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Successful control of an environmental reservoir of NDM-producing *Klebsiella pneumoniae* associated with nosocomial transmissions in a low-incidence setting

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Abstract

Background The hospital wastewater system has been reported as a source of nosocomial acquisition of carbapenemase producing *Enterobacteriaceae* (CPE) in various settings. Cleaning and disinfection protocols or replacement of contaminated equipment often fail to eradicate these environmental reservoirs, which can lead to long-term transmission of CPE. We report a successful multimodal approach to control a New Delhi metallo-beta-lactamase positive *Klebsiella pneumoniae* (NDM-KP) nosocomial outbreak implicating contamination of sink traps in a low-incidence setting.

Methods Following the incidental identification of NDM-KP in a urine culture of an inpatient, we performed an epidemiological investigation, including patient and environmental CPE screening, and whole genome sequencing (WGS) of strains. We also implemented multimodal infection prevention and control (IPC) measures, namely the isolation of cases, waterless patient care, replacement of contaminated P-traps and connecting pieces, and bleach and steam disinfection of sinks for 6 months, followed by patient and environmental screenings for eradication.

Results Between February and May 2022, five NDM-KP cases were identified in an eight-bed neurosurgical intermediate care unit. Among the eight sink traps of the unit, three were positive for NDM-KP. Patient and environmental isolates belonged to multilocus sequence typing ST-268. All isolate genomes were genetically very similar suggesting cross-transmission and a potential role of the environment as the source of transmissions. Following the introduction of combined IPC measures, no new case was subsequently detected and sink traps remained negative for NDM-KP within 6 months after the intervention.

Conclusion The implementation of multimodal IPC measures, including waterless patient care combined with the replacement and disinfection of P-traps and connecting pieces, was successful in the control of NDM-KP after eight

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months. In a low-incidence setting, this approach has made it possible to pursue the objective of zero transmission of carbapenemase-producing *Enterobacteriaceae* (CPE).

Keywords Nosocomial outbreak, Carbapenemase, NDM, Sink traps, Wastewater drains reservoir, Whole genome sequencing

Introduction

Increasing incidence of carbapenemase-producing *Enterobacteriaceae* (CPE) poses a major public-health threat. Effective treatment of CPE infections is often delayed, with limited options available, leading to high mortality. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show large differences in the prevalence of CPE depending on the country [1]. In Switzerland, data from the Swiss Centre for Antibiotic Resistance (ANRESIS) indicate that carbapenem resistance, albeit still rare, is steadily increasing, reflecting trends in neighbouring countries [2]. Infection prevention and control (IPC) measures and the implementation of antimicrobial stewardship programs are crucial for preventing and controlling the emergence and spread of CPE.

The hospital wastewater system has been identified as a potential source of nosocomial acquisition of carbapenemase-producing *Pseudomonas* spp. or *Acinetobacter* spp, as well as CPE, in various settings [3–10]. Reported outbreaks were characterised by a low overall incidence of clinical cases and highly variable durations, likely due to differences in the speed of detection, the type of measures applied, and the duration of follow-up after their implementation [11]. Cleaning and disinfection protocols or replacement of contaminated equipment often fail to eradicate these environmental reservoirs, leading to long-term transmission of CPE [12–15].

We report a successful multimodal approach to control a nosocomial outbreak of New Delhi metallo-beta-lactamase positive *Klebsiella pneumoniae* (NDM-KP) associated with the contamination of sink traps in a neurosurgical intermediate care unit of a Swiss hospital.

Methods

Setting

Lausanne University Hospital (CHUV) is a 1100-bed teaching hospital serving as a primary-level community hospital for Lausanne (catchment population 300'000) and as a secondary and tertiary referral hospital for Western Switzerland (catchment population 1-1.5 million). Among the *Enterobacteriaceae* identified at CHUV, the prevalence of CPE remains less than 1% and the CPE incidence less than 1 case per 1000 admissions, mainly consisting of sporadic cases imported from abroad. The reported outbreak took place in an eight-bed neurosurgical intermediate care unit, including beds for severely brain-impaired patients. Beds are distributed in an open-space area with a central desk for healthcare workers and four sinks. Four additional sinks and one shower are located in adjacent rooms. The map of the unit is shown in Fig. 1.

IPC measures and CPE control policy

After receiving automatic alerts for all patients colonised with multidrug resistance organisms (MDRO), the IPC team ensures that contact isolation measures are in place and carries out investigations of contact patients. Weekly screening for intestinal carriage of MDRO, including CPE, is systematically performed among all intensive care unit (ICU) patients. In non-ICU units, targeted individual screening is performed at admission, based on conventional risk factors such as previous carriage, contact with a positive case (sharing a room or an open space unit with a positive patient before isolation was introduced) or recent hospitalisation abroad [16]. According to the Swiss national IPC guidelines [17], when a new CPE-positive case is identified, contact isolation of the positive patient and its contacts is promptly applied. CPE screening of the contacts on rectal swabs is performed on days 0, 7 and 14 (RT-PCR and culture on day 0, culture only on day 7 and 14). Moreover, chlorhexidine bathing of positive cases is recommended during their hospital stays.

For the reported outbreak, following the identification of a NDM-KP in a urine culture from a patient hospitalised in the neurosurgical intermediate care unit, all the aforementioned epidemiological measures were implemented. Contact precautions were maintained for CPE contact patients until they tested negative on three consecutive weekly screenings. In addition to contacts screening, weekly CPE screenings of all patients were performed on a weekly basis (and then every two weeks) due to the open space architecture of the unit. This continued until discharge of the last positive case, for six months. The IPC team carried out observations of practices and educational rounds to reinforce hand hygiene, material and environment disinfection, and adherence to aseptic care procedures.

Environmental sampling

Environmental sampling for CPE from sink traps (P-trap and connecting pieces) and the shower drain was conducted between May 2022 and May 2023, with a total of 114 samples collected: 102 from eight sink traps and 12 from the shower drain. P-traps samples were collected

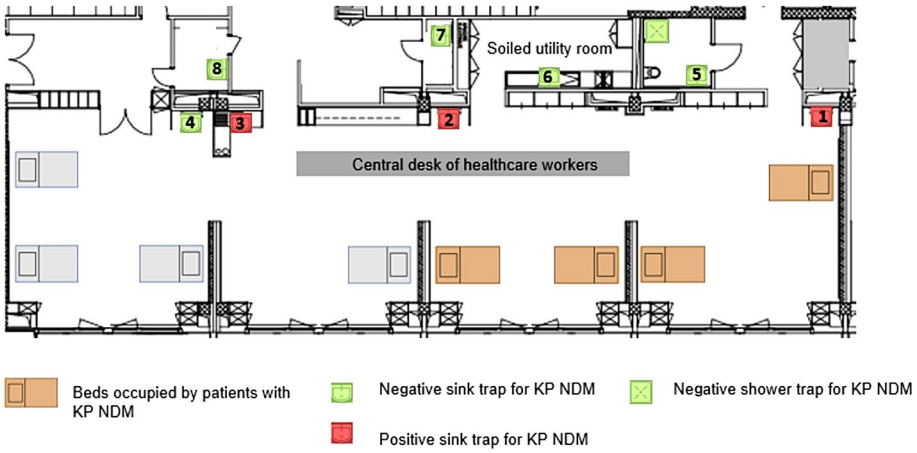

Fig. 1 Map of the intermediate care unit including the location of positive patients and sink traps

using eSwab® (Copan, Italy). The swab was attached to a metal rod before being inserted through the drain into the siphon. Ten back-and-forth motions against the siphon walls were performed before breaking the swab into the eSwab tube containing Amies liquid.

Bacterial identification, molecular characterization, and genomic analysis

Patients and environmental samples were inoculated on selective SuperCarba medium (CHROMagar™, France), and incubated at 37 °C for 24 h. Suspected colonies were identified with MALDI-TOF (Bruker, Germany). The presence of carbapenemase was confirmed using the lateral flow immunoassay NG-Test® CARBA-5 (NG Biotech, France). In parallel to culture, the first screening of contact patients was performed with the Xpert® Carba-R PCR assay (Cepheid, USA).

Whole genome sequencing was performed using an Illumina platform on at least one isolate per patient and environmental site. Sequence reads were analyzed using BioNumerics™ (version 8.1, available at (http://www.applied-maths.com) with default settings, except for the *de-novo* assembly, which was performed using the Unicycler pipeline. Multilocus Sequence Typing (MLST) was determined based on the public scheme available at https://bigsdb.pasteur.fr/klebsiella. For genome comparison, we performed whole genome MLST (wgMLST) using a scheme developed by Applied Maths. Clustering was performed using the categorical-difference coefficient, and a minimum spanning tree for categorical data was built with single and double locus variance priority rules.

Ethical considerations

The data were obtained during service evaluation and outbreak management activities. According to the Swiss national law, this type of work is exempt from the requirement for approval by the competent research ethics committee.

Results

Outbreak description

In February 2022, the first case of NDM-KP was identified in a urine culture from a patient who had been hospitalised for two months in the neurosurgical intermediate care unit. Between February and May 2022, four more patients were identified as newly colonised with NDM-KP, including a patient with a *bla*NDM -positive PCR but a negative culture. All five patients had been hospitalised in the neurosurgical intermediate care unit, and none had been hospitalised abroad. The outbreak unfolded in a biphasic way: three patients were diagnosed in February 2022 (one with a positive urine culture and two contacts with positive rectal samples), and two in April and May 2022 (Fig. 2). The second phase included a new case with a positive urine culture and a contact with a positive rectal sample, both admitted after the first three cases had been discharged. Among the five cases, four were identified during their stay in the neurosurgical intermediate care unit, and one was diagnosed nine days after being transfered to a rehabilitation unit. The time from hospital admission to NDM-KP identification ranged from 13 to 65 days and the duration of hospitalisation in the intermediate care unit spanned from 23 to 99 days. For all patients, acquisition was considered linked to the stay in the intermediate care unit. No digestive decolonisation regimen was proposed to the patients, due to the lack of evidence on the efficacy of such strategies for carbapenemase producing bacteria [14, 18].

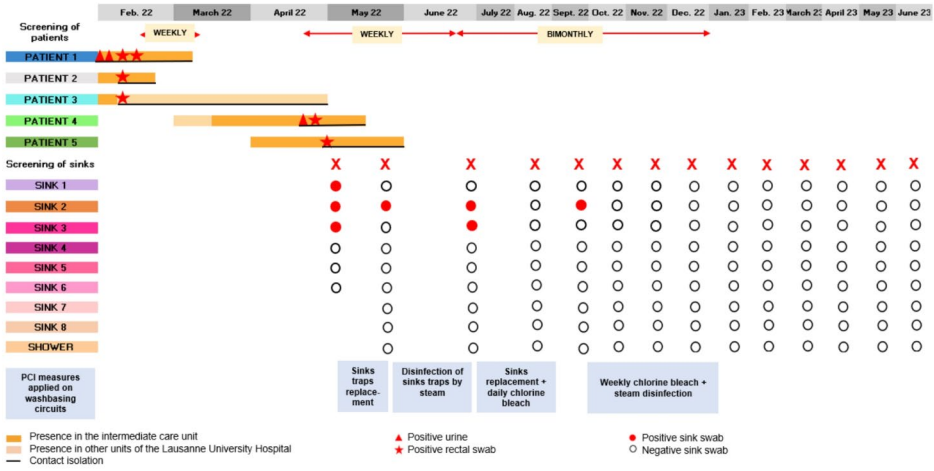

Fig. 2 Summary table of hospital stay of NDM-KP positive patients, environmental investigations and interventions on sink traps

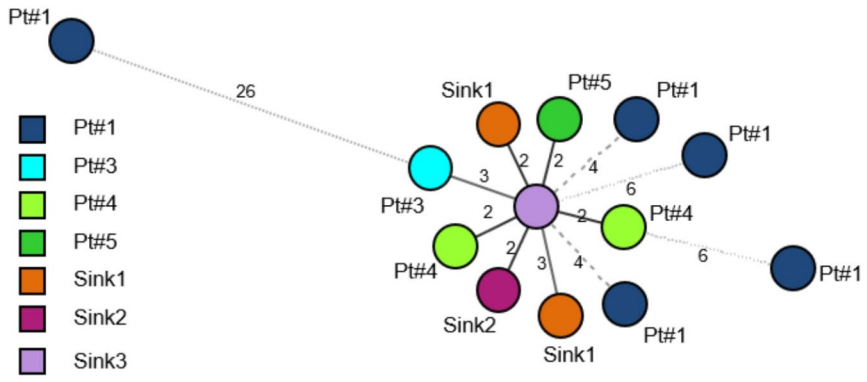

Fig. 3 Minimum spanning tree of ST268 strains. Each circle represents one isolate. The distance between the circles represents the number of different loci

Initial IPC measures

According to Swiss national guidelines [17], IPC measures were applied promptly after the detection of the first NDM-KP case. Upon identification of the fourth and fifth cases in April and May 2022, the IPC team assessed practices using a national standardized method which revealed suboptimal adherence to hand hygiene (51%) and frequent misuse of gloves (no indication for use, not properly changed). Additionally, an improper usage of washbasins during patient care was observed, including the direct spillage in sinks of water used for washing and the use of handwashing basins for cleaning medical devices such as tracheostomy tubes. Following these observations, education rounds to reinforce hand hygiene, and material and environment disinfection were implemented. Weekly screening of inpatients in the unit and enhanced environmental cleaning were also pursued.

Environmental investigations

In view of the new cases found during the second phase of the outbreak, environmental samples from all P-traps and the shower drain were performed in search of a persistent reservoir. Among the six sink traps sampled, three (50%) were positive for NDM-KP. Follow-up testing showed persistent colonisation by the epidemic strain in two sinks in June and in one sink in September 2022 (Fig. 2).

Genomic typing

Genomic typing was performed on NDM-KP isolates successfully cultured from four out of five patients and from all environmental samples. All isolates belonged to MLST ST-268, and their genomes were closely related (0 to 6 loci differences) based on wgMLST (Fig. 3). One of the 5 isolates of case 1 (39087) showed a 26 loci difference, possibly reflecting this patient's

bacterial flora diversity. Moreover, the strains isolated from the P-traps were genetically very close, suggesting cross-transmission.

Enhanced IPC measures

Considering the environmental and genomic evidence suggesting a wastewater-associated reservoir, we implemented "water-free" patient care, a strategy routinely utilized in our ICU. In addition, we replaced the three NDM-KP-positive P-traps (Fig. 2). Follow-up sampling performed two weeks later showed persistent colonisation of a sink trap by the same isolate. At this point, we initiated weekly steam disinfection of all sink traps, performed by the cleaning team. Despite these measures, follow-up sampling one month later revealed persistent colonisation of two P-traps. Given the unsuccessful eradication of the environmental reservoir with the aforementioned measures, we replaced the whole washbasin circuit of colonised sinks and implemented daily disinfection by chlorine bleach (one litre per day per colonised sink followed by sink condemnation during at least 30 min) for two consecutive weeks. New environmental sampling was negative two weeks after the end of the disinfection procedure but turned out positive once again in a previously colonized sink, a month later. Due to the persistent reservoir, we implemented a combined disinfection with chlorine bleach and steam over 3 months and pursued monthly environmental sampling of all P-traps for another 5 months. Follow-up samples of all sink traps and the shower drain, performed until May 2023, remained negative. CPE screening of all inpatients in the unit was continued on a weekly and then bimonthly basis, until December 2022. To date (July 2024) no additional epidemiologically or genomically related NDM-KP-positive patients have been identified. Moreover, no NDM-producing bacteria were found in other environmental samples within our hospital.

Discussion

We report the successful control of an environmental reservoir of NDM-KP in a low-incidence setting, over an eight-month period. During the investigation of this outbreak, WGS analysis allowed us to detect clonal relationships between clinical and environmental isolates, and to identify a probable transmission pathway contributing to the persistence of the epidemic strain in the unit.

Many reports have described outbreaks of MDRO linked to the hospital wastewater systems [10, 11, 15, 19–24]. Highly discriminatory genomic methods, such as WGS, now allow for the confirmation of the key role that wastewater drains play as reservoir [8, 9, 23, 25–27]. Such transmissions have been mainly reported in ICU and hematology-oncology wards, predominantly affecting immunosuppressed patients exposed to several medical devices [6]. In Switzerland, carbapenem resistance remain rare, although numbers are increasing steadily, mirroring the situation in neighbouring countries. *Klebsiella pneumoniae* producing NDM, oxacillinase or *Klebsiella pneumoniae* carbapenemase and *Escherichia coli* producing oxacillinase or NDM are the most frequently observed CPE strains [2, 28, 29]. Recently, Catho et al. [30] published a report on a long-term outbreak of *Pseudomonas aeruginosa* producing Verona integron-encoded metallo-β-lactamase carbapenemase, which was genetically related to the hospital building's wastewater in an intensive care unit in Geneva. This highlights the rising threat of carbapenemase-producing microorganisms in Swiss hospitals.

The biofilm present within the hospital wastewater system creates a particularly conducive environment for the development of a resistant microbiome. Indeed, the repeated exposure to various biological fluids from patients [30] or to antibiotic treatments poured into washbasins encourages the establishment and selection of antibiotic-resistant micro-organisms and therefore the potential horizontal transfer of resistance genes between species. Nevertheless, factors affecting MDRO establishment in the hospital wastewater environment are complex. In the case of carbapenemase-producing *K. pneumoniae*, Park et al. [7] showed that positive patients can seed the wastewater environment in at least 6% of opportunities. Additionally, environmental sites that become colonised are more likely to remain positive, which is congruent with our experience.

Several routes of transmission between patients and aqueous reservoirs have been reported, including retro-contamination via splashes of the healthcare environment [31, 32], healthcare equipment and medical devices [27]. The routes can lead to patient contamination through direct or indirect contact. Sub-optimal design or misuse of sinks may contribute to the dissemination of microorganisms colonizing the washbasin circuit [33, 34]. In our neurosurgical intermediate care unit, washbasins lack devices designed to minimise splashing, such as taps that do not flow directly into the drain and physical barriers that separate the adjacent area. Nevertheless, all waterpoints are located more than one meter far from patients' beds, and patients' clean items are stored far from the sink environment. However, on-site observations revealed several improper uses of washbasins during patient care and suboptimal hand hygiene adherence, which may have contributed to cross-transmission. The role of hand-carried transmission is difficult to evaluate and remains undefined, largely due to the likely contribution of multimodal factors. Nonetheless, several studies have reported a significant reduction in transmission following enhancements in hand hygiene compliance [35].

Therefore, bundled approaches that aims to prevent the seeding of hospital drains with MDROs, by avoiding patient fluid exposition, eradicating colonized reservoirs, and interrupting cross-transmissions between patients and their water environment, have been proposed and led to a more or less satisfactory control of epidemic situations [6]. Modifying or renovating washbasin pipelines, including the revaluation of pipe material (e.g., copper) and the installation of self-cleaning sinks using vibration/heat/ultrasonic [28], can be challenging mostly due to cost and architectural constraints. Other feasible but difficult-to-implement measures include anti-splashing barriers [28, 36] and safe sink practices, such as hand hygiene-dedicated washbasins and disposal of human waste (including water in contact with patients or medical equipment) in a washer-disinfector [34]. Importantly, waterless patient care significantly contributes to the prevention of water environment-to-patient cross-transmission, but is not always accepted by healthcare providers [14, 30, 37].

Regarding the eradication of environmental reservoirs, various drain treatment protocols, primarily involving chemical agents (such as bleach and hydrogen peroxide) [38] or thermal disinfection, have been documented [39]. However, these methods have shown limited, if any, lasting effect on decolonizing MDROs from drain system. The presence of microbial biofilm in sink traps hinders eradication of microorganisms and may warrant the replacement of the whole washbasin circuit. Nevertheless, P-traps replacement alone is often insufficient, as biofilm-derived re-colonization can occur from distal (unchanged) components of the drain system [11]. In our case, partial replacement of the drainage circuit by changing the traps only was not successful, even when combined with weekly steam disinfection. Only the combination of a complete washbasin circuit replacement with bleach and heat disinfection allowed the sustained control of the environmental reservoir, after a 12-month follow-up. The persistent contamination of P-traps after initial replacement highlights the importance of replacing the connecting pieces, while working closely with the plumbing department. More data from prospective studies comparing different replacement and disinfection protocols are required.

Only a few studies reporting success stated the duration of follow-up after the intervention, which ranged, from 2 months to 3.5 years [23, 36, 40, 41]. In our situation, we continued microbiological screening of P-traps for 12 months after the identification of the last positive patient and 5 months after the completion of the sink's disinfection protocol, providing proof for a successful and persistent control of the outbreak. Nevertheless, a possible limitation is the potential undersampling of the aqueous environment, as sampling only sink and shower traps may not suffice to conclusively demonstrate the absence of NDM genes.

The unicentric and observational character of this report represents a key limitation. Moreover, the genomic analysis was limited to the complete sequencing of strains but did not include a plasmid analysis to determine if the NDM plasmids were related. Finally, we used a multimodal approach to control the NDM-KP outbreak and therefore, we were not able to assess each IPC measure separately. Nevertheless, we provide a structured and detailed description of an effective bundle of measures which can be reproduced in other settings.

Conclusion

In conclusion, our comprehensive investigation, spanning several months and utilizing both patient and environmental screenings alongside WGS analysis, was pivotal in the successful control of NDM-KP. This outcome was achieved through the implementation of a multimodal IPC strategy, underscored by waterless patient care and the meticulous replacement and disinfection of P-traps and connecting pieces. Crucially, the success of this endeavor was further enhanced by effective collaboration with plumbing and cleaning teams, demonstrating that in low-incidence settings, proactive and coordinated efforts can substantially mitigate and even completely eliminate the transmission of CPE. This case study underscores the imperative for ongoing vigilance, interdisciplinary collaboration, and innovation in IPC measures to address the evolving challenge of MDROs in healthcare environments globally.

Abbreviations

CPE	Carbapenemase-producing Enterobacteriacea
NDM-KP	Klebsiella pneumoniae producing New Delhi metallo-beta-lactamase carbapenemase
WGS	Whole genome sequencing
wgMLST	Whole genome multilocus sequence typing
IPC	Infection prevention and control
PCR	Polymerase chain reaction
ICU	Intensive care unit
MDRO	Multidrug resistant organism

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Author contributions

EM and PF wrote in an equally contribution the first draft of the manuscript. LS revised the first draft of the manuscript. DSB provided input for the microbiology part and for the part dealing with the molecular characterization and genomic analyses part. All authors revised the final manuscript and approved the final version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate and for publication
Not applicable. This work was conducted in accordance with the local ethic committee's criteria on quality studies.

Competing interests
The authors declare no competing interests.

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