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Nosocomial outbreak of VIM-2 metallo-β-lactamase-producing *Pseudomonas aeruginosa* associated with retrograde urography

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Abstract

Pseudomonas aeruginosa is well adapted to the hospital setting and can cause a wide array of nosocomial infections that occasionally culminate in recalcitrant outbreaks. In the present study, we describe the first nosocomial outbreak of infection caused by bla_{VIM-2}-positive *P. aeruginosa* in Germany. In November and December 2007, highly resistant *P. aeruginosa* isolates were recovered from the urine of 11 patients in the Department of Urology of a University Hospital. Bacterial isolates were typed by multilocus sequence typing and screened for known metallo-β-lactamase (MBL) genes by PCR. Environmental sources of transmission were tested for bacterial contamination using surveillance cultures. Furthermore, a matched case–control study was performed in search of medical procedures significantly associated with case status. Typing of recovered isolates confirmed VIM-2 MBL-producing *P. aeruginosa* of sequence type 175 in all cases. Surveillance cultures did not lead to the identification of an environmental source of the outbreak strain. Case–control analysis revealed retrograde urography as the only exposure significantly associated with case status. The analyses suggest the transmission of a single clone of VIM-2 MBL-producing *P. aeruginosa* leading to the infection of 11 patients within 47 days. Events in temporal proximity to retrograde urographies appear to have facilitated infection in the majority of cases. Department-specific infection control measures, including reinforced hygiene procedures during retrograde urography, quickly terminated the outbreak.

Keywords: Bacterial typing, beta-lactam resistance, infectious disease outbreak, nosocomial infections, *Pseudomonas* infections, urography
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Introduction

Pseudomonas aeruginosa is considered as a classical nosocomial pathogen that is able to prevail in the hospital setting as a result of its resistance against many antibiotics and disinfectants [1]. It can cause a wide array of hospital-associated diseases, including respiratory tract infections, urinary tract infections (UTI), wound infections and bacteraemia [2]. In addition to its intrinsic resistance against anti-infectives, *P. aeruginosa* readily develops resistance against different antibiotic classes by mutation or acquisition of exogenous resistance determinants [3].

The plethora of resistance mechanisms exploited by *P. aeruginosa* include the production of a wide variety of β-lactamases (AmpC cephalosporinases, OXA β-lactamases, etc.), outer membrane protein changes (e.g. OprD loss), production of aminoglycoside-modifying enzymes, mutations in genes encoding topoisomerases (e.g. *gyrA*) and overproduction of efflux pumps (e.g. MexAB-OprM) [1]. As for the β-lactamases, carbapenemase production has caused considerable concern in recent years because it confers resistance to virtually all β-lactam antibiotics, leading to a critical narrowing of therapeutic options. Carbapenem-hydrolyzing enzymes are grouped into several molecular classes. They utilize either serine or zinc at their active sites and are inhibited by clavulanic acid or chelators such as EDTA, respectively [4]. Metallo-β-lactamases (MBLs), as the zinc-dependent enzymes are called, are the most common carbapenemases in *P. aeruginosa* and include the variants of IMP, VIM, SPM, GIM and SIM [4,5]. Although SPM, GIM and SIM MBLs have not been detected beyond their countries of origin, VIM and IMP continue to be detected worldwide [4]. Specifically, VIM-2, first described in France in 1996 [6], has gained particular notoriety as the most-reported MBL worldwide [5].

Horizontal spread of MBL-encoding genes (*bla* genes), as demonstrated by recent inter-species dissemination of bla_{VIM-2} [7], is facilitated through their location on plasmids or transposons as part of gene cassettes within class 1 integrons [8]. At these sites, *bla* genes are frequently associated with genes that confer resistance to aminoglycosides [9,10].

Unfortunately, the accumulation of multiple resistance mechanisms leading to the emergence of virtually pan-resistant isolates has become a reality in many medical centres [1], emphasizing the particular importance of preventive and control measures.

Portable typing methods with sufficient discriminatory power are important for the assessment and reporting of outbreaks and additionally facilitate interlaboratory comparison. Multilocus sequence typing (MLST), initially designed for reproducible and portable typing of *Neisseria meningitidis* [11], was shown to afford a high level of discrimination among *P. aeruginosa* in the hospital setting, yet was expectedly less discriminatory than the poorly portable reference standard pulsed-field gel electrophoresis [12].

The present study describes the evolution and successful management of a recent nosocomial outbreak affecting the Department of Urology in a German university hospital and reveals a significant association of cases with examinations involving the retrograde instillation of contrast dye into the urinary tract (i.e. retrograde cystourethrography and pouchography). Although MBL-producing *P. aeruginosa* isolates have been sporadically reported in Germany [13–15], to our knowledge, outbreaks have not yet been described.

Materials and Methods

Bacterial strains and cases

Bacterial isolates were recovered from the urine of patients at the University Hospital of Wuerzburg in November and December 2007. Urinary leukocytes were quantified by a dipstick method (Combur 2 Test LN; Roche Diagnostics, Mannheim, Germany). In total, 11 representative isolates grown from urine samples from 11 patients were analysed at the Institute for Hygiene and Microbiology. Ten of 11 cases were inpatients at the Department of Urology, whereas one patient (case 7; Table 1) was an inpatient at the Medical Department, but looked after by physicians of the Department of Urology. The age of cases (eight males, three females) was in the range 26–75 years (Table 1).

Species identification was accomplished using Vitek 2 GN cards (bioMérieux, Nürtingen, Germany).

TABLE 1. List of case patients in chronological order of detection and origin of *Pseudomonas aeruginosa isolates*

Case number	Sex	Age	Isolated from (number of isolates)
1	M	56	Urine (7)
2	F	75	Urine (5)
3	M	64	Urine (3)
4	M	62	Urine (2)
5	M	65	Urine (3), skin (1), wound (1)
6	F	67	Urine (1)
7	M	25	Urine (1)
8	M	66	Urine (2)
9	M	72	Urine (4)
10	F	71	Urine (1)
11	M	54	Urine (1)

M, male; F, female.

Susceptibility testing

All strains underwent susceptibility testing using the Vitek 2 AST-N021 panel (bioMérieux). Additional antimicrobials (amikacin, colistin and aztreonam) were tested by disk diffusion on Mueller–Hinton agar. The MIC for colistin was determined with the Etest (AB Biodisk, Solna, Sweden) on Mueller–Hinton agar. The zone diameter and MIC interpretive standards for breakpoints for *P. aeruginosa* were those of the CLSI [16].

Detection of metallo-β-lactamases and typing

After boiling of bacterial suspensions, primers IMP-A, IMP-B, VIM2004A, VIM2004B, SPM-1A and SPM-1B were used for amplification of IMP-, VIM- and SPM-type MLB genes [17], whereas the detection of GIM-type MBLs was attempted with primers GIMF and GIMR [13]. Strains AV65 [18] and TS832347 [19] served as positive controls for the amplification of MBL-genes belonging to IMP and VIM family, respectively. Products obtained with VIM primers were sequenced using amplification primers. VIM-types were identified using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). MLST was performed according to published protocols [20].

Surveillance cultures

For processing of tap water: 500 μL was plated on Columbia blood and MacConkey agar, whereas 1 mL was additionally mixed into 15 mL of thioglycolate broth for enrichment culture. Liquid samples (e.g. liquid soap) were diluted in double-concentrated thioglycolate broth if the total volume was <100 mL or otherwise filtered using sterile membranes (0.45 μm pore size; Millipore, Schwalbach, Germany) followed by transfer of filters onto brain–heart infusion agar. Gels (e.g. ultrasound contact gel) and other semi-solid substances were added to thioglycolate broth at a 1:9 ratio. Microbial surface-monitoring was conducted using 'Replicate

Organism Detection and Counting' plates containing nutrient agar supplemented with disinfectant-inactivating additives (Tween) where appropriate. All samples were incubated for at least 48 h at 37°C without CO_2.

Case–control study

In total, 22 control patients were matched to cases at a 2:1 ratio according to sex, age (by decade), time of admission, medical unit and the availability of urine culture results. Specifically, controls comprised those inpatients at the Urological Department in November and December 2007 who yielded a growth of bacteria other than the outbreak strain from urine samples. Conditional logistic regression analysis of matched case–control groups was performed with special consideration of the small data set according to the method reported by Heinze and Puhr [21]. ORs were estimated from univariable and bivariable models. p-values were corrected for multiple testing using the step-down Bonferroni method.

Results

Timeline of the outbreak

Within 47 days in November and December 2007, multiresistant *P. aeruginosa* was isolated from the urine of 11 inpatients under the care of the Department of Urology. Urine leukocytes of seven patients were quantified as '++' or '+++' [levels '0', '(+)', '+', '++', '+++' designating presence of none, scanty, scanty–moderate, moderate and abundant leukocytes, respectively]. In four of 11 patients (cases 2, 5, 6 and 11), leukocytes could not be quantified because only dip-slides but no native samples were received. In case 5, isolates were additionally obtained from a wound and a skin specimen (Table 1).

Susceptibility testing

All strains showed broad resistance against β-lactams except aztreonam. MICs of imipenem and ceftazidime were ≥16 and ≥64 mg/L, respectively, for all strains. MICs for meropenem and piperacillin were between 8 to ≥16 mg/L and 32 to ≥128 mg/L, respectively. Moreover, the outbreak strain displayed resistance against gentamicin (MIC ≥ 16 mg/L), tobramycin (≥16 mg/L) and ciprofloxacin (≥4 mg/L). Isolates were susceptible to colistin according to Etest (0.75 mg/L), and suceptible to aztreonam and amikacin, as determined by disk diffusion testing. Of note, the above resistance profile (imipenem MIC ≥ 16 mg/L and ceftazidime MIC ≥ 64 mg/L associated with *in vitro* susceptibility to aztreonam, amikacin and colistin) had not been observed previously in our hospital.

Molecular characterization and typing

PCR with primers VIM2004A and VIM2004B amplified a product of 380 bp in all outbreak isolates.

Amplification of IMP-, GIM- or SPM-type MBL genes were negative. Sequencing of the PCR products obtained confirmed the presence of bla_{VIM-2} in all isolates. All strains were of sequence type (ST)-175, as determined by MLST. The presence of VIM-2 represented an epidemiological novelty, and was not detected in bacterial isolates from the University Hospital before November 2007.

Surveillance cultures

In total, 56 contact plates, 37 water samples, four ultrasound contact gels and eight other liquid samples were analysed between 38 and 65 days after recognition of the first case. Contact plates were made from the floor, door handles, stretchers, ultrasound devices, a transrectal ultrasound transducer, X-ray aprons, water sinks, shower heads and surfaces within easy reach of patients (e.g. telephone handsets, bedside lockers) from the two outbreak wards, the urological intermediate care unit and two outpatient offices used for cystoscopy and retrograde urography. Water samples and contact gels were obtained from two urological wards, and the urological intermediate and intensive care units. Finally, other liquid samples comprised liquid soap from one urological ward, rinsing run-off from the biopsy channel of a transrectal probe, disinfection solutions, saline and two opened contrast dyes (Imeron) used for retrograde urography. Notably, the dyes were analysed in week 51 only (Fig. 1). All samples failed to yield multiresistant *P. aeruginosa*.

Matched case–control study

A case–control study was made to evaluate exposures based on data extractable from the hospital information system (HIS). These encompassed chest X-ray, abdominal X-ray, investigations involving retrograde instillation of contrast dye into the urinary tract (retrograde urography), micturition cysturethrogram, any surgery under general anesthesia, sonography, intravenous pyelography, radionuclide imaging of the kidney, abdominal computer-assisted tomography with contrast dye, cystoscopy and contrast dye enema. After identifiying retrograde urography as the strongest univariate predictor (OR 10; Table 2), we performed bivariable analyses including retrograde urography and each of the other variables in turn. Only retrograde urography was significant in each of these models. The strongest confounders of the effect of retrograde urography on case–control status were abdominal X-ray and surgery (Table 3). Nevertheless, retrograde urographies were performed only after the first cultural detection of the outbreak strain in two cases (cases 5 and 10; Fig. 1).

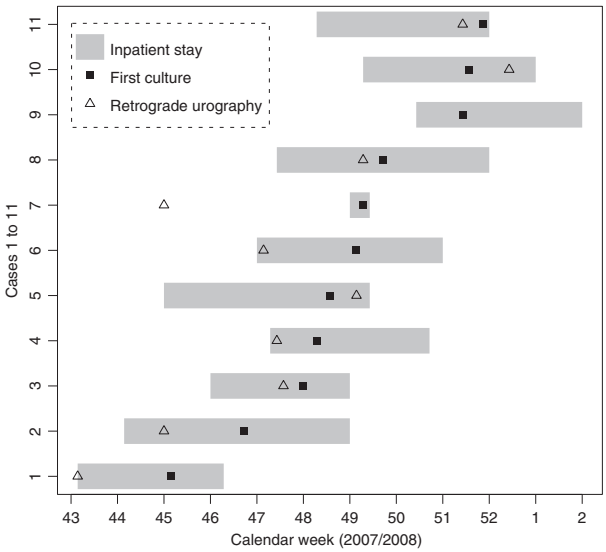

FIG. 1. Temporal succession of culture-based detection of VIM-2 metallo-β-lactamase-producing *P. aeruginosa* in case patients 1–11, tiled vertically. Horizontal grey bars represent the duration of admission. Full black squares and open triangles indicate timepoints of first cultural identification of the outbreak strain and retrograde urography, respectively.

Even though the crude odds ratio remained significant after reanalysis categorizing these cases as 'not exposed' (OR 8.34, 95% CI 1.37–68.85), the reversed temporal sequence in two cases suggests that events in temporal proximity to retrograde investigations are the true sources of transmission.

Implementation of control measures

Specific control measures as listed in Table 4 were proposed at week 50 (Fig. 1) in addition to general measures, including the isolation of cases and strict adherence to hand hygiene guidelines. Actions regarding the performance of retrograde urography (Table 4) were introduced only after conducting a tentative case–control study after case 11. Thereafter, no further transmission related to the outbreak occurred. Despite heightened surveillance, only two additional sporadic MBL isolates were recovered from the previously concerned wards in the 18 months after the outbreak.

Discussion

The present study describes the detection, assessment and successful termination of an outbreak of UTI caused by VIM-2-producing *P. aeruginosa* belonging to ST-175. Given that MBL-producing bacteria are known to cause recalcitrant hospital epidemics lasting months [18] or years [9], the temporally confined emergence of the outbreak strain over <2 months likely reflects the effectiveness of the implemented control measures (Table 4). Successful control was greatly facilitated by an interdisciplinary approach involving medical microbiologists, the infection control team and ward staff.

The isolation of isolates with a unique resistance profile, sequence type (ST-175) and *bla* gene (i.e. *bla* $_{\text{VIM-2}}$) strongly supports the transmission of a single clone between cases. ST-175 has been associated with multidrug-resistance (but not MBL production) in clinical isolates from Hungary [22]. Although sudden increases in bacterial detection rates in one type of specimen can represent pseudo-outbreaks resulting from contaminated equipment used for collection, several points suggest that the described accumulation of cases represents an outbreak of true infections. First, pyuria was confirmed in all cases where native samples were sent for culture. Second, the outbreak strain continued to be

TABLE 2. Univariate OR estimates

Variable	OR	Lower 95% CI	Upper 95% CI	Corrected p-value
retr	10.04	2.26	94.50	0.0156
op	13.33	1.42	1777.44	NS
axr	3.42	0.61	35.47	NS.
ctabd	3.67	0.56	39.06	NS
cxr	2.90	0.57	29.35	NS
cyst	2.68	0.38	29.79	NS
sono	1.53	0.43	5.90	NS
age	0.78	0.22	1.59	NS
rnucl	2.00	0.16	24.62	NS
enema	1.29	0.27	5.47	NS
ivp	1.08	0.26	3.79	NS
mcu	1.00	0.14	7.32	NS

Variables investigated: retr, retrograde urography; op, any surgery involving general anaesthesia; axr, abdominal X-ray; ctabd, computer tomography of the abdomen with contrast dye; cxr, chest X-ray; cyst, cystoscopy; sono, any sonography; age, age in decades; rnucl, radionuclide imaging of the kidney; enema, contrast dye enema; ivp, intravenous pyelography; mcu, micturition cysturography. NS, not significant.

TABLE 3. OR estimates from a bivariable model including 'retr' and adjusting for each of the other variables in turn

Variable	Adjusted by	OR	Lower 95% CI	Upper 95% CI	Corrected p-value
retr	age	9.48	2.06	99.24	0.0161
retr	cxr	8.53	1.86	81.36	0.0161
retr	mcu	7.69	1.96	65.35	0.0161
retr	axr	19.00	2.50	2455.89	0.0120
retr	op	6.01	1.28	55.51	0.0214
retr	sono	14.77	2.73	202.69	0.0066
retr	ivp	8.63	2.09	73.57	0.0144
retr	rnucl	7.41	1.92	48.71	0.0161
retr	ctabd	8.37	1.94	72.90	0.0161
retr	enema	11.01	2.36	109.65	0.0120
retr	cyst	7.46	1.86	62.14	0.0161

retr, retrograde urography; op, any surgery involving general anaesthesia; axr, abdominal X-ray; ctabd, computer tomography of the abdomen with contrast dye; cxr, chest X-ray; cyst, cystoscopy; sono, any sonography; age, age in decades; rnucl, radionuclide imaging of the kidney; enema, contrast dye enema; ivp, intravenous pyelography; mcu, micturition cysturography.

detected in several case patients (but not in other patients of the same department) in follow-up investigations after discharge (data not shown).

We were unable to determine an environmental source of the outbreak by surveillance cultures of samples from hospital wards (e.g. from tap water, water sinks, shower heads, liquid soap, hand disinfection solution), clinical appliances (e.g. ultrasound devices, contact gels) and operating room equipment (e.g. saline for irrigation, contrast dyes for retrograde urography, X-ray aprons). Several studies reported the confirmation by culture of the sources of infection with *P. aeruginosa*, which included a contaminated Bigelow's evacuator used for litholapaxies [23], an inadequately processed cystoscope [24] and a needle guide used for transrectal, ultrasound-guided prostate biopsies [25]. Cystoscopes were not investigated in the present study because hospital guidelines demand automated processing in cleaning and disinfection devices followed by steam and plasma sterilization for rigid and flexible variants, respectively. The are several possible reasons for failure to locate the source of infection by means of surveillance cultures: (i) surveillance cultures were started too late in the course of the outbreak; (ii) dates and locations of sampling had been arranged beforehand with hospital staff; and (iii) contamination might have been temporary and/or light, possibly affecting few disposable items such as irrigation saline or contrast dyes in an early phase of the outbreak. Furthermore, transient colonization of hands, as exemplified in a Belgian outbreak of a panresistant *P. aeruginosa* [26], was not screened for in the present study.

A case–control study was performed in search of exposures significantly associated with case status. Because previous studies have implicated medical procedures in transmission [23–25], we evaluated several investigations extractable from the HIS. Although urinary catheterization represents a recognized mode of infection, it was not included in the analysis, because it cannot be expected to be discriminatory in urological wards, and it is not recorded in the HIS. Analysis revealed only retrograde urographies (retrograde cystourethrographies and pouchographies) to be independently associated with case status. Although this implies transmission of MBL-producing *P. aeruginosa* during retrograde urographies, several arguments put into question a hard and fast causal relationship: (i) only ten of 11 cases underwent this procedure and (ii) two cases had retrograde urographies only after the first positive culture of the outbreak strain (Fig. 1). Although neither point renders the above association insignificant, it demonstrates our inability to sharply delineate a single procedure or investigation responsible for the spread of the outbreak strain. Presumably, the majority of transmission events were directly caused by the procedure or occurred in its temporal proximity. Of note, the substantial temporal overlap of the admission periods of cases (Fig. 1) most likely allowed for further indirect transmissions independent of retrograde urographies. Nevertheless, the termination of the outbreak only after the introduction of control measures specifically targeting retrograde urography provides further support for the assumption that the identified risk factor was responsible for at least a fraction of the transmissions.

TABLE 4. List of specific measures introduced in response to the outbreak

List of department-specific control measures
Hand disinfection prior to tapping bottles with sterile content (contrast dyes, i.v. fluids)
Procurement of additional one-way aprons for isolation rooms to prevent shortage
Standing time of saline used for deblocking of urinary catheters limited to 24 h at room temperature
Mandatory wearing of sterile gloves and fresh surgical gowns when performing retrograde urography
Change of wound dressing to be carried out by surgical officer accompanied by nursing staff
Distribution of the list of case patients to affected wards to facilitate isolation upon readmission

The present report has several limitations: (i) an environmental source of infection could not be determined by culture-based methods and (ii) the low number of cases makes statistical inference difficult (e.g. by obviating the application of multivariate analysis). Furthermore, outbreaks associated with retrograde urographies have not been described, although a small risk, specifically of UTI with *P. aeruginosa*, was suggested in paediatric patients after voiding cystourethrography [27].

In conclusion, the present study provides the first description of a clonal outbreak of MBL-producing *P. aeruginosa* in Germany and highlights the importance of epidemiologic assessment in addition to microbiologic investigations for the formulation of department-specific control measures aiming to limit unwanted nosocomial transmission.

Acknowledgements

JE and UV designed the study and performed molecular strain typing. JE wrote the manuscript and carried out phenotypic analyses. UV devised and implemented infection control measures and lead culture surveillance efforts. CS and GV validated and employed molecular methods for confirmation of metallo-β-lactamase genes. GH performed statistical analyses. EH and HR devised and implemented infection control measures and contributed to the selection of risk factors.

Transparency Declaration

Data were generated as part of routine activities without particular funding at the Institute for Hygiene and Microbiology and the Department of Urology. The authors (JE, CS, GH, GV, EG, HR, UV) do not have a commercial or other association that might pose a conflict of interest.

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