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Sequence Types 235, 111, and 132 Predominate among Multidrug-Resistant *Pseudomonas aeruginosa* Clinical Isolates in Croatia

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A population analysis of 103 multidrug-resistant *Pseudomonas aeruginosa* isolates from Croatian hospitals was performed. Twelve sequence types (STs) were identified, with a predominance of international clones ST235 (serotype O11 [41%]), ST111 (serotype O12 [15%]), and ST132 (serotype O6 [11%]). Overexpression of the natural AmpC cephalosporinase was common (42%), but only a few ST235 or ST111 isolates produced VIM-1 or VIM-2 metallo- β -lactamases or PER-1 or GES-7 extended-spectrum β -lactamases.

Pseudomonas aeruginosa is a major nosocomial pathogen causing infections associated with high mortality rates (1). In addition to intrinsic antimicrobial resistance, it readily acquires resistance to antipseudomonal drugs; of ultimate concern is the increasing prevalence of multidrug-resistant (MDR) strains (1, 2). The MDR phenotype develops mainly through accumulation of mutations affecting efflux, cell permeability, antimicrobial target sites, and expression of the natural β -lactamase AmpC; of note also is the acquisition of resistance genes, including those encoding β -lactamases such as metallo- β -lactamases (MBLs) and class A or class D extended-spectrum β -lactamases (ESBLs) (1, 2). Such genes may spread further horizontally, and their presence promotes clonal dissemination (3–7). The development of multilocus sequence typing (MLST) (8) has intensified the epidemiological analysis of *P. aeruginosa*. Studies on selected strains and regional or national analyses have shown that several successful clones are responsible for the spread of MDR *P. aeruginosa* (3, 9–14), often associated with MBLs and/or ESBLs (12, 14, 15). Our main aim was to describe the clonal structure of MDR *P. aeruginosa* subpopulations circulating in Croatian health care institutions.

The study included 103 consecutive nonduplicate MDR *P. aeruginosa* clinical isolates identified through the network of the Croatian Committee for Antibiotic Resistance Surveillance (16). Between 1 October and 31 December 2008, the laboratories collected all isolates that were not susceptible to at least one agent in 3 or more drug categories, and they sent these isolates to the Reference Centre for Antibiotic Resistance Surveillance in Zagreb, Croatia. The isolates were from 16 laboratories in 9 cities (catchment population, 43.2% of total population), and the majority were from 8 sites in the Zagreb area (47 isolates [45.6%]). The isolates were mainly from elderly patients (median age, 65 years) in internal medicine wards or intensive care units (60.4% of isolates) and were cultured mostly from urine and respiratory tract samples (46.6% and 28.2%, respectively). Susceptibility of the isolates was evaluated by broth microdilution testing according to CLSI guidelines (17). For isolates resistant to all β -lactams, aminoglycosides, and fluoroquinolones, susceptibility to colistin was determined by Etest (bioMérieux, Marcy l'Etoile, France). Almost all isolates were nonsusceptible to at least one cephalosporin and one aminoglycoside (98.0% and 99.0%, respectively) (Table 1). Rates of nonsusceptibility to fluoroquinolones and carbapenems

were also high (90.0% and 83.5%, respectively); 16 isolates (15.5%) were susceptible to colistin only.

The isolates were typed by pulsed-field gel electrophoresis (PFGE) (18), using XbaI and SpeI restriction enzymes separately (Fermentas, Vilnius, Lithuania). PFGE types and subtypes were discerned visually using the criteria described by Tenover et al. (19). In order to construct dendrograms, banding patterns were analyzed with BioNumerics (version 6.01; Applied Maths, Sint-Martens-Latem, Belgium), using the Dice coefficient and clustering by the unweighted pair-group method with arithmetic means (1% tolerance in band position differences). With XbaI, the isolates were classified into 17 pulsotypes (Table 1), with the majority of isolates (53.9%) being clustered into 3 types, i.e., I, B, and H (21, 19, and 15 isolates, respectively). The SpeI analysis discerned 30 types that correlated well with those determined with XbaI (Table 1). Thirty isolates of all SpeI types were subjected to MLST (8); the *P. aeruginosa* MLST database (<http://pubmlst.org/paeruginosa>) was used to assign sequence types (STs). Twelve STs were identified (Table 1; also see Fig. S1 in the supplemental material). The most prevalent ST was ST235, with 42 isolates of 4 XbaI types (including types I and B) from 8 cities. ST111 comprised 15 more-homogeneous isolates (type H) from 6 cities, while ST132 included 11 isolates from 2 cities. The remaining 34 isolates were assigned to 9 STs, with 4 STs having single isolates. One of these, ST966, was a new single-locus variant (SLV) of ST111, classified into clonal complex 111 (CC111). Serotyping was performed using monoclonal O antigen-specific sera (Institute of Immunology, Zagreb, Croatia). Six serotypes were discerned (Table 1), and the 3 major clones, ST235, ST111, and ST132, represented serotypes O11, O12, and O6, respectively, as in previous studies (10, 11, 15).

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TABLE 1 STs, serotypes, PFGE types, susceptibility to selected antimicrobials, and sites of isolation of *P. aeruginosa* isolates

ST	Serotype	PFGE XbaI type (no. of isolates) ^a	MIC (μg/ml) of ^b :								Site(s) ^c (no. of isolates)	
			TZP	CAZ	FEP	IPM	MEM	GEN	AMK	CIP		
235 (n = 42)	O11	I ₁₋₁₂ (21)	T ₁₋₁₁ (11), I ₁₋₄ (4), F ₁₋₂ (3), S ₁₋₂ (2), AD (1)	16 to >256	16 to >128	16 to >128	0.5–1, 8–32	2–32	8 to >256	16–256	≥32	CK (2), KC (3), PU (1), RI (1), VZ (3), ZD (1), ZG1 (2), ZG2 (2), ZG3 (2), ZG4 (2), ZG7 (1), ZG8 (1)
1111 (n = 15)	O12	B ₁₋₁₀ (19)	B ₁₋₇ (13), W ₁₋₂ (2), Y ₁₋₂ (2), V (1), X (1)	16 to >256	16 to >128	16 to >128	0.5–1, 8–128	4 to >64	32 to >256	16–128	8 to >32	RI (8), SB (4), VZ (2), ZD (1), ZG1 (1), ZG3 (2), ZG4 (1)
		N (1)	N (1)	32	>128	32	0.5	4	>256	128	32	ZG7
		P (1)	AB (1)	16	16	16	8	>64	32	32	16	VZ
		H ₁₋₁₁ (15)	U ₁₋₄ (5), Z ₁₋₅ (5), H ₁₋₂ (3), Q (1), AC (1)	64 to >256	4 to >128	32 to >128	2–32	0.5–32	32 to >256	0.5–32	0.125, 2–32	CK (1), RI (2), VT (1), VZ (1), ZD (1), ZG1 (6), ZG2 (3)
132 (n = 11)	O6	L ₁₋₄ (7)	L ₁₋₅ (5), P ₁₋₂ (2)	≥256	8–128	64–128	16–32	32–64	128 to >256	16	32 to >32	ZG1 (1), ZG2 (2), ZG3 (1), ZG5 (2), ZG6 (1)
		E ₁₋₃ (3)	E ₁₋₃ (3)	≥256	64–128	64	4–32	8–32	128–256	8–32	32	ZG1 (1), ZG5 (1), ZG6 (1)
621 (n = 9)	O4	O (1)	O (1)	256	128	64	32	32	>256	32	>32	VZ
		C ₁₋₅	C ₁₋₇	64 to >256	4 to >128	8 to >128	1–2, 16–32	0.5 to >64	≥256	8–64	≥32	KC (2), PU (1), RI (3), ZG1 (1), ZG2 (1), ZG3 (1)
244 (n = 8)	O5	A	A ₁₋₅	16, 128–256	8, 64	8–32	16–32	16–32	32–128	1–8	0.125–1, 16–32	RI (6), ZG3 (2)
292 (n = 8)	O12	D ₁₋₃	D ₁₋₂	32–256	32–64	16–64	8–16	32–64	128–256	8–16	≥32	VZ (3), ZG1 (2), ZG3 (1), ZG4 (1), ZG5 (1)
319 (n = 3)	O11	Q	R	128–256	64–128	32–64	16	32	4	8	2–4	ZD
		M	M	128	8–16	16, 64	16, 128	16, 64	>256	2, 128	32	RI
		F	AA	64	32	32	16	32	2	4	4	CK
		J	J	8	8	32	16	8	64	128	4	ZG1
633 (n = 1)	O6	G	G	256	128	64	16	16	>256	8	0.25	ZG4
		K	K	256	128	64	4	2	16	64	1	CK
MIC ₅₀				128	32	32	16	32	128	16	32	

^a XbaI and SpeI PFGE types were designated by letter symbols independently of each other.
^b TZP, piperacillin-tazobactam; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin. The MICs of netilmicin and norfloxacin are not shown for conciseness. Ranges of MICs refer to the situations in which all MICs between the two values were demonstrated by the study isolates. Two sets of MIC values indicate situations where the isolates of the same ST did not have all values in a range, e.g., ST244 isolates had CIP MICs of 0.125, 0.25, 0.5, 1, 16, and 32 (MICs of 2, 4, and 8 were not demonstrated).
^c CK, Cakovec; KC, Koprivnica; PU, Pula; RI, Rijeka; SB, Slavonski Brod; VT, Virovitica; VZ, Varazdin; ZD, Zadar; ZG, Zagreb (ZG1 to ZG8 indicate 8 different hospitals in Zagreb).
^d NT, nontypeable.

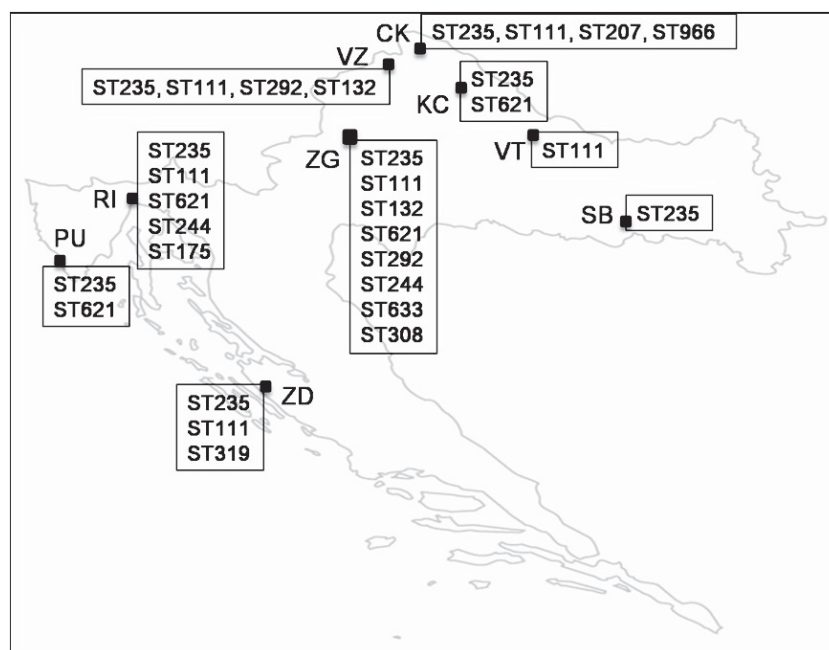


FIG 1 Distribution of clones detected among MDR *P. aeruginosa* clinical isolates in Croatia. CK, Cakovec; KC, Koprivnica; PU, Pula; RI, Rijeka; SB, Slavonski Brod; VT, Virovitica; VZ, Varazdin; ZD, Zadar; ZG, Zagreb.

ST235, ST111, and ST132 accounted for 66.7% of the isolates, and a similar predominance of a few clones in MDR *P. aeruginosa* was observed in other countries (3, 11, 14, 20, 21). ST235 was the most common MDR clone (40.8%), spread mainly by 2 pulsotypes all over Croatia (Fig. 1). It has been ubiquitous worldwide and is associated with various resistance traits, including β -lactamases (3, 9–13, 22–25). In national surveys, ST235 was the most prevalent MDR clone in Japan ($\geq 58.5\%$) and the Czech Republic (39%) (11), for example, and the second most prevalent in France (22%) (3). Enormous spread of MBL-producing ST235 has been observed in Russia over the 2000s (14). ST111 and CC111 have often been reported as well (3, 11, 15, 20, 22, 26, 27). ST111 has been prevalent among MDR *P. aeruginosa* isolates in France (11%) (3) and MBL producers in Greece; recently, MBL-positive ST111 has spread in the Netherlands (27). Its prominent role among MDR isolates in Croatia (15.5%) is due to the broad dissemination of a single pulsotype. The ST132 frequency in our work (10.7%) was comparable to that in the Czech study (10%) (11). Recently, *P. aeruginosa* isolates (82% MDR) from a hospital in Split, a Croatian city not included here, were analyzed; ST111 and ST235 were predominant, and ST111 was the most prevalent clone (22).

Three β -lactamase-mediated resistance mechanisms were analyzed, using all of the study isolates. Derepression of the natural AmpC enzyme (1, 2) was assessed by real-time PCR, using our own primers (Table 2), and interpreted as described previously (28). Forty-three isolates (41.7%) of various clones showed at least 10-fold higher levels of *ampC* mRNA than *P. aeruginosa* PAO1 (data not shown), indicating common overproduction. Class A ESBLs were detected by the double-disk synergy test (DDST), using ceftazidime as the substrate and clavulanate and imipenem as

TABLE 2 Primers used in this study

Gene	Primer name	Sequence (5' to 3')	Source or reference
<i>rpsL</i>	rpsL-1	CCTGCTTACGGTCTTTGA	This study
	rpsL-2	TACATCGGTGGTGAAGGT	
<i>ampC</i>	ampC-1	ATACCAGATTCCCCTGCC	This study
	ampC-2	CTGAAGTAATGCGGTTCTC	
<i>mexA</i>	mexA-1	CATGCGTGTACTGGTTCCG	This study
	mexA-2	TTGAGGATGATGCCGTTTAC	
<i>mexC</i>	mexC-1	GGGTGAAATCCGCATAGAT	This study
	mexC-2	CAGCAGGACTTCGATACCG	
<i>mexE</i>	mexE-1	AACAGTCATCCCCTTCTC	This study
	mexE-2	AATTCGTCCCCTCGTTC	
<i>mexX</i>	mexX-1	CAGGTCGGAGAACAGCAG	This study
	mexX-2	GATCTACGTGAACCTCTCCC	
<i>oprD</i>	oprD-F	CGCCGACAAGAAGAACTAGC	39
	oprD-R	GTGATTACAGGATCGACAG	This study
	oprD-F2	GCCGACCACCGTCAAATCG	
	oprD-R2	AAGTGGTGTGCTGATGTC	
<i>gyrA</i>	gyrA-F	AGTCCTATCTCGACTACGCGAT	40
	gyrA-R	AGTCGACGGTTTCTTTTCCAG	40
<i>parC</i>	parC-F	CGAGCAGGCTATCTGAACTAT	
	parC-R	GAAGGACTTGGGATCGTCCGGA	

TABLE 3 Sequencing analysis of *oprD*, *gyrA*, and *parC* genes^a

Isolate no. ^b	ST	IPM MIC (μg/ml)	OprD size	Amino acid polymorphisms in OprD ^c	Amino acid insertions/deletions in OprD ^{c,d}	CIP MIC (μg/ml)	Amino acid change in GyrA	Amino acid change in ParC
8	111	2	441 ^e	V127L, ^h E185Q, ^h P186G, ^h V189T, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h T276A, ^h A281G, ^h K296Q, ^h Q301E, ^h R310E, ^h G312R, ^h A315G, ^h L347 M, ^h S403A, ^h Q424E	Loop L7-short	NP	ND	ND
16	111	32	441 ^e	V127L, ^h E185Q, ^h P186G, ^h V189T, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h T276A, ^h A281G, ^h K296Q, ^h Q301E, ^h R310E, ^h G312R, ^h A315G, ^h L347 M, ^h S403A, ^h Q424E	Loop L7-short	NP	ND	ND
6	111	32	18 ^f	Q19STOP, ^g V127L, ^h E185Q, ^h P186G, ^h V189T, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h T276A, ^h A281G, ^h K296Q, ^h Q301E, ^h R310E, ^h G312R, ^h A315G, ^h L347M, ^h S403A, ^h Q424E ^h	Loop L7-short ^h	NP	ND	ND
33	111	32	109 ^f	V127L, ^h E185Q, ^h P186G, ^h V189T, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h T276A, ^h A281G, ^h K296Q, ^h Q301E, ^h R310E, ^h G312R, ^h A315G, ^h L347M, ^h S403A, ^h Q424E ^h	1-bp deletion at codon 108 (nt 323), stop codon 110, loop L7-short ^h	NP	ND	ND
20	132	32	>443 ⁱ	D43N, ^h S57E, ^h S59R, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h A267S, ^h A281G, ^h K296Q, ^h Q301E, ^h R310G, ^h V359L	Loop L7-short, 1-bp insertion at codon 402 (nt 1206), undetected stop codon	NP	ND	ND
25	132	32	>443 ⁱ	D43N, ^h S57E, ^h S59R, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h A267S, ^h A281G, ^h K296Q, ^h Q301E, ^h R310G, ^h V359L	Loop L7-short, 1-bp insertion at codon 402 (nt 1206), undetected stop codon	NP	ND	ND
30	175	128	>443 ⁱ	D43N, ^h S57E, ^h S59R, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h A267S, ^h A281G, ^h K296Q, ^h Q301E, ^h R310G, ^h V359L	Loop L7-short, 1-bp insertion at codon 402 (nt 1206), undetected stop codon	32	T83I	S87W
26	292	8	441 ^e	D43N, ^h S57E, ^h S59R, ^h E202Q, ^h I210A, ^h E230K, ^h S240T, ^h N262T, ^h A267S, ^h A281G, ^h K296Q, ^h Q301E, ^h R310G, ^h V359L	Loop L7-short	NP	ND	ND
2	235 (VIM-1)	128	>443 ⁱ	T103S, ^h K115T, ^h F170L, ^h E185Q, ^h P186G, ^h V189T, ^h R310E, ^h A315G	1-bp insertion at codon 402 (nt 1206), undetected stop codon	NP	ND	ND
29	235	0.5	443	T103S, ^h K115T, ^h F170L, ^h E185Q, ^h P186G, ^h V189T, ^h R310E, ^h A315G, ^h G425A		NP	ND	ND
59	235	16	232 ^f	T103S, ^h K115T, ^h F170L, ^h E185Q, ^h P186G, ^h V189T, ^h R310E, ^h A315G, ^h G425A ^h	13-bp deletion at codon 137 (nt 410–422) and 3-bp deletion at codon 142 (nt 425–427), stop codon 233	NP	ND	ND
61	235	32	64 ^f	W65STOP, ^g T103S, ^h K115T, ^h F170L, ^h E185Q, ^h P186G, ^h V189T, ^h R310E, ^h A315G, ^h G425A ^h		NP	ND	ND
64	244	32	137 ^f	W138STOP ^g		0.125		
74	308	16	— ^f	T103S, ^h K115T, ^h F170L, ^h E185Q, ^h P186G, ^h V189T, ^h R310E, ^h A315G, ^h G425A ^h	ISRP10 insertion inside codon 69 (nt 205)	NP	ND	ND
23	633	NP	ND	ND	ND	0.25		
99	319	NP	ND	ND	ND	2	D87N	
1	111	NP	ND	ND	ND	4	T83I	
73	207	NP	ND	ND	ND	4	T83I	
49	235	NP	ND	ND	ND	8	T83I	S87L
4	235	NP	ND	ND	ND	32	T83I	S87L
45	292	NP	ND	ND	ND	32	T83I	S87L
48	244	NP	ND	ND	ND	32	T83I	S87L
75	621	NP	ND	ND	ND	>32	T83I	S87W
84	132	NP	ND	ND	ND	>32	T83I	S87L

^a IPM, imipenem; CIP, ciprofloxacin; NP, not presented; ND, not determined; nt, nucleotide(s).
^b The isolates used in the OprD analysis are ordered according to their STs, whereas the isolates used in the GyrA/ParC analysis are ordered according to increasing ciprofloxacin MICs; isolates 30 and 64 were used in both analyses and are shown in the OprD order.
^c Amino acid polymorphisms represent differences of the sequences obtained in this study from that of the *P. aeruginosa* PAO1 strain (GenBank accession no. AE004091).
^d Loop L7-short indicates modification of the amino acid stretch between position 372 and position 383 from MSDNNVGYKNG to VDSSSSYAGL caused by several nucleotide changes (35).
^e The size of 441 amino acids is due to loop L7-short; the original stop codon is retained.
^f OprD protein size reduced by premature stop codons generated by point mutations.
^g Stop codons generated by point mutations.
^h Theoretical polymorphisms and loop L7-short, located either behind premature stop codons and/or within frames altered by insertions/deletions.
ⁱ The size of >443 indicates situations in which insertions at the end of the *oprD* gene caused frame shifts and extension of the altered frames.
^j —, disruption of *oprD* by ISRP10 caused massive changes in the sequence; therefore, the size of a putative altered protein was not calculated.

inhibitors, on plates without and with 250 µg/ml cloxacillin (AmpC inhibitor) (7). MBLs were detected by the DDST with imipenem and EDTA (29). Identification of various β-lactamase genes, including *bla*_{PER-1}-like and *bla*_{GES}-like ESBL genes and *bla*_{VIM}-like MBL genes, was done by PCRs and sequencing (9, 12, 13, 30). Only 5 isolates of the entire collection were phenotypically positive for the β-lactamase types assessed, and all of those were confirmed to produce such enzymes by molecular analysis. Two isolates expressed ESBLs, i.e., one ST235 from Zagreb with PER-1 and one ST111 from Zadar with GES-7. Three ST235 isolates from different Zagreb sites produced MBLs; one isolate had VIM-1 and two related isolates had VIM-2. The presence of *bla*_{PER-1} in the transposon Tn1213 (31) was checked by PCR (12), and the results confirmed such context. Variable regions of class 1 integrons carrying *bla*_{GES-7}, *bla*_{VIM-1}, and *bla*_{VIM-2} genes were analyzed by PCR mapping and sequencing (30, 32, 33). In contrast to *bla*_{GES-7/IBC-1} carrying integrons in Greece (33), the *bla*_{GES-7} cassette was followed by *aacA4*, and PCRs of the 3' part of the region based on sequences determined to date (33) have failed. The *bla*_{VIM-1} gene cassette array consisted of *bla*_{VIM-1}-*aacA4*-*aadA1* cassettes, followed by the 3'-CS segment. The *bla*_{VIM-2} integrons had *aadB*-*aacA7*-*bla*_{VIM-2}-*dhfrB5*-*aacA5* cassette arrays, adjacent to the *tniC/R* gene of the Tn402 transposition module (32). Links between PER-1, VIM-1, or VIM-2 enzymes and *P. aeruginosa* ST235 have often been reported (3, 9, 12, 14, 23, 24, 34, 35), and in Croatia these were rather occasional. This finding was congruent with those of the study from Split (22) and resembled findings for some other countries (3, 9). The integron with *bla*_{VIM-1} was In110, identified in *Pseudomonas* spp. in Italy and Spain (36, 37) and *Enterobacteriaceae* in Spain (32). The *bla*_{VIM-2} integron was new but differed from In559 only by the additional cassette *aadB*, suggesting direct evolution. In559 has been identified worldwide and is closely associated with ST235 spreading in Russia (14). Interestingly, the ST235 and ST111 isolates with VIM-2 that were identified previously in Split had a totally different integron (22).

Twelve imipenem-resistant (MICs of 8 to 128 µg/ml) and 2 susceptible (MICs of 0.5 to 2 µg/ml) isolates of various STs (including 4 ST235, 4 ST111, and 2 ST132 isolates) were subjected to sequencing of the *oprD* gene, which encodes the porin responsible for imipenem uptake (1, 2). The results are shown in Table 3. A large sequence variety was observed, as seen previously (35). In 5 resistant isolates of ST111, ST235, and ST244, point mutations or frameshift mutations generated premature stop codons, remarkably reducing frames to 19, 65, 110, 138, or 233 codons (including stops). One ST308 isolate had the *ISR10* element inserted inside codon 69. Only the ST244 isolate with the premature stop at codon 138 had otherwise no mutations, compared to *P. aeruginosa* PAO1; all others, including the susceptible isolates, had many polymorphisms with unclear roles, as observed before in a Spanish study, for example (35). Four ST111 isolates had a set of 19 amino acid substitutions (from position 127 to position 424). Four ST235 isolates had 8 changes (from position 103 to position 315), while 4 isolates of ST132, ST175, and ST292 had 14 substitutions (from position 43 to position 359). Other changes found in Spain (35) occurred as well, namely, the distortion of amino acids 372 to 383 ("loop L7-short") in ST111, ST132, ST175, and ST292 and a 1-nucleotide insertion at codon 402 in ST132, ST175, and ST235 isolates. Ten non-ciprofloxacin-susceptible (MICs of 2 to >32 µg/ml) and 2 susceptible (MICs of 0.125 to 0.25 µg/ml) isolates of a variety of STs were analyzed by sequencing the quinolone resis-

tance-determining regions (QRDR) in the *gyrA* and *parC* genes (1); Table 3 shows the results. As expected, the nonsusceptible isolates had mutations in *gyrA* (amino acid substitutions T83I or D81N), either alone (MICs of 2 to 4 µg/ml) or together with mutations in *parC* (S87L/W; MICs of 8 to >32 µg/ml). The overexpression of 4 efflux pumps involved in resistance (1, 2) was studied by real-time PCR, using our own primers specific for genes *mexA*, *mexC*, *mexE*, and *mexX* (Table 2), in all of the study isolates and interpreted as described previously (28). Overexpression of *mexC*, *mexA*, and *mexX* occurred in 21 (20.4%), 11 (10.7%), and 4 (3.9%) isolates, respectively, while *mexE* showed borderline expression at the most (data not shown). These rates were relatively low in comparison with MDR isolates in French and Spanish studies (28, 38).

This study assessed the clonal structure of MDR *P. aeruginosa* across Croatia. Together with the report from Split (22), it revealed the predominance of ST235 and ST111, adding also ST132. Since non-MDR isolates have not been analyzed, it is not clear whether these proportions reflected the overall rates in the *P. aeruginosa* population or concerned MDR organisms only. In the recent Czech and Spanish studies, however, MDR isolates were highly clonal, whereas susceptible isolates were diverse (11, 21). The analysis of selected resistance traits indicated that, while AmpC derepression and OprD and GyrA/ParC modifications were rather common, MBL and class A ESBL production and overexpression of efflux systems were of lower incidence. This means that other mechanisms, not assessed here and/or not yet known, must have played significant roles in the resistance of the study isolates.

Nucleotide sequence accession numbers. The sequences of the integronic gene cassette arrays containing *bla*_{VIM-1} and *bla*_{VIM-2} appeared in the EMBL database under accession numbers KC140564 and KC175287, respectively.

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