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Acetic acid as a decontamination method for sink drains in a nosocomial outbreak of metallo-β-lactamase-producing *Pseudomonas aeruginosa*

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SUMMARY

Background: *Pseudomonas aeruginosa* may colonize water systems via biofilm formation. In hospital environments, contaminated sinks have been associated with nosocomial transmission. Here we describe a prolonged outbreak of a metallo-β-lactamase-producing *P. aeruginosa* (Pae-MBL) associated with sink drains, and propose a previously unreported decontamination method with acetic acid.

Aim: To describe a nosocomial outbreak of Pae-MBL associated with hospital sink drains and to evaluate acetic acid as a decontamination method.

Methods: The outbreak was investigated by searching the microbiology database, microbiological sampling and strain typing. Antibacterial and antibiofilm properties of acetic acid were evaluated *in vitro*. Pae-MBL-positive sinks were treated with 24% acetic acid once weekly and monitored with repeated cultures.

Findings: Fourteen patients with positive cultures for Pae-MBL were identified from 2008 to 2014. The patients had been admitted to three wards, where screening discovered Pae-MBL in 12 sink drains located in the patient bathrooms. Typing of clinical and sink drain isolates revealed identical or closely related strains. Pae-MBL biofilm was highly sensitive to acetic acid with a minimum biofilm eradication concentration of 0.75% (range: 0.19–1.5). Weekly treatment of colonized sink drains with acetic acid resulted in negative cultures and terminated transmission.

Conclusion: Acetic acid is highly effective against Pae-MBL biofilms, and may be used as a simple method to decontaminate sink drains and to prevent nosocomial transmission.

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Introduction

Pseudomonas aeruginosa is a well-known pathogen that causes hospital-acquired infections, especially in immunocompromised patients. Pseudomonas can survive in moist environments, and has the ability to form biofilms that may colonize hospital water systems.[1] Several nosocomial outbreaks of *P. aeruginosa* have been associated with hospital water, sinks, sink drains, faucets, and showers.[2–5] Methods to terminate bacterial outbreaks associated with sink drains have been proposed, including changing of sinks and plumbings, disinfection with chemicals and self-disinfecting siphons.[3,4,6–9] However, further research on methods for eradicating *P. aeruginosa* from plumbing systems is needed.

Acetic acid is a known household remedy for cleaning and has documented effects in topical wound care against pseudomonas infections.[10,11] Here we describe a prolonged outbreak of metallo-β-lactamase-producing *P. aeruginosa* (Pae-MBL) associated with sink drains and propose a previously unreported decontamination method with acetic acid.

Methods

Setting

The survey was conducted at three wards (wards 1, 2, and 3) at the Skåne University Hospital, Lund, Sweden, which is a referral hospital with 800 beds. Ward 1 has capacity for 36 patients, four single rooms and 19 double rooms. Every room has a patient bathroom with one sink. All rooms have a pre-room with a sink for healthcare workers hand hygiene. Ward 2 has capacity for 15 patients with 15 single rooms. The rooms have one sink in the bathroom, one sink in the patient room and one sink in the pre-room for healthcare workers. Ward 3 has capacity for 24 patients with four quadruple rooms, two double rooms and four single rooms. Every room has one sink in the bathroom, one sink in the patient room and one sink for healthcare workers.

All sinks in the patient bathrooms were constructed so that water was directed straight into the sink drains (Figure 1).

Figure 1. A standard sink in the hospital where water is directed straight into the sink drain.

Epidemiological investigation

The clinical microbiology database was searched for patients with Pae-MBL-positive cultures from 2008 to 2014. Patient data on hospital admissions and medical history were extracted from patient journals. The study was approved by the regional ethical Review Board in Lund (no. 2015/293).

Sinks were cultured by swiping a cotton-tipped sterile swab along the lining 5–7 cm down the sink drain so that visible debris was obtained on the cotton tip. In Pae-MBL-positive sinks, a pre-flush sample of 500 mL water was analysed for Pae-MBL growth and faucets were cultured with a sterile cotton swab in the faucet opening and from the aerator.

Species identification and typing

Cultures were analysed at the Department of Medical Microbiology, Laboratory Medicine, Skåne County, Lund. Sink swabs were selectively cultured for Pae-MBL using Uriselect™ agar (Bio-Rad, Hercules, CA, USA) with added vancomycin (10.5 μg/mL, final concentration) (Duchefa Biochemie BV, Haarlem, The Netherlands) with meropenem 10 μg and sulphamethoxazole/trimethoprim 25 μg discs (Oxoid Ltd, Basingstoke, UK). Bacteria with an inhibition zone <25 mm to any of the discs were species-identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS Microflex LT, Bruker Daltonics, Billerica, MA, USA), followed by standardized antibiotic susceptibility testing using the disc diffusion method (Oxoid) and interpretation according to EUCAST breakpoint criteria.[12] MBL was detected with E-test MBL IP/IPI 256/64 (bioMérieux, Marcy l'Etoile, France), and positive strains were confirmed to produce MBL using Check-MDR CT-103 array (Checkpoint B.V., Wageningen, The Netherlands). Water samples were cultured by filtering 100 mL of water, followed by selective Pae-MBL culture of the filter as described above.

Pulsed-field gel electrophoresis (PFGE) analysis was performed at the Public Health Agency Sweden according to standard procedures.[13]

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Susceptibility to acetic acid was determined with a broth dilution method. Pae-MBL bacteria were added at a final concentration of 1×10^6 cfu/mL to acetic acid serially diluted from 24% to 0% in Tryptic Soy Broth (Beckton Dickinson, Franklin Lakes, NJ, USA) with 0.5% glucose (vWR) (TBSG) in a 96-well plate (Thermo Scientific, Waltham, MA, USA). Bacteria were incubated for 24 h at 37°C, and MIC was defined as the minimum concentration of acetic acid preventing visible bacterial growth. A volume of 10 μL from each well was thereafter mixed with 20 μL of phosphate-buffered saline (PBS) and subcultured on TSBG agar. Plates were incubated overnight at 37°C, and colonies were counted. MBC was defined as the concentration of acetic acid that killed >99.9% of the inoculum. Experiments were done in triplicate and repeated at least three times.

Minimum biofilm eradication concentration (MBEC)

MBEC was determined according to a modification of the Calgary Biofilm Device Method.[14] Biofilm was established on plastic pegs by adding 18 μL of an overnight culture of Pae-MBL to 162 μL of TSBG in a microtitre plate. A lid with 96 pegs (MBEC™ assay, Innovotech, Inc., Edmonton, Alberta, Canada) was placed on the plate which was then incubated for 24 h at 37°C on rotation (180 rpm). Pegs were challenged with acetic acid in two-fold dilutions (24% to 0%) for 30 min at room temperature followed by recovery in PBS for 30 min. Pegs were then incubated in TSBG for 24 h at 37°C, and MBEC was defined as the minimum concentration of acetic acid preventing visible growth in the wells. Four independent experiments were performed, each done in triplicate. To verify biofilm formation, pegs were washed three times in PBS, fixed in 100% methanol for 10 min and then stained with Crystal Violet [1% (w/v in water), Sigma–Aldrich, St Louis, MO, USA] for 5 min. Pegs were thereafter rinsed three times in PBS and the dye was extracted with 200 μL ethanol–acetone [80:20 (v/v)]. Absorbance was measured at 550 nm.

Electron microscopy

Bacteria were cultured in TSBG for 24 h at 37°C on rotation (180 rpm) on Thermanox glass coverslips (Agar Scientific, Stansted, UK) in a 12-well plate. Coverslips were then washed once in water and once in PBS, followed by fixation and preparation for scanning electron microscopy as previously described.[15] Specimens were observed in a Philips/FEI XL 30 FESEM at the Core Facility for Integrated Microscopy, Panum Institute, University of Copenhagen.

Results

Clinical cases

In June 2013, two neutropenic haematology patients died from Pae-MBL sepsis. The two patients had been nursed in the same room, one after the other. All contemporaneous patients were cultured from risk sites such as intravenous lines, wounds, stomas, sputum and urinary catheters, but were Pae-MBL negative. However, through environmental screening the bacterium was found in the sink drain of the patients' bathroom. A search in the clinical microbiology database identified another 12 patients with positive Pae-MBL cultures between 2008 and 2014. The patients appeared as single cases with a peak of four patients in the summer of 2013. Strains from patients 1–12 had been stored at the clinical microbiology laboratory allowing further typing. Isolates were susceptible only to colistin and gentamicin and they all produced the same Verona integron-encoded metallo-β-lactamase (VIM) enzyme.

The patients were five women and seven men with a median age of 70 years (range: 37–95) (Table I). Three patients had Pae-MBL-positive blood cultures. All three were neutropenic and treated for haematologic malignancies. Six patients, all of whom had an underlying lung disease, showed Pae-MBL-positive sputum cultures. Three patients had Pae-MBL in wound cultures. Ten of the patients (83%) have died, and in six of these cases Pae-MBL possibly contributed to mortality.

All patients except one had been treated in wards 1, 2, or 3. Three patients had been admitted to both wards 1 and 3, indicating a possible route of transmission. Wards 1 and 2 frequently exchange patients, and transmission of Pae-MBL between wards could have occurred by an undetected patient.

Table I
Case patients' clinical data

Patient	Gender, age (years)	Comorbidities	First positive culture, site of detection	Ward			Stayed in a room with Pae-MBL in the sink drain	Mortality possibly attributable to Pae-MBL
				1	2	3		
01	M, 80	Chronic ulcer	May 8th, 2008, ulcer	×			–	No
02	F, 41	COPD, secret stagnation lungs	Nov 19th, 2008, sputum; Aug 16th, 2009 blood	×			Yes	Yes
03	M, 73	Intensive care, brain tumour	Jan 13th, 2009, tracheal secretion				–	Yes
04	M, 70	COPD	Dec 29th, 2009, sputum	×			–	No
05	M, 95	UTI, dementia, pneumonia	Aug 11th, 2011, urine	×			–	No
06	F, 43	Haematological malignancy, neutropenia	May 2nd, 2012, blood		×		–	Alive
07	M, 73	CVI, secretion stagnation lungs	Sep 8th, 2012, urine; Oct 10th, 2012, sputum	×		×	Yes	Yes
08	F, 76	COPD, myasthenia gravis	Apr 15th, 2013, sputum	×			Yes	Yes
09	F, 55	Haematological malignancy, neutropenia	Jul 1st, 2013, blood		×		Yes	Yes
10	M, 64	Haematological malignancy, neutropenia	Jul 7th, 2013, blood		×		Yes	Yes
11	F, 78	Necrotizing fasciitis (Group A streptococcus)	Jul 24th, 2013, wound	×		×	Yes	No
12	M, 37	Treated tuberculosis, COPD	Aug 5th, 2013, sputum	×		×	Yes	Alive

Pae-MBL, metallo-β-lactamase-producing *Pseudomonas aeruginosa*; COPD, chronic obstructive pulmonary disease; UTI, urinary tract infection; CVI, cerebral vascular injury.

Environmental screening

Initial screening identified six Pae-MBL-positive sinks: four in ward 1 and one each in wards 2 and 3 (Figure 2). All sinks in wards 1 ($N = 51$), 2 ($N = 45$) and 3 ($N = 30$) were then repeatedly cultured, and another two colonized sinks were found in ward 1. In December 2013, ward 2 moved to a different floor. As this ward cares for highly immunocompromised patients, it was decided to culture all sink drains in the entire ward every other month. Initial screenings were negative, but from April 2014 to March 2015 four Pae-MBL-positive sinks were recorded (Figure 2). All patients admitted to these specific rooms since last negative culture were screened for Pae-MBL, but all cultures were negative.

In total, 12 Pae-MBL-positive sink drains were identified. All of them were located in the patient bathrooms (ward 1: six out of 23; ward 2; five out of 15; ward 3: one out of 10). No Pae-MBL growth was detected in cultures of water and faucet in positive sinks. As a control, four wards where none of the patients had been admitted were subjected to repeated culturing of 120 sink drains, which were all negative. Two control wards were located on the floor above ward 1 and one located on the floor beneath ward 2, thus sharing the same main drainpipes.

All sink isolates showed the same antibiotic susceptibility patterns as the clinical isolates and produced the VIM enzyme. Moreover, PFGE typing of the 12 isolates from patients and seven isolates from sinks showed identical or closely related band patterns (Figure 3). Seven patients had available information about rooms, and all of them had stayed in a room with a colonized sink.

Interventions

The initial response was to replace colonized sinks and plumbing, as this has been reported to be a successful method.[6] In ward 1, sinks could not be changed immediately for technical reasons. Awaiting replacement, 250 mL of 24% acetic acid was poured once weekly into colonized sink drains and incubated for 30 min before flushing. Control cultures were done every fourth week immediately before the next treatment. With the exception of one initial positive culture, all control cultures were negative (Figure 2). In May to June 2014, all sinks and plumbings to the wall were changed. Acetic acid treatment was then terminated, as we believed that the bacterial reservoir was eliminated. All positive sinks in wards 2 and 3 were also changed.

In total, 11 Pae-MBL positive sinks were replaced. However, follow-up cultures showed that the bacterium reappeared in three sinks after a mean time of 13 weeks. No known Pae-MBL patient had been admitted to the rooms since replacement. We then cultured the drainpipes going into the wall and found Pae-MBL in four of nine pipes, indicating a reservoir further down. As acetic acid treatment of colonized sinks had previously shown promising results in ward 1, acetic acid treatment of Pae-MBL-positive sinks was restarted. Since the finding of an initial positive culture in one colonized sink, all control cultures have been negative. However, two drainpipes in the wall remained positive even after 10 weeks of acetic acid treatment.

In an effort to completely eradicate Pae-MBL growth, two colonized drainpipes were flushed with hot water (90°C) directly into the pipe in the wall for 5 min with high pressure. Sink drain, siphon, and pipes to the wall were changed at the same time, but one of the pipes became Pae-MBL positive again after five weeks (Figure 2). Since Pae-MBL recurred from a reservoir further down, we decided to treat all patient bathroom sinks with acetic acid from March/April 2015.

In addition to decontamination, 'sink rules' for the patients such as not keeping toothbrushes or toiletries on the sink brim were reinforced.

In 2015, we reduced the frequency of control cultures in ward 1 to once every three or four months due to limited resources and based on our previous experience with continuously negative cultures during acetic acid treatment.

Taken together, 10 Pae-MBL-positive sinks have been treated with acetic acid for a median time of 60 weeks (range: 36–84). During this period, they have had a median of 11 negative cultures (range: 5–14). Two sinks had an initial positive culture after the first week of treatment but have been negative since the second control.

Susceptibility of Pae-MBL to acetic acid

To further study the efficacy of acetic acid against the Pae-MBL strain, susceptibility was tested *in vitro*. Bacteria were highly sensitive to acetic acid with a median MIC of 0.047% (range: 0.047–0.094) and median MBC of 0.19% (range: 0.094–0.19). Two sink isolates and one patient isolate were tested and showed identical MIC values.

As bacteria are believed to establish a biofilm in the sink, the efficacy of acetic acid against biofilm was also studied. The Pae-MBL-positive strain had biofilm-forming ability as visualized by electron microscopy (Figure 4). Next, bacteria were allowed to form biofilm on plastic pegs, which were then exposed to different concentrations of acetic acid for 30 min. Median MBEC was found to be as low as 0.75% (range: 0.19–1.5). Taken together, acetic acid was very potent against Pae-MBL, both for bacteria in planktonic phase and in biofilm.

Follow-up

In July 2015, a 63-year-old woman with haematological malignancy and complicated pneumonia appeared positive for Pae-MBL in sputum (patient 13). Prior to diagnosis, the patient had been admitted to ward 2 in a room with repeatedly positive Pae-MBL cultures from the wall. During her stay at the ward, the acetic acid routine had been omitted for four weeks due to staff changes and lack of communication. Acetic acid MIC of the patient strain was the same (0.047%) as previously tested isolates, indicating no emerging resistance to acetic acid. Acetic acid treatment had restarted at the time of diagnosis and screening of all sinks was negative.

Discussion

In the present study, we describe a prolonged nosocomial outbreak of Pae-MBL where sink drains served as a long-term reservoir. To prevent transmission, we used a simple method to decontaminate colonized sinks with acetic acid treatment.

Sink drains carry a massive bacterial burden and have been described as the reservoir for nosocomial transmission of bacteria.[6,9,16] In a majority of previously described outbreaks, focus has been on healthcare workers in intensive care units

		2013						2014													2015											2016
ward	**Room**	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
ward 1				★★							★					★					✪	🡻			✪				✪			✪
	1			XX	✔	✔✔	✔	✔✔		✔	✔	change sink				✔☑					✔				✔				✔			✔
	4			XX	X	✔✔	✔	✔✔		✔	✔	change sink				X ☑	✔	✔	✔■ HW,CP	✔✔☑	✔✔☑				✔				✔			✔
	6			XX	✔	✔✔	✔	✔✔		✔	✔	change sink				✔☑					X				✔				✔			✔
	24			X✔	✔	✔✔	✔	✔✔		✔	✔	change sink				✔☑					✔				✔				✔			✔
	20										X	change sink				✔■	✔	✔	✔■HW,CP	✔✔■	✔✔■				✔				✔			✔
	19											change sink				X ☑	X	✔	✔☑HW,CP	✔✔☑	✔✔☑				✔				✔			✔
ward 2		★		★		★		★			★		★		✪	★	✪	✪	★	✪	✪	★	✪	★			★		★	✪	✪	★
	1	X change sink ✔✔	✔			✔	ward moved																🡻									
	12										XCP✔✔		✔		✔	✔	✔☑	✔	✔	✔	✔	✔	✔	✔	*		✔		✔	✔	✔	✔
	15												XCP✔✔		XCP✔	✔ ■	✔■	✔	✔	✔	✔	✔	✔	✔	*	**	✔		✔	✔	✔	✔
	9														XCP✔	✔ ■	✔☑	✔	✔	✔	✔	✔	✔	✔	*		✔		✔	✔	✔	✔
	3																					XCP	✔✔	✔	*		✔		✔	✔	✔	✔
ward 3												★																				
	10						X change sink ✔✔					✔																					

★ culture of every sink in the entire ward
✪ culture of all patient bathroom sinks
acetic acid once a week
HW hot water flushing
CP change part, sink drain and drain pipe to the wall
🡻 all patient bathroom sinks treated with acetic acid
✔ negative Pae-MBL culture form sink drain
X positive Pae-MBL culture form sink drain
☑ negative Pae-MBL culture from drain pipe in the wall
■ positive Pae-MBL culture from drain pipe in the wall
* missed acetic acid 4 weeks
** patient 13 Pae-MBL positive

Figure 2. Environmental screening for metallo-β-lactamase-producing *Pseudomonas aeruginosa*.

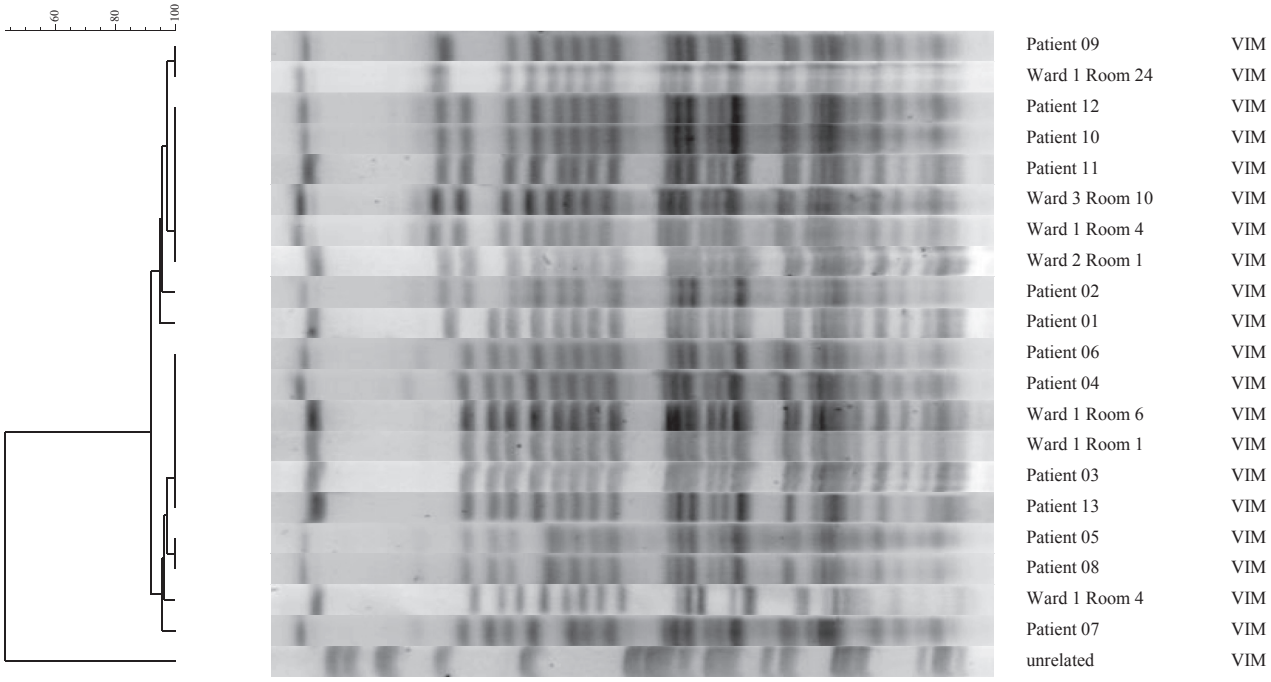

Figure 3. Dendrogram of metallo-β-lactamase-producing *Pseudomonas aeruginosa* (Pae-MBL) isolates. Pulsed-field gel electrophoretic (PFGE) typing showed that all tested isolates from the outbreak (12 clinical and seven sink strains) belonged to the same clade (designated PA-02). As a control, seven unrelated isolates were tested in parallel. Patterns were assigned the same PFGE type if the similarity was ≥90%.

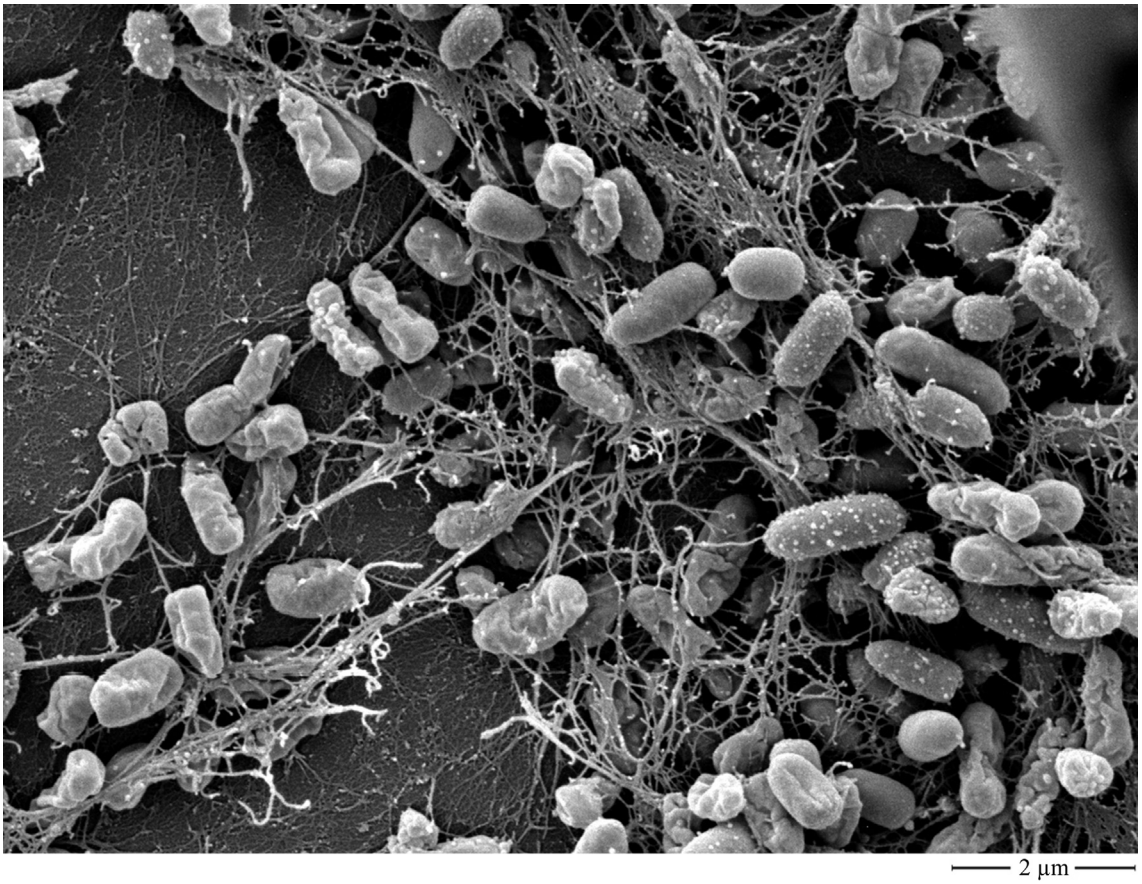

Figure 4. Metallo-β-lactamase-producing *Pseudomonas aeruginosa* (Pae-MBL) biofilm visualized by electron microscopy. The bacteria are embedded in biofilm, visualized as a three-dimensional array of sheet-like structures and fibres.

where patients do not primarily use the sink themselves. In this case, we found Pae-MBL only in sinks used by patients alone. All sinks were constructed so that water directly hit the mesh covering the drain (Figure 1). It has been suggested that this design enhances bacterial transmission as it causes splashing.[4]

Our investigation shows that Pae-MBL not only resides in the sink drain, but also finds a reservoir further down in the pipes in the wall. This explains why the bacterium could reappear despite a complete change of sinks. No Pae-MBL-positive patient was admitted to these rooms after sink replacement, which makes new inoculation unlikely. Acetic acid did not completely decontaminate Pae-MBL growth in the wall. However, weekly treatment of the sink drain, where transmission presumably occurs through splashing, will push back and reduce the bacterial burden significantly. We speculate that the new Pae-MBL case (patient 13) was due to lack of adherence to the acetic acid routine, enabling the bacterium to re-emerge from the colonized pipe in the wall.

The reason why new colonized sinks appeared in wards 1 and 2 is not fully understood. Some rooms, i.e. rooms 19 and 20 in ward 1, share the same drainage and this might explain the late positivity in room 19. However, no Pae-MBL was found in the staff sink for hand hygiene in positive rooms despite sharing drainage systems. Neither did we see retrograde spreading to sinks in wards above or underneath, even though drained through the same main wastepipes. Although an extensive patient screening was negative, we may have missed an unrecognized patient. The frequent exchange of patients between wards 1 and 2 might have contributed to this. It is also possible that we sometimes have false-negative cultures, as we have not excluded the possibility of remaining viable but not culturable bacteria in the drains.

Bacterial biofilms are often difficult to eradicate, but our data show that acetic acid has an exceptional effect against pseudomonas biofilm *in vitro*. Although the in-vitro biofilm may be different from the long-standing biofilm in water systems, our results are supported by a recent publication by Bjarnsholt *et al.*[17] Here they show that a mature pseudomonas biofilm grown for three days in a continuous-flow system may be completely eradicated with acetic acid at a concentration as low as 0.5%.

A shortcoming of the study is the reinforcement of 'sink rules' that may have affected the outcome. However, the fact that colonized sinks have been culture-negative during acetic acid treatment for an observation time of up to 21 months suggests that acetic acid was important for terminating the outbreak. The smell of acetic acid could be a potential limitation but no comments or objections have been made from patients or hospital staff. The present study again emphasizes the importance of proper handling and construction of sinks. Installation of sinks constructed to prevent bacterial transmission would be our preferred response but in a suboptimal setting acetic acid is proposed as a simple and inexpensive method to prevent transmission of pseudomonas while awaiting better long-term solutions.

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Conflict of interest statement
None declared.

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