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A potential role of aminoglycoside resistance in endemic occurrence of *Pseudomonas aeruginosa* strains in lower airways of mechanically ventilated patients

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ABSTRACT

Altogether, 98 *Pseudomonas aeruginosa* isolates from a 5-bed intensive care unit were fingerprinted with pulsed-field gel electrophoresis and tested for aminoglycoside resistance genes *aac(6′)-Ib*, *aac(3″)-IIa*, *ant(2″)-Ia*, *armA*, *rmtA*, and *rmtB* and integrons and virulence genes/operons *phzI*, *phzII*, *phzM*, *phzS*, *apr*, *lasB*, *plcH*, *plcN*, *pilA*, *algD*, *toxA*, *exoS*, *exoT*, *exoY*, and *exoU*. Two major clusters were identified (49 and 19 isolates), harbouring *aac(6′)-Ib*, bla_{PSE-1}, and *ant(3″)-Ia* genes or *ant(2″)-Ia* gene, respectively, on a class I integron. Most virulence genes except for *exoU* and *pilA* were found. Only 1 isolate of the minor cluster (8 isolates) and 1 of the 22 sporadic isolates carried integrons (without gene cassettes); virulence profile was highly variable. Comparing the resistance and virulence patterns of endemic and sporadic isolates suggests that integron-borne aminoglycoside resistance is more closely associated with the frequency than virulence. Consequently, aminoglycoside usage may have played a role in maintenance of the endemic clones.

1. Introduction

Healthcare–associated infections represent the most serious problem of present-day medicine. *Pseudomonas aeruginosa* is a significant pathogen in such infections considering its natural resistance to many antimicrobials and its predisposition for acquired resistance as well (Navon-Venezia et al., 2005). It causes the most severe problems in intensive care units (ICUs), especially among mechanically ventilated patients, where it is among the first pathogens colonizing the respiratory tract (Navon-Venezia et al., 2005). Accordingly, ventilator-associated pneumonia (VAP) is frequently caused by *P. aeruginosa*. Moreover, increased mortality was shown even in patients not meeting the criteria for VAP, but with high burden of *P. aeruginosa* in the airways (Zhuo et al., 2008).

While outbreaks of multiresistant *P. aeruginosa* cause significant morbidity and mortality together with increased healthcare-associated cost and are therefore readily investigated and reported, the driving forces behind an endemic pattern are less well studied (Deplano et al., 2005; Navon-Venezia et al., 2005; Sader et al., 1993). Hypothetically, the selective advantage of a pathogen in a certain setting may derive from its virulence and/or from its resistance to antimicrobials used.

Antibiotic usage frequently serves as a driving force both in outbreaks and in endemicity; outbreaks of multiresistant *P. aeruginosa* occurred multiple times as a consequence of antibiotic overuse. For example, outbreaks of carbapenem- or ceftazidime-resistant strains associated with carbapenem or third generation cephalosporin use have been reported several times (e.g., El Amari et al., 2001; Hsueh et al., 2005). Though the consequences of the selective pressure exerted by other antibiotic groups are less well documented, they may be important, especially the frequently used fluoroquinolones and aminoglycosides. This is especially plausible in case of aminoglycosides, where resistance is frequently associated with resistance integrons, genetic structures which were shown to be associated with nosocomial *P. aeruginosa* strains (Ruiz-Martínez et al., 2011).

Despite a number of virulence factors described, the virulence mechanisms underlying the high mortality of VAP caused by *P. aeruginosa* are poorly understood. Type 3 secretion system and its effectors, especially the exotoxins *exoS* and *exoU*, may be important, but the role of other virulence-associated genes is less unequivocal (Berra et al., 2010).

The present work is concerned with the ecology of *P. aeruginosa* in a non-outbreak situation, i.e., to find and describe differences between successful clones and sporadic isolates in terms of carriage of virulence factors, aminoglycoside resistance genes, and integron-associated gene cassettes at a 5-bed ICU with a pulmonology profile.

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2. Materials and methods

2.1. Setting of ICU

The ICU involved is a newly constructed 5-bed ICU in a building previously also dedicated to healthcare. The ICU is directly connected to the pulmonology clinic of a tertiary care center (university clinic); therefore, the most common admission diagnosis is respiratory failure or pneumonia; the most common underlying disease is end-stage chronic obstructive pulmonary disease (COPD) or lung cancer. The patient population of the ICU is the elderly in general, as suggested by the underlying diseases. The ICU also accepts patients directly from other departments (surgery, internal medicine, etc.) if they are diagnosed with a pulmonology-related illness. Pediatric patients are never admitted to the department.

The study was conducted between September 2008 and February 2010 (546 days); during this period, the occupancy index was 85.6%, and the average ICU stay was 16 (8–33) days. The microbiological monitoring was intensive; critically ill (e.g., intubated) patients were sampled frequently; even daily sampling was performed in some cases. Besides *P. aeruginosa*, *Acinetobacter baumannii*, ESBL-producing, and ESBL-negative *Klebsiella pneumoniae* were the most frequently isolated bacteria. Fungal colonization was also frequent, commonly with *Candida glabrata*. Unexpected increases in the number of *P. aeruginosa* isolations were not detected. Environmental samples (samples from suckers, moisturizers, hospital furniture, fomites used in healthcare, tap surface) and tap water samples collected each month during the study period consistently yielded no Gram-negative bacteria.

2.2. Collection of bacterial isolates

Altogether, 98 isolates of *P. aeruginosa* were included isolated from 37 patients; patient characteristics are shown in Table 1. The majority (87) of isolates originated from the ICU from 28 patients; however, 11 isolates of 11 patients from other wards of the clinic (oncology, pulmonology, and rehabilitation) were also tested. Two patients were sampled both in the ICU and in other wards. Most isolates were cultured from lower airway samples (bronchial washing, sputum, or tracheal aspirate); colony-forming unit (CFU) numbers varied between 10^2 and $>10^5$/mL. Three samples originated from the upper airways, 3 from intravenous cannula and 1 from pleural fluid. Antibiotic susceptibility was determined by the CLSI disk diffusion method against imipenem, meropenem, piperacillin + tazobactam, ceftazidime, cefepime, ciprofloxacin, amikacin, gentamicin, and tobramycin. Species identification was confirmed by PCR specific for *P. aeruginosa* (Spilker et al., 2004).

2.3. Determination of genotype

Pulsed-field gel electrophoresis (PFGE) was used to assess genetic relatedness. Approximately 4×10^8 CFU of bacteria was gently mixed with 2% low melting point SeaPlaque agarose (Lonza) containing 1%

Table 1
Patient characteristics.

Patient	Sex	Year of birth	Ward(s)	Number of hospitalization episodes	Outcome	Main diagnosis	Cumulative dose of aminoglycosides (cumulative number of DDDs administered)				Clusters encountered
							Amikacin	Gentamicin	Streptomycin	Tobramycin	
BGn	F	1923	ICU	1	Died	Respiratory failure	1,0				A
BI	M	1936	ICU	2	Died	COPD	19,0				A
BM	F	1962	ICU	1	Died	COPD	17,0				C
BG	M	1951	ICU	1	Died	Lung cancer, COPD	8,0				B
CsI	M	1937	Pulmonology	1	Survived	COPD	12,0				C
DA	M	1960	ICU	1	Survived	Pneumonia	14,0				A
DSn	F	1928	Pulmonology	1	Survived	Pneumonia					None[a]
DnHI	F	1934	ICU	1	Died	Pneumonia					None
DY	M	1943	ICU, rehabilitation	1	Survived	COPD	11,0				A
JGn	F	1928	ICU	1	Survived	COPD					C
KIB	M	1926	ICU	7	Died	Respiratory failure	15,5				A
FÁ	M	1928	Pulmonology, ICU	1	Survived	COPD					None
FJ	M	1969	Pulmonology	1	Survived	Pneumonia					None
HJ	M	1947	Oncology	2	Survived	Lung cancer	14,0				None
IJ	M	1950	Pulmonology	2	Died	Tuberculosis, COPD	22,0	13,6	83,4		None
JSn	F	1920	ICU	1	Died	Respiratory failure					C
KMn	F	1939	ICU	1	Died	Respiratory failure					B
KG	M	1950	ICU	1	Survived	COPD					A and B
KI	M	1942	ICU	7	Survived	COPD					A and C
KJ	M	1944	Pulmonology	1	Survived	COPD					None
MM	M	1969	ICU	1	Died	Respiratory failure	4,0				C
MJ	M	1990	ICU	1	Survived	COPD					B
MF	M	1953	Rehabilitation	1	Survived	COPD					None
MZ	M	1947	ICU	1	Died	Lung cancer	5,0	4			None
MB	M	1946	ICU	1	Survived	COPD	13,0				None
NGy	M	1932	ICU	3	Died	Lung cancer	8,5				B
NI	M	1938	ICU	6	Survived	COPD	15,0				B-related
Oz	F	1939	ICU	1	Died	COPD					None
PSn	F	1944	Oncology	2	Survived	COPD					None
PJn	F	1942	ICU	1	Survived	COPD					B
PL	M	1941	ICU	10	Survived	COPD				22,0[b]	C
SLn	F	1942	ICU	1	Survived	Respiratory failure	7,0	1,6			C
SF	M	1948	ICU	1	Died	COPD	17,0				A
SzS	M	1957	ICU	5	died	COPD	18,0				B
SzG	M	1944	ICU	3	Survived	COPD	17,0				A and B
TJ	M	1940	ICU	1	Survived	COPD					B
VFn	F	1940	Endoscopy	1	Survived	Pneumonia					None

F = female; M = male.

[a] These patients only harboured *P. aeruginosa* isolates belonging to smaller clusters of with unique patterns.

[b] Inhalational use.

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sodium-lauril-sarcosil and 1 mg/mL Proteinase K (Fermentas). Plugs were incubated overnight in 100 mmol/L EDTA containing 0.2% sodium deoxycholate, 1% sodium lauril-sarcosil-sulphate and 1 mg/mL Proteinase K. On the following day, they were washed for 15 minutes with distilled water, then for 1 hour with Tris-EDTA buffer containing 0.35 mg/mL PMSF, and finally 3 times for 1 hour with Tris-EDTA buffer. Plugs were stored in Tris-EDTA at 4 °C. Before digestion with restriction enzyme, plugs were washed for 1 hour in the buffer recommended by the manufacturer. Digestion of plugs was performed with the enzyme SpeI using the buffer and conditions recommended by the manufacturer. Separation of fragments was performed in a CHEF DRIII machine (BioRad), in 1% SeaKem Gold agarose (Lonza) at 14 °C. The gel ran at 6 V/cm, with a reorientation angle of 120°, switch times were ramped between 5 s and 30 s for 15 hours followed by a 30 to 90 s ramping for 7 hours. Gels were stained with ethidium bromide and visualized under UV light. DNA banding patterns were analysed with the Fingerprinting II software (BioRad), using the Dice coefficient and the UPGMA method with an optimization of 0.5% and a position tolerance of 1–1.5%. Similarity of at least 80% was considered as the threshold of probable genetic relatedness to define clusters.

Isolates belonging to the same cluster were isolated from a patient at the ICU and later in another ward were considered to represent an exportation event. Conversely, importation is defined when a non-ICU isolate precedes a related isolate found in the ICU.

2.4. Aminoglycoside resistance and integron analysis

DNA was extracted by heat treatment; a loopful of bacteria was heated to 98 °C in TE buffer (100 mmol/L Tris, 10 mmol/L EDTA) for 15 minutes, and the supernatant was used as a template. Aminoglycoside modifying enzyme genes *aac(6′)-Ib*, *ant(2″)-Ia* and *aac(3)-IIa* were sought for using the methods described by Frana et al. (2001). Presence of *armA*, *rmtA*, and *rmtB* genes coding for aminoglycoside resistance methylases was tested using the PCR assays reported by Bogaerts et al. (2007). Integron carriage was assessed by the PCR assays of Mazel et al. (2000). Amplification and sequencing of the variable regions were performed for all integron-carrying isolates outside the clusters as well as for selected isolates representing the main PFGE clusters as described by White et al. (2001). Sequences were handled using CLC DNA Workbench 4.0 (CLC Bio), and genes were identified by a GenBank search. After restriction site identification, the identity of integrons within clusters A and B was confirmed by restriction fragment length polymorphism analysis of the variable regions using XbaI and XhoI (Fermentas), respectively, as recommended by the manufacturer.

2.5. Detection of virulence genes.

The genes of virulence factors exotoxin A (*toxA*), alginate (*algD*), type 3 secretion system effectors (*exoT*, *exoS*, *exoU*, *exoY*), phospholipases (*plcH*, *plcN*), proteases (*apr*, *lasB*), type IV pili (*pilA*), phenazin synthesis (*phzI*, *phzII*, *phzS*, *phzM*), and pyoverdin receptors (*fpvA-I*, *fpvA-II*, *fpvA-III*) were sought for by means of previously published PCR assays (de Chial et al., 2003; Finnan et al., 2004; Lanotte et al., 2004).

2.6. Antibiotic consumption.

Consumption of antibiotics (fluoroquinolones as a group, streptomycin, amikacin, gentamicin, and tobramycin) was expressed in the number of defined daily doses (DDDs) per 100 bed-days each year from 2005 to 2009 using the MS Excel application ABC Calc version 3.1. (Monnet 2006). Data on antibiotics used to treat individual patients were collected from the patient records and expressed in number of DDDs used. Cumulative administered aminoglycoside doses per patient are shown in Table 1. Patients were also grouped according to the genotype of *P. aeruginosa* harboured (clusters versus isolates outside the main clusters), and the differences in total consumption of antibiotics as well as in aminoglycoside consumption between these groups were analysed by Kruskal-Wallis test and post-hoc pairwise comparisons with Bonferroni correction using the Past 3.0 software (Hammer et al., 2001). The patient with multidrug-resistant tuberculosis (IJ) receiving prolonged treatment with amikacin and streptomycin was excluded from the statistical analysis.

3. Results

3.1. Aminoglycoside resistance and type of integrons

Out of the 6 aminoglycoside resistance genes sought, only the modifying enzyme genes *aac(6′)-Ib* and *ant(2″)-Ia* were found in the isolates (see below); *aac(3′)-IIa*, *armA*, *rmtA*, and *rmtB* genes were never detected. Only class I integrons were detected.

3.2. Genetic relatedness

Among the 98 isolates tested, 3 main similarity clusters (A-C) were identified; 22 further isolates belonged to 3 smaller clusters (2–3 isolates) or had unique patterns (Fig. 1.) Cluster A included 49 isolates from 12 patients; this cluster was present throughout almost the whole study period. Multiple patients carried the strain simultaneously during certain periods, and returning patients were found colonized at multiple hospitalization events. One isolate was isolated at the rehabilitation ward from a former ICU patient indicating exportation.

Antibiotic susceptibility of the isolates within the cluster was highly variable. While all isolates were uniformly resistant to ciprofloxacin, amikacin, gentamicin, and tobramycin, resistance to other agents was different sometimes even in case of isolates found in the same day, ranging from susceptibility to all beta-lactam agents tested to extensive resistance leaving colistine as the only therapeutic option. All isolates harboured a class I integron with a gene cassette array *aac(6′)-Ib*, bla_{PSE-1}, and *ant(3′)-Ia* (In99 according to the integron nomenclature at the Integrall database, http://integrall.bio.ua.pt). The cluster was characterized by presence of ferripyoverdin receptor type III and lack of *pilA* and *exoU* genes; all other virulence genes were found.

Cluster B included 16 isolates and was present throughout the study period. Though 9 isolates from a single patient dominated this cluster, it affected 11 patients altogether. Antibiotic susceptibility was characterized by ciprofloxacin, gentamicin, and tobramycin resistance, but resistance to beta-lactams was less extended as compared to cluster A; only 5 isolates were resistant to a beta-lactam agent, resistance to all antipseudomonal beta-lactams was not observed. These isolates also carried a class I integron harbouring a single *ant(2″)-Ia* gene (In159). Virulence gene composition was similar to that of cluster A, except for the presence of ferripyoverdin receptor type I instead of type III. Three further isolates were highly similar to this cluster, showing the same virulence profile and carrying the same integron (Fig. 1).

Cluster C included 8 isolates and was present at the ICU from March to December 2009 affecting 6 patients. One isolate (July 2009) was isolated from a patient in the pulmonology ward (potential importation/exportation). Antibiotic susceptibility of the cluster was characterized by imipenem resistance observed in all isolates, except the pulmonology ward isolate. Besides imipenem resistance, 3 isolates (from the same patient) showed a somewhat variable resistance to beta-lactams, ciprofloxacin, and aminoglycosides (Fig. 1). One of these isolates carried class I integrons carrying no gene cassettes (In0). Curiously, another isolate harboured an *aac(6′)-Ib* gene; other aminoglycoside resistance genes were not detected in the cluster. Except *exoS*, all virulence genes sought for were present.

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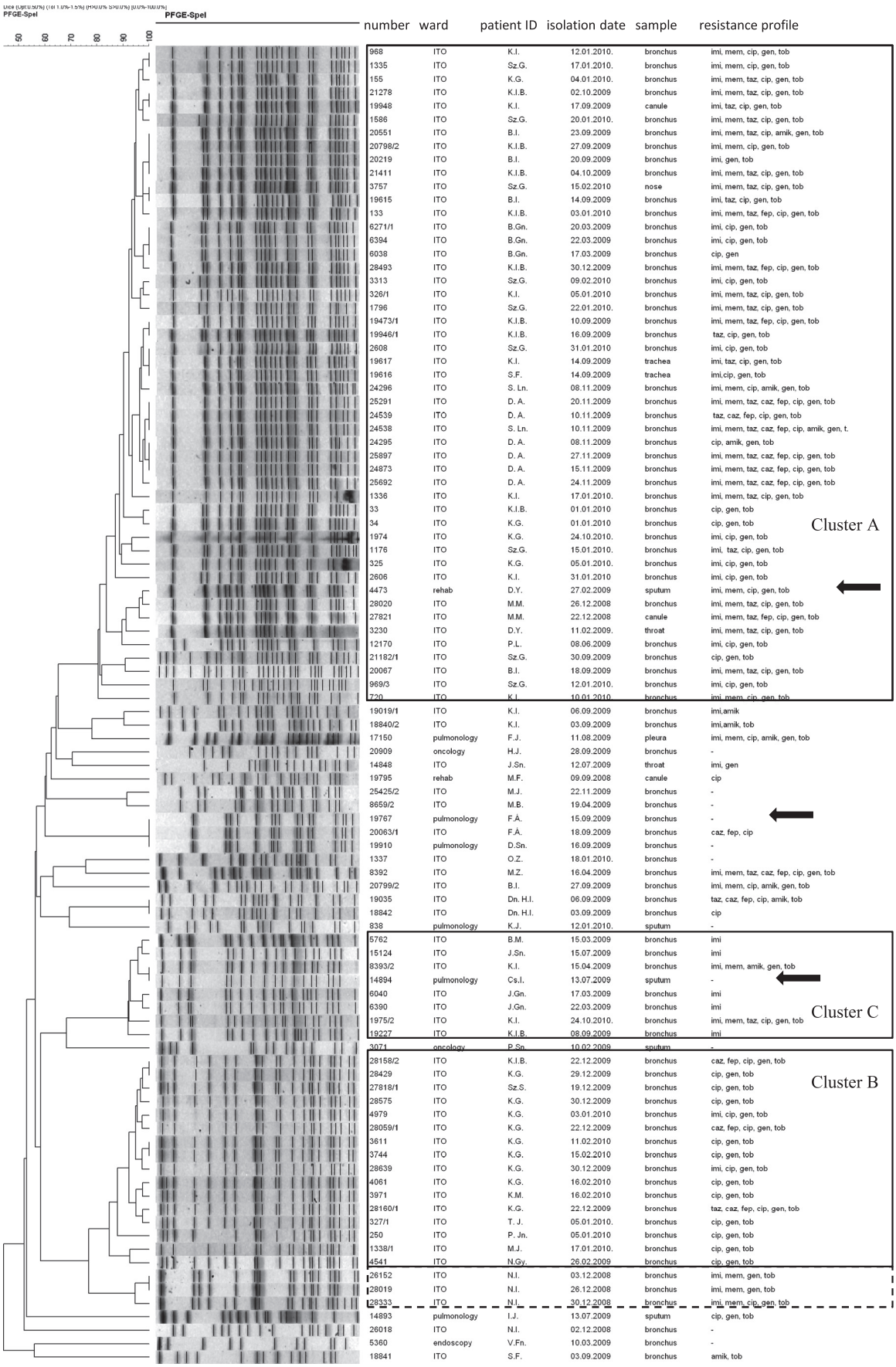

Fig. 1. PFGE patterns and clusters. Clusters are shown in frames; the additional 3 isolates aligned to cluster B are framed with a dashed line. Arrows point to potential importation/exportation events.

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Out of the 22 isolates found in 1 or 2 patients (small clusters and unique patterns), 9 were susceptible to all antipseudomonas antibiotics tested, and only 1 harboured aminoglycoside resistance genes, an *aac(6′)-Ib* gene. (Other aminoglycoside resistance genes were not detected in these 22 isolates.) A single isolate harboured an empty integron (In0). Virulence gene distribution was very heterogeneous; only *phzII*, *apr*, *exoT*, and *plcH* genes were present in all isolates; however, the other genes, except *pilA* and *exoU*, were found in the majority (>16) of isolates. A single isolate harboured all 15 tested virulence determinants.

Out of the 39 patients, 24 had at least 1 isolate belonging to one of the main clusters; all 24 were hospitalized in the ICU. Ten of the remaining 18 patients (with isolates with unique patterns or belonging to small clusters) were not admitted to the ICU. Ten patients had more than 1 strain; these sometimes were carried simultaneously, i.e., isolates belonging to the different strains were isolated only 1 to 3 days apart. In returning patients, both main clusters A and B harbouring class I integrons and aminoglycoside resistance genes were represented.

3.3. Antibiotic consumption

Antibiotic consumption of the pulmonology clinic between 2005 and 2009 was above the university average (97.76 versus 38.78 DDDs/100 bed-days); the most common antibiotics were macrolides, amoxicillin + clavulanic acid, and fluoroquinolones (average yearly consumptions were 29.78, 26.58 and 16.56 DDDs/100 bed-days, respectively). Aminoglycoside consumption similarly was above the university average, 7.46 (5.79–9.90) versus 1.89 (1.91–2.51); beta-lactam therapy was frequently potentiated by aminoglycosides. Aminoglycosides used included streptomycin, netilmycin, tobramycin, gentamicin, amikacin (average consumptions 0.26, 0.02, 1.42, 2.80, and 2.96 DDDs/100 bed-days). A switch from tobramycin to gentamicin in 2006–2007 and then partial replacement of gentamicin by amikacin in 2008–2009 were observed. Fluoroquinolone usage was relatively frequent, 16.58 (12.3–20.7) DDDs/100 bed-days by average, but a marked decrease and the lowest value were observed in 2009 (Fig. 2).

Out of the patients included in the study, only a single patient did not receive any antibiotics. Broad-spectrum beta-lactams (piperacillin + tazobactam and carbapenems) were the most frequently administered antibiotics. Amikacin was the most frequently used aminoglycoside; 19 of the 37 patients, i.e., 16 of the 28 ICU and 3 of the 11 non-ICU patients (one of them for multidrug-resistant tuberculosis) received amikacin (Table 1). Gentamicin was given to 3 patients only; streptomycin, only to the tuberculotic patient; and 1 patient received inhalational tobramycin. Other aminoglycosides were not used.

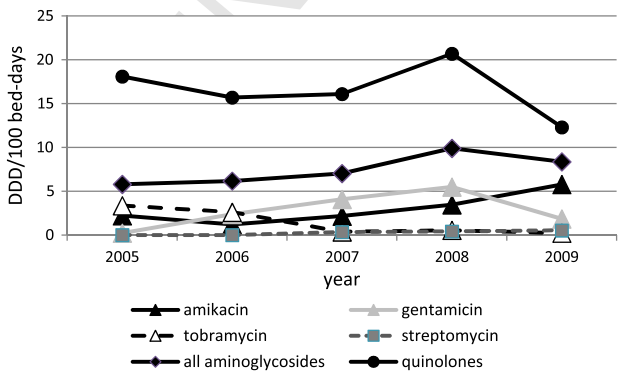

Fig. 2. Aminoglycoside and fluoroquinolone consumption rates of the Department of Pulmonology.

Statistical analysis demonstrated that patients harbouring isolates belonging to cluster A received significantly more amikacin than patients harbouring isolates from other clusters or with unique fingerprints ($P = 0.046$). As overall antibiotic consumptions (as well as consumption of beta-lactams and fluoroquinolones) were comparable among clusters ($P > 0.5$), this was not caused by increased use of antibiotics in general.

4. Discussion

Pattern of isolations and presence of *P. aeruginosa* strains for months suggest an endemic occurrence of clusters A and B, which were transmitted between multiple patients, recolonized returning patients, and persisted for the whole study period. Disappearance of any of the strains from the ICU was not confirmed during the study period. The majority of the ICU isolates (74/87) belonged to clusters A and B, which were present throughout the study period; isolates of cluster C were found less frequently but continuously. Transmission of strains between the ICU and other wards was also detected, but most non-ICU isolates showed unique patterns and were not clonal.

Similar endemic *P. aeruginosa* epidemiology was reported by Foca et al. (2000) in a neonatal intensive care unit, where the source was the healthcare personnel, and patients hospitalized for prolonged periods served as a putative reservoir. Similar findings were reported by Bergmans et al. (1998) and Suarez et al. (2011) in case of adult patients. As the environment repeatedly proved to be negative for *P. aeruginosa*, the reservoir of the strains must have been the healthcare personnel or the patients (e.g., patients KI and SzG for cluster A in January-February 2010 or patient KI for cluster B in the same period) in the present study as well. Cross-transmission between patients was reported to be the major route for colonization earlier (Bergmans et al., 1998; Boyer et al., 2011).

It is also interesting that *P. aeruginosa* isolates of the same patient isolated a few days apart were found to be genetically distinct. This may be explained by the simultaneous colonization by multiple strains (Ortega et al., 2004; Pirnay et al., 2003). Unfortunately, the proportion of patients colonized with multiple strains at the same time could not be assessed from the data in the present study. As routine susceptibility testing is based on a single colony of a homogeneous culture of a species, the possibility of simultaneous presence of multiple strains, possibly with different susceptibilities, must be acknowledged and considered by clinicians in treatment decisions. The impact of colonization with multiple strains on outcome is to be examined by upcoming studies.

Virulence pattern of the isolates was curious; it was not unequivocal that a successful strain (i.e., one that is present in multiple patients for prolonged periods) carries more virulence factors than sporadic isolates (isolated from only 1 or 2 samples of 1 or 2 patients), as could be expected. For example, both frequently isolated strains carried the gene *exoS* coding for a type 3 secretion system effector shown to be important for VAP pathogenesis (Berra et al., 2010), but not *exoU*, which is considered the most harmful effector for host cells (Berra et al., 2010; Veesenmeyer et al., 2009). In contrast, some sporadic isolates did carry *exoU*. Moreover, a sporadic isolate with a wider array of virulence factors than that of the endemic strains was also found. Though presence of a virulence gene is not necessarily equal to its expression in efficient amounts, present data suggest that virulence patterns are not in direct association with success as a colonizer or pathogen. Though one could argue that findings on colonizing isolates are not necessarily applicable to pathogenic isolates, it should also be considered that high mortality was reported in heavily colonized ventilated patients without clinically proven VAP (Zhuo et al., 2008).

Integron carriage is strongly associated with aminoglycoside resistance (Poole 2005). In accordance, the majority of isolates in

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the present study carrying a modifying enzyme gene tested was also class I integron positive.

Both of the 2 most prevalent clones carried integrons and integron-borne aminoglycoside resistance genes, but only 1 of 21 isolates not belonging to major clusters carried an integron, which, in turn, did not harbour any gene cassettes. High variability was observed regarding resistance to beta-lactam agents among isolates of clusters A and B; only aminoglycoside and ciprofloxacin resistance was common to all isolates. A probable explanation may be a synergistic effect of the integron-borne bla_{PSE-1} cassette and a putatively overexpressed efflux pump (cluster A) as reported by Peña et al. (2009) or overexpression of both the chromosomal *ampC* lactamase and efflux pumps (cluster B) as reported by Deplano et al. (2005). Though in many reports the suspicion of clonal spread is raised by a particular resistance pattern shared by the isolates (Hsueh et al., 2005; Lolans et al., 2005), these data show that isolates may be related even when this is not seen. In such situations, an epidemiologically important strain may not be identified, underlining the importance of surveillance studies in settings where *P. aeruginosa* is a frequent pathogen.

The association between aminoglycoside resistance and clonality suggests a role of aminoglycosides in the maintenance and/or spread of successful clones. The pattern of antibiotic consumption, i.e., steadily increasing amikacin usage in the clinic and the statistically significant association of amikacin administration and carriage of cluster A by the same patients points to the role of amikacin usage in the emergence and maintenance of cluster A with integron-borne resistance against amikacin and tobramycin. The role of antibiotic consumption in provocation of resistance is well-known (Hsueh et al., 2005; Iosifidis et al., 2008; Loeffler et al., 2003), and it was reported as a driving force for outbreaks as well (Falagas and Kopterides, 2006; Suarez et al., 2011). In accordance with the above assumption, antibiotic usage was shown to be a risk factor for cross-transmission in case of *P. aeruginosa* as well (Boyer et al., 2011).

The present report shows a complex dynamics of *P. aeruginosa*, characterized by continuous presence of at least 2 genetically unrelated clones suggesting intensive transmission, which were carrying aminoglycoside resistance genes on class I integrons in an endemic epidemiological situation. The data collected indicate that antibiotic usage may also be and sometimes may be the main driving force for maintenance of not only outbreaks but endemic strains as well. Antibiotic resistance in certain situations may be more important than virulence in spread of successful clones.

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