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Pseudomonas aeruginosa carriage, colonization, and infection in ICU patients

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Abstract *Objective:* We evaluated whether *Pseudomonas aeruginosa* associated nosocomial infections in our ICU originate mainly from patients' endogenous flora or from exogenous cross-transmission. *Design and setting:* A 6-month prospective surveillance survey was performed according to standardized protocols at the interdisciplinary ICU of the Azienda Ospedaliera Cannizzaro. *Patients:* The study analyzed 121 patients and focused on three different states: carriage upon admission, colonization of sterile sites, and infections during ICU stay. *Results:* We identified 138 *P. aeruginosa* isolates from 45 patients. The cumulative incidence of *P. aeruginosa* sustained colonization in the ICU was 29.9/100 patients, and the incidence density was 16.2/1,000 patient-days. The cumulative incidence of *P. aeruginosa*-sustained infections in the ICU was 36.7/100 patients, and the incidence density was 19.9/1,000 patient-days. The most frequent infection type was ventilator-associated pneumonia. PFGE analysis of *P. aeruginosa* isolates led to the identification of a major clone represented by 60.8% of isolates involving 45.9% of patients. The impact of cross-transmission, i.e., the preventable proportion of *P. aeruginosa* acquisition, was estimated to be at least 59.5% of all colonization or infection episodes. Acquisition of multidrug-resistant *P. aeruginosa* was significantly associated with cross-transmission. *Conclusions:* Our results suggest that the ICU personnel and environment served as reservoirs for cross-transmission and emphasize the importance of exogenous acquisition of multidrug-resistant *P. aeruginosa*, of reduction in antibiotic pressure, and prompt enforcement of infection control measures.

Keywords Pulmonary nosocomial infections · *Pseudomonas aeruginosa* · Multidrug resistance · Surveillance · Colonization · Carriage

Introduction

Intensive care units (ICUs) worldwide are encountering the highest density of nosocomial infections (NI) and the spread of antibiotic-resistant pathogens responsible for emerging infection problems in the hospital [1–3]. In the rank order of pathogens causing ICU-related infections *Pseudomonas aeruginosa* has held a nearly unchanged position over recent decades [4]. A European survey showed that *P. aeruginosa* is one of the most frequent pathogens isolated from ICU-acquired infections [5, 6], and data derived from recent multicenter surveillance studies place this microrganism as the first Gram-negative species recovered in ICUs in Belgium [7] and Italy [8]. Several patient and pathogen-specific risk factors are associated with acquisition of this pathogen in ICUs, such as length of stay, severity of underlying disease and exposure to invasive procedures, on the one hand [5, 9], and virulence, adherence, and antimicrobial drug resistance on the other [10, 11]. The hospital environment,

particularly moist sites, are known reservoirs of *P. aeruginosa* strains, often multidrug resistant (MDR) due to intrinsic and acquired determinants [12]. Although one possible explanation of the spread of antibiotic-resistant strains in ICUs is the selection exerted by extensive use of antibiotics, increased spread of MDR *P. aeruginosa* may be due to transmissions of resistant clones between patients [13]. Furthermore, it has been suggested that infection represents merely the tip of an iceberg, and that colonization reflects the submerged part. Colonization may be the first step of an endogenous infection, while the colonized patients represent a continuous exogenous source of microrganism for colonization/infection of other patients [10]. The relative importance of both colonization/infection pathways, essential to design appropriate prevention strategies, has rarely been exploited by active surveillance studies. The aim of our survey was to evaluate whether *P. aeruginosa* associated NI in our ICU originate mainly from patients' endogenous flora or from exogenous cross-transmission, by determining: (a) the occurrence of *P. aeruginosa* carriage upon admission, (b) the ICU-acquired *P. aeruginosa* infection and colonization rates by site, (c) the impact of cross-transmission using molecular typing data of the involved microrganisms, and (d) the occurrence of individual risk factors for MDR *P. aeruginosa* acquisition and their clinical impact. Preliminary results were presented in part at the 16th ECCMID [14].

Methods and materials

Patients and setting

During the study period 123 patients were admitted in the ICU for a total of 2,169 days; two patients staying less than 3 days were excluded. Patients characteristics are summarized in Table 1. The study was conducted at the Azienda Ospedaliera Cannizzaro, a 700-bed acute care hospital in Catania, Sicily, with a 12-bed interdisciplinary ICU. Between January and July 2004 all patients who stayed at least 48 h at the ICU were enrolled in the 6-month prospective surveillance survey. The ICU was supported by a single diagnostic microbiological laboratory. Patients were treated either in an eight-bed room or in four single isolation rooms. Each ICU room has its own sink and trolley with equipment for patient care. Hand washing with a detergent skin cleansing solution (clorhexidine) is required between patient contacts; caps, masks, shoe covers, and gloves are required at all times in the patient rooms and bathrooms. Sterile gloves are required for wound care procedures. Standard precautions were in place in the unit. Hand hygiene procedures were routinely emphasized. Other infection control measures consisted of cleaning once daily with tap water of the sink and environment surfaces using glutaraldehyde and sodium hypochlorite solutions.

Table 1 Main characteristics of included patients ($n = 121$)

Age, median (years; range)	51 (2–88)
Sex: male	61.2%
SAPS II score, median (range)	24 (9–58)
Diabetes	18.2%
Cancer	12.4%
Smoking	17.3%
Length of stay, median (days; range)	18 (2–161)
Mechanical ventilation	97.5%
Urinary catheter	97.5%
Central-line catheter	99.2%

Surveillance methodology

A 6-months surveillance prospective survey was performed from January to July 2004, according to the Hospital in Europe Link for Infection Control through Surveillance protocol [15]. An electronic data form was created using the SPSS Data Entry Enterprise Server. The study focused on three different states: carriage upon admission, colonization of sterile sites and infections during ICU stay. Patients with screening cultures testing positives in the absence of, or before isolation of, positive clinical specimens were considered to be carriers. Due to the lack of clinical data confirming infection patients with positive clinical specimens were considered to be colonized. When both clinical screening cultures testing positive on the same day, the patient was considered colonized [10]. Standard definitions of NI were used [15]. We included the following NI sites: pneumonia, bloodstream infections (BSI), central venous catheter-related BSI, and urinary tract infections (UTI). We considered only the first episode of infection or colonization per site and per patient. However, if infection developed after colonization at the same site, only the episode of infection was considered in the analysis. Finally, colonization and infections occurring during ICU stay in the carriers upon admission were not considered. Incidence rates of colonization and of infections were calculated both in terms of cumulative incidence and incidence density [16]. Calculation of National Nosocomial Infections Surveillance (NNIS) System device-associated infection rates and device utilization ratios were carried out in accordance with the methods described for the NNIS System, following the NNIS System report instructions [17]. The Simplified Acute Physiology Score (SAPS) II was used to assess patients' severity of illness at admission.

Bacterial isolates

During the study period routine clinical specimens and screening specimens (oropharyngeal, rectal, nasal, and

axillary swabs) were collected and analyzed to identify *P. aeruginosa*. Screening cultures were collected from each patient on the day of admission. Clinical specimens were collected as requested by the medical staff. All isolates were identified by the Phoenix system (Becton Dickinson Diagnostic System, Pont de Claix, France). Antibiotic susceptibility was determined for the antimicrobial agents listed in Table 2, using an automated Phoenix microdilution system (Becton Dickinson Diagnostic System, Pont de Claix, France). Susceptibility criteria for the test method were in accordance with National Committee for Clinical Laboratory Standards Interpretative Criteria [18]. Isolates showing an intermediate level of susceptibility were classified as resistant. For each antimicrobial agent the resistance rate (RR) was calculated as the number of resistant isolates divided by the total number of isolates for which susceptibility testing had been carried out [19]. Antimicrobial RR were compared with reference RR percentiles distributions as reported by the Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units (SARI) project [13] and by the Intensive Care Antimicrobial Resistance Epidemiology (ICARE) project [17]. MDR was defined as resistance of a *P. aeruginosa* isolate to at least three of the following four drugs: imipenem, ceftazidime, ciprofloxacin, and tobramycin [20]. We identified 138 *P. aeruginosa* isolates from 45 of the 121 patients, while 75 patients remained free of P. aeruginosa during their ICU stay.

Table 2 Antimicrobial resistance rates of *P. aeruginosa* isolates and comparison with reference RR percentiles reported by the Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units (*SARI*) [13, 18] and Intensive Care Antimicrobial Resistance Epidemiology (*ICARE*) and National Nosocomial Infections Surveillance Antimicrobial Use and Resistance (*AUR*) projects [16] (*NA* no data available for comparison)

Antibiotic	RR	SARI	ICARE/AUR
Piperacillin	63.4	NA	> 90th
Piperacillin, tazobactam	64.8	> 75th	NA
Ceftazidime	66.2	> 75th	> 90th
Cefepime	66.9	NA	NA
Meropenem	67.6	NA	NA
Imipenem	72.5	> 75th	> 90th
Ciprofloxacin	65.5	> 75th	> 75th
Levofloxacin	69.7	NA	> 75th
Trimethoprim/sulfamethoxazol	100	NA	NA
Gentamicin	65.5	NA	NA
Amikacin	4.2	> 50th	NA
Aztreonam	73.2	NA	NA

Antibiotic use

The quantity of antimicrobial drugs was standardized by conversion to defined daily doses (DDD) [21]. To control for the population size at risk of receiving antimicrobials we determined the antimicrobial use density (AD) in WHO ATC groups [13].

Macrorestriction analysis and identification of cross-transmission episodes

Molecular typing by pulsed-field gel electrophoresis (PFGE) of the *Spe*I digested genomic DNA was performed as previously described [22]. Macrorestriction fragments were separated using a CHEF-DR III apparatus (Bio-Rad, Hercules, Calif., USA). Interpretation of genomic relatedness was performed using well established criteria [23–25]. The presence of two indistinguishable strains of *P. aeruginosa* in two patients was considered a single episode of cross-transmission [26].

Individual risk factors for MDR *P. aeruginosa*

To identify the individual risk factors for MDR *P. aeruginosa* a case-control study of the patients included in the surveillance was designed. All case patients were those with MDR *P. aeruginosa* while control patients were those with susceptible *P. aeruginosa*. The patients' characteristics and variables that were examined as possible risk factors are listed in Table 3. Patients' mortality and cross-transmission were also evaluated in relation to MDR *P. aeruginosa* infection.

Table 3 Univariate analysis of risk factors for MDR *P. aeruginosa* nosocomial infections

Variables	Cases ($n = 27$)	Control ($n = 18$)	p	OR (95% CI)
Age (years)	56.0	65.3	0.093	–
Gender: male	21 (77.8%)	9 (50.0%)	0.105	3.5 (0.959–12.778)
SAPS II (mean)	27.7	25.9	0.542	–
ICU stay, mean	27.5	25.8	0.744	–
Mechanical ventilation	27 (100%)	18 (100%)	–	–
Length of ventilation, mean	26.1	26.9	0.878	–
Number of antimicrobials, mean	6.0	4.7	0.124	–
Quinolone treatment	11 (40.7%)	10 (55.6%)	0.374	0.5 (0.165–1.836)
Carbapenem treatment	17 (63.0%)	6 (33.3%)	0.071	3.4 (0.971–11.905)
Surgery	21 (77.8%)	17 (94.4%)	0.215	0.2 (0.23–1.880)
Neoplastic disease	5 (18.5%)	0	0.74	–

Statistical analysis

Risk factors for MDR *P. aeruginosa* infections were identified by univariate analysis. The χ^2 test or Fisher's exact test was used for categorical variables. Continuous variables were tested using Student's *t* test. All tests were two-tailed, and $p < 0.05$ was considered statistically significant. A statistical package (SPSS 14.0) was used for all analysis.

Results

Acquisition of *P. aeruginosa* colonization and infection during ICU stay

Two patients (1.7%) were carriers upon admission, and another two were colonized upon admission; these were not included in the surveillance. Thus 117 patients were eligible for follow-up of *P. aeruginosa* colonization and infection during ICU stay. Of these, 26 were colonized in the ICU (22.2%). The cumulative incidence of *P. aeruginosa*-sustained colonization in the ICU was 29.9/100 patients (35 episodes in 117 patients); the incidence density adjusted for the number of patient-days was 16.2/1,000 patient-days (35 episodes in 2,165 patient-days). Thirty patients were infected in the ICU (25.6%). The cumulative incidence of *P. aeruginosa*-sustained infections in the ICU was 36.7/100 patients (43 episodes in 117 patients); the incidence density was 19.9/1,000 patient-days (43 episodes in 2,165 patient-days). The cumulative incidence and the incidence density of infection were significantly higher than the cumulative incidence and the incidence density of colonization ($p < 0.01$). The proportion of each site-specific infection was: 51.2/100 infections for ICU-acquired pneumonia, 18.6/100 infections each for UTI and central line related local infections, 9.3/100 infections for BSI, and 2.3/100 infections for central line associated BSI. Site-specific incidence density values were: 10.2/1,000 patient-days for ICU-acquired pneumonia, 3.7/1,000 each for UTI and central line related local infections each, 1.8/1,000 for BSI, and 0.5/1,000 for central line associated BSI.

Device-associated infection rates and device utilization ratios

Device-associated infection rates and device utilization ratios were compared with the NNIS System reported rates and ratios [17]. Particularly the urinary catheter-associated UTI rate (8.1‰) was equal to the 90th percentile for the NNIS System. The urinary catheter utilization ratio was 0.91, between the 50th and 75th percentiles. The central line associated BSI rate was 4.6‰, between the 50th and 75th percentiles and the central line utilization ratio (0.99) was greater than the 90th percentile. The ventilator-associated pneumonia rate (30.5‰) was greater than the 90th percentile and the ventilator utilization ratio (0.88) was greater than the 90th percentile for the NNIS System.

Surveillance of antimicrobial resistance and antimicrobial use

Most of the *P. aeruginosa* isolates were resistant to multiple antibiotics (100 in 138 isolates, 72.5%); all isolates were resistant to trimethoprim/sulfamethoxazol. Comparison with reference RR percentiles distributions reported by the SARI [13] and ICARE projects [17] revealed high antimicrobial RR, greater than the 75th percentile, for penicillins with lactamase inhibitor, penicillins with extended spectrum, third generation cephalosporins, carbapenems, and quinolones (Table 2). The data on antimicrobial use were collected from the ICU covering 1070.4 DDD during the 6-months surveillance study. Table 4 provides data on the number of DDD,

Table 4 Defined daily doses (*DDD*), antimicrobial use density (*AD*) and comparison with reference AD values percentile distributions reported by the Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units (*SARI*) [18] and Intensive Care Antimicrobial Resistance Epidemiology (*ICARE*) and National Nosocomial Infections Surveillance System Antimicrobial Use and Resistance (*AUR*) projects [16] (*NA* no data available for comparison)

Antimicrobial group[a]	DDD	AD	AD: SARI	AD: ICARE/AUR
Penicillins				
With extended spectrum	2,5	6,8	< 10th	> 90th
With lactamase inhibitor	708,9	1936,8	> 75th	
Cephalosporins				
Third generation	19,6	53,5	< 10th	< 10th
Fourth generation	30,1	82,2	NA	NA
Carbapenems	95,1	43,3	> 50th	> 75th
Quinolones	81	221,3	> 75th	> 50th
Trimethoprim/sulfamethoxazol	16,8	45,9	> 75th	> 50th
Aminoglycosides	70,1	191,5	> 75th	NA
Imidazole derivates	46,3	126,5	> 75th	NA
Total antimicrobial use	1070,4			

[a] The antimicrobials used in the ICU were divided by class and group according to ATC classification employed by the WHO [19]

the AD values, and the comparison with reference AD percentile distributions reported by the SARI [19] and ICARE projects [17]. Comparison revealed high AD values, greater than the 75th percentile, for penicillins with lactamase inhibitor, trimethoprim/sulfamethoxazol, quinolones, aminoglycosides, carbapenems, and imidazole derivates. In addition to, AD values were lower than the 10th percentile for penicillins with extended spectrum and 3rd generation cephalosporins.

Macrorestriction analysis

Among the 138 *P. aeruginosa* isolates 102 collected from 37 patients were analyzed by macrorestriction. PFGE analysis of *P. aeruginosa* isolates led to the identification of 18 unrelated PFGE types, which we termed A–T, and five subtypes of clone A (termed A1–A5), one subtype each of clones D, G, and L (termed D1, G1, and L1, respectively). PFGE types A, D, E, F, G, H, and L were associated with cross-transmission of infection: clone A was represented by 62 of 102 isolates (60.8%) from 17 of 37 patients (45.9%); clone D was present in 4 isolates from 3 patients; clone E was present in 2 isolates from 2 patients; clone F was present in 5 isolates from 3 patients; clone G was present in 2 isolates from 2 patients; clone H was present in 2 isolates from 2 patients; and clone L was present in 7 isolates from 4 patients. The remaining PFGE types were single patterns associated with sporadic strains.

Identification of cross-transmission episodes

The total number of cross-transmission episodes was 15, thus the impact of *P. aeruginosa* cross-infection was estimated to be at least 44.1% (15 over 34 infections), but this figure was higher when cross-colonization was included (59.5%, 25 over 42 colonizations or infections), thus defining the preventable proportion of all *P. aeruginosa*-sustained cross-transmission episodes.

Antimicrobial susceptibility patterns of *P. aeruginosa* PFGE types

The analysis of antimicrobial susceptibility patterns showed that *P. aeruginosa* strains of PFGE profiles A, L, and S were all MDR. In addition to, strains of PFGE types A and S were resistant to all antimicrobials tested except to amikacin. Generally, all *P. aeruginosa* PFGE types were susceptible to amikacin and resistant to trimethoprim/sulfamethoxazol. Strains of PFGE types B, H, M, N, P, and R were susceptible to all antimicrobials tested except to trimethoprim/sulfamethoxazol. During the 6-month surveillance study 27 patients with MDR *P. aeruginosa* (case group) and 18 patients with susceptible *P. aeruginosa* (control group) were obtained. Proportions and means for the different variables in the two groups are listed in Table 3. Univariate analysis showed that MDR *P. aeruginosa* infections were only significantly related to cross transmission of infections ($p = 0.002$). Mortality was not significantly higher in MDR *P. aeruginosa* patients (32.0%) than in controls (33.3%) (odds ratio 1.063, 95% confidence interval 0.292–3.863). The results of individual risk factors univariate analysis are displayed in Table 3. No variable was found to be a significant risk factor for infection with MDR *P. aeruginosa*.

Discussion

Our study provided an epidemiological *scenario* of *P. aeruginosa* acquisition during ICU stay using a standardized protocol intended for advanced risk-adjusted comparison of rates with those reported by international networks such as NNIS, HELICS, and SARI. The low proportion of patients positive upon admission was consistent with the findings of other studies [10, 27, 28] while the cumulative incidence of *P. aeruginosa* acquisition during ICU stay was as high as 35.0/100 patients (42 colonized or infected patients in 117).

In our study we adopted the sophisticated approach of the *P. aeruginosa* surveillance program of Bertrand et al. [10]. Thus instead of limiting surveillance to infection, merely the tip of the iceberg, we also included surveillance of colonization [29]. Rates of infection were significantly higher than those of colonization. According to the definitions of Bertrand et al., if infection developed after colonization at the same site, only the episode of infection was considered in the analysis. This might explain the higher rate of infection than the colonization rate in our survey. However, colonization represented a substantial part of the bacterial load within the ICU and a continuous potential source of cross-transmission [10, 27].

Device-associated infection rates and device utilization ratios should be evaluated together for appropriate interpretation [17]. The most frequent infection type was ventilator-associated pneumonia, with a rate and a ventilator utilization ratio both exceeding the 90th percentile for the NNIS System, highlighting the need of reducing the use or limiting the duration with which such device is used in order to lower the ventilator-associated pneumonia rate in the unit. The urinary catheter associated UTI rate was equal to the 90th percentile for the NNIS System, but the urinary catheter utilization ratio was lower than the 75th percentile; the central-line utilization ratio exceeded the 90th percentile, but the central line associated BSI was lower than the 75th percentile. As such, device associated UTI and BSI cannot be defined as a problem in our ICU, but merely as an area for further investigation.

It has been suggested that high resistant rates of pathogens associated with NI in the ICUs are correl-

ated with high rates of antibiotic use, but increased RR may also be due to transmission between patients [13]. Thus high RR of sporadic strains in the presence of high AD, as observed for penicillins with lactamase inhibitor, trimethoprim/sulfamethoxazol, quinolones, and carbapenems, would primarily suggest an overuse of these antimicrobials; on the other hand, low AD for third-generation cephalosporins and for penicillins with extended spectrum, in the presence of high RR, i.e., to ceftazidime, cefepime, and piperacillin, shown by *P. aeruginosa* strains of pulse type A, may be explained by cross-transmission. Evidence of such spread is reflected by the predominance of clone A, 60.8% of isolates involving 45.9% of patients. High AD in the presence of high RR of epidemic clones may indicate that both explanations hold true. In our study the acquisition of MDR *P. aeruginosa* was consistently associated with cross-transmission ($p = 0.002$).

Infections that seem to be most amenable to infection control measures are those that result from transmission between patients. However, efforts to measure the frequency of these events are scarce [3]. The aim of our survey was to evaluate, based on microbiological routine investigations, whether *P. aeruginosa*-associated NI in the ICU originate mainly from patients' endogenous flora or from exogenous cross-transmission. The impact of cross-transmission in our ICU, representing the preventable proportion of *P. aeruginosa* acquisition, was estimated to be at least 59.5% of all colonization/infection episodes, even higher than the estimate of about 50% reported in recent studies [3, 10]. In general, the estimated proportion of cases resulting from transmission vary depending on the occurrence of outbreaks in the period of study [30]. This study provides evidence that an outbreak of *P. aeruginosa* has occurred in our ICU.

In conclusion, although no periodical surveillance cultures were performed, our results suggest that the ICU personnel and environment serve as reservoirs for cross-transmission, and emphasize the importance of exogenous acquisition of MDR *P. aeruginosa*. Active surveillance of NI and antibiotic use is of paramount importance to address guidelines for empirical antibiotic regimens, reduction in antibiotic pressure and prompt enforcement of infection control measures in an evidence-based way.

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