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Sink drains as reservoirs of VIM-2 metallo-β-lactamase-producing *Pseudomonas aeruginosa* in a Belgian intensive care unit: relation to patients investigated by whole-genome sequencing

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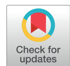

SUMMARY

Background: Hospital-acquired infections caused by VIM-encoded metallo-β-lactamase-positive *Pseudomonas aeruginosa* are a major problem in intensive care units (ICUs) worldwide. A previous study conducted in the UZ Brussel hospital revealed that sink drains of the ICU were a possible source of various multidrug-resistant pathogenic bacteria.
Aim: To investigate the presence and persistence of VIM *P. aeruginosa* in the sink drains of the four adult ICUs and their role in nosocomial infections, emphasizing sink-to-patient transmission.
Methods: Thirty-six sinks located in the ICUs of the UZ Brussel were sampled and screened for the presence of VIM *P. aeruginosa* in August and October 2019. Whole-genome sequencing (WGS) was performed on all positive sink drain isolates together with 61 isolates from patients who were retrospectively selected (ICU patients 2019–2020, $N = 46$; non-ICU patients 2019, $N = 6$).
Findings: Twenty sinks were found positive for *P. aeruginosa* at both sampling time-points. WGS revealed that the predominating environmental cluster belonged to sequence type ST111. Ten additional STs were identified. VIM-2 was detected among all ST17 ($N = 2$) and ST111 ($N = 14$) sink drain isolates. Based on whole-genome multi-locus sequence typing analysis of all genomes, 15 clusters of highly related isolates were identified, of which seven included both sink drain and clinical isolates.
Conclusion: Our findings confirm that sink drains are a possible source of VIM-2 *P. aeruginosa*, probably after being contaminated with clinical waste from patients.

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Patients could be exposed to VIM-2 *P. aeruginosa* dispersed in their environment because of colonized sink drains.

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Introduction

Pseudomonas aeruginosa infections are not very common in the general population, but infections and colonization can occur in high-risk individuals such as people with cystic fibrosis, chronic lung obstructive disease, or an immunodeficiency. Hospitalized patients often have wounds, indwelling catheters, or are ventilated and could therefore be a potential host for *P. aeruginosa*. [1]. In addition, *P. aeruginosa* has been considered as an important causative agent of nosocomial infections worldwide, and, since this bacterium is intrinsically resistant or less susceptible to several antimicrobial agents, infections caused by *P. aeruginosa* are challenging to eradicate. Moreover, *P. aeruginosa* clones resistant to almost every antibiotic tested, including carbapenems, are widely disseminated. Carbapenem resistance among *P. aeruginosa* may be caused by the acquisition of carbapenemases, mainly Ambler class B metallo-β-lactamases (MBLs) [2]. Oliver *et al*. described the worldwide existence of antibiotic-resistant high-risk clones such as ST235, ST111, and ST175 [3].

A large number of *P. aeruginosa* outbreaks have been linked to sources in the environment, especially water systems [4–6]. Wash hand basin drains in hospitals may contain enormous numbers of bacteria such as *P. aeruginosa*, which may then be transmitted to patients. When water runs into the sink drain, aerosols may contaminate the environment and the hands of healthcare workers. The sink is thus an open, actively emitting pathogen reservoir [4–6]. As early as 1991, Döring *et al*. suggested that sinks could play a role in the transmission of *P. aeruginosa* [5]. Upon entering the hospital, hand cultures of all studied personnel were *P. aeruginosa* negative. However, during duty, 42.5% of personnel members acquired different *P. aeruginosa* strains on their hands. They identified identical genotypes of *P. aeruginosa* on the hands of the personnel and in the sink, suggesting a transmission route from sink to hands [5]. We also previously reported that sinks were involved in the transmission of multi-resistant carbapenemase-producing Enterobacterales in the intensive care unit (ICU) [7].

In 2019, we noticed an increasing prevalence of multi-resistant *P. aeruginosa*, such as Verona integron-encoded MBL (VIM)-positive strains, in clinical or screening isolates of patients in our adult ICU. The goal of the present study was to verify whether patients could be colonized or infected by micro-organisms present in the sink drains and to investigate whether high-risk clones of *P. aeruginosa* are present in our ICU. To achieve this goal, whole-genome sequencing (WGS) was employed.

Methods

Setting

The University Hospital Brussels is a teaching hospital with more than 700 beds. There are four ICUs for adults, each containing six beds. Every unit has nine sinks: one for every patient, one in the anteroom, one in the utility room, and one placed centrally. In total, 36 sinks are available in the ICUs.

Infection control measures and environmental cleaning policy

Patients at the ICU are screened rectally for the presence of resistant Gram-negative bacteria on admission, every week, and at discharge. Respiratory, blood, urine, and other clinical samples are taken when there is a suspicion of infection. Patients colonized or infected with multi-resistant *P. aeruginosa* are placed in contact isolation precautions in a single room with use of gloves and a disposable overcoat. The room is cleaned daily with Incidin® Plus (0.5% glucoprotamin) (Microtek, Zutphen, The Netherlands). At discharge, the room is cleaned intensively and unused consumables, such as gloves and hand alcohol, are discarded. Periodic checks of the quality of terminal cleaning are performed with the Glowcheck® (Hartmann, Heidenheim, Germany). The sinks are flushed once a week with Incidin Plus. The water supply to the ICU is unfiltered and is tested quarterly for the presence of *P. aeruginosa*.

Sink sampling and microbiological methods

Pseudomonas aeruginosa isolates were recovered by taking swabs in 36 sink drains (10–15 cm depth) (eSwab; Copan, Brescia, Italy). The sinks were sampled twice at a 1.5-month interval (August 2019 and October 2019). After sampling, 1 mL of Fastidious Organisms Broth (FB, own preparation) was added to the eSwab, and the tubes were incubated aerobically for 48 h at 37°C. Presumptive *P. aeruginosa* isolates were recovered on MacConkey agar (bioMérieux, Marcy l'Etoile, France) (48 h, aerobic incubation at 37°C) and identified by matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Microflex LT mass spectrometer with MALDI Biotyper 3.0 software and Reference Library 3.2.1.0 (Bruker Daltonik GmbH, Bremen, Germany). The presence of the VIM β-lactamase enzyme was investigated with the RESIST-4 O.K.N.V. immunochromatographic lateral flow assay (Coris BioConcept, Gembloux, Belgium). Confirmed *P. aeruginosa* strains were further selected for WGS.

Clinical isolates

The *P. aeruginosa* isolates collected as described in 'Infection control measures and environmental cleaning policy' were stored at −80°C. From this collection, *P. aeruginosa* isolates were retrospectively selected to perform WGS. The study consisted of different subgroups (Appendix A):

- 59 clinical or rectal screening isolates from 44 randomly selected patients without cystic fibrosis, residing at least once at the ICU (January to December 2019);

– six isolates from six randomly selected patients, not residing at the ICU (May to August 2019);
– two VIM-positive isolates from clinical samples of two COVID-19 patients residing at the ICU (October to December 2020).

If, within the same short hospitalization period, multiple isolates with the same antibiotic resistance profile were obtained from a patient, only a single one was included for WGS.

Identification of strains was performed as described in 'Sink sampling and microbiological methods'. Antibiotic susceptibility testing was investigated both by the disc diffusion method and by MIC susceptibility testing using the interpretative criteria of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) combined with recommendations of the National Reference Center (NRC) and BAPCOC (Belgian Commission for the Coordination of the Antibiotic Policy). The presence of the VIM β-lactamase enzyme was determined as described in 'Sink sampling and microbiological methods'.

Whole-genome sequencing

Genomic DNA was extracted using the Maxwell RSC Cell DNA purification kit (Promega Corporation, Madison, USA). Fragmentation of genomic DNA was carried out using the NEBNext® Ultra™ II FS module. Sequencing libraries, with an insert size of on average 550 bp, were prepared using the KAPA Hyper Plus kit (Kapa Biosystems, Wilmington, USA) and a Pippin Prep size selection. In order to avoid PCR bias, the PCR amplification step was excluded and a 500 ng input of genomic DNA was used. After equimolar pooling, libraries were sequenced on a Novaseq 6000 instrument (Illumina, San Diego, CA, USA) using an SP-type flow cell with 500 cycles. A 1% PhiX control library was included in each sequencing run. Sequence quality was assessed with FastQC (version 0.11.4) software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). De-novo assembly was performed using SPAdes genome assembler (http://bioinf.spbau.ru/spades).

In-silico identification of serotypes, resistance genes, and virulence factor-related genes

Identification of serotypes and acquired antibiotic resistance genes was performed using tools available from the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/) (ResFinder 3.2, PAst 1.0) [8]. The presence of resistance genes was determined with a percentage identity (ID) threshold of 90% over the length of the reference sequence whereas β-lactamase variants were determined with a percentage ID threshold of 100%. In addition, potential virulence factor-related genes, from which a selection was made based on previous studies, were identified by the virulence factor database (VFDB, http://www.mgc.ac.cn/VFs/) [9–12].

Whole-genome multi-locus sequence typing (wgMLST) analysis

The sequencing data was analysed using the wgMLST scheme for *P. aeruginosa* available in BioNumerics v.7.6.3. (Applied Maths, Sint-Martens-Latem, Belgium). This scheme consists of 15,143 loci, including the seven classic MLST loci [13]. Both assembly algorithms were used for allele calling, i.e. the assembly-free k-mer-based approach using the raw reads and the assembly-based BLAST approach. The default settings were used for both the assembly-free and assembly-based algorithms. The quality of the sequence read sets, the de-novo assemblies, the assembly-free and the assembly-based allele calls were verified using the quality statistics window in BioNumerics. The MLST profile of each isolate was determined using the PubMLST allele mapping experiment incorporated in BioNumerics. Minimal spanning trees (MSTs) were generated using the wgMLST allelic profiles as input data in BioNumerics. Branch lengths reflect the number of allele differences between the isolates in the connected nodes. For clustering, the maximum distance between nodes was set at <14 [14].

Results

P. aeruginosa from sink drains

Pseudomonas aeruginosa was recovered from 20 out of the 36 sink drains at both sampling time-points. These positive sink drains were spread over all four ICUs. *P. aeruginosa* was absent from the sink drains of the utility rooms in all four ICUs. One isolate, Env. ICUC_PAUZB105, was excluded from the wgMLST analysis due to impurity of the DNA extract.

P. aeruginosa STs and serogroups among sink drain and clinical isolates

In total, 11 distinct STs were identified among the sink drain isolates (ST17, ST27, ST111, ST164, ST244, ST253, ST348, ST395, ST446, ST1058, and ST1074) (Figure 1). Seven of these STs were also identified in the clinical isolates (ST17, ST27, ST111, ST244, ST253, ST446, and ST1058) (Figure 1). ST111 was the predominating ST, accounting for 36% of the sink isolates ($N = 14/39$), followed by ST395 and ST446, each accounting for 13% of the sink isolates ($N = 5/39$) (Figure 1). Sink drain ST111 isolates were retrieved in all four ICUs whereas ST395 and ST446 isolates were found in three ICUs (Figure 2).

Twenty-eight different STs were observed among the 59 clinical isolates from 44 ICU patients and the six clinical isolates from six non-ICU patients from 2019, suggesting different chains of transmission (Figure 1). The most frequently identified STs, sorted by ascending order of ST number, were ST17 (five isolates from five ICU patients; one isolate from a non-ICU patient), ST27 (four isolates from four ICU patients), ST111 (nine isolates from eight ICU patients), ST175 (three isolates from two ICU patients; one isolate from a non-ICU patient), ST 235 (six isolates from three ICU patients), ST244 (eight isolates from four ICU patients), ST253 (one isolate from an ICU patient; two isolates from two non-ICU patients) and ST446 (four isolates from three ICU patients) (Appendix A). Isolates with ST175 and ST235 were found among clinical isolates only. Interestingly, the two clinical isolates from 2020 were ST17 and ST111 (Figure 1).

All ST111 isolates were associated with serogroup O12 (Appendix A). Isolates with ST175 and ST235 were linked to serogroups O4 and O11, respectively (Appendix A).

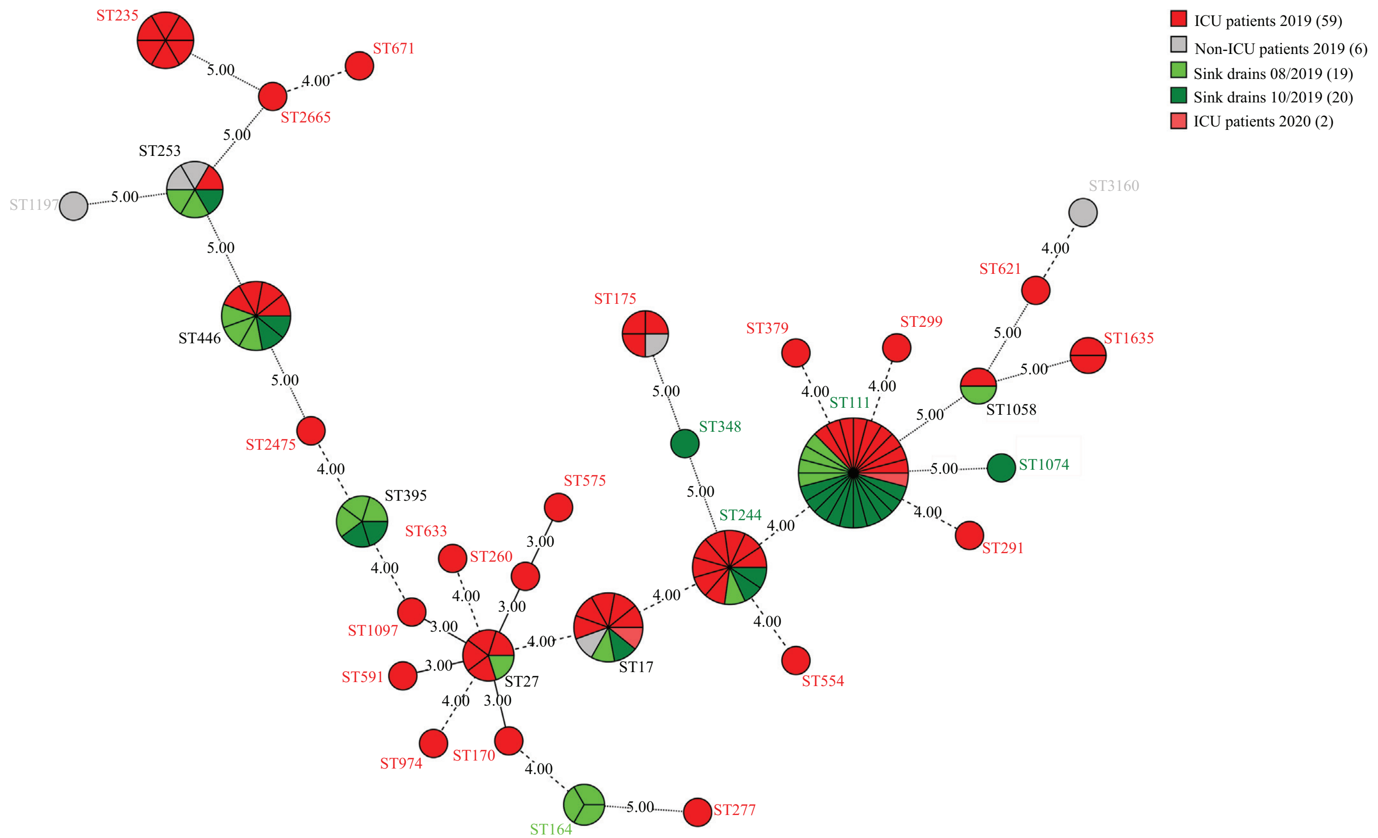

Figure 1. Minimum spanning tree based on PubMLST allelic profiles of 106 *P. aeruginosa* genomes built from PubMLST analysis, with indication of the year of isolation. The analysis was carried out in BioNumerics, using the *P. aeruginosa* wgMLST scheme. Thirty-two distinct MLST STs were identified among all isolates, i.e. 39 sink isolates, 61 clinical isolates from 46 ICU patients and six clinical isolates from six non-ICU patients. Nodes are colour-coded per type of isolate and their respective numbers as labelled. Numbers of allelic differences are indicated on the lines connecting various STs.

wgMLST analysis

Eight clusters were identified based on the wgMLST analysis of the 39 sink drain isolates (Figure 2). The isolates within the predominant environmental cluster, cluster 1 ($N = 11$), belonged to ST111. These isolates were retrieved from three out of the four ICUs (B, C, and D; Appendix A and Figure 2). The three remaining ST111 isolates clustered together (cluster 2: range: 0—6) and were all retrieved from ICU A (Figure 2). Consequently, the wgMLST analysis showed that ST111 sink drain isolates were subdivided into two clusters of closely related isolates. Also the ST17 (cluster 6: no allelic differences), ST164 (cluster 5: no allelic differences), and ST395 isolates (cluster 4: range: 0—1) were closely related within their respective clusters (Figure 2). The latter belong to the second predominant environmental cluster, cluster 4 ($N = 5$), and were retrieved from three ICUs (B, C, and D; Appendix A and Figure 2). ST446 isolates, however, were more diverse (range: 0—119). Still, three ST466 isolates, retrieved from different ICUs (A and C; Figure 2), were highly similar (cluster 7: no allelic differences). The same was seen for ST244 and ST253 isolates (range: 1—35 and 0—31, respectively) (Figure 2). For each ST, however, two isolates were closely related and belonged to clusters 3 and 8, respectively (Figure 2).

After performing wgMLST analysis on all 106 *P. aeruginosa* genomes (39 sink drain and 67 clinical isolates) seven additional clusters of highly related isolates were identified, accounting for a total of 15 clusters (Appendix B). Clusters 16* and 17* cannot be considered as clusters because the isolates were retrieved from the same patients (patients 13 and 29, respectively). Seven out of the 15 clusters included genomes of both sink drain isolates and clinical isolates (clusters 1 (ST111), 2 (ST111), 3 (ST244), 6 (ST17), 10 (ST446), 11 (ST446) and 12 (ST1058)), suggesting human-to-environment-to-human transmission. The patients within each cluster generally stayed at the ICUs from which corresponding sink drain isolates were detected. The clinical isolates within cluster 1 were retrieved from patients staying at ICU B, C or D, for example (Appendix A). Yet, within cluster 3, one isolate was retrieved from a patient that remained at ICU C during the hospital stay (patient 40), while the two sink isolates were retrieved at ICU B (Env. ICUB_PAUZB011 and Env. ICUB_PAUZB017). Interestingly, a lag of several months was observed between the collection date of some clinical samples and the environmental sampling date. Cluster 10, for example, includes one sink drain isolate (Env. ICUB_ PAUZB013) and three clinical isolates from two patients (patients 07 and 33) staying at ICU B, that showed no allelic differences although the isolates were retrieved approximately

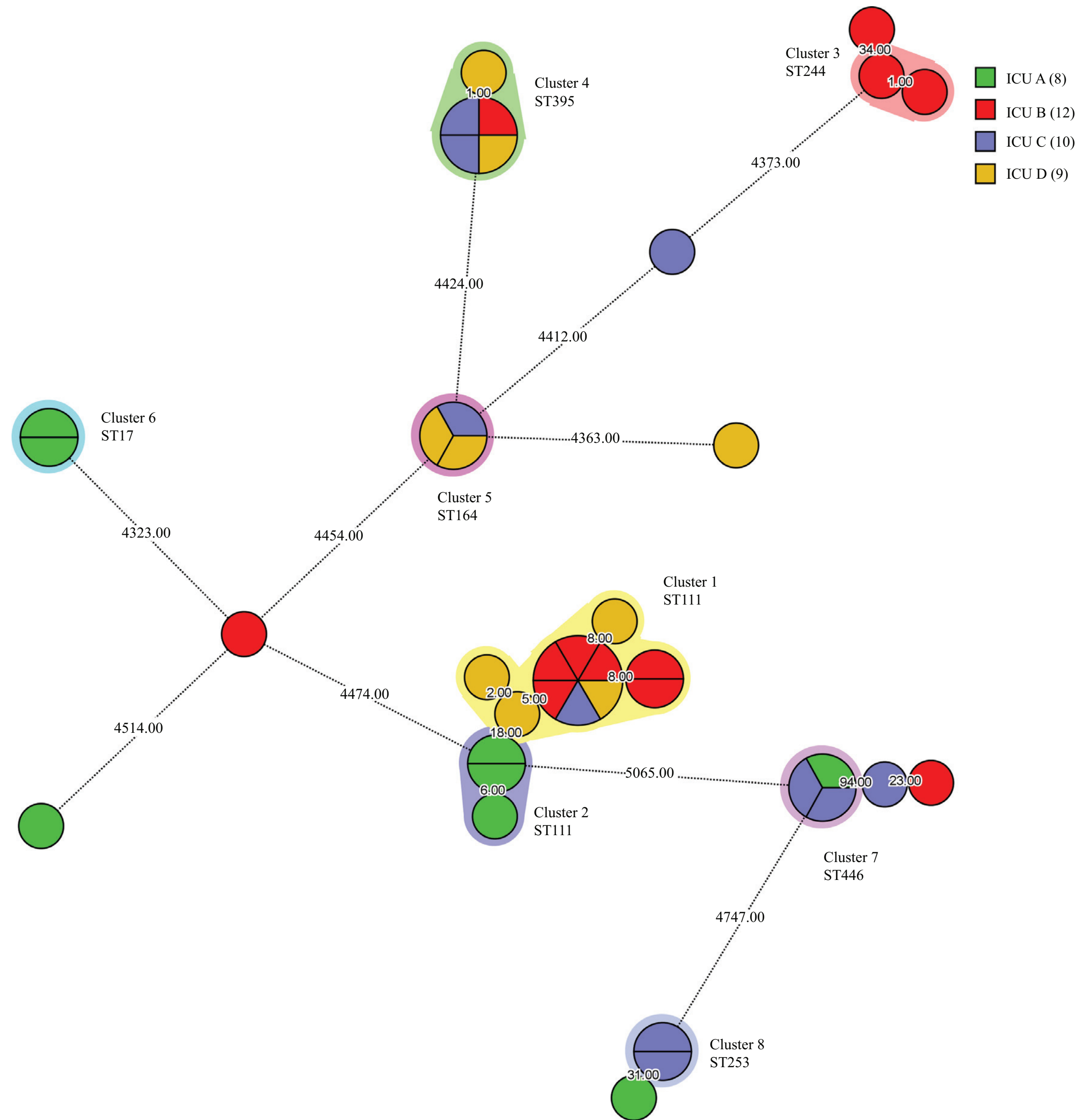

Figure 2. Minimum spanning tree based on wgMLST allelic profiles of 39 *P. aeruginosa* genomes built from wgMLST analysis. The analysis was carried out in BioNumerics, using the *P. aeruginosa* wgMLST scheme. Eleven distinct STs were identified among all sink isolates. A fixed threshold of <14 allelic differences was used for clustering of isolates. Nodes are colour-coded per originating intensive care unit and their respective numbers as labelled. Numbers of allelic differences are indicated on the lines connecting the various STs.

six months apart (Appendices A and B). The same was observed for cluster 11, where the sink drain isolate (Env. ICUC_ PAUZB024) was retrieved six months after the clinical isolate from ICU C, while the clinical isolate was retrieved from a patient staying at ICU D (patient 31). These two isolates showed eight allelic differences. Also, two patients (patients 16 and 17) were admitted with a negative rectal screening and became positive for VIM *P. aeruginosa* during their hospital stay, which was after the second sink sampling time-point in October 2019. In line with this, one year later, two COVID-19 patients (patients 51 and 52) became positive for VIM *P. aeruginosa* during their hospital stay. The isolates from both patients belonged to clusters 6 (ST17) and 2 (ST111), respectively. The presence of biofilms in the drainage pipes connecting the sinks might be an explanation for the obtained results.

Within four clusters composed of sink drain isolates, no links to clinical isolates were identified (clusters 4 (ST395), 5 (ST164), 7 (ST446), and 8 (ST253)). The sink drain isolates within each cluster were closely related with 0–1 allelic differences. Four clusters comprised clinical isolates only and showed no link to the environment (clusters 9 (ST27), 13 (ST175), 14 (ST253), and 15 (ST235)). Two of these clusters comprised clinical isolates from patients staying at the ICU and non-ICU patients (clusters 13 and 14) (Appendix B). The ICU patient from cluster 13 (patient 28) had previously been hospitalized in 2018 already, staying in non-ICU wards also, where the patient might have acquired the isolate. However, the ICU

patient from cluster 14 (patient 02) did not leave the ICU during the hospital stay.

All isolates recovered from a single patient (patients 01, 10, 13, 15, 28, 29, and 33) were highly similar and belonged to the same ST, with only 0–5 allelic differences. Yet, isolates with different STs were also retrieved from some ICU patients (patients 02, 25, and 29), showing the ease with which *P. aeruginosa* infects fragile patients (Appendix A).

Phenotypic resistance profiles of clinical P. aeruginosa isolates

The phenotypic resistance profiles were investigated for the clinical samples only (Appendix C).

Antibiotic resistance and virulence gene profiles of P. aeruginosa isolates

The presence or absence of antibiotic resistance and virulence genes found in the 39 sink isolates and the 67 clinical isolates are presented in Appendices D and E, respectively.

Sixteen out of the 39 sink drain isolates (clusters 1 (ST111), 2 (ST111), and 6 (ST17)) carried the acquired β-lactamase gene bla_{VIM-2} together with the intrinsic resistance genes *aph(3′)-Iib* (chromosomal aminoglycoside phosphotransferase), bla_{PAO} (β-lactamase resistance), and catB7 (chloramphenicol resistance) (Appendix D). The intrinsic resistant genes $bla_{OXA-395}$ (β-lactamase resistance; ST111) or bla_{OXA-50} (β-lactamase resistance; ST17) were also detected in these sink drain isolates as well as the *crpP* (ciprofloxacin-modifying enzyme), *fosA* (fosfomycin resistance), and *sul1* (sulfonamide resistance) genes. All but one of these sink drain isolates carried the acquired aminoglycoside-resistance genes *aac(6′)-29a* and *aac(6′)-29b*, which are flanking the bla_{VIM-2} gene on a class 1, In59-like integron. Exactly the same genes, depending on the clusters, were found in the clinical isolates carrying bla_{VIM-2} (13 isolates from 12 patients) (Appendix D).

Interestingly, one of the clinical isolates in cluster 13 carried the bla_{VIM-4} gene (patient 28_PAUZB078; ST175). Another clinical isolate, outside a cluster, had a bla_{IMP-13} gene (patient 11_PAUZB056; ST621). Also these genes are present on class 1 integrons (Appendix D).

A wide variety of putative virulence genes involved in survival and persistence were identified among the *P. aeruginosa* genomes as expected (Appendix E). The presence of type III secretion system (TTSS) exotoxin *exoS* gene was observed in all isolates except those from ST235, ST253, ST446, ST671, ST1197, and ST2665. These isolates were the only isolates containing *exoU*, which is known to be mutually exclusive with *exoS*. The *exoY* gene was present in all isolates, except for one ST17 isolate (patient 45_PAUZB099).

The quorum sensing genes *lasI* and *lasR* were present within all but one *P. aeruginosa* genome (patient 48_PAUZB102) (Appendix E). Similarly, all except three ST17 isolates possessed the quorum sensing genes *rhlI* and *rhlR* (patient 28_PAUZB077, patient 28_PAUZB078, and patient 50_PAUZB104). Both regulatory systems are known to regulate the production of many virulence factors and the formation of biofilms.

Discussion

The prevalence of infections with multidrug-resistant Gram-negative bacteria is increasing worldwide. In 2017 the World Health Organization classified the carbapenem-resistant *P. aeruginosa* as a 'priority 1 pathogen', which means that the bacterium may pose a particular public health threat. As studies show, VIM *P. aeruginosa* occurs in a wide range of environmental reservoirs and objects in the hospital, such as floors and walls, the beds of patients, door handles and various other objects [15,16]. However, it appears that the sink drain is probably the most important reservoir with a huge number of colony-forming units. A previous study conducted in our hospital revealed that sink drains of the ICU were a possible source of various multidrug-resistant pathogenic bacteria and suggested that transmission from these drains could indeed play a role in nosocomial infections [7].

The results of the current study confirm that sink drains are a possible source of VIM *P. aeruginosa* and that patients could become colonized or infected from sinks. Indeed, taking both sampling moments together, 55% of the sampled sink drains ($N = 36$) tested positive for *P. aeruginosa*. Of these positive samples, 41% ($N = 16/39$) carried the bla_{VIM-2} gene. Fifteen clusters were distinguished among all the analysed isolates. In seven of these, a genetic link was found between clinical and environmental isolates. At least 25 of the 61 (41%) clinical samples of patients from the ICUs can be linked to isolates found in the sink drains. All patients included in this study who carried a VIM-2 *P. aeruginosa* ($N = 12$) were linked to environmental samples of ST111 (clusters 1 and 2) or ST17 (cluster 6). Seven of the 12 (58%) patients died. No single link was seen between environmental isolates and non-ICU clinical samples ($N = 6$). However, as seen in clusters 13 and 14, a link was seen between ICU and non-ICU patients. This can probably be explained by the fact that patients and staff can move between the wards leading to the spread of resistant micro-organisms. It was not always clear in our study whether the sink drains were contaminated by the patients or the other way around. We noticed that two patients with negative rectal screening on admission became VIM-2 positive during their hospital stay, after the second sink sampling time-point. One year later, the same was observed for two COVID-19 patients. This suggests transmission from sinks to patients. Actually, the latest audit performed in the ICUs, in the summer of 2020, showed that clinical waste from patients had been flushed through the sinks instead of being evacuated via waste containers meant for that purpose. This practice had already started before 2020 but continued due to the COVID-19 outbreak as the personnel wanted to keep the clinical waste of the patients as much as possible in the isolation rooms. Consequently, the ideal situation would be rooms without sinks, which will be kept in mind when the new ICUs is built. In the meantime, siphons with a separate drain for clinical waste will be installed. Consistently disinfecting the sink drains with agents active against bacterial biofilms, such as acetic acid, could be a cheap additional measure [17].

Focusing on the STs, we can conclude that ST111 is present throughout the year in patients. Moreover, we found a great diversity of STs in the sink drains, meaning that there is a broad community of *P. aeruginosa* harboured in the ICUs and that

they can circulate for months at least. Indeed, it is reported that nosocomial *P. aeruginosa* infections or colonizations are generally characterized by polyclonality [18]. ST175 and ST235, known to be MDR international high-risk clones, were found among clinical isolates only [3]. ST111, however, was isolated from both patient and sink samples.

In this study, there are some missing parts and unexplainable links. For example, multiple very closely related strains were found in different sink drains among different ICUs, without one single link to a patient (clusters 4, 5, 7, and 8). A possible hypothesis is that *P. aeruginosa* can traffic between hospital U-bend sinks via biofilms in the wastewater network as shown in a study by Moloney *et al*. [19]. It is also possible that healthcare workers are carriers of resistant strains and can transmit them between patients without a link with the environment. It is notable that the water supply to the ICU is monitored four times a year to evaluate the presence of *P. aeruginosa*. During the study, levels remained less than one colony-forming unit per 100 mL, indicating that the presence of *P. aeruginosa* is coming from the ICU itself, and not being delivered via the water supply.

Besides the identification of the STs and serogroups, additional information about virulence factors and resistance genes was provided by WGS. The acquired aminoglycoside-resistance genes *aac(6′)-29a* and *aac(6′)-29b*, the intrinsic aminoglycoside-resistance gene *aph(3′)-Iib*, the β-lactamase-resistance genes bla_{PAO}, $bla_{OXA\text{-}395}$, and $bla_{VIM\text{-}2}$, the chloramphenicol-resistance gene *catB7*, and ciprofloxacin-resistance gene *crpP* were frequently identified in the isolates. All but two VIM-2 isolates carried the $bla_{VIM\text{-}2}$ gene in between two aminoglycoside-resistance gene cassettes in an In59-like integron structure, which is the same organization as described by Van der Bij *et al*. [20]. They pointed out that this integron structure is the most frequently found in outbreaks with VIM *P. aeruginosa* in the Netherlands.

Considering the virulence factors, Newman *et al*. described the negative association between the $exoU^+$ genotype and XDR phenotypes [11]. As seen in our results as well, the isolates of the international high-risk clones ST175 and ST111 were all $exoU^-/exoS^+$. However, a third international high-risk clone, ST235, which has caused numerous outbreaks worldwide and is associated with a particularly poor outcome, has an $exoU^+/exoS^-$ genotype [21,22]. Our clinical isolates also include isolates of this ST.

One limitation of this study is that it is possible that the real proportion of sink drains positive for *P. aeruginosa* is higher than we found, because we did not use selective or enrichment media. Another limitation of the study is that some sinks may have been sampled shortly after cleaning, which could explain why sometimes no bacterial growth at all was obtained. In addition, we acknowledge that no phenotypic testing of the environmental isolates was performed, i.e. the presence of genes does not necessarily reflect gene expression. Moreover, we only investigated acquired antibiotic resistance genes. The mutational resistome, affecting cell wall permeability, antibiotic efflux systems and point mutations, was not investigated [23,24].

In conclusion, this study confirms that sink drains are an important reservoir of VIM-2 *P. aeruginosa* strains, probably after being contaminated with clinical waste from patients. These bacteria can be transmitted to patients directly and indirectly via healthcare workers. Moreover, VIM-2 *P. aeruginosa* is known to lead to increased mortality. Since patients on the ICU are already vulnerable to infections, this can pose huge problems. WGS has been of great value in our outbreak investigation. It could help in the rapid recognition of an outbreak with accurate mapping of the spread and identification of potential sources, facilitating the implementation of infection control measures.

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Conflict of interest statement
None declared.

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Appendices A–E. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2021.05.010.

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