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First outbreak of VIM-2 metallo- β -lactamase-producing *Pseudomonas aeruginosa* in The Netherlands: microbiology, epidemiology and clinical outcomes

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ABSTRACT

This study was designed to investigate the prevalence and characteristics of metallo- β -lactamase (MBL)-producing *Pseudomonas aeruginosa* in a tertiary care centre in The Netherlands, a country that is considered to have a low prevalence of antibiotic-resistant bacteria. Imipenem-resistant *P. aeruginosa* isolates cultured from clinical specimens during 2008–2009 were analysed phenotypically and molecularly by polymerase chain reaction (PCR) with sequencing. Genotyping was performed by multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA). Clinical information was obtained by electronic chart review for all patients infected or colonised with an imipenem-resistant *P. aeruginosa* isolate that was included in the study. In total, 106 imipenem-resistant *P. aeruginosa* isolates were included. The *bla*_{VIM-2} gene was detected in 35/106 isolates (33%) and was associated with integrons. Compared with non-MBL-producing imipenem-resistant *P. aeruginosa*, VIM-2 MBL-producing isolates showed higher rates of multidrug resistance. Patients with VIM-2 MBL-producing isolates were more likely to be admitted to the Intensive Care Unit (ICU) and had a higher risk of invasive infection, including development of bacteraemia. MLVA identified two separate VIM-2 MBL-producing clones, responsible for outbreaks in the ICU but also affecting 10 other departments. This is the first reported outbreak of VIM-2 MBL-producing *P. aeruginosa* in The Netherlands. Once introduced, VIM-2 MBL-producing *P. aeruginosa* cause significant infections and are easily spread within the hospital setting.

1. Introduction

In The Netherlands, identification of metallo- β -lactamases (MBLs) among *Pseudomonas aeruginosa* has been extremely rare. A recent study at a large centre in The Netherlands that screened 650 isolates for MBL genes found only 2 isolates that produced MBLs [1]. Worldwide, however, there has been a substantial increase in the reporting of MBLs among *P. aeruginosa* isolates since the 1990s [2].

MBLs are capable of hydrolysing most of the β -lactam antibiotics, including carbapenems, and are inhibited by metal chelators such as ethylene diamine tetra-acetic acid (EDTA). MBL-encoding genes are frequently located on integrons, which are associated with mobile elements and often carry genes encoding for additional resistance determinants. As a consequence, MBL-producing *P. aeruginosa* isolates are often resistant to different groups of antimicrobial agents and this resistance can be transmitted to other *P. aeruginosa* strains or to unrelated types of bacteria. In addition, MBL-producing *P. aeruginosa* cause significant infections that are associated with higher case fatality rates compared with infections caused by carbapenem-resistant *P. aeruginosa* that do not produce MBLs [3,4].

Until now, six types of MBL genes have been described among *P. aeruginosa* (IMP, VIM, SPM, SIM, GIM and AIM). Although the last four MBL genes have rarely been detected beyond their region of initial detection, i.e. the spread of SPM types is limited to Brazil, whilst GIM, SIM and AIM types are rare and restricted to Germany, Korea and Australia, respectively [2,5,6], VIM and IMP genes show a global distribution and continue to be detected at increasing rates worldwide [5]. In

particular, VIM-2 emerged as the predominant MBL variant among *P. aeruginosa*, and VIM-2 MBL-producing *P. aeruginosa* has been responsible for clonal spread and nosocomial outbreaks [2,7–11].

In 2008–2009, we noticed a high prevalence of imipenem-resistant *P. aeruginosa* isolates at the Erasmus University Medical Centre in Rotterdam (The Netherlands), a 1300-bed teaching hospital. A retrospective study was therefore undertaken to assess the molecular and clinical epidemiology of imipenem-resistant isolates at this hospital.

2. Material and methods

2.1. Bacterial isolates

Consecutive imipenem-resistant *P. aeruginosa* [imipenem minimum inhibitory concentration (MIC) >8 $\mu\text{g/mL}$ or disk zone diameter ≤ 13 mm] isolated from clinical specimens during the period January 2008 to November 2009 were selected for this study. Only one isolate per patient was included. Resistance to imipenem was confirmed by Etest (bioMérieux, Marcy-l'Étoile, France) for all isolates. Isolates with an imipenem MIC ≤ 8 $\mu\text{g/mL}$ were excluded.

2.2. Bacterial identification and antimicrobial susceptibility

Identification to species level and antibiotic susceptibility were determined using VITEK[®] 2 (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO). Identification of isolates from lower respiratory tract specimens of patients with cystic fibrosis (CF)

was performed by oxidase activity (DrySlide™ Oxidase; Becton Dickinson, Franklin Lakes, NJ) and resistance to C390 (DIATABS™; Rosco Diagnostica A/S, Taastrup, Denmark). Antibiotic susceptibility of these isolates was determined by disk diffusion. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria for broth dilution and disk diffusion [12].

2.3. Identification of metallo- β -lactamases

The presence of *bla*_{VIM} and *bla*_{IMP} genes was determined by polymerase chain reaction (PCR) for the simultaneous detection of *bla*_{VIM} and *bla*_{IMP} β -lactamase genes using primers and conditions as described previously [13]. Sequencing of the MBL genes was performed using the class 1 integron primers 5CS (5'-GGC ATC CAA GCA GCA AG-3') and 3CS (5'-AAG CAG ACT TGA CCT GA-3') in combination with VIM or IMP primers on a Thermal Cycler 9700 instrument (Applied Bio-systems, Norwalk, CT). Two different amplicons per isolate were sequenced in an overlapping fashion to ensure that the entire MBL allele was obtained [8]. Automated sequencing was performed on the PCR products using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Norwalk, CT) and sequence analysis software.

2.4. Multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA)

Isolates were cultured on trypticase soy agar II + 5% sheep blood plates (Becton Dickinson, Breda, The Netherlands) and were incubated overnight at 37 °C. Cell lysates were made from a few colonies as described previously [14]. Subsequently, using the MLVA9-Utrecht protocol, a touchdown PCR with 2.5 μ L of each lysate was

performed with primers for variable-number tandem-repeats (VNTRs) as described previously [14,15]. PCR products were separated on a 2% agarose gel by electrophoresis next to a 100 bp DNA ladder (Invitrogen, Breda, The Netherlands) and the number of repeats was deduced by applying the MLVA alleles assignment table for *P. aeruginosa* genotyping (<http://minisatellites.u-psud.fr/MLVAnet/>). Loci that repetitively did not yield a PCR product were assigned allele '99' in order to include these isolates in the genotype analysis.

2.5. Epidemiological and clinical data

Microbiological data (i.e. site of isolation and susceptibility to antimicrobial agents) were obtained for all imipenem-resistant isolates. The electronic medical records of all patients with an imipenem-resistant isolate were reviewed systematically and included the collection of demographic information (i.e. age, sex, outpatient visit or hospital admission and hospital department) and clinical information (i.e. infection or colonisation, development of bacteraemia with the imipenem-resistant isolate, underlying disease, antimicrobial treatment in the 14 days before specimen collection and 30-day mortality after specimen collection). Infection was defined as a positive sample together with clinical evidence of infection according to standard US Centers for Disease Control and Prevention (CDC) criteria [16]. Colonisation was defined as a positive sample without clinical evidence of infection. Patients were deemed to have an hospital-acquired isolate if the culture was performed >48 h after admission. Patients were deemed to have a community-acquired isolate if the culture was performed on an outpatient visit or if the culture was performed within the first 2 days of hospital admission; these patients were further classified as having either

community-acquired isolates or healthcare-associated community-acquired isolates [17].

2.6. Statistical analysis

Differences between MBL- and non-MBL-producing isolates as well as between patients with a MBL-producing isolate and patients without a MBL-producing isolate were analysed univariately using Student's *t*-test or Wilcoxon signed-rank test in the case of a non-normal distribution for continuous variables, and χ^2 tests with Fisher's exact test in the case of small numbers for non-continuous variables. Logistic regression analysis was performed to assess patient predictors for isolation of a MBL-producing *P. aeruginosa* isolate. A multivariate logistic regression model was constructed using backward selection to identify significant variables. Variable selection was based on the likelihood ratio test. A *P*-value of <0.05 was considered statistically significant. In addition, it was determined whether isolation of a MBL-producing *P. aeruginosa* isolate was a predictor of infection, development of bacteraemia or mortality by univariate and multivariate logistic regression analyses. In multivariate analysis, the following potentially confounding variables were corrected for: age; gender; hospital admission, including the number of days admitted; Intensive Care Unit (ICU) admission; primary diagnosis; type of antimicrobial treatment in the previous 14 days; and elevated C-reactive protein (CRP) and leukocytosis or leukopenia (i.e. CRP > 10 mg/L and leucocytes >10 × 10⁹/L or <4.5 × 10⁹/L). A confounding variable remained in the final model if its inclusion resulted in a >10% change in the effect size of the primary association of

interest. Additionally, the same analyses were performed restricted to patients without a diagnosis of CF.

3. Results

3.1. Bacterial isolates

During the study period, 5167 *P. aeruginosa* isolates were cultured from 1491 patients. A total of 161 patients (11%) were infected or colonised with an imipenem-resistant isolate; an isolate was retrospectively available for MBL testing from 111 (69%) of the 161 patients. Imipenem resistance was confirmed by Etest in 106 isolates: 74 (70%) were isolated from respiratory tract specimens, 6 (6%) from urine, 5 (5%) from blood, 8 (8%) from soft tissue or bone, 7 (7%) from intra-abdominal specimens and 6 (6%) from various other specimens. The majority of isolates (69%) were hospital-acquired, 23% were acquired in the community but were healthcare-associated, and 9% of the isolates were community-acquired.

Of the 106 isolates confirmed by Etest, 35 (33%) were positive for *bla*_{VIM}. None of the isolates were positive for *bla*_{IMP}. VIM MBL-producing isolates were more often isolated than non-MBL-producing isolates from urine (14% vs. 1%), blood (11% vs. 1%) and intra-abdominal specimens (20% vs. 0%), whilst non-MBL producing isolates were more often isolated from the respiratory tract (78% vs. 22%) and soft tissue or bone specimens (11% vs. 0%). Almost all of the MBL-producing isolates were hospital-acquired (32/35; 91%).

3.2. Antimicrobial susceptibility

Results of antimicrobial susceptibility testing are shown in Table 1. Except for susceptibility to aztreonam, MBL-producing isolates were more resistant to the antibiotics tested than non-MBL-producing isolates. Colistin resistance was detected in one MBL-producing isolate and one non-MBL-producing isolate. Resistance to colistin was confirmed by Etest in both isolates.

3.3. Multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA)

Among the MBL-producing isolates, MLVA identified one large cluster of 24 patients (MLVA 1), a cluster of 7 patients (MLVA 2), a cluster of 3 patients (MLVA 3) and one patient with a non-related isolate (Fig. 1A). Twelve (50%) of the isolates belonging to MLVA 1 and six (86%) of the seven isolates belonging to MLVA 2 were isolated from patients admitted to one of the two ICUs. The patient with the unrelated isolate and one patient belonging to MLVA 3 were also admitted to the ICU at the time of isolation (Fig. 1B). Most of the other patients with VIM MBL-producing isolates were admitted to various internal medical or surgical wards.

3.4. Identification of metallo- β -lactamases and the integron

PCR with integron primers 5C and 3C and the different combinations of VIM primers of random selected isolates among all three distinct MLVA genotypes ($n = 12$) amplified various amplicons ranging from 800 bp to 1.2 kb. Sequence analysis of the products revealed 100% identity with the sequence of the *bla*_{VIM-2} allele [18]. The following gene cassettes were detected in all 12 VIM-2 MBL-producing *P.*

aeruginosa: *aacA29a*–*bla*_{VIM-2}–*aacA29b* in the same order as previously described [19].

3.5. Patient characteristics

The majority of patients were male (62%) and their median age was 43 years [interquartile range (IQR) 21–64 years]; 77% of patients had already been admitted at the time of specimen collection and the median length of hospitalisation at that time was 12 days (IQR 0–37 days). A large proportion of patients (37%) was diagnosed with CF. Of the 106 patients with an imipenem-resistant *P. aeruginosa* isolate, only 31 (29%) had clinical evidence of an infection at the site of isolation. In three patients, the first imipenem-resistant *P. aeruginosa* was isolated from blood culture. Eleven other patients developed bacteraemia with the imipenem-resistant isolate during their admission. The overall mortality rate 30 days after specimen collection was 15% (Table 2).

Table 3 shows the risk factors for isolation of a VIM-2 MBL-producing *P. aeruginosa* isolate in the univariate analysis. In multivariate analysis, admission to the ICU [odds ratio (OR) = 2.6, 95% confidence interval (CI) 1.0–7.3], clinical evidence of infection at the isolation site (OR = 2.6, 95% CI 1.0–6.9) and antimicrobial therapy with fluoroquinolones in the 14 days before isolation (OR = 3.0, 95% CI 1.0–9.2) were borderline significantly associated with isolation of a VIM-2 MBL-producing isolate. Patients who were diagnosed with CF as an underlying disease were less likely to have a VIM-2 MBL-producing *P. aeruginosa* isolate than patients with a malignancy or cardiac, renal, neurological or gastrointestinal disease as an underlying disease

(OR = 0.1, 95% CI 0.1–0.6). However, two CF patients with a VIM-2 MBL-producing isolate were identified. One of the patients belonged to the large cluster MLVA 1. However, this patient had no history of previous ICU admission and had only been admitted for 5 days to the Pulmonary Department at the time of specimen collection. The other patient was a child transferred from another hospital with a VIM-2 MBL-producing isolate that belonged to the small cluster MLVA 3.

3.6. Clinical outcome

In the univariate logistic regression analysis, isolation of a VIM-2 MBL-producing *P. aeruginosa* was significantly associated with infection (OR = 4.7, 95% CI 1.9–11.6). However, statistical significance was not maintained in the multivariate analysis (OR = 2.5, 95% CI 0.9–6.8). There was a strong association between isolation of a VIM-2 MBL-producing isolate and development of bacteraemia in the univariate analysis (OR = 41, 95% CI 5–334). Multivariate analysis to assess the association between isolation of a VIM-2 MBL-producing isolate and development of bacteraemia was, however, not possible owing to the small numbers of patients with bacteraemia by a non-MBL-producing isolate. There was no significant association between isolation of a VIM-2 MBL-producing isolate and mortality after 30 days in univariate and multivariate analysis (OR = 2.3, 95% CI 0.8–6.9). Results were similar when analysis was restricted to non-CF patients only.

4. Discussion

This is the first outbreak of VIM-2 MBL-producing *P. aeruginosa* in The Netherlands, a country that is renowned for its low level of antimicrobial resistance [20]. In this study, a high prevalence of VIM-2 MBL-producing *P. aeruginosa* among imipenem-resistant isolates was found. The majority of the *P. aeruginosa* isolates belonged to a single cluster, suggesting patient-to-patient transmission. VIM-2 MBL-producing isolates were mostly identified in patients with clinical evidence of infection and with underlying diseases other than CF. However, two CF patients with an isolate that tested positive for VIM-2 by PCR and sequencing were identified, a finding that is extremely rare since carbapenem resistance among *P. aeruginosa* isolates in CF patients is usually caused by mutational changes in the regulation of AmpC β -lactamase, OprD porin and efflux pumps and not by acquisition of MBL genes [21]. A recent case report from Portugal in combination with our remarkable finding, however, suggest that the VIM-2 gene has now spread into the CF *P. aeruginosa* population [22].

Worldwide, VIM-2 is the dominant MBL gene associated with nosocomial outbreaks due to MBL-producing *P. aeruginosa* [2,7–11]. These outbreaks are usually restricted to specific patient groups and locations, such as the ICU and haematology wards where patients are often exposed to broad-spectrum antimicrobial agents and with an increased risk of cross-transmission of resistant bacteria between patients [23]. At our hospital, the outbreak was mainly among patients admitted to the ICU, but ten different hospital departments were also affected, and cluster MLVA 1 caused a small outbreak involving three patients admitted to a surgical ward in 2008. In addition, a

retrospective analysis of *P. aeruginosa* isolates cultured from blood specimens from 2000 to 2007 was performed and demonstrated that VIM-2 MBL-producing *P. aeruginosa* isolates have been present at our hospital since 2003. The eight retrospective isolates that tested positive for *bla*_{VIM-2} belonged to the major cluster MLVA 1 (data not shown) and were isolated from patients admitted to various departments.

All of the VIM-2 MBL-producing *P. aeruginosa* isolates harboured an integron structure that showed remarkable similarity to the integron-associated structure for VIM-2 MBL-producing *P. aeruginosa*, i.e. In59, previously described in France and Sweden [19,24]. Interestingly, the VIM-2 MBL-producing *P. aeruginosa* that was responsible for a nosocomial outbreak during 2003 and 2004 in Canada [8] showed an MLVA genotype similar to our major outbreak strain (data not shown). However, the *bla*_{VIM-2} from the Canadian strain was associated with a different type of integron, i.e. In100, that was first described in Portugal [25]. Previous reports from different areas in the world have shown that *bla*_{VIM} among *P. aeruginosa* strains are associated with various integron-associated structures in specific major clonal complexes [26–29]. This suggests that the emergence of VIM MBL-producing *P. aeruginosa* might occur independently in various parts of the world via the acquisition of different *bla*_{VIM}-containing integrons in isolates of similar clonal lineage in combination with clonal dissemination.

Limited studies have addressed the effect of a MBL gene on morbidity and mortality [3,4,30–32]. These studies showed higher infection and mortality rates among patients with a MBL-producing *P. aeruginosa* compared with patients with a non-

MBL-producing *P. aeruginosa*. The current results also suggest that isolation of VIM-2 MBL-producing isolates is associated with invasive infection, including bacteraemia. However, this study lacked power in multivariate analysis to reach statistical significance. To further evaluate the threat of MBL-producing *P. aeruginosa* and its potential to cause severe infections, well designed prospective epidemiological studies are urgently required. Given the limited therapeutic options, these studies should also include clinical predictors that are associated with improved survival.

This study shows that the global dissemination of VIM-2 MBL-producing *P. aeruginosa* has reached The Netherlands and that these isolates are an important cause of carbapenem resistance. Results from molecular typing illustrate that once VIM-2 MBL-producing clusters are introduced into a hospital setting they are easily spread within the hospital. In the absence of new agents for the treatment of infections caused by multiresistant Gram-negative bacteria, the spread of these multiresistant bacteria will lead to treatment failures with increased morbidity and mortality. Clinical microbiological laboratories must therefore be capable of distinguishing MBL-producing strains from those with other mechanisms responsible for carbapenem resistance in order to implement timely and adequate infection control practices.

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Competing interests

None declared.

Ethical approval

Not required.

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Fig. 1. (A) Multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) genotypes of VIM-2 metallo- β -lactamase (MBL)-producing *Pseudomonas aeruginosa* isolates from 35 patients at Erasmus University Medical Centre (Rotterdam, The Netherlands), 2008–2009, showing one large cluster of 24 patients, a smaller cluster of 7 patients, a cluster of 3 patients, and 1 patient with a strain unrelated to any of the three clusters. (B) MLVA genotypes of VIM-2 MBL-producing *P. aeruginosa* isolates from 35 patients at Erasmus University Medical Centre (Rotterdam, The Netherlands), 2008–2009. Patients admitted to the Intensive Care Unit (ICU) at the time of specimen collection or <48 h before specimen collection are shown by grey shading.

Table 1

Resistance rates according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12] for imipenem-resistant *Pseudomonas aeruginosa* isolated at Erasmus University Medical Centre (Rotterdam, The Netherlands) during 2008–2009

Antibiotic	No. (%) of resistant isolates			<i>P</i> -value
	All isolates (<i>N</i> = 106) ^a	Non-MBL-producer (<i>N</i> = 71)	MBL-producer (<i>N</i> = 35) ^b	
Ceftazidime	81 (76)	50 (70)	31 (89)	0.05
Ciprofloxacin	60 (57)	27 (38)	33 (94)	<0.001
Tobramycin	56 (53)	22 (31)	34 (97)	<0.001
Amikacin	55 (52)	29 (41)	26 (74)	0.001
Aztreonam	56 (53)	44 (62)	12 (34)	0.007
Colistin	2 (2)	1 (1) ^c	1 (3) ^c	1.0

MIC, minimum inhibitory concentration.

^a Imipenem MIC > 8 µg/mL.

^b All isolates produced *bla*_{VIM-2}.

^c Colistin MIC = 8 µg/mL.

Table 2

Characteristics of patients with an imipenem-resistant *Pseudomonas aeruginosa* isolated at Erasmus University Medical Centre (Rotterdam, The Netherlands) during 2008–2009

Characteristic	No. (%) of patients			P-value
	Total patients (N = 106)	Patients with non-MBL-producer (N = 71)	Patients with MBL-producer (N = 35) ^a	
Male sex [<i>n</i> (%)]	66 (62)	45 (63)	21 (60)	0.7
Age (years) [median (IQR)]	43 (21–64)	30 (19–63)	57 (38–66)	0.002
Hospital admission at time of culture [<i>n</i> (%)]	82 (77)	48 (68)	34 (97)	<0.001
Days admitted [median (IQR)]	12 (0–37)	7 (0–30)	25 (10–44)	0.002
Admission to ICU [<i>n</i> (%)] ^b	34 (32)	14 (20)	20 (57)	<0.001
Underlying disease [<i>n</i> (%)]				<0.001
Malignancy	16 (15)	7 (10)	9 (26)	
Pulmonary/cardiac disease	9 (8)	7 (10)	2 (6)	
Gastro-intestinal/renal disease	23 (22)	7 (10)	16 (46)	
Cystic fibrosis	39 (37)	37 (52)	2 (6)	
Other	19 (18)	13 (18)	6 (17)	
Site of infection [<i>n</i> (%)]				0.006
Lower respiratory tract	8 (8)	5 (7)	3 (9)	
Urinary tract	4 (4)	0 (0)	4 (11)	
Intra-abdominal	10 (9)	1 (1)	9 (26)	
Primary bloodstream	3 (3)	1 (1)	2 (6)	
Other infection	6 (6)	6 (8)	0 (0)	

No infection/colonisation	75 (71)	58 (82)	17 (49)	
Infection markers [<i>n</i> (%)] ^c	25 (24)	11 (15)	14 (40)	0.005
Bacteraemia [<i>n</i> (%)] ^d	14 (13)	1 (1)	13 (37)	<0.001
30-day mortality rate [<i>n</i> (%)]	16 (15)	8 (11)	8 (23)	0.12

IQR, interquartile range; ICU, Intensive Care Unit.

^a All isolates produced *bla*_{VIM-2}.

^b At time of specimen collection or <48 h before specimen collection.

^c C-reactive protein >10 mg/L and leukocytes >10 × 10⁹/L or <4.5 × 10⁹/L.

^d Presentation with or development of bacteraemia during admission with the imipenem-resistant isolate.

Table 3

Patient predictors for isolation of a metallo- β -lactamase (MBL)-producing

Pseudomonas aeruginosa in the univariate logistic regression analysis ^a

Predictor	OR (95% CI)	P-value
Age >50 years	2.8 (1.2–6.4)	0.017
Male sex	0.9 (0.4–2.0)	0.7
Hospital admission	16.3 (2.0–126)	0.008
>10 days hospitalised	3.5 (1.4–8.6)	0.006
Admission to ICU ^b	5.4 (2.2–13.2)	<0.001
Cystic fibrosis	0.1 (0.1–0.3)	<0.001
Infection at isolation site	6.0 (2.4–14.6)	<0.001
Antimicrobial therapy in the previous 14 days		
Penicillins/(clavulanic acid)	1.9 (0.7–5.1)	0.2
Piperacillin/tazobactam	1.9 (0.7–5.1)	0.2
2GC or 3GC with no activity against <i>P. aeruginosa</i>	5.3 (1.6–17.0)	0.005
Ceftazidime	0.8 (0.2–4.3)	0.8
Carbapenems	1.7 (0.7–4.0)	0.3
Fluoroquinolones	4.1 (1.5–11.3)	0.006
Aminoglycosides	0.4 (0.1–1.3)	0.1
Colistin	0.9 (0.3–3.1)	0.8
Other	3.5 (1.5–8.3)	0.004
Infection markers ^c	3.6 (1.4–9.2)	0.007

OR, odds ratio; CI, confidence interval; ICU, Intensive Care Unit; 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin.

^a All isolates produced *bla*_{VIM-2}

^b At time of specimen collection or < 48 hours before specimen collection.

^c C-reactive protein >10 mg/L and leukocytes >10 × 10⁹/L or <4.5 × 10⁹/L.

Figure 1A MLVA genotyping of VIM-2-producing *P. aeruginosa* isolates of 35 patients of the Erasmus University Medical Centre, Rotterdam, The Netherlands, 2008-2009, show one large cluster of 24 patients, a smaller cluster of 7 patients, a cluster of 3 patients and one patient with a strain unrelated to any of the 3 clusters.

Figure 1B MLVA genotypes of VIM-2-producing *P. aeruginosa* isolates of 35 patients, Erasmus University Medical Centre, Rotterdam, The Netherlands, 2008-2009. Patients admitted at the intensive care unit (ICU) at the time of specimen collection or ≤ 48 hours before specimen collection are shown by grey shading.