malto.			-
20	Pseudo. aeruginosa			Yes
21	Sterile			-
22	Entero. rog.	Acineto. ursingii		Yes, both
23	Steno. malto.			-
24	Pseudo. nitroreducens			-
25	sterile			-
26	Pseudo. putida	Comamonas terringa		-
27	Klebsiella pneumoniae	Pseudo. mont.		Yes, species 1
28	Pseudo. mont.	Pseudo. sp.		-
29	sterile			-
30	Pseudo. mont.	Elizabethkingia miricola		-
31	Pseudo. putida			-
32	Steno. malto.			-
33	Steno. malto.			-
34	Pseudo. Plecoglossicida			-
35	Pseudo. oitidis			-
36	Entero. cloacae	Entero. kobei	Pseudo. mont.	Yes, species 1 and 2
37	Steno. malto.			-
38	Steno. malto.			-
39	Pseudo. aeruginosa			Yes
40	Pseudo. nitroreducens	Steno. malto.		-
41	Steno. malto.			-
42	Entero. rog.	Steno. malto.	Acineto. pittii	Yes, species 1 and 3
43	sterile			-
44	Pseudo. oleovorans			-

Abbreviations Table 1	
Acineto. = Acinetobacter	Pseudo. = Pseudomonas
Entero. = Enterobacter	malto. = maltophilia
Steno = Stenotrophomonas	mont. = monteilii
rog. = roggenkampii	

Table 2. Bacterial species identified by MALDI-TOF that warranted additional resistance testing. This is due to their known potential to harbour plasmid-encoded carbapenemases.

Klebsiella pneumoniae	From the *Enterobacteriaceae* family, therefore a possible CPE. (Adeolu *et al.*, 2016)
Acinetobacter pittii and ursingii	Both species belong to the Acinetobacter calcoaceticus-baumannii complex. They are very similar to A. baumanii. (Nemec *et al.*, 2011)
Enterobacter roggenkampii, cloacae and kobei	E. cloacae and kobei are members of the Enterobacter cloacae complex group. This means that they share many genetic features. E. roggenkampii is a recently discovered strain that also belongs to this group. (Davin-Regli *et al.*, 2019)
Serratia marcescens	A Gram-negative bacterium and member of the ESCPM group. (Zivkovic Zaric *et al.*, 2023)
Citrobacter freundii	From the *Enterobacteriaceae* family (Ranjan & Ranjan, 2013; Yao *et al.*, 2021)and member of the ESCPM group. According to: (Zivkovic Zaric *et al.*, 2023)
Pseudomonas aeruginosa	Among Gram-negative bacteria, Pseudomonas aeruginosa is one of the biggest triggers of hospital-acquired infections, and can also exhibit multi-drug resistance. (Spagnolo *et al.*, 2021)

Discussion

Absence of Detected Carbapenemase-Producing Organisms in Sink Traps

The results of this study indicate that none of the 44 sink traps sampled from high-risk rooms of the CHUV harboured detectable carbapenemase-producing bacteria. This is a reassuring finding, especially given that the rooms had previously been occupied by patients known to be CPE carriers. The selective CHROMagar™ mSuperCarba medium ensured that only carbapenem-resistant strains could grow, and the absence of positive resistance test results confirms that no carbapenemase-producing organisms were present at the time of sampling.

However, several bacterial species commonly associated with acquired resistance mechanisms – particularly plasmid-mediated carbapenemases – were identified. These included typical ESKAPE group members, underlining the importance of continued environmental monitoring.

If any of the resistance tests had returned a positive result, the recommended protocol would have involved genotypic and plasmid profiling of the isolates and comparison with the patient strains from the same room. A full match between patient and environmental strains would have suggested direct transmission. A match limited to the plasmid level would have indicated horizontal gene transfer within the sink's microbial community. In all

such cases, infrastructure interventions such as replacement of contaminated plumbing would have been necessary, as this remains the only reliable method to eliminate CPOs from hospital drainage systems.

This work highlights the complexity of tracking and controlling nosocomial pathogens, especially those able to transmit resistance via mobile genetic elements. It also illustrates the utility of combining selective culture media, MALDI-TOF identification, and rapid resistance assays to efficiently screen hospital environments.

Limitations

The patient list included only patients identified as CPE carriers during their stay at the hospital between January and May 2024. It is therefore possible that transmission may have occured before this period and remained undetected. Additionally, not all rooms associated with these patients could be investigated: the rooms located on the hospital's 17th floor were excluded from sampling due to time constraints.

It should also be noted that, although no carbapenemase-producing organisms were detected, several bacterial colonies were still able to grow on the selective CHROMagar™ mSuperCarba medium. This suggests that these strains may exhibit alternative mechanisms of resistance to carbapenems, which were not investigated in the present study. As a result, the absence of carbapenemase production does not rule out the presence of other clinically relevant resistance traits in the sampled sink traps.

Conclusion

Despite sampling in 44 sinks associated with the highest-risk patients during the January-May 2024 period, no carbapenemase-producing organisms were detected. These findings suggest that the CPE outbreak observed in previous years at the CHUV did not extend beyond the ICU. While the presence of other resistant bacteria was confirmed, no carbapenemase activity was detected, suggesting the maintenance of localised control.

These results support the continued use of rigorous surveillance protocols. Given the capacity of bacteria to acquire and disseminate resistance genes, routine environmental monitoring remains essential. Further studies should aim to expand the temporal and spatial scope of sampling and explore complementary methods such as metagenomics for earlier detection of resistance spread.

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