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Large, protracted, multi-species and multi-clonal spread of VIM-type metallo-β-lactamase-producing Enterobacterales in an Italian hospital

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SUMMARY

Background: Carbapenem-resistant Enterobacterales, particularly those producing carbapenemase (CPE), pose a major threat to human health, being listed among critical-priority resistant pathogens by the World Health Organization.
Aim: To report on a large nosocomial spread of CPE of different species producing Verona integron-encoded metallo-β-lactamase (VIM)-type carbapenemases, and on the infection prevention and control measures that were adopted to combat the spread.
Methods: Conventional culture and molecular methods were used for detection and identification of VIM-positive CPE (VIM-CPE) causing infections or colonizing patients or present in environmental specimens. Whole-genome sequencing analysis of selected isolates was performed to investigate clonal relatedness. Basic (active surveillance, contact precautions, close contact screening, cohorting of patients, surface cleaning, hand hygiene) and advanced (weekly point-prevalence surveys for rectal colonization, additional training of healthcare workers, extraordinary ward sanitization, extraordinary maintenance interventions, and environmental microbiological screening, single-use equipment, ward relocation) infection prevention and control (IPC) measures were implemented to combat the spread.
Findings: Spread of VIM-CPE involving 151 patients (mostly colonizations) was documented in a single hospital ward from November 2021 to December 2023. The spread involved several different species of Enterobacterales, with clonal expansion documented in some cases. Implementation of basic and advanced IPC measures was temporarily successful at

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mitigating the spread, but multiple relapses were observed, suggesting the presence of an unidentified environmental reservoir.

Conclusion: VIM-CPE has the potential to cause large and complex nosocomial outbreaks in hospital environments, underscoring the challenges to their control by IPC practices.

Introduction

Among resistant bacteria, carbapenem-resistant Enterobacterales (CRE) have been identified in recent years as one of the major threats to human health and are considered among top-ranking critical-priority pathogens for research in drug development by the World Health Organization (WHO) [1]. In CRE, the major mechanism of resistance is represented by the production of carbapenemases, including serine-β-lactamases of molecular class A (e.g. KPC-type) and D (e.g. OXA-48 like), or metallo-β-lactamases (MBLs) of molecular class B (e.g. NDM-type, VIM-type, and IMP-type) [2].

Carbapenemase-producing Enterobacterales (CPE) have the potential to spread rapidly and colonize patients in healthcare settings, causing potentially large-scale epidemics [3,4]. Moreover, horizontal transfer of carbapenemase genes has resulted in rapid interspecies dissemination of these mechanisms of carbapenem resistance [5,6]. Globally, these enzymes are becoming more frequent in healthcare and community settings and are considered highly problematic, since CPE are associated with a high burden in terms of mortality and disability-adjusted life-years [7].

Substantial variability in the prevalence of carbapenemase types exists in continental, national, and regional settings [8]. In Italy, the CRE epidemic observed since 2010 has mostly been driven by the dissemination of *Klebsiella pneumoniae* strains producing KPC-type carbapenemases (KPC-Kp) [9—11]. However, the epidemiological scenario has been compounded by an increasing proportion of MBL producers [12—15]. This trend is concerning, since MBL-producing Enterobacterales exhibit a spectrum of resistance also including the recently licensed β-lactam/β-lactamase inhibitor combinations (e.g. ceftazidime—avibactam, meropenem—vaborbactam, and imipenem—relebactam) [16—18].

Effective infection prevention and control (IPC) practices are critical for CPE containment in healthcare settings. The core IPC practices for CPE include: early identification of infections and active surveillance through rectal screening for CPE carriage, hand hygiene, contact precautions, patients isolation, patient and staff cohorting, environmental cleaning, proper reprocessing of non-disposable medical devices, staff education, and antimicrobial stewardship [19—21]. However, implementation of standard IPC measures, in some cases, is not sufficient to fully control the outbreaks, due to the potential contribution of environmental reservoirs [22—24].

VIM-type MBLs, first detected in *Pseudomonas aeruginosa* isolates from Europe in the late 1990s, have subsequently been reported also in Enterobacterales [25,26]. However, with the notable exception of Greece, VIM-producing CPE (VIM-CPE) have remained sporadic or limited to small-scale clonal outbreaks in other European countries including Italy [27—31].

In this work, we describe the unexpected occurrence of a large and protracted nosocomial spread of VIM-CPE, involving different species, observed in a single ward of a hospital in an area where VIM-CPE have so far remained sporadic. We also report on the difficulties in containing the spread of VIM-CPE, despite the implementation of basic and advanced IPC measures, to underscore the challenges to control dissemination of similar resistant pathogens in healthcare settings.

Methods

Setting

The internal medicine unit (IMU) of Siena University Hospital (a 700-bed tertiary-care university hospital located in central Italy) houses 30 beds, of which six are placed in double rooms and the remaining in quadruple rooms. Each quadruple room is equipped with a bathroom with two sinks, one WC, a shower, and a bidet, while each double room is equipped with a bathroom with one sink, one WC, and a shower. Adjunctively, the ward is equipped with four handwashing sinks reserved to personnel and one sink for waste disposal in the 'dirty' utility room. Patients admitted to IMU are mostly elderly, with acute or subacute medical problems, and with high prevalences of invasive devices, induced immunosuppression, and comorbidities (e.g. diabetes, cardiac impairment, oncologic diseases, renal impairment).

Definitions

'Colonization' was defined by detection of rectal carriage of one or more types of CPE. Colonized patients were identified through CPE rectal screening undertaken on admission, and thereafter on a weekly basis. 'Imported colonization' was defined when CPE colonization was detected on admission to the ward during the observation period (November 1st, 2021 to December 31st, 2023). 'Incident colonization' was defined when CPE colonization was detected in a patient that was negative at admission in the ward, during the same period. 'Infection' by CPE was defined according to Centers for Disease Control and Prevention criteria, after review by an infectious diseases specialist [32]. 'Molecular-positive only (MPO) colonization' was defined when a rectal molecular screening positive for $bla_{\text{VIM-type}}$ was associated with a negative rectal swab culture for a bla_{VIM}-type CPE.

Standard CPE colonization surveillance workflow

All patients in the IMU, as a high-risk setting, were screened for CPE carriage on admission and weekly, and before transfer to another healthcare facility, in accordance with Italian

guidelines as well as established local routine practice [33,34]. The laboratory protocol for screening of CPE carriage is based on a combined approach using molecular-based testing at the admission (Allplex™ Entero-DR Assay, Seegene, Seoul, South Korea; or Xpert Carba-R kit, Cepheid, Sunnyvale, CA, USA) and culture-based testing using ChromID™ Carba Smart selective medium (bioMérieux, Marcy-l'Etoile, France) for subsequent monitoring. CPE were identified at the species level by matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry (Bruker Daltonics, Billerica, MA, USA), and the type of carbapenemase was determined by lateral flow immune assay (LFIA) using the O.K.N.V.I. Resist-5 test (Coris BioConcept, Gembloux, Belgium). Specimens positive for one or more carbapenemase genes by molecular testing were always subjected to culture for identification of the CPE species.

Infection control policy

At the IMU, the standard institutional procedure was activated as response to detection of a nosocomial outbreak. The procedure included the following 'basic' control measures (BCM) implemented in response to occurrence of at least two linked incident cases: (i) activation of a crisis team, including the general leadership of the hospital; (ii) contact precautions for colonized/infected patients with use of gloves and a disposable overcoat; (iii) 'close contacts' identification for screening and pre-emptive contact precautions; (iv) improvement of hand hygiene adherence monitoring by direct observations according to WHO method [35]; (v) cohorting of cases or care organization based on a go-forward approach; (vi) reinforcement of standard cross-transmission control measures such as dedicated patient care equipment (e.g. blood pressure cuff, thermometers, oximeters); (vii) cleaning and sanitization three times a day of rooms hosting colonized/infected patients with terminal steam cleaning after patient discharge or transfer. All surfaces, except medical devices, were cleaned with sodium hypochlorite-based products. Medical devices were cleaned with disposable disinfection wipe pre-saturated with 70% alcohol and 2% chlorhexidine gluconate. Additional infection control measures (ACMs), implemented in response to a lack of significant impact of BMC, based on the crisis team evaluation, included: (i) weekly point prevalence surveys for rectal colonization by CPE using the same molecular method used for screening at admission (Allplex™ Entero-DR Assay); (ii) IPC (infection prevention control) training of healthcare workers; (iii) extraordinary ward sanitization (cleaning and disinfection of the unit by closing successively each of its rooms and subjecting each one to terminal cleaning) and implementing daily shared medical devices disinfection; (iv) extraordinary maintenance interventions including replacement of drain pipes; (v) environmental microbiological screening; (vi) adoption of single-use jugs for patient hygiene; (vii) enhanced training of cleaning staff and staff turnover reduction; (viii) temporary relocation of the ward (Table I).

Environmental microbiological screening

Environment microbiological screening was undertaken twice during the outbreak. Environmental samples were collected from all sinks ($N = 20$) and bed surfaces ($N = 30$) during the first round, and from replaced sink drain siphons ($N = 7$) of some rooms during the second round of environmental investigations. Environmental samples were taken with sterile, rayon-tipped swabs (Transystem™ 108C; Copan, Brescia, Italy) moistened with sterile saline when the sampled area appeared to be dry. The presence of CPE was assessed as follows: on the first round, samples were plated on to Columbia blood agar medium (bioMérieux) and incubated for 48 h at 35 ± 2 °C in aerobic atmosphere; on the second round, samples were plated on ChromID™ Carba Smart medium (bioMérieux) after an enrichment step in brain–heart infusion (BHI) broth (24 h incubation on 35 ± 2 °C in aerobic atmosphere in both cases). Identification of suspected colonies was performed by MALDI-ToF mass spectrometry (Bruker Daltonics). Enterobacterales isolates underwent testing for the presence of carbapenemase genes by polymerase chain reaction (Allplex™ Entero-DR Assay), followed by testing for carbapenemase production by LFIA O.K.N.V.I. Resist-5 test (Coris BioConcept).

Whole-genome sequencing and bioinformatics analysis

A subset of VIM-CPE from patients and environmental samples were subjected to whole-genome sequencing (WGS) analysis. The selection was made to cover temporal and species distribution of clinical and environmental isolates. A VIM-CPE collected from the rectal swab of a patient admitted to the IMU in September 2021 (i.e. prior to the first wave of the outbreak), available at the Laboratory of Microbiology and Virology of Siena University Hospital, was also subjected to WGS analysis for comparison. A detailed description of WGS and bioinformatics analysis methods is provided in the Supplementary Appendix.

Results

Description of the outbreak and control measures

A large spread of VIM-positive cases involving the IMU ward emerged in November 2021 and continued for the 26-month observation period evolving in four major waves. Overall, 151 of the 1685 patients (8.9%) admitted to the IMU ward during the outbreak period were involved (mean age: 76.1 years; median age: 80 years; male-to-female ratio: 1:1; mean length of stay: 20.7 days). During the observation period, patient characteristics did not change, except for a seven-week interval in which the ward was dedicated to admission of COVID-19 patients (Supplementary Figure S1).

Overall, 17 patients were imported cases (overall prevalence: 1.0% of admissions), while the remaining 134 patients were incident cases, being CPE negative at admission and testing positive for VIM-CPE after 3–49 days (mean: 14.7 days). The prevalence of incident cases during the observation period was 7.9%. In all cases, VIM positivity was detected from surveillance specimens (rectal swabs), revealing intestinal carriage. In two cases a VIM-CPE was also cultured from clinical specimens: in one case *Klebsiella oxytoca* from blood (one month after rectal swab positivity by VIM-CPE of the same species), and in the other case *K. oxytoca* from urine (one day after rectal swab positivity by a VIM-positive *Enterobacter cloacae*).

Concerning the imported cases, one or two species of VIM-CPE (in 11 and two cases, respectively) were isolated from rectal swabs of the 17 VIM-positive imported cases, yielding a

Table I
Matrix of 'additional' control measures (ACMs) and relationship with outbreak waves

Measures	ACMs	Wave 1 ($N = 28$) Nov 21st–Feb 22nd (weeks 1–14)	Wave 2 ($N = 30$) May 22nd–Jul 22nd (weeks 1–13)	Wave 3 ($N = 44$) Sep 22nd–Mar 23rd (weeks 1–26)	Wave 4 ($N = 32$) Apr 23rd–Dec 23rd (weeks 1–37)
PPSs	Molecular-based weekly point-prevalence surveys for CPE colonization by rectal swabs	ACMs	ACMs	ACMs	ACMs
SR	Drain pipes (siphons) replacement of all bidet and sinks drains in three rounds; replacement of shower drains was not possible	ACMs	–	–	–
ES	Environmental microbiological screening in two rounds: – round 1 from sinks and bed surfaces – round 2 from replaced bidet and sinks, drains, siphons	ACMs	–	–	–
IPC training	Healthcare workers' infection prevention control training by educational sessions	ACMs	–	ACMs	ACMs
WS	Extraordinary ward sanitization: cleaning and disinfection of the unit by closing successively each of its rooms and subjecting each one to terminal cleaning, daily shared medical devices disinfection	ACMs	ACMs	ACMs	ACMs
Ward relocation	Temporary ward transfer to allow extraordinary maintenance measures	ACMs	–	–	–
EM	Extraordinary maintenance; painting of the walls and the elimination of medications and fomites (medication vials, gloves, and containers)	ACMs	–	–	–
SDD	Sink and shower drain decontamination by 0.5% sodium hypochlorite-based effervescent chlorine tablets, on a weekly basis	–	ACMs	ACMs	ACMs
PHDD	Single-use jugs (disposable devices) for patient hygiene	–	–	ACMs	ACMs
CS training	Enhanced training of cleaning staff and staff turnover reduction	–	–	ACMs	

N = number of VIM-CPE incident cases.

total of 15 VIM-positive isolates of five different species including *K. oxytoca* ($N = 2$), *E. cloacae* complex ($N = 5$), *E. coli* ($N = 4$), *C. freundii* ($N = 2$), and *K. pneumoniae* ($N = 2$). In four cases a rectal molecular screening positive for $bla_{\text{VIM-type}}$ was associated with a negative rectal swab culture for a VIM-CPE (MPO cases).

Concerning the incident cases, one, two or three species of VIM-CPE (in 95, 13, and one case, respectively) were isolated from rectal swabs, yielding a total of 124 VIM-positive isolates of eight different species including *K. oxytoca* ($N = 29$), *E. cloacae* complex ($N = 23$), *Citrobacter* spp. (*C. freundii*, $N = 46$; *C. farmeri*, $N = 9$; *C. koseri*, $N = 1$), *Escherichia coli* ($N = 6$), *K. pneumoniae* ($N = 8$), and *Raoultella ornithinolytica* ($N = 2$). The diversity of species apparently increased with ongoing waves. An MPO result was observed in 25 cases. The proportion of different species and MPO cases over the four waves is represented by the heat map in Supplementary Table S1.

The majority of incident cases clustered in room 4 ($N = 28/134$, 20.9%) and room 11 ($N = 25/134$, 18.6%), with a relative prevalence of *Citrobacter* spp. ($N = 10/28$, 35.7%) and *Klebsiella* spp. ($N = 10/25$, 40.0%), respectively. None of the imported cases but one were hospitalized in these rooms. Notably, the rooms with the higher number of isolates were those more distant from the waste disposal (dashed square in Figure 1).

The four major waves were separated by windows ≥6 weeks free of incident cases: a first wave, from early November 2021 to early February 2022 ($N = 28$ incident cases, $N = 2$ imported cases); a second wave from early May to late July 2022 ($N = 30$ incident cases, $N = 5$ imported cases); a third wave from mid-September 2022 to early March 2023 ($N = 44$ incident cases, $N = 4$ imported cases); and a fourth wave from late April 2023 to December 2023 ($N = 32$ incident cases, $N = 6$ imported cases). Each wave started with an incident case of carriage detected in absence of other positive patients in the ward (Supplementary Figure S1).

Wave I

The first incident case of VIM-CPE occurred in early November 2021 and was identified by a weekly monitoring sample. The BCMs were therefore activated. In January 2022, considering the occurrence of 11 new incident cases despite

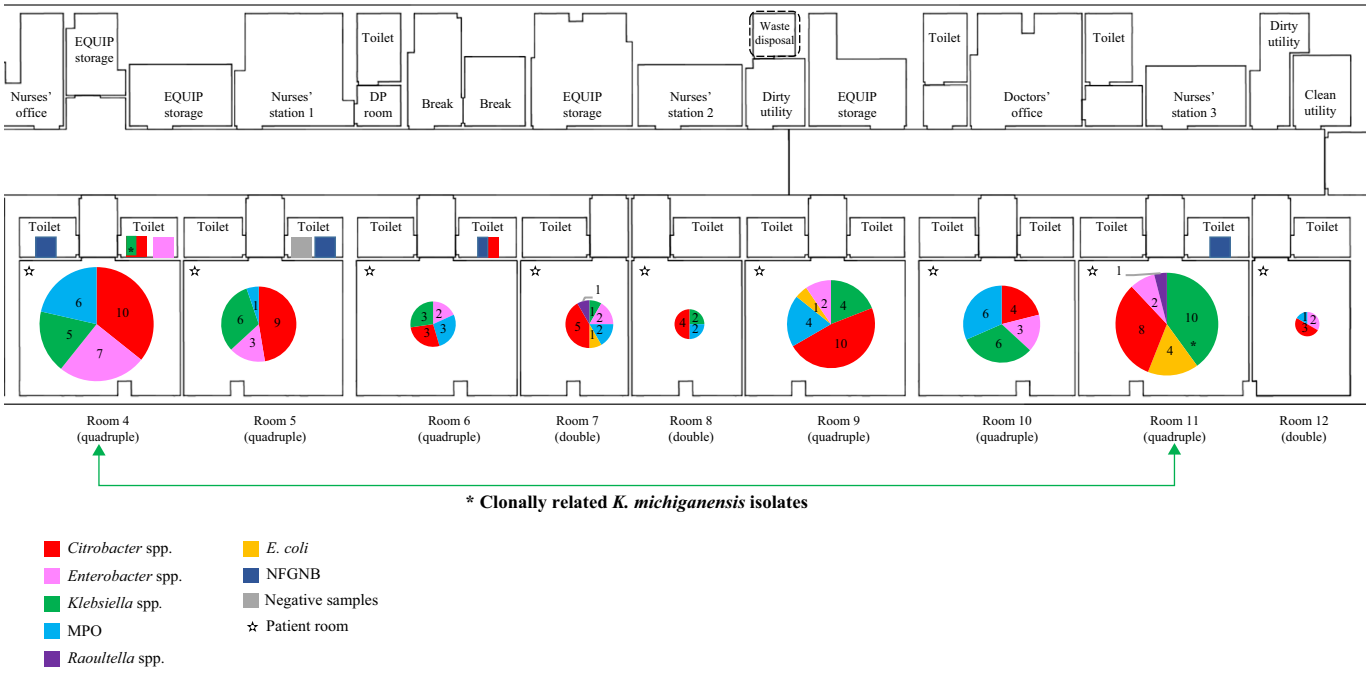

Figure 1. Distribution of *bla*$_{\text{VIM-type}}$-positive outbreak and environmental samples by patient's room. Legend: squares represent environmental samples; circles represent patient isolates; circle diameter is directly proportional to number of isolates. NFGNB, non-fermenting Gram-negative bacilli; EQUIP storage, equipment storage; DP room, drug preparation room.

BCMs (Supplementary Figure S1), ACMs, including ward relocation, were also implemented (a detailed description of ACMs implemented is shown in Table I). Thereafter, since no new cases had been detected for a six-week period, outbreak eradication was presumed and the ACMs were terminated in February 2022.

Wave II

In early May 2022, new cases of incident colonizations were reported (Supplementary Figure S1). ACMs were re-adopted (Table I) until early July 2022 when the ward was converted into a COVID-19 unit for seven weeks with ward sanitization (WS) upon opening and closing. Universal contact and droplet precautions were adopted, and family visits were restricted during the whole period. As no new cases were registered for six weeks, the outbreak was again considered to be over.

Wave III

In early September 2022, VIM-CPE were again isolated from monitoring weekly samples of two patients admitted in the same room. Concomitantly, KPC-producing *Klebsiella pneumoniae* were also isolated from routine rectal monitoring of six IMU inpatients (Supplementary Figure S1). All BCMs were again implemented with a rapid effect on the KPC outbreak, that was controlled in a few weeks. Conversely, new cases of VIM-CPE were found and, therefore, some ACMs were re-activated (Table I). The last case was registered in early March 2023, and seven weeks after their discharge the outbreak was considered to be over.

Wave IV

In late April 2023, a new incident case of VIM-CPE was observed, and additonal cases were subsequently reported, with variable incidence over time. Wave IV was still ongoing by the end of the study period (December 2023).

During the outbreak, staff cohorting could not be adopted due to staff shortages. However, multiple lapses in infection control measures were detected by direct observation of hand hygiene compliance and adherence to contact precautions. Lapses were corrected when observed, and systematic changes were recommended based on these observations.

Microbiological and genomic analysis of the spread

Environmental investigation

The first round of environmental sampling, performed in January 2022 (ES1; Supplementary Figure S1), yielded no CPE-positive cultures. To enhance the sensitivity of the culture, the second round of sampling (February 2022, ES2) was performed with a modified protocol including an enrichment step followed by plating on selective medium. This second round yielded 16 VIM-producing isolates including 10 non-fermenting Gram-negative bacilli (NFGNB) (*Pseudomonas alcaligenes*, $N = 2$; *Pseudomonas monteilii*, $N = 2$; *Pseudomonas putida*, $N = 2$; *Pseudomonas stutzeri*, $N = 1$ and *Stenotrophomonas maltophilia*, $N = 3$) and six Enterobacterales (*E. cloacae* complex, $N = 2$; *C. freundii*, $N = 2$; *K. oxytoca*, $N = 1$; *K. pneumoniae*, $N = 1$). At least one VIM-producing organism was obtained from all rooms ($N = 4$) subjected to ES2, while Enterobacterales were detected only from two of them (rooms four and six; Figure 1).

WGS analysis

A selection of 57 VIM-CPE ($N = 52$ from incident cases and $N = 5$ from imported cases) collected during the four epidemic

waves, plus two environmental isolates collected during ES2 and a pre-outbreak VIM-CPE (isolated in September) available in collection were subjected to WGS analysis.

WGS analysis confirmed identification for isolates identified by MALDI-ToF as *E. coli*, *C. freundii*, *C. farmeri*, and for most isolates identified by MALDI-ToF as *K. pneumoniae* (except two, identified as *K. quasipneumoniae* and *K. variicola*, respectively by WGS). On the other hand, isolates identified by MALDI-ToF as *E. cloacae* complex were identified by WGS as *Enterobacter hormaechei*, all belonging to subsp. *hoffmannii*, except for the environmental isolate, that belonged to subsp. *oharae*. The isolate identified as *C. koseri* by MALDI-ToF was found to be *C. freundii* by WGS analysis. The isolates identified by MALDI-ToF as *K. oxytoca* were either *Klebsiella grimontii* or *Klebsiella michiganensis* by WGS. Distribution of isolates investigated by WGS by species and sources is reported in Supplementary Table S2.

Comparative genomic analysis of isolates of the same species revealed a variable scenario. An essentially monoclonal population was observed for *K. grimontii*, *C. farmeri*, *C. freundii*, and *E. hormaechei* from incident cases, suggesting that these cases were associated with expansion of a single clone which became established in the ward. However, the *E. hormaechei* isolates from imported cases or from the environmental source were different from the resident clone associated with incident cases, suggesting that they had not been the source for that. On the other hand, the isolate of *K. michiganensis* from the environmental sample was clearly related with an isolate from an incident case, supporting a possible role of the environment as a direct source of VIM-CPE. Finally, notable clonal diversity was observed among isolates of *K. pneumoniae* and *E. coli*, regardless of their imported or incident source (Supplementary Table S2 and Supplementary Figure S2).

All strains investigated by WGS carried the $bla_{\text{VIM-1}}$ gene, together with a variable array of other resistance determinants among the different circulating clones and a variable plasmidome (with IncA-, IncF-, and Col-type replicons being the most common) (Supplementary Table S2).

Discussion

Italy has been among European countries that have experienced rapid and massive dissemination of CPEs since 2010, with prevalent dissemination of KPC-Kp, subsequently compounded by New Delhi metallo-β-lactamase (NDM)-CPE, while CPEs producing other carbapenemases have remained relatively uncommon [12,36,37]. Despite emergence since the late 1990s, the prevalence of VIM-CPE has remained very low [25,37—39]. According to the most recent data by the Italian ARISS surveillance network, VIM-type carbapenemases were responsible for 1.2% of the total of CR *K. pneumoniae* and *E. coli* obtained from invasive infections in 2023 [40].

In this work we describe one of the largest outbreaks of VIM-CPE reported so far, observed in a single hospital ward. The spread was rapidly intercepted thanks to the active surveillance programme for CPE enforced in our hospital, which mandates for rectal screening of CPE carriage by molecular testing for all hospital admissions at risk for CPE (as recommended by the Tuscan Regional Government) and, subsequently, on a weekly basis.

Unlike previous reports, the spread of VIM-CPE was sustained by a VIM-CPE of several different species of Enterobacterales, and WGS analysis revealed that the phenomenon consisted mostly of clones of some species that apparently had become endemic in the ward environment, but was also compounded by the horizontal dissemination of the carbapenemase gene among different clones of different species. Although plasmid analysis was beyond the scope of this study, it will be of interest in future investigations to confirm the contribution of plasmid dissemination in the complex microbiological landscape underlying this spread of VIM-CPE.

Notably, despite the large number of patients involved (151 cases), an infection by VIM-producing CRE was reported in two cases only, while the remaining patients were colonized at the intestinal level, without signs of infection. This observation would support an overall low-virulence behaviour of VIM-CPE, mostly represented by species other than *K. pneumoniae* and *E. coli*.

The implementation of increasingly aggressive IPC practices (from standard precautions to temporarily ward relocation) was apparently able to temporarily mitigate the spread of VIM-CPE but not to eradicate the phenomenon, which was likely supported by an environmental reservoir of VIM-positive strains. Unfortunately, it was impossible to identify a clear source for the spread within the ward. However, several elements are highly suggestive for the existence of an environmental reservoir. In fact, the lack of a known patient reservoir at the beginning of the four waves and the relapses occurring after relatively long intervals free of incident cases would support the existence of environmental reservoirs, possibly contributing to occasional transmissions of VIM-CPE strains or VIM-encoding plasmids in the clinical setting. Indeed, this possibility was confirmed by the finding of clonally related isolates of VIM-positive *K. michiganensis* obtained from an incident human case sample and from a sink drain. Moreover, the longer time free from incident cases observed following ward relocation was obtained, maintaining the same healthcare staff and patients case mix, enabling us to reasonably exclude the existence of a human reservoir (e.g. intestinal colonization) among healthcare workers. Since common IPC measures were sufficient to control a concomitant spread of KPC-CPE without an eradicating effect on the VIM-CPE spread in the same setting, this would suggest the need for tailored interventions adapted to the nature and the origin of the outbreak.

Outbreaks involving MDR *K. oxytoca* complex strains have been previously linked to environmental reservoirs, such as handwashing sinks and wastewater drainage systems [41,42]. Furthermore, the finding of VIM-positive *Pseudomonas* spp. strains in almost all siphons sampled during ES2 suggests that water pipes could represent an ecological niche where carbapenemase-producing strains of Gram-negative non-fermenters could survive and act as sources for transfer of MBL genes to Enterobacterales. Finally, the report of an epidemiologically unrelated *E. hormaechei* strain, isolated before the study period, displaying a clear genetic correlation with isolates from the spread, supports the existence of indirect transmission dynamics within the ward.

In our study all *K. michiganensis* and *K. grimonti* strains were initially misidentified as *K. oxytoca*, confirming the limitations of the MALDI-ToF in discriminating highly related species within the *K. oxytoca* complex, as reported in the literature [43,44].

Interestingly, environmental VIM-positive isolates found in this study were ubiquitous in sinks in the IMU but with a

prevalent distribution in the rooms most distant from the waste disposal. This could indicate an improper use of sinks for the disposal of body fluids from colonized patients with subsequent sink colonization, as described elsewhere [45,46].

Recently, the role of the hospital environment as a hidden reservoir for CPE has been explored [47]. In particular, antibiotic resistance mechanisms can persist and be transferred between bacterial species in hospital wastewaters and moist surfaces of hygienic services. The role of wastewater drainage systems (drains, sink/shower traps, toilets, drainage pipes) has been demonstrated in various studies, while water splashes from the contaminated drains can lead to dissemination of MDRO-containing droplets, which in turn may contaminate the adjacent surfaces or the skin of nearby healthcare personnel and patients [41,45,48,49]. Interestingly, VIM is among the most frequently reported carbapenemases responsible for contamination of the hospital water environment, which may also be a source for new acquisitions [50—53]. Recent outbreak studies using advanced genomic epidemiological methods, including WGS, have revealed hospital environmental reservoirs of several species of bacteria, harbouring plasmids conferring resistance to carbapenems and transferable to pathogenic Enterobacterales strains [54].

One strength of this study is the availability of population-based CPE surveillance data, while a possible limitation is the narrow environmental sampling. Given the limited number of samples the extent of the environmental contamination may have been underestimated.

Antibiotic consumption in the studied ward could have influenced the dynamics of the VIM-producing organisms' spread. However, during the study period, no significant changes occurred in the overall quantity (all antibiotics and carbapenems) nor in the pattern of most prescribed molecules (data not shown).

In conclusion, the present findings underscored the possibility that large and complex outbreaks of VIM-CPE can occur in hospitals, likely fuelled by the presence of environmental reservoirs, which can be recalcitrant to eradication by conventional IPC practices recommended for CPE. A better knowledge of environmental colonization sites might help in guiding surveillance strategies, outbreak investigations, and IPC interventions.

Ethics statement

Rectal screening was performed according to the hospital IPC guidelines, and collection of clinical data as part of outbreak investigations for implementation of appropriate IPC practices. This study focused on distribution and characteristics of bacterial isolates, and of IPC management. Consequently, ethical approval was not required.

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.2024.12.003.

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