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Prevalence of ESBL and PMQR Genes in *Serratia marcescens* Isolated from Clinical Samples at a University Hospital

Bir Üniversite Hastanesindeki Klinik Örneklerden İzole Edilen *Serratia marcescens* İzolatlarında ESBL ve PMQR Genlerinin Prevalansı

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ABSTRACT

Introduction: *Serratia marcescens is a gram-negative bacterium that causes severe infections and contributes to increased morbidity and mortality due to rising antimicrobial resistance. This study evaluated the prevalence of extended-spectrum beta-lactamase (ESBL) and plasmid-mediated quinolone resistance (PMQR) genes.*

Materials and Methods: *A total of 640 S. marcescens strains isolated from various clinical samples over five years were included in this study. The strains were identified by mass spectrometry, and their antimicrobial susceptibility was determined using an automated system (Becton, Dickinson and Company, Franklin Lake, USA) and the Kirby-Bauer disk diffusion method, with evaluation based on The European Committee on Antimicrobial Susceptibility Testing 2022 criteria. Seventy strains that exhibited intermediate (I) or resistant (R) profiles to third-generation cephalosporins or quinolones were further screened by in-house polymerase chain reaction for beta-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}), and PMQR genes (qnrA, qnrB, qnrS, aac(6')-Ib-cr, and qepA), as well as for integron content. The blaCTX-M gene and the variable region of class 1 integrons were analyzed by Sanger sequencing.*

Results: *The detected resistance genes included 5.7% (n= 4) bla_{TEM}, 2.9% (n= 2) bla_{SHV}, 1.4% (n= 1) $bla_{CTX-M-15}$, 4.3% (n= 3) qnrS, 2.9% (n= 2) aac(6')-Ib-cr, and 4.3% (n= 3) intI1. The bla_{OXA}, qnrA, qnrB, qepA, and intI2 genes were not detected. Two strains harboring class 1 integrons contained gene cassettes including aadA2, dfrA12, and ANT(2")-Ia.*

Conclusion: *This study provides valuable epidemiological data on ESBL production and quinolone resistance genes among S. marcescens strains in our region, indicating an overall low level of resistance to carbapenems and aminoglycosides, but a noticeable increase in resistance to third-generation cephalosporins and quinolones over the years. Further studies and surveillance are needed to better guide the use of antibiotics in S. marcescens infections.*

Key Words: *Serratia marcescens; ESBL; PMQR; Integron; Antibiotic resistance*

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ÖZ

Bir Üniversite Hastanesindeki Klinik Örneklerden İzole Edilen *Serratia marcescens* İzolatlarında ESBL ve PMQR Genlerinin Prevalansı

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Giriş: *Serratia marcescens ciddi infeksiyonlara yol açan ve artan antimikrobiyal direnç profili nedeniyle mortalite ve morbidite artışına neden olan bir gram-negatif bakteridir. Bu çalışmada, S. marcescens'te genişlemiş spektrumlu beta-laktamaz (ESBL) ve plazmid aracılı kinolon direnci (PMQR) genlerinin prevalansı değerlendirildi.*

Materyal ve Metod: *Bu çalışmaya beş yıl boyunca çeşitli klinik örneklerden izole edilen toplam 640 S. marcescens suşu dahil edildi. Suşlar MALDI-TOF ile tanımlandı ve suşların antimikrobiyal duyarlılıkları otomatize sistem (Becton, Dickinson and Company, Franklin Lake, ABD) ve Kirby-Bauer disk difüzyon yöntemi kullanılarak belirlendi ve The European Committee on Antimicrobial Susceptibility Testing 2022'ye göre değerlendirildi. Üçüncü kuşak sefalosporinlere veya kinolonlara karşı orta duyarlı (I) veya dirençli (R) yetmiş suş beta-laktamaz genleri (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}), PMQR genleri (qnrA, qnrB, qnrS, aac(6')-Ib-cr, qepA) and integron içeriği in-house polimeraz zincir reaksiyonu yöntemiyle tarandı. bla_{CTX-M} ve sınıf 1 integron içeriğinin Sanger sekans analizi yapıldı.*

Bulgular: *Tespit edilen antimikrobiyal direnç genleri %5.7 (n= 4) bla_{TEM}, %2.9 (n= 2) bla_{SHV}, %1.4 (n= 1) $bla_{CTX-M-15}$, %4.3 (n= 3) qnrS, %2.9 (n= 2) aac(6')-Ib-cr ve %4.3 (n= 3) intI1 idi. bla_{OXA}, qnrA, qnrB, qepA ve intI2 genleri tespit edilmedi. Sınıf 1 integronları bulunan iki suş, aadA2, dfrA12 ve ANT(2")-Ia gen kasetleri içeriyordu.*

Sonuç: *Bu çalışma, bölgemizdeki S. marcescens suşları arasında ESBL üretimi ve kinolon direnç genleri hakkında önemli epidemiyolojik veriler sunarak, karbapenemlere ve aminoglikozitlere karşı genel olarak düşük bir direnç seviyesi olduğunu ancak yıllar içinde üçüncü kuşak sefalosporinlere ve kinolonlara karşı dirençte belirgin bir artış olduğunu göstermektedir. S. marcescens infeksiyonlarında antibiyotik kullanımının daha iyi yönetilebilmesi için daha fazla sürveyans çalışmalarına ihtiyaç vardır.*

Anahtar Kelimeler: *Serratia marcescens; ESBL; PMQR; İntegron; Antibiyotik direnci*

INTRODUCTION

Serratia marcescens is a ubiquitous gram-negative, oxidase-negative, rod-shaped opportunistic pathogen belonging to the family *Yersiniaceae* within the order *Enterobacterales*. More than 20 *Serratia* species have been described, *S. marcescens* being the most commonly associated with human infections, including pneumonia, urinary tract infection, keratitis, and sepsis. *S. marcescens* frequently causes infections and outbreaks, especially in pediatric patients and immunosuppressed adults[1,2]. For a long time, *S. marcescens* was considered a nonpathogenic saprophytic organism. However, in the 1950s, it was recognized as a human pathogen and has since developed significant resistance. Global surveillance studies are needed to monitor the antimicrobial resistance of this microorganism[3,4].

Antibiotic resistance is a global health issue, leading to increased morbidity and mortality. Acquired resistance may arise from chromosomal mutations or horizontal gene transfer[5]. The occurrence of chromosomally encoded AmpC beta-lactamase is the intrinsic resistance mechanism to certain beta-lactams, such as ampicillin and amoxicillin/clavulanate, in this bacterium[1]. Extended-spectrum beta-lactamases (ESBLs) confer resistance to penicillin, first-, second-, and third-generation cephalosporins, and monobactams, but not to cephamycin or carbapenems. ESBL genes can be encoded on plasmids or chromosomes, and mobile genetic elements may carry multiple resistance genes simultaneously, such as those for ESBL and plasmid-mediated quinolone resistance (PMQR)[6]. Quinolone resistance can result from chromosomal mutations or the presence of PMQR genes. There are three determinants of PMQR:

qnr proteins, *aac*(6')-Ib-cr, and *qep*A. However, quinolone resistance is primarily attributed to chromosomal mutations[7]. Integrons are mobile genetic elements carried on chromosomes, plasmids, and transposons, and their transfer is responsible for the spread of multidrug resistance among bacteria[8] .

The difficulty in treating *S. marcescens* infections is largely due to its intrinsic and acquired resistance to antimicrobials. Therefore, surveillance of acquired antimicrobial resistance is crucial. This study aimed to investigate ESBL production, resistance to quinolones, and the presence of class 1 and class 2 integrons in *S. marcescens* strains over the years.

MATERIALS and METHODS

Design and Settings

Karadeniz Technical University, Faculty of Medicine, Farabi Hospital is an 831-bed tertiary care hospital. In this study, 1377 clinical samples from which *S. marcescens* was isolated were analyzed. When repeated reproductions were excluded, 640 antimicrobial susceptibility test results for *S. marcescens* were analyzed between January 2018 and December 2022. This study focused on 70 *S. marcescens* strains that exhibited intermediate (I) or resistant (R) profiles to third-generation cephalosporins or quinolones. These strains were further analyzed for the presence of ESBL and PMQR genes, as well as integron content, using polymerase chain reaction (PCR). All strains were identified using conventional microbiological methods and mass spectrometry [matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), Bruker, Bremen, Germany]. The *pfs* and *lux*S genes were also screened. Antimicrobial susceptibility tests were performed using and automated system (Becton, Dickinson and Company, Franklin Lake, USA) and Kirby-Bauer disk diffusion method, evaluated based on The European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2022 criteria[9]. Beta-lactamase genes (bla_{TEM}, bla_{SHV}, $bla_{CTX-M,}$ and bla_{OXA}), PMQR genes (*qnr*A, *qnr*B, *qnr*S, *aac*(6')-Ib-cr, and *qep*A), and class 1 and 2 integrons (*int*I1 and *int*I2) were also screened. This study aimed to contribute to the treatment of *S. marcescens* infections by identifying phenotypic antibiotic resistance, resistance genes, and integrons.

Inclusion and Exclusion Criteria

S. marcescens strains isolated from clinical samples between January 2018 and December 2022 were included in the study. If multiple isolates were obtained from the same patient, only the first strain was included.

Sample Collection, Identification, and Antimicrobial Susceptibility Tests

Seventy strains isolated from clinical samples were identified as *S. marcescens* using MALDI-TOF mass spectrometry. Antimicrobial susceptibility tests were performed on clinical strains identified as *S. marcescens* on the BD Phoenix automated identification/antimicrobial susceptibility testing system (Becton, Dickinson and Company, Franklin Lake, USA) per the manufacturer's recommendations. The Kirby-Bauer disk diffusion method was used with ciprofloxacin (5 µg), levofloxacin (5 µg), cefotaxime (5 µg), and ceftazidime (10 µg) disks (Oxoid, Basingstoke, UK), following EUCAST guidelines[9]. Antimicrobial susceptibility test results were interpreted according to the EUCAST 2022 breakpoint table[10].

Polymerase Chain Reaction

The *pfs* and *lux*S genes were screened for *S. marcescens* identification as described by Zhu et al[11]. Antimicrobial resistance genes (bla_{TEM}, $bla_{SHV,}$ bla_{CTX-M}, bla_{OXA}, *qnr*A, *qnr*B, *qnr*S, *qep*A, and *aac*(6')-Ib-cr), and class 1 and class 2 integrons, integrase 1 and integrase 2 genes (*int*I1 and *int*I2) were also screened by in-house PCR. For strains positive for integrase genes, PCR was performed with 3'CS and 5'CS primers to analyze the variable regions. The primers used are listed in Table 1. Positive controls for the PCR assays were sourced from the Karadeniz Technical University, Medical Microbiology Laboratory and Prof. Dr. O. B. Özgümüş.

PCR conditions for *pfs* and *lux*S genes were as follows: 94 °C for five min; 30 cycles of 94 °C for 45 sec, 55 °C for 30 sec, 72 °C for 15 sec, followed by a final extension at 72 °C for 10 minutes. For antimicrobial resistance genes, the PCR conditions were: 94 °C five min; 30 cycles of 94 °C one min, 55 °C one min, 72 °C one min, followed by 72 °C for

Table 1. Primers used in the study

Primer	Sequence (5′ → 3′)	Target Gene	PCR product size (bp)	References
Fpfs1 Rpfs2	CCG GCA TCG GCA AAG TCT ATC TGG CCC GGC TCG TAG CC	*pfs*	193	[11]
FluxS1 RluxS2	GCT GGA ACA CCT GTT CGC ATG TAG AAA CCG GTG CGG	*lux*S	102	[11]
TEM-F TEM-R	ATG AGT ATT CAA CAT TTC CG CTG ACA GTT ACC AAT GCT TA	bla_{TEM}	867	[35]
SHV-F SHV-R	TCG GGC CGC GTA GGC ATG AT AGC AGG GCG ACA ATC CCG CG	bla_{SHV}	626	[36]
CTX-M-F CTX-M-R	CGC TTT GCG ATG TGC AG ACC GCG ATA TCG TTG GT	bla_{CTX-M}	550	[37]
OXA-F OXA-R	ACA CAA TAC ATA TCA ACT TCG C AGT GTG TTT AGA ATG GTG ATC	bla_{OXA}	885	[35]
qnrA1 qnrA2	ATT TCT CAC GCC AGG ATT TG GAT CGG CAA AGG TTA GGT CA	*qnr*A	516	[38]
qnrB1 qnrB2	GAT CGT GAA AGC CAG AAA GG ACG ATG CCT GGT AGT TGT CC	*qnr*B	469	[38]
qnrS1 qnrS2	GCA AGT TCA TTG AAC AGG GT TCT AAA CCG TCG AGT TCG GCG	*qnr*S	428	[39]
aac6IbcrF aac6IbcrR	TTG CGA TGC TCT ATG AGT GGC TA CTC GAA TGC CTG GCG TGT TT	*aac*(6′)-Ib-cr	482	[40]
qepAF qepAR	AAC TGC TTG AGC CCG TAG AT GTC TAC GCC ATG GAC CTC AC	*qep*A	596	[39]
IntI1F IntI1R	GGT CAA GGA TCT GGA TTT GG ACA TGC GTG TAA ATC ATC GTC	*int*I1	500	[41]
IntI2F IntI2R	CAC GGA TAT GCG ACA AAA AGG T GTA GCA AAC GAG TGA CGA AAT G	*int*I2	740	[41]
5′-CS 3′-CS	GGC ATC CAA GCA GCA AG AAG CAG ACT TGA CCT GA	5′-CS 3′-CS	variable	[42]

Bp: Base pair.

10 minutes. PCR conditions for the *int*I1 and *int*I2 genes were: 94 °C five min; 34 cycles of 94 °C 45 sec, 57 °C one min, 72 °C one min, followed by 72 °C for 10 minutes. For class 1 integron variable region analysis, the PCR conditions were: 94 °C for three min; 35 cycles of 94 °C for one min, 55 °C for one min, and 72 °C for 2.5 min, followed by a final extension at 72 °C for five minutes. Electrophoresis was performed on 2% agarose gel containing 0.5 µg/mL ethidium bromide (Sigma Aldrich, St. Louis, USA), and bands were visualized using a transilluminator (Vilber, Eberhardzell, Germany).

DNA Sequencing

PCR products of the variable regions of integrons and bla_{CTX-M} were sent to Macrogen Inc. (Amsterdam, Netherlands) for DNA Sanger sequencing. Sequence similarity searches were conducted using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov).

Conjugation and Transformation Assays

Conjugation assays were performed using the broth mating method[12,13]. *S. marcescens* strains positive for bla_{TEM}, bla_{SHV}, or bla_{CTX-M} were used as donors, while rifampicin-resistant *Escherichia coli* J53-2 (*met pro*) served as the recipient. Equal volumes (1 ml each) of recipient and donor cells were mixed gently in a tube and incubated at 37 °C overnight without shaking. A 100 µl aliquot of this mixture was plated on Luria-Bertani (LB) agar (Becton, Dickinson and Company, Franklin Lake, USA) supplemented with 2 µg/mL of ceftazidime and 150 µg/mL

of rifampicin (Sigma Aldrich, St. Louis, USA) to select for transconjugants.

Total plasmid DNA was purified from the strains using the alkaline extraction method[14] and transformed into competent *E. coli* DH5α cells by the heat shock method, as previously described[15]. Transformants were selected on LB agar containing 100 µg/mL of ampicillin (Sigma Aldrich, St. Louis, USA). Plasmid DNA was electrophoresed on a 0.9% agarose gel containing 0.5 µg/mL ethidium bromide and visualized under UV light.

RESULTS

Of the patients from whom the 70 *S. marcescens* strains were isolated, 27.1% were female and 72.9% were male, with a mean age of 55.3 years (standart deviation= 31.1; range= 0–98). The strains were most frequently isolated from the pediatrics (18.6%) [which included pediatric departments (5.7%), pediatric outpatient clinic (7.1%), pediatric intensive care unit (ICU) (2.9%), newborn ICU (2.9%)], surgical department (18.6%), surgical outpatient clinic (11.4%), internal medicine departments (14.3%), internal medicine outpatient clinic (20%) and ICU (17.1%) departments. Of all 70 patients, 61.4% were hospitalized inpatients, including those in the ICU. The most common sources of clinical samples were respiratory tract samples (42.9%), urine (31.4%), wounds (10%), blood cultures (5.7%), and other sources.

Over five years, *S. marcescens* was isolated from 1377 clinical samples. After excluding recurrent isolates, 640 antimicrobial susceptibility test results for *S. marcescens* were analyzed (Table 2). Seventy *S. marcescens* isolates with intermediate (I) or resistant (R) profiles to third-generation cephalosporins or quinolones were further analyzed for the presence of ESBL and PMQR genes, as well as integron content, using PCR. Antimicrobial susceptibility tests were conducted using an automated system and the disk diffusion method, with the results

Table 2. The antimicrobial resistance profiles of *S. marcescens* (n= 640) between 2018-2022

Date	Number of strains		AK	GN	CIP	CAZ	CRO	PTZ	SXT	IMP	MEM	ERT
2018	122	R	0.8%	0.8%	0.8%	0.8%	2.5%	2.5%	0.8%	2.5%	0%	0%
		I	0%	0%	0%	1.6%	3.3%	0%	0%	0%	0%	0%
		S	99.2%	99.2%	99.2%	97.6%	94.2%	97.5%	99.2%	97.5%	100%	100%
2019	131	R	0.8%	0.8%	6.1%	8.4%	9.2%	2.3%	1.5%	1.5%	1.5%	3.8%
		I	0%	0%	4.6%	0%	0.8%	0%	0%	1.5%	0%	0%
		S	99.2%	99.2%	89.3%	91.6%	90%	97.7%	98.5%	97%	98.5%	96.2%
2020	123	R	0.8%	4.1%	8.1%	4.9%	8.2%	2.5%	3.3%	0.8%	0%	0.8%
		I	0%	0%	2.5%	4.1%	1.6%	0.8%	0%	9%	0%	0%
		S	99.2%	95.9%	89.4%	91%	90.2%	96.7%	96.7%	90.2%	100%	99.2%
2021	142	R	0.7%	4.2%	12.7%	11.3%	16.9%	10.6%	5.6%	0.7%	0%	6.3%
		I	0%	0%	3.5%	4.2%	0%	1.4%	0%	7%	2.8%	0%
		S	99.3%	95.8%	83.8%	84.5%	83.1%	88%	94.4%	92.3%	97.2%	93.7%
2022	122	R	0%	2.5%	8.2%	3.3%	8.2%	3.3%	1.6%	0.8%	0%	4.1%
		I	0%	0%	2.5%	3.3%	2.5%	1.6%	0%	3.3%	0.8%	0%
		S	100%	97.5%	89.3%	93.4%	89.3%	95.1%	98.4%	95.9%	99.2%	95.9%
Total	**640**	**R**	**0.5%**	**2.5%**	**7.3%**	**5.9%**	**9.2%**	**4.4%**	**2.7%**	**0.8%**	**0.3%**	**3.1%**
		I	**0.1%**	**0%**	**2.7%**	**2.7%**	**1.6%**	**0.8%**	**0%**	**4.7%**	**0.8%**	**0%**
		S	**99.4%**	**97.5%**	**90%**	**91.4%**	**89.2%**	**94.8%**	**97.3%**	**94.5%**	**98.9%**	**96.9%**

AK: Amikacin, GN: Gentamicin, CIP: Ciprofloxacin, CAZ: Ceftazidime, CRO: Ceftriaxone, PTZ: Piperacillin/Tazobactam, SXT: Trimethoprim-Sulfamethoxazole, IMP: Imipenem, MEM: Meropenem, ERT: Ertapenem.

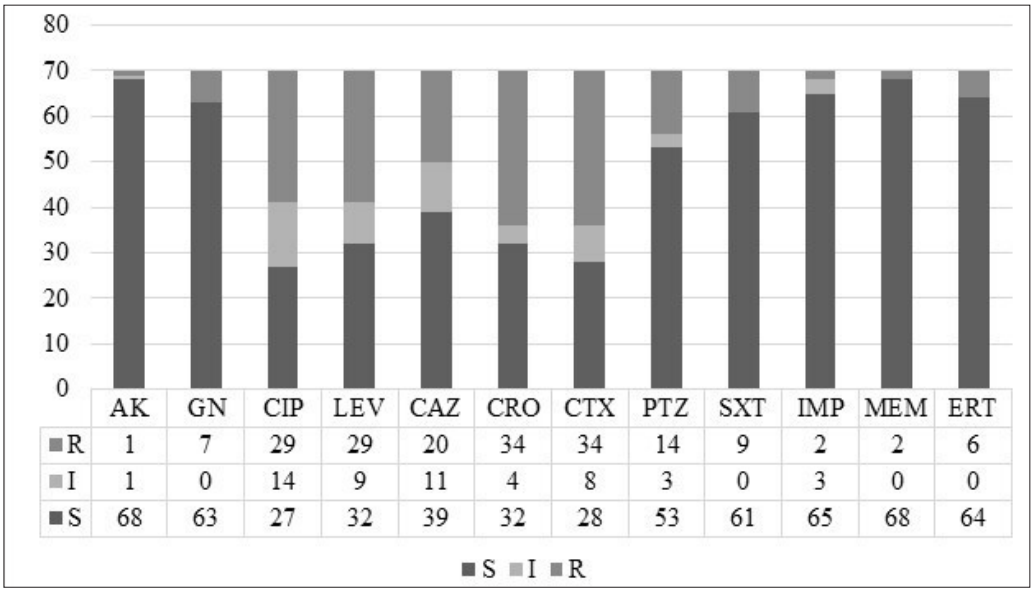

Figure 1. Antimicrobial susceptibility test results in 70 *S. marcescens* strains.

AK: Amikacin, GN: Gentamicin, CIP: Ciprofloxacin, CAZ: Ceftazidime, CRO: Ceftriaxone, PTZ: Piperacillin/Tazobactam, SXT: Trimethoprim-Sulfamethoxazole, IMP: Imipenem, MEM: Meropenem, ERT: Ertapenem.

for the 70 *S. marcescens* strains (Figure 1). Among these 70 selected strains, 21.4% were multidrug-resistant (MDR). These strains were initially identified using MALDI-TOF and using PCR targeting the *pfs* and *lux*S genes. All *S. marcescens* strains tested positive for both genes.

Isolate TRSM 5, which was resistant to ceftriaxone and cefotaxime, showed positive phenotypic ESBL confirmation using the cefepime/cefepime-clavulanate gradient strip test. Genotypic confirmation of ESBL was performed by sequencing the $bla_{\text{CTX-M}}$ PCR product, which identified the presence of $bla_{\text{CTX-M-15}}$.

The following genes were detected by PCR: 5.7% (n= 4) bla_{TEM}, 2.9% (n= 2) bla_{SHV}, 1.4% (n= 1) $bla_{\text{CTX-M-15}}$, 4.3% (n= 3) *qnr*S, 2.9% (n= 2) *aac*(6')-Ib-cr, and 4.3% (n= 3) *int*I1. The bla_{OXA}, *qnr*A, *qnr*B, *qep*A, *and int*I2 genes were not detected. PCR was then performed with 3'CS and 5'CS primers for variable region analysis on strains with positive *int*I1 genes. The analysis revealed that two out of the three strains (TRSM 107 and TRSM 119) carried gene cassettes. The variable region of class 1 integrons was characterized as follows: TRSM 107 carried the *aad*A2 and *dfr*A12 gene cassettes, while TRSM 119 carried the *dfr*A12 and *ANT*(2")-Ia gene cassettes. Isolate TRSM 66 carried the bla_{TEM} and *aac*(6')-Ib-cr genes; TRSM 107 carried the bla_{TEM}, *aad*A2, and *dfr*A12; TRSM 119 carried the *aac*(6')-Ib-cr, *dfr*A12, and *ANT*(2")-Ia genes concurrently (Table 3). No plasmid-mediated resistance was detected in any of the beta-lactamase gene-containing strains.

DISCUSSION

Over time, *S. marcescens* rapidly acquires resistance to many antimicrobial agents, leading to treatment failure. Beta-lactams and quinolones are frequently used to treat infections caused by gram-negative bacteria. The production of ESBL and PMQR is a significant factor in antimicrobial resistance[16]. The spread of antimicrobial resistance genes among bacteria via mobile genetic elements increases morbidity and mortality rates in hospitals[17]. Surveillance of acquired antimicrobial resistance, along with epidemiological data, is crucial. It contributes to the development of both empirical treatment protocols and regional or national antibiotic resistance profiles. Additionally, identifying resistant bacteria in the laboratory is essential for guiding appropriate treatment[18].

Table 3. Epidemiological properties of resistance genes and integron-containing *S. marcescens* strains

Strain	Date	Source	Hospital unit	Antimicrobial resistance profile	Resistance genes	Integron content	Gene cassette array
TRSM 1	2020	Blood culture	Pediatric department	CAZ (I), CTX (I), IMP (I)	bla_{TEM}		
TRSM 5	2020	Sputum	Surgical department	CIP, LEV, CAZ, CRO, CTX	$bla_{CTX\text{-}M\text{-}15}$		
TRSM 106	2020	Urine	Pediatric outpatient clinic	GN, CIP, LEV, CRO, CTX	bla_{TEM}		
TRSM 107	2020	Endotracheal aspirate	ICU	GN, CIP, LEV, CAZ, CRO, CTX, PZT, SXT	bla_{TEM}	Class 1 integron	*aad*A2, *dfr*A12
TRSM 31	2021	Tissue biopsy	Surgical department	CIP (I)	*qnr*S		
TRSM 32	2021	Endotracheal aspirate	Pediatric department	CIP, LEV	*bla*		
TRSM 33	2021	Wound	Surgical department	CIP (I)	*bla*		
TRSM 58	2021	Endotracheal aspirate	ICU	CAZ, CRO, CTX	bla_{SHV}		
TRSM 66	2021	Urine	Surgical department	GN, CIP, LEV, CAZ (I), CRO, CTX, PZT, SXT, ERT	bla_{TEM}, *aac* (6')-Ib-cr		
TRSM 119	2022	Urine	Internal medicine outpatient clinic	GN, CIP, LEV, CAZ (I), CRO, CTX, PZT	*aac* (6')-Ib-cr	Class 1 integron	*dfr*A12, *ANT* (2")-Ia
TRSM 120	2022	Urine	Internal medicine department	CIP, LEV, CRO, CTX, SXT	bla_{SHV}		

AK: Amikacin, GN: Gentamicin, CIP: Ciprofloxacin, CAZ: Ceftazidime, CRO: Ceftriaxone, PTZ: Piperacillin/Tazobactam, SXT: Trimethoprim-Sulfamethoxazole, ICU: Intensive care unit, IMP: Imipenem, MEM: Meropenem, ERT: Ertapenem.

When antimicrobial susceptibility test results were analyzed over five years, resistance rates to aminoglycosides, carbapenems, piperacillin-tazobactam, and sulfonamides were found to be lower than other antibiotics. Gonzalez et al. and Yang et al. reported findings consistent with this study. Samonis et al. and Celejewski et al.[19-21] observed that carbapenems were the least resistant. Notably, resistance to third-generation cephalosporins and quinolones showed a tendency to increase and fluctuate. Therefore, strains with reduced susceptibility to third-generation cephalosporins and quinolones were further analyzed.

In the 70 *S. marcescens* strains included in the study, the majority of patients were male, with a mean age of 55.3 (range 0-98). The patient ages ranged from newborn to 98 years old. The respiratory system was the most common source of *S. marcescens* infection. *S. marcescens* infections frequently affect pediatric patients; in this study, it was most frequently isolated from the pediatrics department[22]. Ferreira et al. demonstrated that *S. marcescens* strains were more frequently found in males and were isolated from the respiratory system. In their study, the mean age of the patients was 57 years (range= 0-93). Clinical samples in studies may vary depending on hospital facilities.

According to the EUCAST guidelines, intermediate and/or resistant susceptibility to ceftazidime and/or cefotaxime (or resistance to cefpodoxime)

in members of the order *Enterobacterales* indicates a positive ESBL screening result[23]. In inducible chromosomal AmpC-producing bacteria such as *S. marcescens*, ESBL confirmation is performed using the cefepime/cefepime-clavulanate combination.

Most ESBL enzymes are encoded on plasmids and spread via horizontal gene transfer. These plasmids may also carry resistance genes to multiple other antimicrobials. Consequently, resistance to other antimicrobials may develop alongside ESBL production, leading to treatment failure and increased mortality. It is, therefore, important to identify ESBL-producing bacteria early and implement appropriate treatment protocols[6,24,25].

McCann et al., Samonis et al., Elsherbiny et al., Jean et al., Ferreira et al. and Tamma et al. reported that the frequency of ESBL production in *S. marcescens* ranged from 11%, 7.9%, 45.5%, 16%, 16.7% and <1% in different countries, respectively[20,22,26-29]. Generally, CTX-M is the most common enzyme.

TRSM 5 was the only strain shown to be a phenotypic and genotypic ESBL producer, carrying the $bla_{CTX\text{-}M\text{-}15}$ gene. The rate of ESBL-producing *S. marcescens* was 1.4% in this study. CTX-M-15 has been reported as the most common enzyme in CTX-M type beta-lactamases in *S. marcescens*[4].

Quinolone resistance is not caused by PMQR genes alone. Chromosomal mutations (such as *gyr*A, *gyr*B, *par*C, and *par*E) are the primary mechanisms that cause quinolone resistance. However, the spread of genes carried on plasmids poses a serious problem for treatment, as they have a greater capacity to drive epidemics[24,25,30]. The *aac*(6')-Ib-cr gene may be associated with resistance to fluoroquinolones and aminoglycosides. Ferreira et al. reported consistent findings[22]. In this study, TRSM 66 and TRSM 119 were both *aac*(6')-Ib-cr positive. These two strains were also resistant to gentamicin and ciprofloxacin we tested.

Yang et al. reported that the frequency of PMQR genes in *S. marcescens* was 4.8%[31]. In this study, 4.3% (n= 3) strains carried the *qnr*S gene, and 2.9% (n= 2) carried the *aac*(6')-Ib-cr gene. The *qnr*A, *qnr*B, and *qep*A genes were not detected.

Yang et al. showed that five of seven ESBL-producing strains (71.4%) carried the PMQR gene[16]. The co-production of these genes suggests that caution should be exercised when using quinolones in ESBL-producing strains. Consequently, treatment options are limited in ESBL-producing bacteria.

Many resistance genes can be carried on the same plasmid[24,25]. Multiple studies have shown that ESBL and PMQR genes are often carried together in *Enterobacterales*[16,32,33]. TRSM 66 carried both the bla_{TEM} and *aac*(6')-Ib-cr genes simultaneously.

In the *S. marcescens* strain TRSM 5, third-generation cephalosporin resistance was not transferred to rifampicin-resistant *E. coli* J53-2 recipient in the conjugation assay, indicating that the $bla_{CTX\text{-}M\text{-}15}$ gene is likely located on the chromosome. Total plasmid DNA preparations from the remaining strains containing the beta-lactamase gene were transferred to *E. coli* DH5α by heat shock. However, no transformants were obtained in ampicillin selection, suggesting that these genes may also be located on the bacterial chromosome[6].

Integrons are genetic elements capable of capturing gene cassettes, which can be found on plasmids, transposons, and chromosomes. Gene cassettes encode many genes, including antimicrobial resistance genes, and play a role in the spread of antimicrobial resistance via horizontal gene transfer. Integrons are more common in gram-negative bacteria and may be responsible for multidrug resistance[8]. *S. marcescens* most frequently carries class 1 integrons but can also carry class 2 or 3 integrons. Celejewski et al. showed that 28 of 120 *Serratia* spp. carried the integron gene cassettes, with class 1 integrons being the most common[21].

In this study, three strains were integrase 1 positive. One of them was an empty cassette, while the other two carried gene cassettes. TRSM 107 had a gene cassette with *aad*A2 (aminoglycoside-3"-adenyltransferase), responsible

for streptomycin/spectinomycin resistance, and *dfr*A12 (dihydrofolate reductase), responsible for trimethoprim resistance. TRSM 119 had a gene cassette with *dfr*A12 and *ANT*(2")-Ia (aminoglycoside-2″-O-nucleotidyltransferase), responsible for aminoglycoside resistance[8,34]. Two different gene cassettes containing aminoglycoside resistance and one cassette containing trimethoprim resistance were detected in all strains.

The limitation of this study is that, although it was conducted using clinical isolates, all strains were included without distinguishing between colonization and infection.

CONCLUSION

In this study, ESBL production, quinolone resistance, and the presence of integrons in *S. marcescens* were observed. Our findings highlight the critical importance of antimicrobial resistance surveillance. Antimicrobial resistance is a significant public health issue, particularly in hospital settings where it can easily spread. Monitoring epidemiological data will aid in determining both regional and international resistance profiles. Treatment protocols should be adjusted according to the identified resistance profiles. Every healthcare institution should closely monitor its own resistance patterns and enforce strict infection control measures. Further studies are necessary to enhance the surveillance of antimicrobial resistance, especially in AmpC-producing bacteria.

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ETHICS COMMITTEE APPROVAL

Ethics committee approval for the study was granted by the Karadeniz Technical University, Faculty of Medicine Scientific Research Ethics Committee (Approval No. 2022/253).

CONFLICT of INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: AOK, SAS, OBÖ, CKB, EÖ

Analysis/Interpretation: AOK, SAS, OBÖ, CKB, EÖ

Data Collection or Processing: AOK, SAS, OBÖ, AR, ER

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