Original Article

Determination of the Antibiotic Resistance Rates of *Serratia marcescens* Isolates Obtained from Various Clinical Specimens

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Abstract

Aim: *Serratia marcescens* clinical isolates are increasingly resistant to antibiotics. Therefore, the treatment of infections caused by *S. marcescens* becomes difficult. The aim of this study was to examine the antimicrobial resistance profiles of *S. marcescens* bacteria isolated from various clinical specimens according to body regions and clinics and to evaluate for enzyme production rates associated with antibiotic resistance of these isolates. **Materials and Methods:** Blood culture samples were incubated in a fully automated BACTEC-FX system. Identification and antibiogram processing was carried out by fully automated VITEK 2 identification and antibiogram system. The obtained results were retrospectively screened. **Results:** *S. marcescens* was identified in a total of 158 clinical specimens. The departments where *S. marcescens* was most commonly identified were the Anesthesia Intensive Care Unit (25.9%), followed by Chest Diseases (19.6%). Serratia isolates were most commonly determined in blood culture (35.4%) and sputum culture (24.6%). Resistance rates to ceftriaxone and ceftazidime were 22.7% and 19.6%, respectively. However, the rate of resistance detected to cefotaxime and gentamicin (0.6%) was very low. **Conclusion:** Clinical isolates of *Serratia* exhibited highest resistance to ceftriaxone, ceftazidime, and piperacillin/tazobactam. However, it was found that the tested *Serratia* strains did not exhibit high resistance to other antibiotics. Our results suggest that cefotaxime and gentamicin are the most suitable antibiotics for treatment. The extended-spectrum β-lactamase and inducible β-lactamase ratios were found to be decreased by 6%–7%. Although different results may be obtained from different hospitals and regions, it should not be forgotten that *Serratia* strains may be resistant to many antibiotics and that the results of antibiotic susceptibility testing may help plan antibiotic treatment.

Keywords: *Antibiotic resistance, antibiotic susceptibility, Serratia marcescens*

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Introduction

Serratia is a bacterium found in the family Enterobacteriaceae that can cause opportunistic infections even though it is usually a weak pathogen.[1,2] *Serratia*, unlike the other members of the Enterobacteriaceae family, is less involved in the gastrointestinal tract and has lipase, gelatinase, and DNase enzymes. Currently, 14 species of *Serratia* are recognized.[3-5]

Serratia marcescens is among the most common infectious agents in infections associated with *Serratia*. They cause infections with significant mortality and morbidity in newborns.[3-6] Furthermore, *S. marcescens* is an important infectious agent that causes hospital-acquired respiratory and urinary tract infections in neonatal-adult intensive care unit and immunodeficient patients. In addition, respiratory

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tract, surgical wound, skin, and soft tissue infections associated with *S. marcescens* may cause bacteremia.[5-7]

S. marcescens strains are able to produce inducible β-lactamase (IBL) and extended-spectrum β-lactamase (ESBL), so they can develop resistance to many beta-lactam antibiotics. However, beta-lactam group antibiotics are among the most basic antibiotics used in the treatment of bacterial infections. Resistance of *S. marcescens* to this groups of antibiotics will be a disadvantage in the treatment. Treatment of nosocomial infections caused by *S. marcescens* is quite difficult. This bacterium can exhibit multidrug resistance to beta-lactam, aminoglycoside and quinolone group antibiotics as well as natural resistance to many antibiotics.[8] In case of *S. marcescens* as a causative agent in hospital infections, planning of the treatment according to antibiotic susceptibility test results is of great importance in terms of treatment success.[9-15]

Determination of IBL and ESBL ratios will have vital importance in the treatment of infections associated with *S. marcescens*, in evaluating antibiotic susceptibility and in selecting antibiotic therapy.

The objective of this study was to evaluate the distribution of these isolates according to clinics and isolated body regions as well as the susceptibility of these bacteria to antibiotics. For this purpose, *S. marcescens* isolates were isolated from samples from various clinics of our hospital and sent for culture to be examined in this study.

Materials and Methods

Ethical approval for this retrospective study was obtained from the local ethics committee of Afyon Kocatepe University.

Antibiotic susceptibility results of *S. marcescens* strains isolated from various clinical specimens were evaluated in this study. The distribution of isolates of *Serratia* according to the samples like blood culture, sputum culture, wound culture, urine culture, tracheal aspirate, tissue culture, superficial skin example, aspirate culture, catheter culture, pleural culture, and various clinics was evaluated.

Between 2014-2018, culture and antibiogram results of various samples obtained from various clinics of Afyon Kocatepe University Hospital were included in the study.

A fully automated BACTEC-FX (Becton Dickinson, Sparks, MD, USA) blood culture incubation system was used for bacterial isolation from blood cultures.

Samples from blood culture bottles giving positive signals were cultured on 5% sheep blood agar and EMB agar media.

Pediatric blood culture specimens were additionally cultured on chocolate agar. Subsequently, the medium was incubated at 37°C for 24–48 hours. Urine specimens were quantitatively cultured on blood agar and chromogen agar media. The media were left to incubate at 37°C for 18–24 hours. A colony count of 100,000 CFU/mL, which is a uniform type of colony, was generally considered significant for urine samples. All other clinical specimens from our laboratory were cultured on 5% sheep blood, EMB, and chocolate agar media and left to incubate at 37°C for 24–48 hours.

Identifications of Gram-negative bacilli grown in culture and antibiotic susceptibility were investigated using the VITEK 2 (bioMérieux, Inc. Hazelwood, MO, USA) fully automated identification and antibiogram system, as recommended by the manufacturer. Disc induction test was used to determine the ratio of IBL. Suspensions of *S. marcescens* strains were prepared at 0.5 McFarland turbidity and spread on a Mueller–Hinton agar medium. Imipenem was used as a strong inducer and ceftriaxone, ceftazidime, and cefotaxim discs were used as weak inducers.

Ceftriaxone, ceftazidim, and cefotaxim discs were placed 2 cm away from the imipenem in the center of the medium. Flattening or indentation of the growth inhibition zone of the cephalosporins disc at the side of the disc imipenem containing the test strain has indicated the release of AmpC β-lactamase. ESBL ratios were determined using the VITEK 2 system.

To prevent contamination, for each patient sample, the presence of *S. marcescens* in two different blood culture bottles that are routinely given to the laboratory at 2-day interval was confirmed by culturing on tryptic soy agar plates containing 5% sheep blood. Same way, the presence of *S. marcescens* in two different clinical samples was confirmed by culturing on tryptic soy agar plates containing 5% sheep blood for each of the other patient samples.

Prior to testing, the frozen isolates were subcultured twice, and the fresh isolates subcultured once, on tryptic soy agar plates containing 5% sheep blood. In order to check the accuracy of the study, external (Oneworld Accuracy Company, Turkey) and internal (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213 strains were used) quality control studies are carried out regularly for VITEK 2 system. At the same time, the results obtained in the fully automated system were repeated with conventional biochemical tests and the results were confirmed. In the study to determine the ESBL ratio, the results obtained from the VITEK 2

system were confirmed by using double-disk synergy method.[16,17]

Results

S. marcescens was identified in 158 of the various clinical specimens. Bacteria (158) were mostly isolated from the anesthesia intensive care unit (25.9%), the chest diseases department (19.6%), and from the pediatric department (6.9%), respectively.

Clinics and rates that *S. marcescens* have been isolated are detailed in Table 1.

Table 1: Clinics and rates of *S. marcescens* isolates

Clinics	Number of investigated strains (*n*)	Rate of investigated strains (%)
Anesthesia Intensive Care	41	25.9
Chest Diseases Department	31	19.6
Pediatric Health and Diseases	11	6.9
Plastic and Reconstructive Surgery	10	6.3
Orthopedics and Traumatology	9	5.6
Neurosurgery	8	5
Neonatal Policlinic and Intensive Care	8	5
Department of Infectious Diseases	7	4.4
Nephrology	6	3.7
Physical Therapy and Rehabilitation	5	3.1
Cardiovascular surgery	4	2.5
Neurology	4	2.5
Medical Oncology	4	2.5
Internal Medicine	3	1.8
General Surgery	2	1.2
Endocrinology	1	0.6
Chest Surgery	1	0.6
Gynecology and Obstetrics	1	0.6
Cardiology	1	0.6
Total	158	100

Table 2: Clinical sample types and rates of *S. marcescens* isolates

Sample type	Number of investigated strains (*n*)	Rate of investigated strains (%)
Blood culture	56	35.4
Sputum culture	39	24.6
Wound culture	25	15.8
Urine culture	15	9.4
Tracheal aspirate	12	7.5
tissue culture	4	2.5
Superficial skin example	3	1.8
Aspirate culture	2	1.2
Catheter culture	1	0.6
Pleural culture	1	0.6
Total	158	100

S. marcescens were determined mainly in 35.4% of blood cultures, 24.6% of sputum cultures, and 15.8% of lesion cultures, respectively. Clinical sample types and the rates that *S. marcescens* has been isolated are detailed in Table 2.

According to the antibiotic susceptibility tests, the antibiotic with the highest resistance rate was found to be ceftriaxone (22.7%). Antibiotics with the lowest resistance rates were determined as cefotaxime and gentamicin (0.6%). The antibiotic resistance rates of *S. marcescens* isolated from various clinical specimens are detailed in Table 3.

The ratio of IBL producers from a total of 158 *S. marcescens* isolates grown in various clinical specimens was found to be 6.3% and the ratio of ESBL producers was determined as 6.9%. The ratios of IBL and ESBL for *S. marcescens* are detailed in Table 4.

Table 3: The antibiotic resistance rates of *S. marcescens* strains

Antibiotics	Number of resistant strains (*n*)	Resistance rates (%)
Ceftriaxone	36	22.7
Ceftazidime	31	19.6
Piperacillin/tazobactam	31	19.6
Imipenem	21	13.2
Meropenem	21	13.2
Ertapenem	19	12
Netilmicin	17	10.7
Tigecycline	16	10.1
Cefixime	14	8.8
Cefepime	11	6.9
Ticarcillin/klavulunat	11	6.9
Amikacin	10	6.3
Ciprofloxacin	7	4.4
Piperacillin	6	3.7
Tobramycin	5	3.1
Cefoperazone/sulbactam	4	2.5
Trimethoprim/sulfamethoxazole	4	2.5
Aztreonam	3	1.8
Fosfomycin	2	1.2
Levofloxacin	2	1.2
Cefotaxime	1	0.6
Gentamicin	1	0.6

Table 4: IBL and ESBL rates of *S. marcescens*

n/%	ESBL (+)	ESBL (−)	Total
IBL (+)	6/3.7%	4/2.6%	10/6.3%
İBL (-)	5/3.2%	143/90.5%	148/93.7%
TOTAL	11/6.9%	147/93.1%	158/100%

IBL=Inducible β-lactamase, ESBL=Extended-spectrum β-lactamase

Discussion

Studies on *Serratia* infections indicate that they are encountered more frequently in newborn units and in immunocompromised individuals.

In one study in Turkey, *S. marcescens* was most commonly isolated from the pediatric age group (41.3%) and determined as the most common cause of urinary tract infections (34.79%). The same study asserted that *S. marcescens* grew in blood cultures (21.74%), which may cause sepsis.[17]

In this study, *Serratia* strains were isolated most frequently from blood cultures from samples from the Anesthesia Intensive Care Unit (25.9%). This was followed by the frequency of *Serratia* isolated from Chest Diseases samples (19.6%) and the Children's Health and Diseases samples ranked third (6.9%) [Table 1].

Bozkurt *et al.* found that *Serratia* strain isolates were sensitive to amikacin 95.7%, ciprofloxacin 91.5%, imipenem 88. 9%, cefotetan 85.3%, gentamicin 82.2%, ampicillin/sulbactam 5.9%, cefazolin 6.8%, tetracycline 9.1%, ampicillin 9.1%, and cefuroxime 10%, respectively.[17] In another study conducted in our country, 10 strains of *S. marcescens* were isolated in an epidemic in a newborn unit and all strains were reported to be susceptible to imipenem, meropenem, amikacin, and ciprofloxacin. Most of the infected babies were premature and half of the patients perished.[18]

In a study conducted in Taiwan, bacteraemia due to *S. marcescens* was detected in 22 patients. Furthermore, 68%, 14%, and 9%, of hospital outbreak cases were found to have primary bacteremia, pneumonia, and urinary system infections, respectively. A minor proportion of these infections has been found to have suppurative thrombophlebitis and surgical wound infections and some cases resulted in death. All isolates were resistant to moxalactam, ciprofloxacin and imipenem, ampicillin, and cephalothin.[19]

In one study in the United States, antibiotics susceptibility rates of *S. marcescens* were detected as 100% for meropenem, 97.2% for imipenem, 91.7% for ceftazidime, 87.3% for gentamycin, and 83.1% for piperacillin/tazobactam.[20]

According to a study conducted in Brazil, 53 infants were found to have an infection caused by *S. marcescens*, and some cases were fatal. These strains were determined to produce beta-lactamase and to be resistant to cephalosporins.[21]

According to a survey in Japan, 114 cases of *S. marcescens* infections were detected in a hospital within 2 years.

They have been detected to be highly resistant to piperacillin, third- and fourth-generation cephalosporins, new quinolones, and aminoglycosides in particular. Most of these strains were isolated from urinary system samples and respiratory samples.[22]

In Taiwan, Hsueh *et al.* determined that 34% of *S. marcescens* were resistant to cefotaxime.[23] In our study, the resistance rate against cefotaxime was determined as low as 0.6%. *S. marcescens* is resistant to narrow spectrum penicillin and cephalosporins, cefuroxime, cefamycin, macrolide, tetracycline, nitrofurantoin, and colistin.[8]

S. marcescens is also frequently resistant to new fluoroquinolones and third generation cephalosporins used in the treatment of infections.[13-24]

Some strains of *S. marcescens* produced beta-lactamases and some have production potential of ESBL. It has also been shown that some strains may exhibit resistance to carbapenems by generating metallo-beta-lactamases. On the other hand, beta-lactamase inducible in the form of ampC may cause failure in cephalosporin treatment, which is considered to be effective *in vitro*.[25-28]

The fact that the antibiotic resistance of *Serratia* strains isolated from the patient samples in this study was not high is quite, bewildering.

This may be attributed to the isolation of strains at different times and from different clinics.

In different studies, the reason for the presence of more resistant *Serratia* strains is usually the isolation of bacteria from the same clone that causes hospital infections during outbreak.

Bacteria of the same genus can exhibit different antibiotic resistance profiles according to different regions and hospitals.

For example, in a hospital, high-level aminoglycoside resistance in *Serratia* strains was reduced within 10 years due to a decrease in the use of aminoglycoside in *Serratia* infections.[19]

In different studies, *S. marcescens* strains have been found to be highly resistant to the beta lactam group and especially cephalosporin group antibiotics; therefore, treatment should be decided according to antibiotic susceptibility tests every time. In this study, clinical isolates of *Serratia* had high resistance to ceftriaxone, ceftazidime, and piperacillin/tazobactam. However, cefotaxime and gentamicin were found to be the most suitable antibiotics for treatment. Furthermore, it should not be ignored that serratia strains with different resistance profiles have been detected in different studies.

Cephalosporin treatment is likely to fail in cases where *S. marcescens* strains produce inducible beta lactamase. All *Serratia* strains were found susceptible to gentamicin except one. However, it is a known fact that some *S. marcescens* strains have aminoglycoside-modifying enzyme.[18]

When the immunocompromised patients infected with this bacterium are taken into account, it becomes clear that effective and correct antibiotic combinations are vital to treatment. Antibiotic susceptibility tests must be applied absolutely for efficient treatment of patients with infections associated with *S. marcescens*. Treatment with antibiotic combinations considering the results of an antibiotic susceptibility test is of great importance for the success of treatment and the eradication of outbreak, depending on the infections.

Conclusion

Serratia isolation, identification, and antibiotic susceptibility tests cannot be performed from clinical samples in some health institutions. Therefore, the results of this study and other similar studies will help in the selection of antibiotics for newborn unit's patients and immunocompromised patients. In this study, resistance rates of *S. marcescens* to antibiotics were lower than expected. In this retrospective study that was evaluated different clinical specimens and contained a wide interval of time, *S. marcescens* strains were generally isolated from different cases. Generally, strains were isolated from the outbreaks are evaluated in others studies and more resistant *Serratia* strains were detected in outbreaks. Nevertheless, we believe that the data of this study will guide to the clinicians that perform empirical treatment.

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Conflicts of interest

There are no conflicts of interest.

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