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Spread of Multidrug-Resistant *Pseudomonas aeruginosa* Clones in a University Hospital

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An outbreak of multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) infections in a university hospital is described. Phenotypic and genotypic analysis of 240 isolates revealed that 152 patients, mainly in the intensive care unit (ICU), were colonized or infected with MDRPA, the majority with O11. All metallo-β-lactamase (MBL)-positive isolates carried the bla_{VIM-2} or bla_{VIM-1} gene. One or more type III secretion system toxin genes were detected in most isolates. Five dominant pulsed-field gel electrophoresis (PFGE) types were characterized, associated with ST235, ST111, ST253, ST309, and ST639.

Pseudomonas aeruginosa is an opportunistic pathogen causing severe invasive disease in critically ill and immunocompromised patients. Because of its ubiquitous nature, ability to survive in moist environments, and innate resistance to many antibiotics and antiseptics, it constitutes a common pathogen in hospitals, particularly in intensive care units (ICUs). Its treatment is a therapeutic challenge because of the intrinsic resistance and the ability to easily acquire resistance determinants (1). Multidrug-resistant *P. aeruginosa* (MDRPA) infections occur mainly in ICU patients (2). The prevalence and epidemiology of MDRPA have become the focus of numerous single- and multicenter surveillance studies (3). The large number of secreted and cell-associated virulence factors is implicated in the pathogenesis of severe infections. The type III secretion system (TTSS), a complex of three proteins, is associated with lung injury, sepsis, and a 6-fold-greater risk of mortality, constituting an important virulence determinant (4, 5). Results on emergence and spread of MDRPA isolates in the University Hospital of Patras (UHP) and their phenotypic and genotypic characteristics are presented in this study.

During a 2-year period, a total of 952 *P. aeruginosa* isolates were recovered from 430 patients hospitalized in our tertiary-care hospital, located in southwestern Greece, with 700 acute-care beds and about 100,000 admissions annually. Two hundred and forty, the first 10 from every month with no replicate isolates (one isolate per patient), from different wards and a variety of clinical specimens, including true infections and carriage, were selected for further study. Colonizing isolates were recovered from stool and respiratory tract specimens from patients without signs of infection.

P. aeruginosa was identified by standard methods. Colonizing (83) and infection-related (157) isolates were compared for their phenotypes and genotypes. For further analyses, isolates were divided into two groups: those recovered from ICU patients (92) and those from non-ICU patients (148).

Antibiotic susceptibility testing was performed by the agar disk diffusion method against antipseudomonal agents according to CLSI guidelines (6). All isolates resistant to at least three classes of antibiotics were defined as MDRPA (7). The MIC of colistin was determined by the Etest (AB Biodisk, Solna, Sweden). All imipenem-nonsusceptible (IMP-NS) isolates (MICs $>$ 1 mg/liter) were examined for metallo-β-lactamase (MBL) production using the Etest MBL assay (AB Biodisk).

Serotyping was performed by 16 monovalent antisera (Bio-Rad, Marne's-la-Coquette, France) as previously described (8).

Among the IMP-NS isolates, the bla_{VIM} gene was detected using the multiplex PCR-enzyme-linked immunosorbent assay (ELISA) system (hyplex MBL ID PCR module Hyb-module test system; BAG Health Care, Lish, Germany) (9). Types of bla_{VIM} genes were identified by sequencing analysis among selected bla_{VIM}-positive strains after comparison with a data bank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In all isolates, TTSS genes (*exoS*, *exoT*, *exoU*, *exoY*) were investigated by PCR (10).

Clones were defined by pulsed-field gel electrophoresis (PFGE) of *speI* (Roche, Penzberg, Germany) DNA digests. Banding patterns were compared by Fingerprinting II Informatics Software (Bio-Rad, Berkeley, California), and clones were defined according to already established criteria (11). A dendrogram comparing molecular weights of strains' DNA fragments to those from a previous collection by using FPQuest software (Bio-Rad; catalog number 1709300) was computed. Clustering was based on ≥75% similarity. Selected strains of the main PFGE types were characterized by multilocus sequence typing (MLST) (http://pubmlst.org/paeruginosa).

Pearson's chi-square test was used to evaluate the differences in the frequencies of variables in ICU and non-ICU wards, conducted by PASW Statistics 18, release version 18.0.0 (SPSS, Inc., Chicago, IL; www.spss.com). Results were considered significant at a P value of ≤0.05.

A comparison of nonsusceptibility to antibiotics between ICU and non-ICU *P. aeruginosa* isolates is presented in Fig. 1. All isolates were susceptible to colistin (MICs ≤ 2 mg/liter). During the study period, a high frequency of MDRPA was detected (152/240 isolates; 63.33%), mainly in ICU isolates (Table 1). There were no

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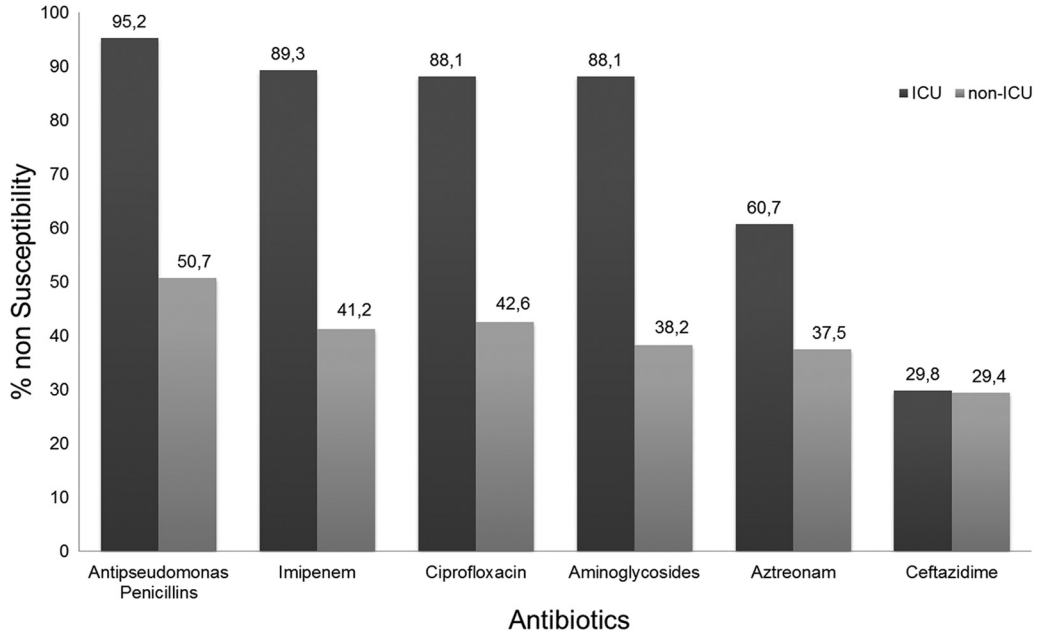

FIG 1 Percentages of *P. aeruginosa* isolates nonsusceptible to various classes of antibiotics among ICU and non-ICU patients. Antipseudomonas penicillins include azlocillin, carbenicillin, piperacillin, and ticarcillin-clavulanic acid. Aminoglycosides include amikacin, netilmicin, and tobramycin.

statistically significant differences in antibiotic resistance patterns between infecting and colonizing isolates. A bla_{VIM} gene was detected in all 49 MBL-positive isolates. No statistically significant difference was observed between ICU and non-ICU isolates (17.4% versus 22.3%, respectively) (Table 1). The majority of the isolates carried bla_{VIM-2}, while only three strains carried the bla_{VIM-1} gene.

Serogroup O11 predominated in the ICU compared to all other wards (Table 1) as well as among MDRPA isolates (75% [63/84] in the ICU and 57.4% [39/68] in non-ICU wards [$P = 0.021$]).

The majority of isolates (227/240; 94.6%) carried one or more toxin genes, while only 79 (33%) carried all four. Ninety-one isolates (91/240; 37.9%) carried both *exoU* and *exoS* genes. *exoU* was detected mainly among ICU patients, while *exoS* from non-ICU patients was found with a statistically higher frequency (Table 1). Also, the *exoS* gene was statistically significant among infection isolates compared to carriage (53.50% versus 31.32%; $P = 0.001$).

PFGE exhibited five predominant pulsotypes (Table 1; Fig. 2). PFGE type a strains were identified as ST235; type b strains carrying the bla_{VIM-1} gene belonged to ST111, while those carrying bla_{VIM-2} were identified as ST235; type c strains were ST253 and type d strains were ST235, while those of type s were classified as ST309 and ST639. The remaining isolates were classified into 78 PFGE types including 1 to 3 strains each. Polyclonality was observed mainly among non-ICU strains (Table 1). Common clones among infecting and colonizing isolates were identified. The MDRPA strains, including MBL-positive strains, belonged mainly to pulsotypes a and d, both characterized as ST235.

Surveillance studies have documented increases in the frequency of outbreaks, especially in ICU infections caused by strains resistant to multiple classes of antibiotics (12, 13). *P. aeruginosa* was related to 8% of total infections and carriage during the study period, while among ICU patients, an outbreak occurred due to the spread of one main clone (ST235) of MDRPA. In our hospital setting, MDRPA accounted for 49.5% of *P. aeruginosa* infections before the present study, reached 63.3% during the studied period, and dropped afterwards to 38.5%.

A higher prevalence of MBL production was observed among IMP-NS *P. aeruginosa* isolates (33%) than in other studies from Greece and other European countries (14, 15). All MBL-positive isolates in the present study carried the bla_{VIM} gene and were spread in all hospital wards, especially among non-ICU patients (Table 1). VIM-type MBLs are predominant in Europe, particularly in the Mediterranean region, and have been associated with large outbreaks of MDRPA (3, 16, 17). More specifically, bla_{VIM-2}

TABLE 1 Characteristics of ICU and non-ICU *P. aeruginosa* isolates[a]

Characteristic	No. (%) of isolates with each characteristic		*P* value
	ICU isolates ($n = 92$)	Non-ICU isolates ($n = 148$)	
MDRPA	84 (91.3)	68 (46.0)	<0.001
MBL positive	16 (17.4)	33 (22.3)	0.359
Serotype O11	68 (73.9)	50 (33.8)	<0.001
bla_{VIM}	16 (17.4)	33 (22.3)	0.359
exoS	25 (27.2)	86 (58.1)	<0.001
exoT	76 (82.6)	116 (78.4)	0.427
exoU	83 (90.2)	118 (79.7)	0.032
exoY	81 (88.0)	125 (84.5)	0.438
Clone a (ST235)	59 (64.1)	22 (14.9)	<0.001
Clone d (ST235)	15 (16.3)	18 (12.2)	0.365
Clone b (ST111 or ST235)	3 (3.3)	7 (4.3)	0.745
Clone c (ST253)	1 (1.0)	5 (3.4)	0.410
Clone s (ST309 or ST639)	0 (0.0)	6 (4.0)	0.084
All other clones	14 (15.2)	90 (60.8)	<0.001

[a] ICU, intensive care unit; MDRPA, multidrug-resistant *P. aeruginosa*; MBL, metallo-β-lactamase.

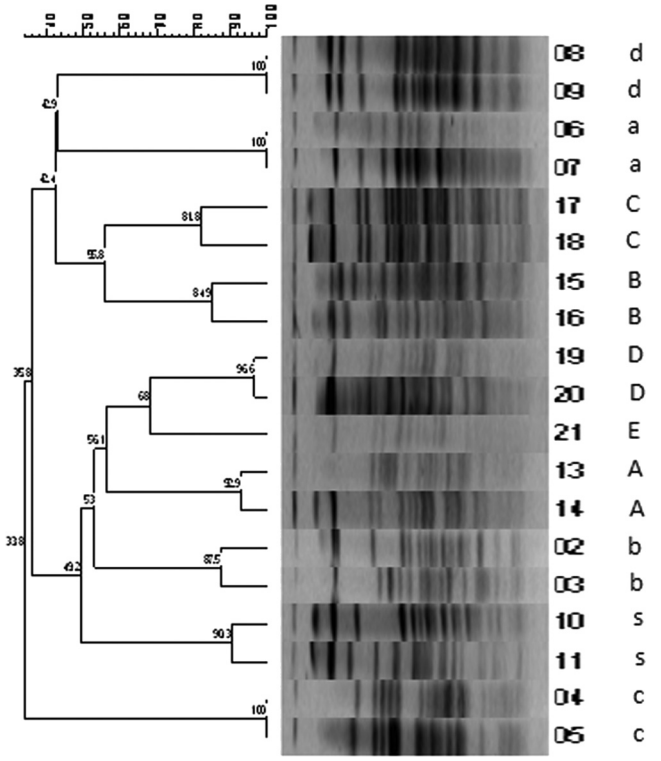

FIG 2 Dendrogram of *P. aeruginosa* isolates after digestion of DNA with SpeI and PFGE. Comparison of clonal types identified in the present study with previous ones, recovered from patients in the same hospital. No relationship was detected between the recent and the older clones. Lines 8, 9, 6, 7, 2, 3, 10, 11, 4, and 5 are from the present study (clones d, d, a, a, b, b, s, s, c, and c, respectively). Lines 17, 18, 15, 16, 19, 20, 21, 13, and 14 are from a previous study (clones C, C, B, B, D, D, E, A, and A, respectively).

is the most frequent type in southern European countries (3), while in Greece, bla_{VIM-17}, a variant of bla_{VIM-2}, was identified in another outbreak (16).

Serotype O11 is common in hospital outbreaks and associated with multidrug resistance (2, 12), as is also shown in the present study.

In our collection, 94.6% of *P. aeruginosa* isolates carried one or more TTSS genes, as reported elsewhere (18). *exoS* was more frequent in isolates from urinary tract and wound infections from non-ICU patients, a finding that is in accordance with those by other investigators (18). *exoU* was associated with serotype O11, as reported also by Faure et al. (4), and detected mainly among ICU isolates ($P = 0.032$). Expression of *exoU* correlates with acute cytotoxicity and accelerated lung injury playing a role in the development of septic shock in ICU high-risk patients (18, 19).

MDRPA strains belonged mainly to PFGE types a and d of serotype O11 and ST235. A comparison of the recently identified clones with previous ones revealed no relationship (Fig. 2) (20). Studies have reported clonally related nosocomial outbreaks of MDRPA producing IMP-13 MBL (13) and panantibiotic-resistant *P. aeruginosa* in ICUs (12). The observation that the majority of pulsotype d strains (ST235) carry the bla_{VIM-2} gene reinforces the theory that clonal spread may have played a role in the outbreak of IMP-NS *P. aeruginosa*. bla_{VIM} gene spread was identified among clonally related strains in the last decade (1, 17, 21).

The observation that most carriage isolates belonged to the two predominant PFGE types a (38/83) and d (11/83) and to the same clone, ST235, indicates that colonization during ICU hospitalization contributes to infection and spread to other wards. Clinical isolates of ST235 (serotype O11) harboring acquired β-lactamases have been reported worldwide (22). *P. aeruginosa* ST235 (serotype O11) strains from bloodstream infections were among the three predominant epidemic clones in the Czech Republic (22).

This study describes a clonal outbreak during a 2-year period of MDRPA serotype O11 of the ST235 clone in a university hospital which occurred mainly in the ICU. *P. aeruginosa* clearly represents one of the most challenging pathogenic bacteria, since MDRPA isolates spread clonally quite frequently. The monitoring of MBL- and exotoxin gene-carrying isolates has epidemiological significance in the identification of drug-resistant and virulent *P. aeruginosa* isolates, especially in high-risk patients. Our work shows the need for clonal identification, since MDRPA outbreaks require targeted infection control measures.

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The authors have no conflict of interest to declare.

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