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Molecular Epidemiology of Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa* Isolates from Norway and Sweden Shows Import of International Clones and Local Clonal Expansion▽

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Scandinavia is considered a region with a low prevalence of antimicrobial resistance. However, the number of multidrug-resistant (MDR) Gram-negative bacteria is increasing, including metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa*. In this study MBL-producing *P. aeruginosa* isolates identified in Norway ($n = 4$) and Sweden ($n = 9$) from 1999 to 2007 were characterized. Two international clonal complexes (CC), CC111 ($n = 8$) and CC235 ($n = 2$), previously associated with MBL-producing isolates, were dominant. CC111 isolates (ST111/229; serotype O12; bla_{VIM-2}) included clonally related isolates identified in Skåne County, Sweden ($n = 6$), and two isolates associated with importation from Greece and Denmark. In all CC111 isolates, bla_{VIM-2} was located in integron In*59.2* or In*59* variants. The two CC235 isolates (ST235/ST230; serotype O11; bla_{VIM-4}) were imported from Greece and Cyprus, were possibly clonally related, and carried bla_{VIM-4} in two different integron structures. Three isolates imported from Ghana (ST233; serotype O6; bla_{VIM-2}), Tunisia (ST654; serotype O11; bla_{VIM-2}), and Thailand (ST260; serotype O6; bla_{IMP-14}) were clonally unrelated. ST233 was part of a new CC (CC233) that included other MBL-producing isolates, while ST654 could also be part of a new CC associated with MBL producers. In the isolates imported from Ghana and Tunisia, bla_{VIM-2} was part of unusual integron structures lacking the 3′ conserved segment and associated with transposons. The bla_{VIM} gene was found to be located on the chromosome in all isolates. Known risk factors for acquisition of MBL were reported for all patients except one. The findings suggest that both import of successful international clones and local clonal expansion contribute to the emergence of MBL-producing *P. aeruginosa* in Scandinavia.

Metallo-β-lactamases (MBLs) comprise one of the most clinically important families of β-lactamases in Gram-negative bacilli (30, 43), largely due to their association with mobile genetic elements that often carry other resistance genes, resulting in multidrug resistance (MDR) (30, 43). Moreover, the hydrolytic spectrum of MBLs includes all β-lactams with the exception of monobactams, and they are not inhibited by classical serine β-lactamase inhibitors (30, 43). The acquired MBLs include the VIM and IMP families (30, 43), SPM-1 (30, 43), GIM-1 (2), SIM-1 (17), AIM-1 (44), KHM-1 (36), NDM-1 (45), and DIM-1 (29). In particular, VIM-2 has emerged as a dominant MBL variant worldwide. Multilocus sequence typing (MLST) has identified international clonal complexes (CCs) responsible for the dissemination of MBL-producing *Pseudomonas aeruginosa*, particularly in European countries (7, 8, 12, 18, 20, 33), but also in Japan (15), Singapore, and Brazil (http://pubmlst.org/paeruginosa/).

Infections with MBL-producing *P. aeruginosa* isolates have been shown to be associated with higher mortality rates than infections with MBL-negative *P. aeruginosa* isolates (16, 46, 48) and a higher incidence of invasive disease (16). Risk factors associated with infections by MBL-producing *P. aeruginosa* isolates include recent use of β-lactams or fluoroquinolones, renal failure, indwelling urinary catheters, neurological disease, antineoplastic chemotherapy, corticosteroid therapy, and/or intensive care unit stay (14, 47).

The majority of the observed carbapenem resistance in Nor-

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TABLE 1. Clinical data, MBL allele, serotype, PFGE-type, and MLST results for MBL-producing *P. aeruginosa* isolates from Norway and Sweden

Isolate	Yr of isolation	Specimen	Risk factor(s)[a]	Place of isolation[b]	Hospitalization abroad,[c] place	MBL allele	Serotype	ST	Clonal complex	PFGE type
U9-19005	1999	Urine	Renal failure	Malmö, SWE	N	VIM-2	O12	111	111	A1
PA66[d]	2001	Urine	ICU, ND	Stockholm, SWE	Y, Greece	VIM-4	O11	230	235	D
AK-5493[d]	2004	Eye secretion	ICU, ND	Stockholm, SWE	Y, Greece	VIM-2	O12	229	111	C
B4-25753	2004	Blood	ICU	Malmö, SWE	N	VIM-2	O12	111	111	A1
K34-7[d]	2006	Tracheal secretion	ICU	Oslo, NOR	Y, Ghana	VIM-2	O6	233	233	F
K34-73	2006	Tracheal secretion	ICU	Akershus, NOR	Y, Cyprus	VIM-4	O11	235	235	D1
BU-20287	2007	Urine	Urinary catheter	Lund, SWE	N	VIM-2	O12	111	111	A2
BU-43038	2007	Urine	Urinary catheter	Lund, SWE	N	VIM-2	O12	111	111	A2
BU-36178	2007	Urine	Urinary catheter	Lund, SWE	N	VIM-2	O12	111	111	A3
BNL-1681	2007	Sputum		Lund, SWE	N	VIM-2	O12	111	111	A1
OS-210	2007	Urine	ICU	Stockholm, SWE	Y, Tunisia	VIM-2	O11	654		E
K44-24	2007	Urine	ICU	Tønsberg, NOR	Y, Thailand	IMP-14	O6	260		G
K45-32	2007	Surgical site	ICU	Oslo, NOR	Y, Denmark	VIM-2	O12	111	111	A1

[a] Risk factors according to Hirakata et al. and Zavascki et al. (14, 47), ICU, intensive care unit; UTI, urinary tract infection; ND, neurological disease.
[b] SWE, Sweden; NOR, Norway.
[c] Y, yes; N, no.
[d] MBL allele, serotype, and ST were determined previously (12, 32).

way and Sweden is due to the overexpression of efflux pumps and decreased permeability and not to carbapenemases (11, 31). The emergence of new mobile resistance mechanisms often originates from an exogenous source, exemplified by the first two MBL-producing isolates identified in Norway and Sweden, which were both associated with hospitalization abroad (13, 32).

In this study, we examined the molecular epidemiology of MBL-producing *P. aeruginosa* isolates identified in Norway and Sweden from 1999 to 2007, along with the genetic context of the bla_{MBL} genes.

(Part of this study was presented at the 18th European Congress of Clinical Microbiology and Infectious Disease [ECCMID], Barcelona, Spain.)

MATERIALS AND METHODS

Bacterial isolates. The clinical isolates investigated in this study are listed in Table 1. Submissions were based on resistance to carbapenems, ceftazidime, and/or piperacillin-tazobactam as stated in the national guidelines (http://www.unn.no/afa/category10274.html and http://www.srga.org/RAFMETOD/betamas.htm). Bacterial identification was performed using Vitek 2 (bioMérieux, Marcy l'Etoile, France). *P. aeruginosa* RON-2, harboring $bla_{\mathrm{VIM\text{-}2}}$ as part of integron *59* (In*59*) (28), was used for comparison in pulsed-field gel electrophoresis (PFGE) and MLST. Rifampin-resistant *P. aeruginosa* PAO1 and *Escherichia coli* J53-2 were used in transfer experiments. *P. aeruginosa* 303-03 and R22 carrying $bla_{\mathrm{VIM\text{-}4}}$ and $bla_{\mathrm{VIM\text{-}2}}$ on an ~400-kb (26) and an ~100-kb plasmid (unpublished results), respectively, were used as controls in plasmid analysis. *P. aeruginosa* 170-01 was used as a template for preparation of the bla_{VIM}-probe (26).

Identification of MBL-producing isolates. The presence of MBLs was investigated using the MBL Etest (AB bioMérieux, Solna, Sweden) and by qualitative spectrophotometric analysis of crude cell extracts for imipenem hydrolysis and subsequent inhibition with EDTA as previously described (41). Identification of the MBL-encoding genes was done using multiplex real-time PCR as described by Mendes et al. (22).

Susceptibility testing. MICs were determined with Etest (AB bioMérieux) according to the manufacturer's instructions and interpreted with clinical breakpoints from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/clinical_breakpoints/).

Serotyping. All isolates were serotyped using monoclonal O-antigen sera (Bio-Rad, Marnes-La-Coquette, France) as described in the manufacturer's instructions.

PFGE. PFGE was performed on genomic DNA digested with SpeI (New England Biolabs, Hirts, United Kingdom) in agarose plugs at 37°C until completion, and DNA fragments were separated as previously described (12). Dendrograms were generated by the unweighted-pair group method using average linkages (UPGMA) with the band position tolerance set to 1.0%. A cutoff value of an ≥80% level of similarity, corresponding to a maximum six-band difference, was used to determine the genetic relatedness among isolates (12).

MLST. MLST was performed according to the method of Curran et al. (4), and the resulting PCR products were sequenced using BigDye 3.1 terminator chemistry on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Experimentally determined nucleotide sequences were compared to existing alleles in the MLST database (http://pubmlst.org/paeruginosa/) and assigned allelic numbers, and sequence types (STs) were obtained. Isolates having five or more identical alleles were considered part of the same clonal complex (10). The BURST algorithm was used to analyze the MLST findings.

Determination of MBL genes and their adjacent genetic structures. The MBL gene, the surrounding genetic structure, and its linkage to integrons and Tn*21*-like transposons were analyzed by PCR and sequenced using previously described primers and custom-designed primers (Table 2). Sequence analysis was performed using SeqManII software (DNAStar, Madison, WI) and compared with sequences deposited in the GenBank database (http://www.ncbi.nlm.nih.gov).

Genomic localization of bla_{VIM}. The genetic localization of bla_{VIM} genes was examined with S1 nuclease-treated total DNA separated by PFGE, followed by in-gel hybridization using a bla_{VIM} probe as previously described (26).

Transfer of resistance. Conjugal-transfer experiments were carried out by filter mating with a donor/recipient ratio of 1:1 at 37°C. Rifampin-resistant *E. coli* J53-2 and *P. aeruginosa* PAO1 were used as recipient strains. Transconjugants were selected on Luria-Bertani (LB) agar plates (Becton Dickinson, Sparks, MD) supplemented with 150 μg/ml rifampin (Sigma-Aldrich, St. Louis, MO) and 4 μg/ml ceftazidime (Sigma-Aldrich).

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this study have been deposited in GenBank with accession numbers FN397623, FN397626, FN397624, FN397625, FN397622, FN397621, FN397620, FN397619, FN397618, FN397628, and FN397627.

RESULTS

Origin of isolates. In Norway, all four MBL-positive isolates identified were linked to import from Ghana (32), Cyprus, Thailand, and Denmark (Table 1). In Sweden, three isolates were imported (Greece [$n = 2$] and Tunisia). However, six isolates were shown to be endogenous to Skåne County (Malmö/Lund), Sweden, from patients with no history of recent international travel. The first two of these isolates (U9-19005 and B4-25753) were identified at Malmö University Hospital in 1999 and 2004, while four isolates were identified at Lund University Hospital in 2007. Several of the patients

TABLE 2. Primers used to determine the genetic structures surrounding the bla_{MBL} genes in this study and linkage with Tn*21*

Primer	Sequence (5′→3′)	Target gene/region	Reference
5′ CS	GCC TGT TCG GTT CGT AAG CT	*intI1*	42
3′ CS	CGG ATG TTG CGA TTA CTT	*qacEΔ1*	42
tniCF	CGA TCT CTG CGA AGA ACT CG	*tniC*	42
VIMgen-F2	GTT TGG TCG CAT ATC GCA AC	bla_{VIM}	22
VIMgen-R2	AAT GCG CAG CAC CAG GAT AG	bla_{VIM}	22
VIMgen-F2Seq	GTT CGC ATA TGC GAC CAA AC	bla_{VIM}	This study
VIMgen-R2Seq	CTA TCC TGG TGC TGC GCA TT	bla_{VIM}	This study
VAR	TCA ATC TCC GCG AGA AGT GC	bla_{VIM}	This study
tnpRF	GAT ACA GGG TTT CGC GAC TG	*tnpR* (Tn*21*)	41
IMPgen-F1	GAA TAG RRT GGC TTA AYT CTC	bla_{IMP}	22
IMPgen-R1	CCA AAC YAC TAS GTT ATC	bla_{IMP}	22
IMP-X-F	AAC ACG GTT TGG TGG TTC TT	bla_{IMP}	This study
blaPSE-1-F	ACC GTA TTG AGC CTG ATT TA	bla_{PSE-1}	This study
blaPSE-1-R	ATT GAA GCG TGT GTT TGA GC	bla_{PSE-1}	This study
PSE-Seq	GGA AGC GCT GAT TGC CAT TGT AA	bla_{PSE-1}	This study
aadB-F	GGC GAG CTC GAG GCA ATA GT	*aadB*	This study
aadB-FR	AAG CAG GTT CGC AGT CAA GT	*aadB*	This study
aadB-RF	CTT TCA GGT CGC GAT ATG CG	*aadB*	This study
aacA4-Seq1	ACC CGT CGC CGA GCA ACT T	*aacA4*	This study
aacA7-R	TTC CGG AAG CAG CGT ACT TG	*aacA7*	41
aacA7-FR	TTC AAC AGG CCT GAC GAG CG	*aacA7*	This study
aacA7-RF	TGC TCA AGT ACG CTG CTT CC	*aacA7*	This study
arrSeq-1	TAG GTG ACT TGC TTT CGC CT	*arr-7*	This study
arrSeq-2	TAG GAC TTG GTT GGA TTG CC	*arr-7*	This study
aac(6′)-lb-F	TTG CGA TGC TCT ATG AGT GGC TA	*aac(6′)-Ib/aacA4*	25
aac(6′)-lb-R	CTC GAA TGC CTG GCG TGT TT	*aac(6′)-Ib/aacA4*	25
aac(6′)-lb-seq	CGT CAC TCC ATA CAT TGC AA	*aac(6′)-Ib/aacA4*	25
sul1-mF	ACG AGA TTG TGC GGT TCT TC	*sul1*	1
sul1-F	CGG CGT GGG CTA CCT GAA CG	*sul1*	38
sul1-R	GCC GAT CGC GTG AAG TTC CG	*sul1*	38
orf5-F	AGG TTG TGC GGC TGA TGC	*orf5*	19
tnpATn5501-pr2	CTT CTC GCT GAC TAT GAG ATC G	*tnpA* (Tn*5501*)	This study
tnpATn5501-pr3	GGA ATA CTT GGC GTT GAC TG	*tnpA* (Tn*5501*)	This study
tnpATn5501-pr4	ATG TCA GCT TCT GCG TGT CCT T	*tnpA* (Tn*5501*)	This study
tnpATn5501-pr5	AAA GCA ATC CTT TGT CGC CGA G	*tnpA* (Tn*5501*)	This study
tnpATn5501-pr6	TCC ACG ATC TAC GCA ACC TGA A	*tnpA* (Tn*5501*)	This study
tnpATn5501-3Seq	CCG ATC TGC AAG ATG CAA TA	*tnpA* (Tn*5501*)	This study
strAstrB-F	TAT CTG CGA TTG GAC CCT CTG	*strA*	39
strAstrB-R	CAT TGC TCA TCA TTT GAT CGG CT	*strB*	39
strAstrB-RF	AGC CGA TCA AAT GAT GAG CAA TG	*strB*	This study

had previous risk factors associated with infections due to MBL-producing *P. aeruginosa* (14, 47), such as intensive care unit stay, renal failure, neurological disease, or urinary catheters (Table 1).

Antimicrobial susceptibility profiles. All isolates displayed an MDR phenotype, with two isolates resistant to all antibiotics tested, including colistin (MIC, 4 μg/ml). The carbapenem MIC was >32 μg/ml in all isolates, while the MICs of penicillins and cephalosporins varied but were all in the resistance range (piperacillin-tazobactam MIC, 32 to >256 μg/ml; ceftazidime MIC, 64 to >256 μg/ml; and cefepime MIC, 32 to >256 μg/ml). Five isolates were intermediately susceptible to aztreonam (MIC, 8 to 16 μg/ml), while the remaining seven were resistant (MIC, 32 to >256 μg/ml). All isolates were resistant to tobramycin (MIC, 32 to >256 μg/ml) and amikacin (MIC, 32 to >256 μg/ml), while six isolates were susceptible (MIC, 2 to 4 μg/ml) and seven isolates were resistant (MIC, 8 to >256 μg/ml) to gentamicin. Resistance to ciprofloxacin (MIC, 16 to >32 μg/ml) was observed in all isolates.

MBL allele and genetic context. Sequencing of the integrons carrying the MBL genes showed that the six isolates endogenous to Skåne County, AK-5493 (imported from Greece), and K45-32 (imported from Denmark) all carried bla_{VIM-2} in In*59*-like structures consisting of two aminoglycoside resistance gene cassettes (*aacA29a* and *aacA29b*) and bla_{VIM-2} (Fig. 1A and B). The In*59*-variant in isolates B4-25753, BU-20287, BU-43038, AK-5493, and BU-36178 were identical to In*59.2* from Greece (GenBank accession no. EU118149) (37), while isolates U9-19005 and BNL-1681 had a 59-base element (59-be) of *aacA29a* identical to that of In*59.2* but had a 59-be of *aacA29b* identical to that of In*59* (GenBank accession no. AF263519) (28) (1 nucleotide difference from the 59-be of *aacA29b* in In*59.2*). The In*59* variant in isolate K45-32, on the other hand, had the same gene cassette (*aacA29b*) on each side of the bla_{VIM-2} gene. In addition, the 59-be of the *aacA29b* gene cassette in the first position (normally occupied by *aacA29a*) was identical to the 59-base element of *aacA29a* in In*59.2*. In both K34-7 (32) and OS-210, derived from patients hospitalized in African countries, bla_{VIM-2} was part of integrons lacking a standard 3′ conserved segment (3′ CS) consisting of fused *qacΔE1-sul1* genes (Fig. 1C and F). In OS-210, bla_{VIM-2} was located in a complex structure as the first gene cassette of a class 1 integron, followed by the gene cassette *aadB*. The 59-be of *aadB* was followed by another short section

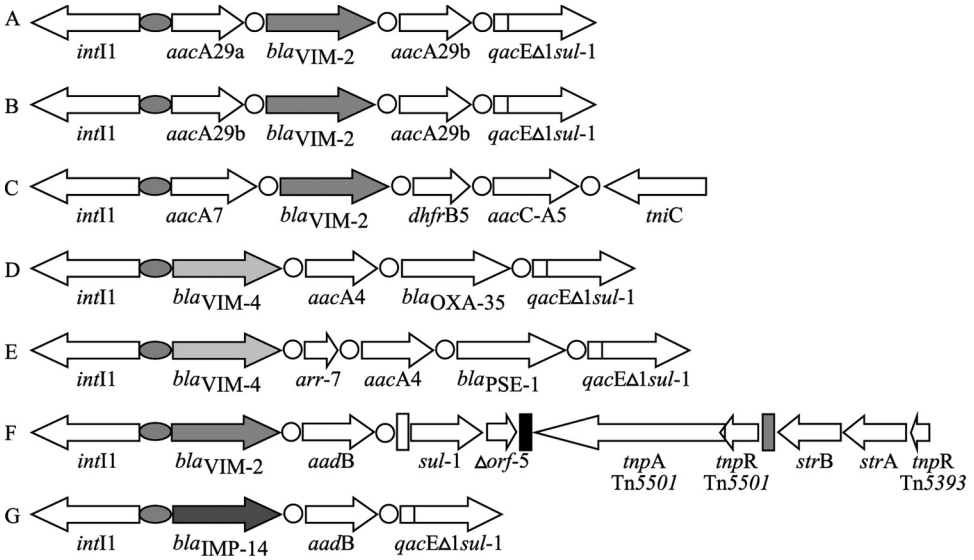

FIG. 1. Schematic view (not to scale) of the genetic context surrounding the MBL genes in Norwegian and Swedish MBL-producing *P. aeruginosa* isolates. (A) Isolates U9-19005, AK-5493, B4-25753, BU-20287, BU-43038, BU-36178, and BNL-1681 (GenBank accession numbers FN397626, FN397624, FN397621, FN397619, FN397622, FN397620, and FN397625). (B) Isolate K45-32 (GenBank accession number FN397618). (C) Isolate K34-7 (GenBank accession number FM165436). (D) Isolate PA66 (GenBank accession number AY866525). (E) Isolate K34-73 (GenBank accession number FN397623). (F) Isolate OS-210 (GenBank accession number FN397628). (G) Isolate K44-24 (GenBank accession number FN397627). The genetic structures of isolate PA66, AK-5493, and K34-7 were determined previously (12, 32). Open reading frames are represented by arrows indicating the orientation. MBL genes are shown in grey. The 59-be of each gene cassette is represented by an open circle and the *attI* site by a gray oval. The partial 5′ CS in front of the *sul1* gene of OS-210 (F) is represented by an open rectangle. The inverted repeat of Tn*5501* is indicated by a black rectangle, and the inverted repeat of Tn*5393* is indicated by a gray rectangle.

of the 5′ CS, including a part of the promoter region and a truncated *attI* site fused to *sul1*. No *qacE* gene was identified. The original start codon of *sul1* was mutated due to the fusion with the *attI* site. However, this fusion introduced a putative start codon for *sul1* four codons upstream. The *sul1* gene was followed by a truncated *orf5* and two transposons. A Tn*5501*-related transposon (34, 35) was inserted into *orf5*. The inverted repeat left (IR-L) of Tn*5501* in pGNB-1 was also identified on the left side, while the IR-R was not found in the sequenced region. On the right side of Tn*5501*, the sequence data show insertion of a Tn*5393* variant (3). The IR-R of Tn*5393* was identified 4 bp to the right of *tnpR* of Tn*5501*, followed by the streptomycin resistance genes *strB* and *strA* and 62 bp of *tnpR* of Tn*5393*.

In two isolates, PA66 (12) and K34-73, $bla_{\text{VIM-4}}$ was identified in the first position as part of class 1 integrons (Fig. 1D and E). The *aacA4* gene cassette was present in both integrons, but they carried two different β-lactamase genes, $bla_{\text{OXA-35}}$ and $bla_{\text{PSE-1}}$. The integron in isolate K34-73 also carried a new putative *arr* gene (named *arr-7*), encoding rifampin resistance, that was most similar to the Arr-4 gene (90% amino acid identity; GenBank accession number EF660562) (5).

In the only isolate that carried an IMP enzyme (K44-24), $bla_{\text{IMP-14}}$ was identified in the first position in a class 1 integron, followed by the *aadB* gene cassette (Fig. 1G).

In six isolates, bla_{VIM} was linked to Tn*21* (data not shown). The data indicate the same insertion site of the transposon in four of the isolates with $bla_{\text{VIM-2}}$ as part of an In*59*-like integron (U9-19005, AK-5493, B4-25753, and K45-32). In the other isolates carrying an In*59*-like integron, $bla_{\text{VIM-2}}$ was not linked to Tn*21*. $bla_{\text{VIM-4}}$ in isolates PA66 and K34-73 was also linked to Tn*21*, but with different insertion sites.

Location of bla_{VIM} and transferability of resistance. The bla_{VIM} gene was found to be located on the chromosome in all isolates by S1 nuclease PFGE and in-gel hybridization (data not shown). Transfer of the bla_{VIM} genes to *P. aeruginosa* PAO1 or *E. coli* J53-2 was not successful.

Epidemiological typing of isolates by serotyping, PFGE, and MLST. The typing results are summarized in Table 1. All isolates from Skåne County (Malmö/Lund, Sweden) and K45-32 (imported to Norway from Denmark) were clonally related by PFGE (Table 1 and data not shown), were designated type A1 to A3 (Dice coefficient, ≥95%), belonged to ST111, and were of the O12 serotype. AK-5493, previously typed as ST229, a double-locus variant (DLV) of ST111 and serotype O12 (12), had a PFGE profile, designated type C, indicating possible clonal relatedness to ST111 isolates (Dice coefficient, 76%). MLST showed that RON-2, which also carried In*59* (28), displayed ST111 and had a PFGE profile designated type B (Dice coefficient, 79%). Three isolates were of the O11 serotype (PA66, K34-73, and OS-210). PA66 had previously been typed as ST230 (12). K34-73 (PFGE type D1) showed genetic relatedness to PA66 (PFGE type D; Dice coefficient, 86%) and was typed as ST235, a single-locus variant (SLV) of ST230. The last serotype O11 isolate, OS-210, was typed as ST654 and was clonally unrelated (PFGE type E) to the other isolates. Two isolates, K34-7 and K44-24, imported from Ghana and Thailand, respectively, were of serotype O6 and were clonally unrelated (designated PFGE types F and G,

respectively). K34-7 was typed as ST233 (32) and K44-24 as ST260.

DISCUSSION

The global dissemination of MBL-producing *P. aeruginosa* isolates has reached Scandinavia, a region renowned for its low level of antibiotic resistance (9, 23, 24). All 13 MBL-producing *P. aeruginosa* isolates identified in Norway and Sweden from 1999 to 2007 showed MDR profiles with two isolates resistant to all antibiotics tested, including colistin.

Seven isolates were derived from patients recently hospitalized abroad, suggesting that the import of such strains is significant and that human travel contributes to their dissemination. The different countries (Greece, Ghana, Cyprus, Tunisia, Thailand, and Denmark) associated with import also underscore the global dissemination of MBL-producing *P. aeruginosa*. However, the finding of clonally related, endogenous isolates in a geographically limited area in Sweden (Skåne County) over a protracted period of time indicates a background level and the establishment of MBL-producing *P. aeruginosa* with the potential of further dissemination. The identification of four of these isolates at the same hospital (Lund) in 2007 indicates local reservoirs and transmissions. Retrospective analysis of patient histories showed that the isolates were identified over a 6-month period and that none of the patients had been admitted to the same department at the same time. However, possible patient-to-patient, health care personnel-to-patient, or environment-to-patient transmissions cannot be ruled out. Interestingly, isolate U9-19005 was isolated in 1999, which makes it the third-earliest VIM-2-positive *P. aeruginosa* isolate to be reported.

Two previously described clonal complexes, CC111 (previously described as CC4/BG4) and CC235 (previously described as CC11/BG11), have been shown to dominate among MBL-producing *P. aeruginosa* isolates (7, 8, 12, 15, 18, 20, 33), and the same CCs dominate in the isolates from Norway and Sweden. All isolates from Skåne County, along with isolates imported from Denmark and Greece, were of serotype O12 and displayed ST111/ST229, which is part of CC111, and all harbored $bla_{\text{VIM-2}}$ as part of In*59* variants. In*59* was first described in France in an isolate (RON-2; GenBank accession number AF263519) from 1998 (28) and has subsequently been identified in Austria (7), while In*59* variants have been identified in Greece (GenBank accession numbers EU118148 and EU118149) (37). The differences in the In*59* structures in the isolate from France and isolates from Greece lie in the 59-bes of the *aacA29* gene cassettes (28, 37) and the bla_{VIM} gene ($bla_{\text{VIM-2/-17}}$). MLST showed that RON-2 also displayed ST111, and it was possibly related by PFGE. In addition, a representative strain of the major European O12 clone also belongs to CC111 (20).

The STs of the two O11 isolates imported from Greece and Cyprus belonged to CC235, further underscoring the importance of the CC in the dissemination of MBLs, as it includes VIM-producing *P. aeruginosa* of serotype O11 in Europe (8, 12, 18, 20, 33) and IMP- and SPM-1-producing *P. aeruginosa* isolates from Japan (15), Brazil, and Singapore (http://pubmlst.org/paeruginosa).

Both isolates (K34-7 and OS-210) imported from an African country harbored $bla_{\text{VIM-2}}$ in unusual integron structures linked to transposons (Fig. 1C and F). Russian isolates with the same TniC-like transposon as K34-7 have been typed as ST235, part of CC235 (33). However, the ST233 of K34-7 (32) could be part of a new CC (CC233) that includes the SLVs ST742 and ST743 associated with IMP-1-producing *P. aeruginosa* from Singapore and the DLV ST375 associated with SPM-producing *P. aeruginosa* from Brazil (http://pubmlst.org/paeruginosa). The integron structure of isolate OS-210 was associated with two transposons, Tn*5501* and Tn*5393*, in which the TnpA and TnpR protein sequences were identical to TnpA and TnpR of the Tn*5501*-related transposon found in the IncP-1β plasmid pGNB-1 (35). Interestingly, the sequence downstream of *aadB* consisted of a partial 5′ CS and *sul1*, as observed in the In*104* region of genomic island SGI1-J found in *Salmonella enterica* serovar Emek (19). The mutated start codon of *sul1*, along with the putative new start codon, could result in a Sul1 protein with four additional amino acids at the N terminus that has previously been inappropriately annotated as *sul3* (27). Whether the *sul1* gene is expressed from the new putative start codon is uncertain. Tn*5393* harboring *strA-strB* genes found at the end of the integron structure in OS-210 has also been detected in the genomic island SGI1 of *Salmonella enterica* serovar Kentucky (6), and Tn*5393* has also been found to be associated with the extended-spectrum β-lactamase PER-1 in *Alcaligenes faecalis* (21) and has been identified in many other bacterial species, both environmental and clinical (40). OS-210 displayed ST654, which has been shown to be associated with IMP-type MBL in Singapore (http://pubmlst.org/paeruginosa/), and the DLV ST741, associated with IMP-1 in Singapore, suggesting a possible new successful CC that is widespread (http://pubmlst.org/paeruginosa/).

The chromosomal location of bla_{VIM} and the lack of transfer of bla_{VIM} support the clonal dissemination of MBL-producing *P. aeruginosa*. Previous studies have also shown that the majority of MBL genes are chromosomally located (26).

Interestingly, K44-24 was the only isolate that harbored an IMP enzyme (IMP-14) and was imported to Norway from Thailand, the only country where $bla_{\text{IMP-14}}$ had been described previously (GenBank accession no. AY553332, FJ257650, and FJ257651). The gene cassette combination of $bla_{\text{IMP-14}}$ and *aadB* found in K44-24 was also identical to GenBank accession no. FJ257651. K44-24 was typed as ST260, an ST in which none of the associated SLVs and DLVs were associated with MBL producers (http://pubmlst.org/paeruginosa/), indicating that MBL-producing isolates with other genetic backgrounds are circulating.

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