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

Julianna Mózes [a], Ildikó Szűcs [b], Dávid Molnár [a], Péter Jakab [a], Ebrahimi Fatemeh [a], Mária Szilasi [b], László Majoros [a], Piroska Orosi [c], Gábor Kardos [a,*]

[a] Department of Medical Microbiology, Medical and Health Science Center, University of Debrecen, H-4032 Debrecen Nagyerdei krt. 98, Hungary
[b] Department of Pulmonology, Medical and Health Science Center, University of Debrecen, H-4032 Debrecen Nagyerdei krt. 98, Hungary
[c] Department of Hospital Hygiene and Infection Control, Medical and Health Science Center, University of Debrecen, H-4032 Debrecen Nagyerdei krt. 98, Hungary

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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*, $bla_{PSE\text{-}1}$, 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.

* Corresponding author. Tel.: +36-52-255-425; fax: +36-52-255-424.
E-mail address: kg@med.unideb.hu (G. Kardos).

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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*, extended-spectrum beta-lactamase-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

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 or with unique patterns.
[b] Inhalational use.