

Molecular Characterization of Class 1, 2 and 3 Integrations in *Serratia* spp. Clinical Isolates in Poland – Isolation of a New Plasmid and Identification of a Gene for a Novel Fusion Protein

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Purpose: Gram-negative rods of the genus *Serratia* play an increasing role as etiological agents of healthcare-associated infections (HAI) in humans. These bacteria are characterized by natural and acquired resistance to several groups of antibacterial agents. The aim of the study was to characterize class 1, 2 and 3 integrations in the clinical isolates of *Serratia* spp. in Poland.

Methods: The study comprised 112 clinical strains of *Serratia*, isolated from patients hospitalized in Poland in 2010–2012. Identification of strains was confirmed using MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) system. Detection of class 1, 2 and 3 integrase DNA sequence was performed by multiplex-PCR. Amplicons obtained in the PCR reactions were purified and then sequenced bidirectionally.

Results: Among the analyzed strains, *Serratia marcescens* was a predominant species (103/112, 92.0%). All three classes of integrase DNA sequence were detected in the analyzed strains of *Serratia* spp. DNA sequence of class 3 integron, besides integrase gene, revealed three gene cassettes (*dfrB3*, *bla*_{GES-7}, *bla*_{OXA/aac(6')-Ib-cr}). BLAST analysis of DNA sequence revealed that class 3 integron was carried on 9448 bp plasmid which was named pPCMI3 – whole sequence of its DNA was submitted to GenBank NCBI (National Center for Biotechnology Information) – NCBI MH569711.

Conclusion: In this study, we identified a new plasmid pPCMI3 harboring class 3 integron. This is the first report of a gene *oxa/aac(6')-Ib-cr* coding for a novel fusion protein, which consists of OXA β-lactamase and acetyltransferase *aac(6')-Ib-cr*. In the analyzed strains, class 1 and 2 integrations were also detected. Among the strains with class 1 integron, nine contained cassette array 5'CS-aadA2-ORF-dfrA12-3'CS, and two – cassette array 5'CS-aacC1-ORF-ORF-aadA1-3'CS, which were not previously reported in *Serratia* spp.

Keywords: *Serratia marcescens*, gene cassettes, antibiotic resistance, carbapenemase, fusion protein

Introduction

Gram-negative rods of the genus *Serratia* are environmental bacteria which play an increasing role as etiological agents of healthcare-associated infections (HAI). These bacteria were previously classified in the family *Enterobacteriaceae*, but due to 2016 taxonomy change, the genus *Serratia* at present belongs to the newly formed family *Yersiniaceae* in the order *Enterobacterales*.¹

Bacteria *Serratia* spp. are widely distributed in the external environment, mainly in water and soil, but also in plants and animals.² Since the mid-1970s, they have

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been increasingly recognized as human pathogens. At present, 30 species of *Serratia* are recognized, out of which *S. marcescens* is the species most important in medicine, causing a wide range of diseases, particularly in neonates and small children as well as in hospitalized and/or immunocompromised adult patients.^{2–6} *Serratia marcescens* affects, most often, the urinary tract, skin and soft tissue, the central nervous system (CNS), the eye, or leads to pneumonia or sepsis.^{2,7,8}

Antimicrobial resistance of *S. marcescens* is mediated by the resistance genes located on the bacterial chromosome and on the mobile genetic elements, eg, plasmids and integrons. A set of mobile genes (so-called “mobilome”) enables horizontal transfer of genes contributing to the spread of antibiotic resistance between bacteria of the same or even different species.⁹

Integrons have a wide distribution among bacterial isolates obtained from clinical samples of hospitalized patients. One of the most crucial problems is activity of integrons leading to increased antibiotic resistance in clinical strains.¹⁰ Until now, five classes of integrons have been characterized, based on analysis of the nucleotide sequence of the integrase gene, out of which the first three (class 1, 2 and 3) are involved in spreading the resistance gene cassettes.¹¹

Class 1 integrons are the most prevalent type of integrons and have been frequently reported in clinical isolates of Gram-negative bacteria.¹² The structure of class 1 integrons consists of two conserved regions, including 3' conserved segment (3' CS) and 5' conserved segment (5' CS), as well as internal gene cassettes which encode antimicrobial resistance genes. Class 1 integrons carry a variety of resistance gene cassettes, and most of them contain one of *aadA* genes, which confers resistance to streptomycin and spectinomycin. On Integrall website, which provides easy access to integron DNA sequences and genetic arrangements, 66 unique sequences obtained from *Serratia* spp. were submitted (date of release 05.08.2021).¹³ Most of them belong to class 1 integrons, there are two sequences of integrons class 3 and one of class 2. Among 95 cassettes carrying recognized antibiotic resistance genes within class 1 integrons, 47 (49.5%) contain resistance to aminoglycosides, 29 (30.1%) resistance to β -lactams, and 10 (10.5%) resistance to trimethoprim.

Class 2 integrons are less common in *Enterobacterales* and are less diverse.¹⁴ They are associated with transposons which belong to Tn7 family.¹⁵ Crowley et al reported class 2 integrons in 9/30 (30.0%) clinical strains of *S. marcescens*,

which contained the gene cassettes *dfrA1*, *sat1* and *aadA1*, conferring resistance to trimethoprim, streptothricin, and streptomycin/spectinomycin, respectively.¹⁶

Class 3 integrons are rare and had been discovered only in a few species, including *S. marcescens*.⁹ Arakawa et al isolated class 3 integron from a carbapenem-resistant *Serratia marcescens* strain.¹⁷ Its module was characterized by Collis et al as consisting of the *intI3* gene, *attI3* site, and P_c promoter – in the same way as the class 1 integron module.¹⁸ Correia et al reported that an *intI3* integrase gene contained in class 3 integron isolated from a strain of *Klebsiella pneumoniae* showed 98.8% identity to that of *Serratia marcescens* AK9373.¹⁹

The presence of integrons in *S. marcescens* isolated from clinical specimens in Poland has been documented in only one study.²⁰ Thus, the aim of the present study was to characterize class 1, 2 and 3 integrons in the clinical isolates of *Serratia* spp. in Poland.

Materials and Methods

Collection of Clinical Isolates of *Serratia* spp.

The study comprised a total of 112 strains, which were isolated from patients hospitalized in 6 hospitals in two cities in Poland (Warsaw and Otwock) in 2010–2012. All isolates were initially tested by Vitek-2 Compact system, using GN-ID card dedicated to identification of Gram-negative bacteria. Antimicrobial susceptibility testing was performed simultaneously using AST-N-259 card for evaluation of MIC (minimal inhibitory concentration) value of 16 different antibiotics and other chemotherapeutics, including β -lactams, aminoglycosides, fluoroquinolones, tetracyclines, colistin and trimethoprim/sulfamethoxazole. MALDI-TOF MS was performed (MALDI Biotyper, Bruker) as a conclusive method for identification of *Serratia* spp. strains.²¹

DNA Extraction

Extraction of genomic DNA was performed by Genomic Mini kit (A&A Biotechnology) following the manufacturer's instructions. The quantity of DNA was analyzed by gel electrophoresis and compared with GeneRuler DNA Ladder MIX (Thermo Fisher Scientific).

Detection of Integrase DNA Sequence

Detection of class 1, 2 and 3 integrase DNA sequence was performed by multiplex-PCR (polymerase chain reaction) in accordance with the protocol published by Su et al.²² DNA

Table 1 Primers Used for Detection of Integrase DNA Sequence²²

Primer	Sequence	Amplicon Size [bp]	T [°C]
Int1-Fw	ACGAGCGCAAGGTTTCGGT	565	58
Int1-Rv	GAAAGGTCTGGTCATACATG	565	58
Int2-Fw	GTGCAACGCATTTTGCAGG	403	58
Int2-Rv	CAACGGAGTCATGCAGATG	403	58
Int3-Fw	CATTGTGTTGTGGACGGC	717	58
Int3-Rv	GACAGATACGTGTTTGGCAA	717	58

Abbreviation: bp, base pairs.

sequences of 6 primers used for investigation are shown in [Table 1](#).

DNA amplification was performed in DNA thermal cycler (S1000 Thermal Cycler, BIO-RAD) in a final volume of 50 µL, containing 5 µL of genomic DNA (500 ng) extract mixed with 5 µL of 10X Taq buffer (with ammonium sulfate) (Thermo Fisher Scientific), 5 µL of MgCl₂ 25 mM solution (Thermo Fisher Scientific), 1 µL of dNTPs 10 mM solution (Thermo Fisher Scientific), adequate volume of aqueous solution of primers (final concentration of each primer should amount to 40 pmol per reaction), 0.5 µL Taq DNA Polymerase (Thermo Fisher Scientific), deionized water up to 50 µL (Direct Q-3 UV Millipore). The conditions of amplification reaction were as follows: initial denaturation at 95°C for 3 minutes, followed by 30 cycles of DNA denaturation at 95°C for 30 seconds, primers annealing at 58°C for 1 minute, primer extension at 72°C for 1 minute and a final elongation at 72°C for 5 minutes. Positive and negative controls were included in PCR assays and Gene Ruler DNA Ladder MIX (Thermo Fisher Scientific) was used as a molecular size standard. PCR products were separated by DNA electrophoresis on 3% NuSieve 3:1 agarose gel (Lonza) in 1X TAE buffer, stained with ethidium bromide and visualized by VersaDoc Imaging System version 1000 (BIO-RAD).

Amplification of Variable Regions of Class 1 and 2 Integrons

Amplification of the variable region of class 1 and 2 integrons was performed by PCR reaction only for isolates

known to contain respective 1 and 2 integrase sequences. DNA sequences of primers are shown in [Table 2](#).

DNA amplification was performed in DNA thermal cycler (S1000 Thermal Cycler, BIO-RAD) in a final volume of 50 µL, containing 1 µL of genomic DNA (1 ng) extract (100X diluted in deionized water) mixed with 10 µL of 5X iProof buffer (BIO-RAD), 1 µL of dNTPs 10 mM solution (Thermo Fisher Scientific), adequate volume of aqueous solution of primers (final concentration of each primer should amount to 300 pmol per reaction), 0.5 µL iProof High-Fidelity DNA Polymerase (BIO-RAD), deionized water up to 50 µL (Direct Q-3 UV Millipore). The conditions of the amplification reaction were as follows: initial denaturation at 98°C for 1 minute, followed by 35 cycles of DNA denaturation at 98°C for 30 seconds, primers annealing (temperature in accordance to data shown in [Table 2](#)) for 15 seconds, primer extension at 72°C for 2 minutes and a final elongation at 72°C for 10 minutes. Positive and negative controls were included in PCR assays, Gene Ruler DNA Ladder MIX (Thermo Fisher Scientific) was used as a molecular weight standard. PCR products were separated by DNA electrophoresis on 1% agarose gel (Sigma Aldrich) in 1X TAE buffer, stained with ethidium bromide and visualized by VersaDoc Imaging System version 1000 (BIO-RAD).

Sequencing of PCR Products and Sequence Analysis

Amplicons obtained in the PCR reactions before sequencing were purified by ChargeSwitch PCR Clean-UP kit (Thermo Fisher Scientific) in accordance with the

Table 2 Primers Used for Amplification of the Variable Region of Class 1 and 2 Integron DNA Sequence

Primer	Sequence	Amplicon Size [bp]	T [°C]	Reference
REN-INT-P	ATCGATGTTTGATGTTATGGAGC	Variable	52	This study
REN-INT-M	ATCGAGACTTGACCTGATAGTTTG	Variable	52	This study
Hep-74	CGGGATCCCGGACGGCATGCACGATTGTGA	Variable	64	[15]
Hep-51	GATGCCATCGCAAGTACGAG	Variable	64	[15]

manufacturer's instructions. Purified PCR products were sequenced bidirectionally by the Laboratory of DNA Sequencing and Oligonucleotide Synthesis in the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences using sequencer 373/x1 DNA Analyzer (Applied Biosystems). The chromatogram analysis and alignment were performed by Clone Manager. Homology analysis was carried out using the BLAST program provided by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

Sequencing of Plasmid Carrying Class 3 Integron by Next-Generation Sequencing (NGS)

Genomic DNA of strain carrying class 3 integrase gene was sequenced on the Genome Sequencer GS FLX Titanium. The results of sequencing were matched by Newbler program. Further work for determination of the final DNA sequence was performed by Seqman program (DNASTar).

Results

Identification and Antimicrobial Susceptibility

Among 112 tested strains, more than 90% (103 isolates) were identified as *S. marcescens*, 7 strains as *S. liquefaciens*, 1 as *S. fonticola*, and 1 as *S. ureilytica*. Summary of the antimicrobial susceptibility patterns of the analyzed strains is shown in Figure 1. Complete results of identification and antimicrobial susceptibility tests are shown in the [Appendix A](#) attached to this article.

Detection of Class 1, 2 and 3 Integrase

In the strains analyzed in this study, the presence of all three classes of integrase was confirmed. Class 1 was the most common type present in 26 isolates, which represented more than 23% of all tested strains. Class 2 was confirmed in 10 strains, 9 of them simultaneously carried class 1 integrase DNA sequence. Just one strain contained a single class 2 integrase DNA sequence. Only one isolate carried class 3 integrase DNA sequence. Results are shown in the attached [Appendix A](#).

Characterization of Variable Regions of Class 1 and 2 Integrons

Variable regions of class 1 and 2 integrons were amplified by iProof polymerase which has a high thermostability and

ability for fast synthesis of long DNA products of PCR reactions. Compilation of gene cassettes characterized in tested strains of *Serratia* spp. is shown in Table 3. Samples no. 64, 91, 96 and 99 did not show any products of PCR reactions.

All 10 isolates containing class 2 integrons were identical and they had the same gene cassette arrangement: *aadA1*, *sat2* and *dfrA1* (Figure 2), which are responsible for resistance to aminoglycosides, streptomycin and trimethoprim, respectively.

Characterization of Class 3 Integron

Strain 89 was of particular interest due to the presence of class 3 integron, therefore a search for plasmid DNA carrying the integron was performed. Genomic DNA of strain 89 was used as a template in NGS sequencing. BLAST analysis of DNA sequence revealed that class 3 integron is carried on 9448 bp plasmid which has been named pPCMI3. Whole DNA sequence of plasmid pPCMI3 was submitted to GenBank NCBI (NCBI MH569711).

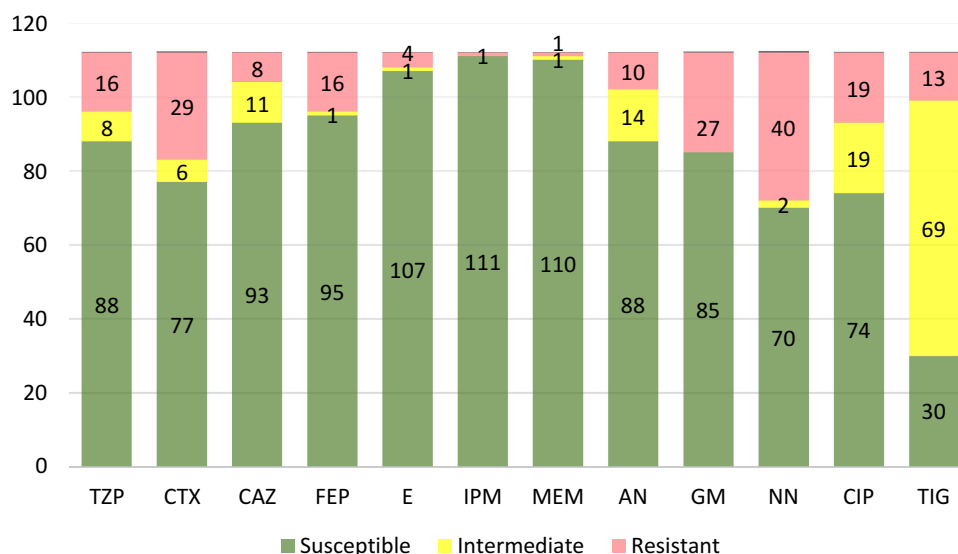
DNA sequence of class 3 integron, besides integrase gene, harbors three gene cassettes (*dfrB3*, *bla_{GES-7}*, *bla_{OXA/aac(6')-Ib-cr}*) (Figure 2). The *aac(6')-Ib-cr* is a variant of *aac(6')-Ib* with two amino acid substitutions (Trp87Arg and Asp164Tyr) allowing it to acetylate and reduce the activity of ciprofloxacin.²³

Discussion

Gram-negative rods of the genus *Serratia* are members of the order *Enterobacterales*, the significance of which, in medicine remains underestimated. The most important species within the genus *Serratia* is *S. marcescens*, due to a high frequency of isolations from humans and resistance to many antimicrobial agents. Nowadays, *S. marcescens* is unquestionably regarded as an etiological agent of opportunistic infections in humans, particularly in neonates and small children, but also in severely immunocompromised adults.^{2–5}

Samonis et al showed that more than 88% of *Serratia* spp. strains isolated from patients in Cretan hospitals between 2010 and 2015 were identified as *S. marcescens*.²⁴ In our study as many as 90% of analyzed strains of the genus *Serratia* were identified as *S. marcescens*.

Almost 29% of strains tested in this study (32 isolates) were recovered from the lower respiratory tract, followed by 26 (23.2%) isolates from wounds, and 25 (22.3%) strains recovered from urine. Ferreira et al reported that respiratory tract was the major source of *S. marcescens* isolates from patients hospitalized in intensive care units.²⁵



	TZP**	CTX	CAZ	FEP	E	IPM	MEM	AN	GM	NN	CIP	TIG
% R	23%	26%	7%	14%	4%	1%	1%	21%	24%	36%	33%	90%
% I*	0%	5%	10%	1%	0%	0%	1%	0%	0%	2%	3%	0%
% S	77%	69%	83%	85%	96%	99%	98%	79%	76%	62%	64%	10%

Figure 1 The antibiotic resistance patterns of *Serratia* spp. (n=112) measured by MIC value according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) recommendations.²²

Notes: *According to the decision of the EUCAST in July 2019 (implemented in January 2020) at present letter "I" means "susceptible with increased exposure". **n=104.
Abbreviations: TZP, piperacillin/tazobactam; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; E, ertapenem; IPM, imipenem; MEM, meropenem; AN, amikacin; GM, gentamicin; NN, tobramycin; CIP, ciprofloxacin; TIG, tigecycline.

Analysis of over 18,000 of the clinical isolates previously classified in the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*, collected from 85 US hospitals, showed domination of strains recovered from urine (34.5%), followed by the respiratory tract (28%), and isolates from wounds (18%).²⁶ Liou et al analyzed 403 nonduplicate *S. marcescens* strains – isolates from respiratory samples comprised 39.0%, followed by the urinary tract samples (22.3%).²⁷ These differences should be ascribed to various profiles of hospital wards that were the sources of analyzed strains.

According to EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria (version no. 11), all tested isolates of *Serratia* spp. were reported as resistant to colistin, despite the fact that strains nr 31, 51, 78, 98, 107, 108, 109, 114 and 115 examined in this study showed the MIC value meeting the criteria of "susceptible".²⁸ Strains of *Serratia* spp. are characterized by intrinsic resistance to amoxicillin/clavulanic acid, first and second generation cephalosporins due to the presence of a chromosomal AmpC beta-lactamase enzyme. These bacteria are also intrinsically resistant to colistin through

the genes (eg, *arnBCADTEF*) that encode the LPS (lipopolysaccharide)-modifying enzymes (addition of pEtN and L-Ara4N to LPS).²⁹ Therefore, regardless of the MIC value of mentioned antibiotics, all *Serratia* spp. strains have to be reported as "resistant" to these antibiotics because of the lack of clinical effect.

Among third generation cephalosporins, cefotaxime was the least effective against *Serratia* spp. strains. Less than 70% of tested strains were susceptible to this antibiotic in contrast to ceftazidime (83% of susceptible strains) and cefepime (85% of susceptible strains), which were the most effective cephalosporins (in our study the MIC values of carbapenems showed that only 5 (4%) strains were not susceptible to ertapenem), 1 (1%) to meropenem, and 1 (1%) to imipenem (Figure 1). Liou et al revealed that only 3 from 403 strains (0.7%) isolated in Taiwan between 2002–2010 were resistant to carbapenems.²⁷ Number of strains resistant to amikacin is convergent with data from Polish hospitals where around 10% of tested strains were resistant to amikacin.^{30,31} Of note, in our study 33% of strains were characterized by resistance to ciprofloxacin. Similar rate

Table 3 Compilation of Gene Cassettes in Class I Integrons

Strain	Source	Gene Cassette	Gene Cassette	Gene Cassette	Gene Cassette	NCBI Number
16	Respiratory tract	<i>aac(6')-Ib</i>	<i>blaVIM-4</i>			[JF905459.1]
18	Respiratory tract	<i>aac(6')-Ib</i>	<i>blaVIM-4</i>			[JF905459.1]
22	Wound	<i>aacC1</i>	ORF	ORF	<i>aadA1</i>	[KR028107.1]
27	Wound	<i>aacC1</i>	ORF	ORF	<i>aadA1</i>	[KR028107.1]
28	Wound	<i>aacC1</i>	ORF	ORF	<i>aadA1</i>	[KR028107.1]
31	Nose	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
32	Respiratory tract	<i>aadA1</i>				[KU948605.1]
33	Wound	<i>aadA1</i>				[KU948605.1]
40	Throat	<i>aadA1</i>				[KU948605.1]
41	Respiratory tract	<i>aadA1</i>				[KU948605.1]
44	Nose	<i>aac(6')-Ib</i>				[KU839731.1]
54	Wound	<i>aacC1</i>	ORF	ORF	<i>aadA1</i>	[KR028107.1]
61	Wound	<i>aacC1</i>	ORF	ORF	<i>aadA1</i>	[KR028107.1]
64	Urine	Lack of amplified variable region				
66	Liver	<i>aacC1</i>	ORF	ORF	<i>aadA1</i>	[KR028107.1]
81	Urine	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
82	Urine	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
83	No data	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
85	No data	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
87	No data	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
90	No data	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
91	No data	Lack of amplified variable region				
96	Urine	Lack of amplified variable region				
98	Urine	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]
99	No data	Lack of amplified variable region				
105	Urine	<i>aadA2</i>	ORF	<i>dfrA12</i>		[KY862013.1]

(39.6% of resistant strains) was reported by Liou et al and these authors postulated the need for continued surveillance of antimicrobial resistance of *Serratia* spp. clinical isolates, particularly for fluoroquinolones.²⁷ Hornsey et al reported that in therapy of infections caused by *S. marcescens*, the use of tigecycline is limited because of up-regulation of the SdeXY-HasF efflux pump which is associated with reduced susceptibility to this antibiotic and also to ciprofloxacin.³² In our study 90% of strains of

Serratia spp. were resistant to tigecycline. In a study by Magiorakos et al, 21 isolates (19%) were resistant to at least 3 different groups of antibiotics and chemotherapeutics and were reported as multidrug-resistant (MDR) *S. marcescens* strains.³³ Most of them (19 strains) harbored at least one integrase DNA sequence.

Among 26 tested strains carrying the class 1 integrase DNA sequence, for 22 strains PCR products were obtained as a result of amplification of the variable region of class 1

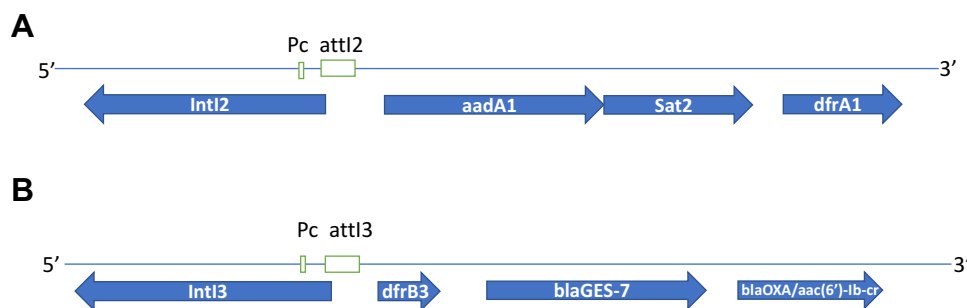


Figure 2 Diagram of integrons. Blue arrows mark open reading frames. Green boxes mark regulatory items: Pc- promoter and attI2 or attI3 – attachment site. **(A)** Class 2 integron: integrase (IntI2 – 977 bp) and antibiotic resistance gene cassettes (aadA1 – 852 bp, Sat2 – 583 bp, dfrA1 – 473 bp). **(B)** Class 3 integron: integrase (IntI3 – 1040 bp) and antibiotic resistance gene cassettes (dfrB3 – 236 bp, bla_{GES-7} – 863 bp, bla_{OXA/aac(6')-Ib-cr} – 554 bp).

integrons. All of them had at least one aminoglycoside resistance gene cassette, and all (except strain no. 41) showed resistance to gentamicin and tobramycin ([Appendix A](#)).

In this study we detected four different cassettes containing resistance genes to aminoglycosides, only one cassette with a resistance gene to β -lactams and one cassette with resistance genes to trimethoprim ([Table 3](#)). Among 39 cassettes containing genes with a known function, 28 (71.8%) comprised cassettes determining resistance to aminoglycosides, 9 (23.0%) carried genes of resistance to trimethoprim, and only 2 (5.1%) were cassettes with the genes of resistance to β -lactams. We observed similar proportions of cassettes as reported by Xia et al who analyzed different types of gene cassette arrays of integrons from clinical bacterial isolates in China, 2000–2014.³⁴

Nielsen et al analyzed coexistence of gene cassettes linked to bacterial resistance to antimicrobials and integrase class 1 sequence in almost 16,000 nucleotide sequences of bacterial genomes.³⁵ These authors showed that the most common mechanism of resistance linked to integron class 1 were gene cassettes coding for aminoglycoside nucleotidyltransferase.³⁵ Among 22 variable regions of integrons class 1 characterized in this study, only three (no. 16, 18 and 44) did not contain a gene cassette coding for aminoglycoside nucleotidyltransferase. Ten strains carried cassette aadA1, and nine – cassette aadA2.

Among the analyzed strains of *Serratia* spp., there were 5 which carried only a single cassette (aadA1), 2 strains – carried two cassettes 5'CS-aac(6')-Ib-bla_{VIM-4}-3'CS, 8 strains had three cassettes 5'CS-aadA2-ORF-dfr12-3'CS, and 5 strains – carried 5'CS-aacC1-ORF-ORF-aadA1-3'CS. It is often reported that cassette aac(6')-Ib is the first or the only one in the array. It is also typical for cassette aadA1 to be the lone one, or the last in the array.³⁶

Cassette array 5'CS-aadA2-ORF-dfr12-3'CS is atypical, not reported in *Serratia* spp. so far, however it was reported in *K. pneumoniae* strains isolated in Russia [KY862013.1] and China [DQ323053]. Similarly, cassette array 5'CS-aacC1-ORF-ORF-aadA1-3'CS is rarely detected – it was isolated in Russia [KR028107.1], Ireland [AJ784787] and Australia [EF015496], solely in the strains of *Acinetobacter baumannii*.

Two strains (number 16 and 18) carried two identical gene cassettes encoding metallo- β -lactamase bla_{VIM-4} and aminoglycoside 6'-N-acetyltransferase aac(6')-Ib ([Table 3](#)). BLAST analysis of these two sequences revealed their identity with integron sequences found in Hungarian strains of *S. marcescens* [NCBI JF905459.1], *K. pneumoniae* and *K. oxytoca*.³⁷ In Poland, VIM-4 carbapenemase was originally observed in *P. aeruginosa* strains isolated from hospitalized children.³⁸ Comparison of amino acid sequences of integrons no. 16 and 18 with VIM-4 from *P. aeruginosa* revealed a single substitution in the repeat region. Valine present in protein of *P. aeruginosa* was substituted by glycine in *S. marcescens* isolates no. 16 and 18 (Val168Gly). Between 2006 and 2012 Izdebski et al identified 20 *S. marcescens* strains producing VIM/IMP-carbapenemase, including 11 isolates carrying bla_{VIM-4} gene cassette.²⁰ All of them possess In238 variant of class 1 integron containing the following array of gene cassettes: 5'CS-aacA4-bla_{VIM-4}-3'CS which are identical to DNA sequence of integrons no. 16 and 18. We can speculate with high probability that Izdebski analyzed isolates identical to no. 16 and 18, but no sufficient data (antimicrobial resistance, source of strains) are available to conclude it definitely.

In view of the nonsense mutation in 179 codon Int2 sequence, class 2 integrons are less varied than class 1 integrons.¹⁴ Isolates no. 16, 18, 22, 27, 28, 43, 54, 61, 66 and 85 had identical nucleotide sequence of gene cassettes located in class 2 integrons ([Figure 2](#)). Separate analysis

focusing on occurrence and characteristics of class 2 integrons was not conducted on Polish clinical isolates. Kotlarska et al analyzed *E. coli* isolated from wastewater and reported that three of four analyzed class 2 integrons were identical to integrons investigated in this paper.³⁹

Nowadays class 3 integrons undergo a dynamic worldwide expansion, however it could not be explored quickly by PCR reaction due to the lack of 3' conserved region in DNA sequence. For *S. marcescens* strain no. 89 with confirmed presence of DNA sequence of class 3 integrase, plasmid DNA sequencing was performed because previously class 3 integrons were reported throughout plasmid DNA.^{17,40–42} The sequence of pPCMI3 plasmid, which is 9448 bp in size, was submitted to NCBI GenBank (NCBI MH569711).

Plasmid pPCMI3 harbors class 3 integron with three gene cassettes. The first of these, *dfrB3*, which is responsible for resistance to trimethoprim,⁴³ and the second gene cassette encoding β -lactamase GES-7, had been already described in the DNA sequences of integrons.^{44–46} The last one is a gene cassette coding the fusion protein OXA/aac(6')-Ib-cr, it consists of β -lactamase OXA responsible for resistance to β -lactams and acetyltransferase aac(6')-Ib-cr responsible for resistance to aminoglycosides and fluoroquinolones. Gene *aac(6')-Ib-cr* has been detected in many clinical strains of bacteria.^{47,48} Fusion oxa/aac(6')-Ib has also been described in the structure of plasmid classified in the group IncQ carrying integron class 3.⁴⁹ Whereas fusion oxa/aac(6')-Ib-cr was detected for the first time in this study.

Piotrowska et al developed molecular characterization and comparative genomics of IncQ-3 plasmids isolated from raw wastewater of "Czajka" Wastewater Treatment Plant in Warsaw, Poland.⁴⁵ Plasmid p115_p2 hosted in *Raoultella* sp. and isolated from raw wastewater has identical size as plasmid pPCMI3 and, moreover, BLAST analysis revealed high level homology (more than 99.9%) between them. The occurrence of IntI3 in pPCMI3, an IncQ plasmid, provides possibilities for widespread dissemination, an ability to replicate in diverse hosts and carrying of diverse antibiotic resistance genes conferring resistance to different antibiotics and chemotherapeutics, eg, carbapenems, quinolones, β -lactams, sulphonamides, and tetracyclines.⁵⁰

In conclusion, although strains of *Serratia* spp. are opportunistic pathogens which cause nosocomial infections, they still remain outside the mainstream interest of scientists. The results obtained in this study constitute a comprehensive analysis of the integrons in the clinical isolates of *Serratia* spp. in

Poland. Among tested isolates of *Serratia* spp., 28 (25%) harbored at least one integron DNA sequence with antimicrobial resistance gene cassettes. Carbapenems remain a group of antibiotics active against the the highest number of tested isolates, however their use should be restricted to avoid emergence of resistance. These observations indicate the need for ongoing research to monitor the prevalence of integrons in the clinical isolates of *Serratia* spp.

Ethics Statement

The clinical samples were part of the routine hospital laboratory procedure and therefore approval of the institutional review board or ethics committee was not applicable.

Funding

The study was supported by the grant funded by the National Science Centre of Poland. (No. DEC-2011/01/N/NZ7/05336).

Disclosure

We declare that there are no conflicts of interest associated with this study.

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