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Prolonged use of carbapenems and colistin predisposes to ventilator-associated pneumonia by pandrug-resistant *Pseudomonas aeruginosa*

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Abstract *Objective:* We present our experience with five cases of pandrug-resistant *Pseudomonas aeruginosa* ventilator-associated pneumonia (VAP) and analysis of risk factors. *Design and setting:* Case-control study in a 15-bed intensive care unit (ICU). *Patients and participants:* The study included 5 cases and 20 controls. Each case patient was matched to four contemporary controls according to gender, prior hospital admissions, hospitalization duration, ICU admission cause, Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Function Assessment (SOFA) scores on ICU admission, and length of ICU stay, and mechanical ventilation duration until first VAP episode by a multidrug-resistant bacterium. *Measurements and results:* Recorded variables included age, gender, daily APACHE II and SOFA scores, patient medication, treatment interventions, positive cultures and corresponding antibiograms, occurrence of infection, sepsis, and septic shock, other ICU-associated morbidity, length of ICU stay and mechanical ventilation, and patient outcome. Healthcare worker and environmental cultures, and a hand-disinfection survey were performed. Pandrug-resistant *P. aeruginosa* isolates belonged to the same genotype and were $bla_{\mathrm{VIM-1}}$-like gene positive. The outbreak resolved following reinforcement of infection-control measures (September 27). The sole independent predictor for pandrug-resistant *P. aeruginosa* VAP was combined use of carbapenem for more than 20 days and colistin use for and more than 13 days (odds ratio 76.0; 95% confidence interval 3.7–1487.6). An additional risk factor was more than 78 open suctioning procedures during 6–26 September (odds ratio 16.0; 95% confidence interval 1.4–185.4). *Conclusions:* Prolonged carbapenem-colistin use predisposes to VAP by pandrug-resistant *P. aeruginosa*. Cross-transmission may be facilitated by open suctioning.

Keywords Disease outbreaks · *Pseudomonas aeruginosa* · Carbapenems · β-Lactamases · Colistin

Introduction

Hospital-acquired infections due to multidrug-resistant Gram negative bacteria constitute a growing problem [1–3]. Colistin treatment is a useful option [1–3], but may promote emergence of pandrug-resistant bacteria [4]. In the 15-bed second medical-surgical intensive care unit (ICU) of Evaggelismos General Hospital we prospectively collect patient data to determine risk factors for infections by multidrug-resistant bacteria. This project has started after an outbreak of glycopeptide-resistant *Enterococcus faecium* [5]. During an 18-day period we identified five cases of ventilator-associated pneumonia (VAP) by pandrug-resistant *Pseudomonas aeruginosa*. Because

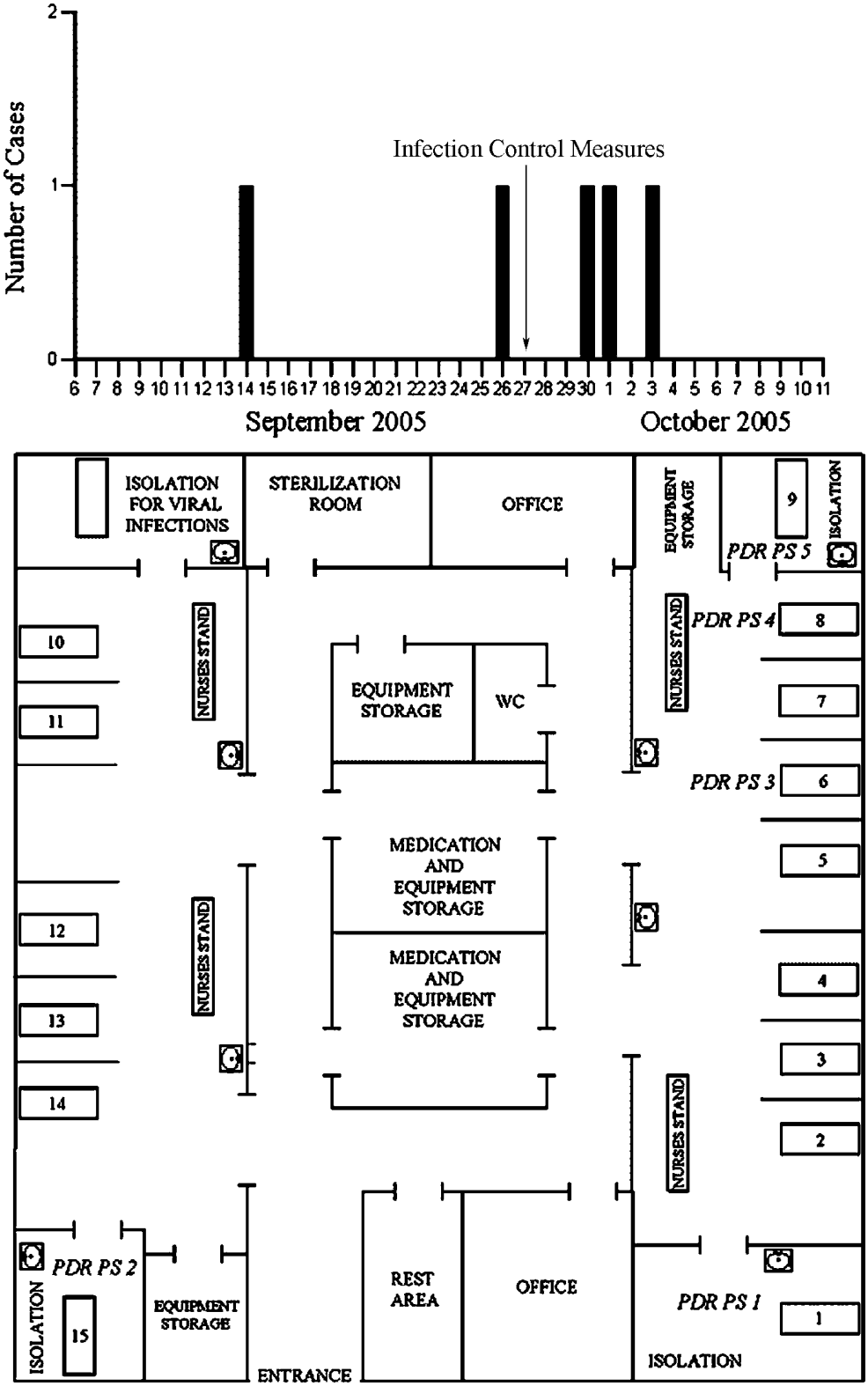

Fig. 1 *Above* Histogram for the pandrug-resistant *Pseudomonas aeruginosa* outbreak. Case patient no. 1 (index case) died on 22 September, and case patient no. 2 fulfilled the criteria for ventilator-associated pneumonia on 26 September. Thus an incubation period of at least 4 days was assumed. *Below* Schematic representation of the 15-bed intensive care unit. The beds of the case patients are indicated as pandrug-resistant (*PDR*) *P. aeruginosa* (*PS*) *1–5*

the clinical importance of preventing such infections is undisputed, we conducted a case-control comparison to identify relevant risk factors. Preliminary results of this study were presented at the 19th Annual Congress of the European Society of Intensive care Medicine.

Materials and methods

We obtained institutional approval for the study and informed consent from the patients' next of kin to use clinical and laboratory data from patient charts. Between 14 September and 3 October 2005 five patients developed VAP by pandrug-resistant *P. aeruginosa* (i.e., resistant to all antibiotics tested, including colistin; Fig. 1). Relevant molecular typing included extraction of genomic DNA, repetitive extragenic palindromic sequence-based polymerase chain reaction, and detection of $bla_{\mathrm{VIM-1}}$-like genes (detailed methods are presented in the Electronic Supplementary Material, ESM). Pandrug-resistant *P. aeruginosa* isolates belonged to the same genotype (clone; Fig. 2) and were positive for $bla_{\mathrm{VIM-1}}$-like genes (Fig. 3). On 14 and 26 September, patients 1 and 2, respectively, had already occupied two ICU isolation rooms for over 28 days. Patient 1 had persistent right hemithorax empyema despite decortication. Patient 2 was admitted to an isolation room for burns covering 18% of body surface area and acute lung injury secondary to smoke inhalation. Patients 3 and 4 suffered multiple trauma; they were transferred to isolation rooms on October 2 and 3, respectively. Patient 5 was admitted to an isolation room for severe hospital-acquired pneumonia and received renal replacement therapy for the first 10 days in the ICU. Attending physicians and nursing staff were the same for patients 3–5 and for 3 and 4, respectively. Between July and October there were 51 healthcare workers, including two physiotherapists. There was only one physiotherapist during the day and night shifts for all 15 ICU beds.

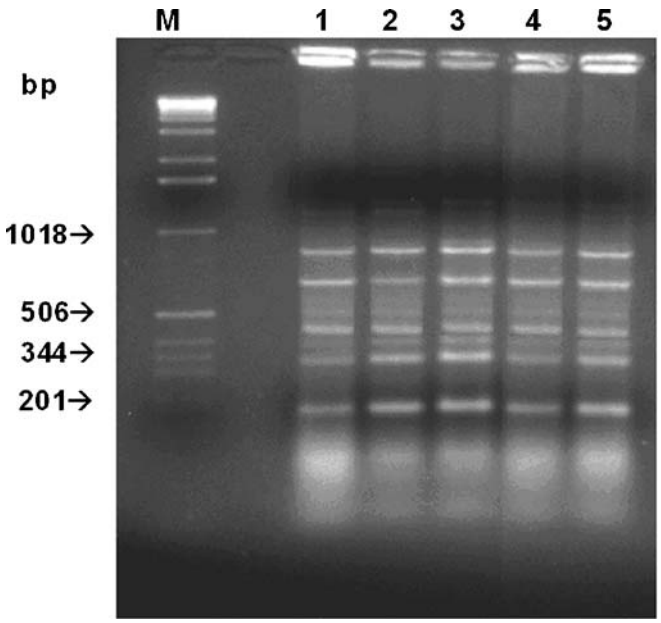

Fig. 2 Repetitive extragenic palindromic sequence-based polymerase chain reaction profiles of the five pandrug-resistant *P. aeruginosa* isolates. *M* 1-kb DNA ladder (Invitrogen)

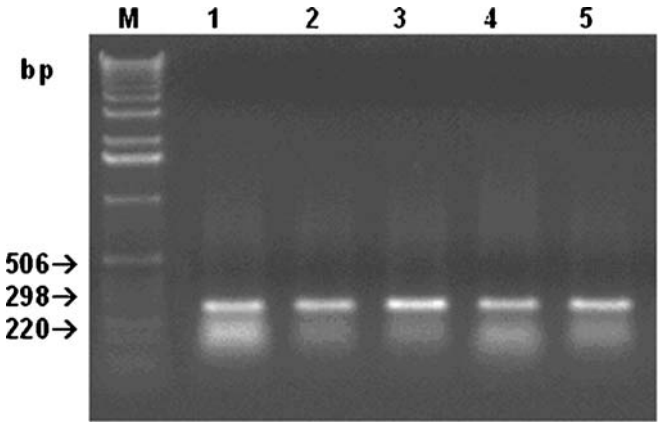

Fig. 3 Polymerase chain reaction for the detection of the $bla_{\mathrm{VIM-1}}$ gene. *Lanes 1–5* The five $bla_{\mathrm{VIM-1}}$ gene positive *P. aeruginosa* isolates (polymerase chain reaction product of 261 bp). *M* 1-kb DNA ladder (Invitrogen)

Each case patient was matched to four contemporary controls according to gender, number of prior hospital admissions, hospitalization-duration before ICU admission, ICU admission cause (medical or surgical), Acute Physiology and Chronic Health Evaluation (APACHE) II score on ICU admission, and Sequential Organ Dysfunction Assessment (SOFA) score on first ICU day. Controls' ICU length of stay was 10 days or longer, and they had at least one VAP episode (Table 1). Time from ICU admission and mechanical ventilation duration to first VAP episode were similar in cases and controls (Table 1). The first VAP episode was caused by a Gram-negative bacterium susceptible only to carbapenems and colistin in all case patients and 17 controls; such bacteria were defined as carbapenem sensitive. In the other three controls the first VAP episode was caused by a bacterium susceptible only to colistin (defined as carbapenem-resistant). These patients were already receiving carbapenems for bacteremia/septic shock. On initiating colistin administration carbapenems were not discontinued because of their in vitro synergistic activity with colistin against carbapenem-resistant bacteria [6] (see below). The same antibiotic policy was employed for all VAP episodes caused by cerbapenem-resistant bacteria [7]. Consequently all case patients and controls experienced VAP episodes treated with carbapenems with/without colistin, according to antibiograms and/or synergy studies. The use of other antibiotics did not differ significantly between case and control groups. Relative to controls the case patients had longer ICU stay, mechanical ventilation duration, and exposure to carbapenems and

Table 1 Patient characteristics of pandrug-resistant *P. aeruginosa* cases and controls; values are mean ± SD, or number (percentage), or median (interquartile range) (*PDR* pandrug-resistant, *APACHE* Acute Physiology and Chronic Health Evaluation, *SOFA* Sequential Organ Dysfunction Assessment, *IMV* invasive mechanical ventilation, *ALI* acute lung injury, *ARDS* acute respiratory distress syndrome, *RRT* renal replacement therapy, *P-RBC* packed red blood cells, *CS* carbapenem sensitive, *CR* carbapenem resistant, *VAP* ventilator-associated pneumonia, *CS* susceptibility only to carbapenems and colistin, *CR* susceptibility only to colistin)

Characteristic [a]	Cases ($n=5$)	Controls ($n=20$)	p
Factors used for case-control matching			
Gender: male	4 (80%)	13 (65%)	0.64
Previous hospitalization (range)	1.0 (1.0–1.0)	0.5 (0.0–1.0)	0.49
ICU admission cause			
Medical	2 (40%)	8 (40%)	1.00
Surgical	3 (60%)	12 (60%)	1.00
Open surgical wound	1 (20%)	3 (15%)	0.87
APACHE II score [b]	19.8 ± 5.8	21.7 ± 5.2	0.49
SOFA score [c]	7.2 ± 2.0	8.2 ± 2.1	0.50
Hospital stay pre-ICU (days)	8.8 ± 11.1	10.1 ± 12.7	0.47
Length of ICU stay until first VAP episode (days)	6.0 ± 3.0	6.2 ± 3.6	0.93
Duration of IMV until first VAP episode (days)	6.0 ± 3.0	6.1 ± 3.7	0.98
Potential risk factors			
Age (years)	53.4 ± 22.1	45.5 ± 20.8	0.50
Length of ICU stay (days)	53.6 ± 19.3	20.4 ± 10.7	0.02
Duration of IMV (days)	44.0 ± 19.8	16.3 ± 11.8	0.03
Chronic obstructive pulmonary disease	1 (20%)	2 (10%)	1.00
Diabetes	1 (20%)	1 (5%)	0.38
Poor glycemic gontrol	1 (20%)	2 (10%)	0.17
ALI, ARDS	4 (80%)	10 (50%)	0.34
Acute renal failure, RRT	2 (40%)	8 (40%)	1.00
Units of tranfused P-RBC/patient	2.2 ± 2.4	1.7 ± 1.6	0.70
Steroid use [d]	4 (80%)	10 (50%)	0.34
Central venous catheter use (patient-days)	43.6 ± 23.8	29.2 ± 16.6	0.26
Swan-Ganz catheter use (patient-days)	1.2 ± 2.7	0.6 ± 1.2	0.65
Sepsis, septic shock	4 (80%)	11 (55%)	0.62
Sepsis, septic shock due to CS bacteria	3 (60%)	6 (30%)	0.31
Sepsis, septic shock due to CR bacteria	2 (40%)	5 (25%)	0.60
Sepsis, septic shock due to to CS and CR bacteria	1 (20%)	3 (15%)	0.87
VAP due to CS bacteria	5 (100%)	17 (85%)	0.59
VAP due to CR bacteria	4 (80%)	10 (50%)	0.34
Episodes of VAP due to CS and CR bacteria	4 (80%)	6 (30%)	0.12
Drugs administered (patient-days)			
β-Lactam use (patient-days) [e]	3.8 ± 3.0	3.0 ± 3.0	0.58
Third-generation cephalosporin	1.0 (0.0–8.0)	0.0 (0.0–0.5)	0.22
Quinolone	0.0 (0.0–1.0)	3.0 (0.0–4.0)	0.78
Aminoglycoside [f]	3.8 ± 3.0	3.0 ± 3.0	0.58
Vancomycin	13.0 (0.0–23.0)	0.0 (0.0–6.25)	0.22
Antifungal	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.62
Carbapenem use	44.2 ± 26.0	14.7 ± 7.6	< 0.001
Colistin use	30.2 ± 23.5	4.8 ± 4.7	< 0.001
Open suction procedures			
6–26 Sept. 2005	100.0 ± 47.4	32.5 ± 34.2	< 0.001
27 Sept.–11 Oct. 2005	54.8 ± 37.9	28.9 ± 31.2	0.12

[a] No other ICU associated morbidity and chronic comorbidity (see also "Risk Factors" in ESM) exhibited significant differences between cases and controls (data not shown)
[b] Determined on ICU admission, with maximal case-to-control difference of 3 points
[c] Determined on the first day of ICU stay, with maximal case-to-control difference of 1 point
[d] 300 mg/day for 5–7 days followed by gradual taper
[e] Other than carbapenems
[f] Before pandrug-resistant *P. aeruginosa* isolation

colistin, and more frequent exposure to open suctioning during 6–26 September; the latter was due to more VAP days during this period in the case group than in controls (11.8 ± 2.9 vs. 3.8 ± 3.7).

Recorded variables included age, gender, daily APACHE II, and SOFA score, prescribed medication and other treatment interventions (e.g., placement of central venous catheters, daily number of open suctioning

procedures), positive cultures and antibiotic susceptibility, occurrence of infection and sepsis/septic shock, other ICU-associated morbidity (e.g., acute lung injury, acute renal failure), length of ICU stay, mechanical ventilation duration, and survival to ICU discharge.

Definitions

Standard definitions were used for hospital-acquired infections, chronic comorbid conditions and ICU stay-related complications (see ESM). Based on patient data and our antibiotic policy for VAP by multidrug-resistant bacteria (see ESM) we defined prolonged carbapenem use as carbapenem administration longer than 20 days. In the case group prolonged carbapenem use was always associated with colistin use for longer than 13 days; "the vice versa association" held for the entire ICU population between July and October 2005.

Cultures

In patients with infection/sepsis [8, 9] we obtained reference (initial) and follow-up culture samples [venous blood, intravascular catheter-tips, endotracheal aspirates, urine, bronchoalveolar lavage fluid (in suspected VAP), cerebrospinal fluid (in suspected meningitis), chest/abdominal drainage fluid, maxillary sinus drainage fluid, and surgical wound swabs]. Follow-up blood, endotracheal aspirate, urine, and cerebrospinal fluid cultures were repeated at least twice until ICU discharge. Immediately after obtaining reference culture samples empirical antibiotic therapy with two broad-spectrum agents (e.g., piperacillin/tazobactam or third-generation cephalosporin plus vancomycin) was started. Subsequently, antiobiotic therapy was readjusted according to obtained antibiograms. Blood culture samples were processed with BACTEC 9240 (Becton Dickinson, Franklin Lakes, N.J., USA). Subcultures were carried out in blood, blood anaerobic, MacConkey, Sabouraud, and chocolate agar. Other specimens were also inoculated in in additional media (e.g., Fildes enrichment, Brucella agar with blood, hemin and vitamin K). Cultures were incubated at 35 °C. Micro-organisms were identified by VITEK II system (Biomérieux, Vitek II, Hazelwood, Mo., USA).

Antimicrobial susceptibility testing

Minimum inhibitory concentration (MIC) values were determined by broth microdilution (VITEK II). Standard breakpoints were used [10]. Tested antibiotics included amikacin, gentamicin, netilmicin, tobramycin, aztreonam, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanic acid, ceftriaxone, ceftazidime, cefepime, imipenem/cilastatin, meropenem, colistin, ciprofloxacin, ofloxacin, pefloxacin, and moxifloxacin. Intermediate susceptibility was defined as resistance. Synergy studies were conducted as previously described [11] (see ESM). MICs of imipenem, meropenem and colistin were also determined by E-test (Biodisk, Solna, Sweden). MIC breakpoint for susceptibility to colistin was 2 mg/l. Bacteria with MIC of 4 mg/l or higher were considered resistant. Employed colistin formulation was colistin sulfomethate sodium (Colistin, Norma, Athens) with 1 mg being equivalent to 13,333 IE. Colistin was always administered intravenously.

Control and investigation of factors of cross-transmission

On confirmation of the second case of pandrug-resistant *P. aeruginosa*. VAP (Fig. 1) standard infection control measures (use of disposable gowns, masks, and gloves, and hand hygiene before and after patient contact) were reinforced. Furthermore, weekly lessons on infection control were organized. ICU areas and equipment employed for case patients nursing (Fig. 1) were decontaminated and remained out of use for at least 48 h following case patient discharge or death.

Surveillance cultures of healthcare workers and hand disinfection survey

During 16–30 September 2005 all healthcare workers had a semiquantitative broth rinse hand culture [12, 13] performed on arrival for work and prior to performing hand hygiene or on entering the ICU [14] (Fig. 1). Also healthcare worker compliance with appropriate hand washing and glove use was quantified by two observers. There were ten 2-h observational periods (five during day shift and five during night shift) with healthcare workers being unaware of the survey [12]. Observation was repeated within the week following outbreak resolution (Fig. 1).

Environmental and surveillance cultures

During the above time periods culture samples were also taken from moist areas (e.g., sinks, faucets, and water samples), equipment (ventilators, bedside monitors, and suction apparatus), and containers of hand lotions and soaps [12, 13]. Samples from case patients' environment were taken immediately before and after decontamination. Samples were immediately transported to the microbiology laboratory and processed within 2–4 h of arrival. Lastly, from October 2005 to October 2006 biweekly patient surveillance cultures (tracheobronchial aspirates) were performed.

Risk factors

Potential risk factors for pandrug-resistant *P. aeruginosa* VAP included age over 55 years [15], chronic comorbid conditions, hospital and/or ICU stay-related complications, and treatment-related factors. Risk factors are presented in detail in ESM.

Statistical analysis

Data analysis was performed with the Statistical Package for Social Sciences version 12.0 (SPSS, Chicago, Ill., USA). Dichotomous or categorical variables were compared by χ^2 or Fisher's exact test. Continuous variables were compared using the two-tailed, independent-sample *t* test or the Mann-Whitney exact *U* test; distribution normality was tested by the Kolmogorov-Smirnov test. Binary, forward, stepwise logistic regression analysis was carried out after entering variables statistically significant on univariate logistic regression analyses and excluding confounders. Confounding variables were those associated with a risk factor and causally related to outcome [16]. We sought to derive a model with the smallest set of independent variables to predict VAP by pandrug-resistant *P. aeruginosa* [17]. Odds ratios and 95% confidence intervals were used for statistically significant risk factors. Statistical significance was set at $p < 0.05$. Values are reported as number (percentage), mean ± SD, or median (interquartile range) as appropriate.

Results

In the case group sequential isolation of carbapenem-sensitive and carbapenem-resistant bacteria led to prolonged, combined use of carbapenems and colistin because synergy study results against carbapenem-resistant bacteria (see also ESM) revealed fractional inhibitory concentration index values of 0.5 or less. Full culture results from the case group are presented in Table E1 (ESM). Between 14 September and 3 October 2005 pandrug-resistant *P. aeruginosa* was isolated at least once from culture specimens from all case patients. Repeated isolation occurred only in patient no. 1. MICs for imipenem, meropenem, amikacin, and colistin were 16 mg/l, 8 mg/l, 16 mg/l, and 8 mg/l, respectively. Synergy studies revealed fractional inhibitory concentration indices of 0.5–1.0 for meropenem and amikacin, indicating additive activity of these two antibiotics against pandrug-resistant *P. aeruginosa* [11]. Fractional inhibitory concentration indices for antibiotic pairs including colistin were indifferent (range > 1.0 to ≤ 4.0) [11]. Consequently in case patients colistin was replaced by amikacin (1 g every 24 h infused over 30 min) and meropenem (dose increased from 1 to 2 g every 8 h) was continued (patient no. 1), or imipenem was replaced by meropenem (dose, 2 g every 8 h; patients nos. 2–5). Each 2-g meropenem dose was infused over 3 h. Peak serum concentrations of amikacin (determined within 45–60 min postinfusion) ranged within 38.3–69.4 mg/l. Patient no. 1 developed multiple organ failure and died on 22 September. In the other four case patients (80%) cure (body temperature normalization, VAP resolution, and negative follow-up cultures) was confirmed within the next 10 days in the ICU and was followed by ICU discharge. Fourteen (70%) of the controls also survived to ICU discharge (n.s.).

Cultures from healthcare workers were sterile. This result was not considered conclusive because healthcare workers were screened just once before infection control measures reinforcement [12]. Hand hygiene was significantly improved after infection control measures reinforcement (Table 2). Accordingly, alcoholic rub (Sterilium, Bode Chemie, Hamburg, Germany) consumption increased by 31.2%. Environmental cultures yielded isolation of pandrug-resistant *P. aeruginosa* and carbapenem-resistant *Acinetobacter baumannii* on wall suction apparatus corresponding to case patients beds before but not after decontamination with 0.1% hypochlorite [18]. Carbapenem-resistant *A. baumannii* and *P. aeruginosa* were isolated from another eight wall suction apparatus and one tap, which were also decontaminated. Patient surveillance cultures have remained negative so far (i.e., for 1 year) for pandrug-resistant *P. aeruginosa*. During the same period there were sporadic new cases of respiratory colonization ($n = 4$) and VAP ($n = 3$) by carbapenem-resistant *A. baumannii*.

Factors significantly associated with VAP due to pandrug-resistant *P. aeruginosa* (univariate logistic regression analysis) were combined use of carbapenem

Table 2 Results of the hand disinfection survey performed on 51 healthcare workers before and after infection control measures. Lack of compliance with standard hand hygiene even once during a 2-h observation period (see text) was classified as "no compliance"

	Before		After		*p*
	n	%	*n*	%	
Hand disinfection [a] before patient contact	19	37	31	61	0.03
Hand disinfection [a] after patient contact	41	80	46	90	0.26
Glove removal immediately after patient encounter	12	24	29	57	0.001

[a] Performed with alcoholic rub

Table 3 Factors significantly associated with infection due to pandrug-resistant *P. aeruginosa* in pandrug-resistant *P. aeruginosa* cases and controls (univariate analysis) (*PDR* pandrug-resistant, *CI* confidence interval)

	Cases ($n = 5$)	Controls ($n = 20$)	Odds ratio	95% CI	p
Carbapenem use > 20 days and colistin use > 13 days [a]	4 (80%)	1 (5%)	76.0	3.9–1487.5	0.004
> 78 open suction procedures 6–26 Sept. 2005	4 (80%)	4 (20%)	16.0	1.4–185.4	0.03
Carbapenem use > 20 days [b]	4 (80%)	5 (25%)	12.0	1.1–134.1	0.04

[a] Also identified as the sole independent predictor of PDR *P. aeruginosa* infection by binary, stepwise logistic regression results, with an identical odds ratio, 95% CI, and p value

[b] Considered a confounder

for more than 20 days and colistin longer than 13 days, more than 78 open suction procedures during 6–26 September, and carbapenem use longer than 20 days (Table 3). As the last factor fulfilled the definition of a confounder [16], binary logistic regression was performed with the first two factors as independent variables. The sole independent predictor of pandrug-resistant *P. aeruginosa* pneumonia was the prolonged exposure to the carbapenem-colistin combination (Table 3). The model predicted correctly the occurrence of VAP due to pandrug-resistant *P. aeruginosa* in 23 (92.0%) of the 25 patients, with sensitivity and positive predictive value of 80.0% and specificity and negative predictive value of 95.2% (see also ESM).

Discussion

We describe the first ICU outbreak of VAP by a pandrug-resistant, $bla_{\mathrm{VIM-1}}$ gene positive strain of *P. aeruginosa* in patients without cystic fibrosis. Prolonged exposure to carbapenems and colistin independently predicted pandrug-resistant *P. aeruginosa* pneumonia. The major factor of cross-transmission was the number of open suctioning procedures from outbreak onset to infection control reinforcement. Due to personnel shortage open suctioning was performed by only two physiotherapists in the entire ICU. The infection was effectively treated by meropenem and amikacin in four case patients. The outbreak resolved by improving hand hygiene and isolating all case patients.

Sporadic cases [4, 15] or small outbreaks [19] of infections by colistin-resistant *P. aeruginosa* have recently been reported in patients without and with cystic fibrosis [4, 15, 19]. According to current and previous [15] results, colistin resistance has been confirmed in metallo-β-lactamase producing *P. aeruginosa*. Colistin is a polypeptide antibiotic with two major components: colistin A (polymyxin E_1) and colistin B (polymyxin E_2). It exhibits hydrophobic and lipophilic moieties and disrupts bacterial cell membrane by interacting with phosphates and fatty acids of the lipopolysaccharide core and lipid A moieties [20–22]. Carbapenems are also cell-wall acting agents. However, their substituted β-lactam structure differs from colistin structure. Thus molecular mechanisms of carbapenem and colistin resistance probably differ as well.

Our results suggest that prolonged colistin exposure was a prerequisite for resistance development in this particular strain of pandrug-resistant *P. aeruginosa*. As with β-lactams [23], colistin may induce synthesis of enzyme(s) conferring colistin resistance [20]. Polymyxin exposure causes addition of aminoarabinose to lipid A [22, 24]. The mechanism involves a mutated PmrAB locus activated by a sensor phosphokinase, with concomitant selective suppression of the corresponding, deactivator PmrB phosphatase. This results in constitutive activation of the PmrA regulon which stimulates aminoarabinose synthesis [22]. Other resistance mechanisms involve peptide-degrading elastases [25] and substitution of protein OprH for magnesium in the outer membrane [20].

Pandrug-resistant *P. aeruginosa* VAP was treated with meropenem plus amikacin for 10 days (treatment-duration titrated to clinical [26] and microbiological cure) because (a) MICs for the two antibiotics were among the lowest determined, (b) meropenem dose and administration technique (2 g/8 h, infusion duration 3 h) would maximize time above MIC in serum [27, 28] and possibly in respiratory epithelial lining fluid and alveolar cells [29], (c) single-daily dose of 1 g amikacin consistently resulted in peak serum concentrations greater than 2.5–3 times the MIC, with mean bronchial secretions concentration probably approaching MIC level [30], (d) fractional inhibitory concentration indices indicated additive activity for meropenem and amikacin, and (e) combination therapy is recommended for *P. aeruginosa* pneumonia [26, 31, 32].

Recently two large ICU outbreaks of multidrug-resistant *P. aeruginosa* producing IMP-type metallo-β-lactamases [33] or exhibiting decreased OprD porin expression and AmpC overexpression [34] were partly attributed to personnel shortage [33] or contaminated healthcare worker hands [34]. Personnel reinstatement [33] and improved contact precautions and patient isolation [34] facilitated outbreak resolution. In another report [35] patients with multidrug-resistant *P. aeruginosa* infection received potent antipseudomonal agents for prolonged periods for infections by less resistant Gram-negative bacteria. Also a prior, large outbreak of *A. baumanii*

pneumonia was attributed partly to ceftazidime selection pressure; healthcare worker compliance with standard hand washing recommendations was deemed poor and subject to improvement [14]. Poor hand hygiene in conjunction with open suctioning procedures resulting in aerosolization and contamination of healthcare worker hands have recently been suggested as a factor in *A. baumannii* cross-transmission [18]. Thus in concordance with prior results and current findings the outbreak reported herein can be attributed to (a) carbapenem-colistin selection pressure, (b) increased exposure to open suctioning procedures (performed by the same physiotherapist due to personnel shortage; Table 3), and (c) relatively poor compliance to standard contact precautions (Table 2).

The small number of "positive" events (occurrence of pandrug-resistant *P. aeruginosa* VAP) per variable tested ($n = 5$ events/2 variables = 2.5) increased the risk of inaccurate and/or biased estimation of the logistic regression coefficients and odds ratios [36]. However, after excluding the weaker risk factor due to collinearity (see also ESM) "positive" events per variable were doubled ($n = 5$), thereby reducing the likelihood of inaccurate estimation [36]. Also, model accuracy was 92%, and model usefulness was evident since it correctly classified 80% of the case patients (i.e., 30% above than what would be expected by chance alone; see also ESM). Lastly, combined univariate and bivariate logistic regression results effectively provided a plausible explanation for the pandrug-resistant *P. aeruginosa* outbreak. Nevertheless, ten or more positive events per variable tested would be recommended to minimize bias risk in parameter estimates [36]. However, the validity of logistic regression models is also affected by total events per variable tested and sample size (here "positive" plus "negative" events = 25, sample size = 25) [36]. The case group size was probably restricted by the improved infection control. Surveillance culture results indicate eradication of pandrug-resistant *P. aeruginosa*, with carbapenem-resistant *A. baumannii* VAP still constituting a recurrent problem [13]. Additional measures comprise personnel increase (two physiotherapists per shift) [33] and consideration of closed suctioning [18].

Just prior to the occurrence of pandrug-resistant *P. aeruginosa* VAP, case patients had already survived a protracted ICU-stay and considerable ICU-related morbidity (Table 1). At this time case patients were clinically stable and had lower SOFA scores than at ICU admission (4.6 ± 1.4 vs. 7.2 ± 2.0, $p = 0.007$, paired t test). Also, for case patients nos. 2–5 survival to ICU discharge was considered probable by their attending physicians. Thus case patients could be regarded as "potential" survivors from an ICU subpopulation with mortality rate of 40% or higher. Subsequent pandrug-resistant *P. aeruginosa* pneumonia had an attributable 20% mortality. This is consistent with prior mortality data on effectively treated VAP by multidrug-resistant bacteria [1–3].

Conclusions

The prolonged combined use of carbapenems and colistin predisposes to VAP by pandrug-resistant *P. aeruginosa*. Our results emphasize the need for (a) limiting the duration of treatment of multidrug-resistant bacterial infections with carbapenems and colistin and (b) control of healthcare worker hand and/or environmental contamination by open suctioning. Pandrug-resistant *P. aeruginosa* pneumonia may be treatable by a 10-day course of two antipseudomonal antibiotics with the lowest MICs and additive activity.

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