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ORIGINAL ARTICLES

WOUND INFECTIONS CAUSED BY *SERRATIA MARCESCENS*: A MICROBIOLOGY SURVEY

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ABSTRACT

INTRODUCTION: *Serratia marcescens* wound infections are a concern in healthcare settings, associated with delayed healing, prolonged hospital stays, higher costs, and sepsis in severe cases.

AIM: This study aimed to evaluate the antimicrobial susceptibility of *S. marcescens* wound isolates from patients in St. Marina University Hospital, Varna, Bulgaria (2016–2023) and to explore the genetic mechanisms responsible for the 3rd generation cephalosporin resistance in these isolates.

MATERIALS AND METHODS: Over the 8-year period, 131 isolates were collected. Identification and antimicrobial susceptibility testing were performed using Phoenix (BD, USA) and Vitek 2 (BioMerieux, France), with results interpreted by EUCAST guidelines. The genetic mechanisms responsible for beta-lactam resistance were studied by PCR.

RESULTS: A total of 98.5% of the patients were diagnosed with nosocomial *S. marcescens* infections. Diabetes and a cardiovascular disease were found as accompanying risk factors in 42.8% and 71%, respectively. Resistance rates were: ceftriaxone—14.5%; gentamicin—13.7%; cefepime/trimethoprim-sulfamethoxazole—12.9%, and ciprofloxacin/levofloxacin—12.2%. No carbapenem resistance was found. Bla_{CTX-M} was present in 72.7% of cephalosporin-resistant isolates.

CONCLUSION: *S. marcescens* remains a significant nosocomial pathogen, with less than 15% resistance to main antimicrobials. We found the leading role of bla_{CTX-M} for the 3rd generation cephalosporin resistance in *S. marcescens*, confirming the global trends.

Keywords: *Serratia marcescens, wound infections, antibiotic resistance, bla genes*

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INTRODUCTION

Serratia marcescens is a Gram-negative bacterium, classified in family *Yersiniaceae*, order *Enterobacterales* (1). First recognized as a human opportunistic pathogen in 1913 (2), nowadays *S. marcescens* is associated with a variety of infections, but mainly in immunocompromised individuals, newborns, and patients after catheterization or different medical interventions. Its role as an important etiologic agent of

hospital-acquired infections, such as wound and urinary tract infections, respiratory and bloodstream infections, is well documented (3). Outbreaks caused by *S. marcescens* have been reported in surgical departments, in patients undergoing wound care (4,5).

S. marcescens wound infections are a significant concern in the healthcare settings, usually associated with delayed wound healing, prolonged hospitalization, increased healthcare costs, and in the severe cases—with septic conditions (6,7). In addition, the role of *S. marcescens* as an important opportunistic pathogen is enhanced by its acquired resistance to widely used antimicrobial agents in the medical practice.

AIM

The aim of this study was to evaluate the antimicrobial susceptibility of *S. marcescens* wound isolates, collected during the period 2016–2023, as well as to study the genetic mechanisms responsible for 3rd generation cephalosporin resistance in these isolates.

MATERIALS AND METHODS

During the period 2016–2023, a total of 131 non-duplicate consecutive clinical isolates of *S. marcescens* were obtained from wound samples of 131 hospitalized patients in various wards of St. Marina University Hospital, Varna, Bulgaria. The automated systems Phoenix (BD, USA) and Vitek 2 (BioMerieux, France) were used for species identification and antimicrobial susceptibility testing (AST) and the results were interpreted according to EUCAST guidelines (8). The detection of extended-spectrum beta-lactamase production (ESBL) was done by the double-disk synergy test (DDST) (8). The DNA extraction was carried out according to a protocol already mentioned elsewhere (9). Standard PCR protocols were used for detection of bla_{ESBL} genes (CTX-M, TEM, SHV types) in the 3rd generation cephalosporin-resistant isolates as previously described (10). The primers for each gene, annealing temperatures, and product sizes used are shown in Table 1.

Table 1. PCR protocols.

Target	Primer Sequence	T	Products size
TEM	F: ATA AAA TTC TTG AAG AC R: TTA CCA ATG CTT AAT CA	43˚C	1075 bp
SHV	F: ACT GAA TGC GGC GCT TCC R: TCC CGC AGA TAA ATC A	61˚C	297 bp
CTX-M	F: CVA TGT GCA GYA CCA GTA A R: ARG TSA CCA GAA YMA GCG G	61˚C	585 bp

T—annealing temperature

RESULTS

During the period 2016–2023, a total 131 wound isolates, collected from 131 patients with wound infections, were studied. The isolates' distribution according to the hospital department is shown in Table 2. A total of 129 patients were diagnosed with hospital-acquired infections (HAIs) (98.5%). Diabetes and a chronic cardiovascular disease were found as accompanying risk factors in 42.8% and 71% of the patients, respectively.

The following resistance rates in the collected group of 131 *S. marcescens* isolates were found: ceftriaxone, 14.5%; gentamicin, 13.7%; cefepime and trimethoprim/sulfametoxazole, 12.9%; ciprofloxacin and levofloxacin, 12.2%; amikacin, 9.7%. Isolates resistant to carbapenems (imipenem, meropenem) were not detected. All nineteen 3rd generation cephalosporin- resistant isolates demonstrated positive DDST (Fig. 1).

Eleven 3rd generation cephalosporin-resistant isolates of *S. marcescens* (11/19), based on the ward of isolation and resistance profile, were subjected to PCR to identify the genetic mechanisms of beta-lactam resistance. Bla_{CTX-M} was found in 72.7% (8/11). Among these, 4 isolates were also bla_{TEM} positive. Despite the positive DDST, three isolates did not give any PCR product with the used primers.

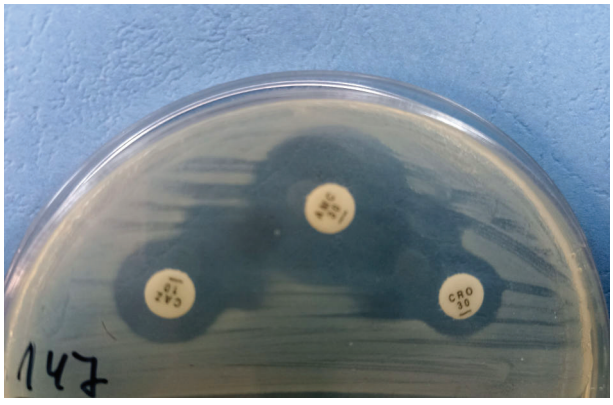

Fig. 1. Positive double-disk diffusion synergy test for detection of ESBLs: a zone of synergism between amoxicillin/clavulanic acid 20/10 (AMC 30), ceftriaxone 30 μg (CRO 30), and ceftazidime 30 μg (CAZ 30) disks.

Table 2. Distribution of 131 isolates of S. marcescens according to the hospital department.

Department of Hospitalization	Total n=131 n (%)
Vascular Surgery	79 (60.3%)
Internal Medicine	15 (11.5%)
Orthopedics	7 (5.4%)
Abdominal Surgery	7 (5.4%)
Maxillofacial Surgery	7 (5.4%)
Intensive Care Unit	5 (3.8%)
Otorynolaryngology	3 (2.3%)
Haematology	2 (1.5%)
Urology	2 (1.5%)
Others	3 (2.3%)

DISCUSSION

Surgical site infections (SSIs) associated with *Serratia* spp. are the most common postoperative complications worldwide and are mostly exogenous in their origin (11). Documented outbreaks of *S. marcescens* infections have occurred in patients receiving wound care, particularly in surgery departments and among patients with neurosurgical site infections (11). Gery et al. describe an outbreak involving eight patients in a digestive surgery ward who developed *Serratia*-associated SSIs due to the use of a contaminated T-shaped intraoperative probe (12). In 2022 another outbreak of SSIs was identified in a neurosurgery and orthopedic department due to contaminated brushes impregnated with chlorhexidine (13). These infections, as highlighted by O'Horo et al., are associated with alarmingly high mortality rates, underscoring the gravity of the issue (14). In the context of these facts, over 90% of our isolates are associated with nosocomial acquisition and SSIs; and over 75% are related to surgical clinics, particularly to the vascular surgery clinic (60.3%). The annual epidemiological report of the European Center for Disease Prevention and Control for 2018–2020 identifies *Enterococcus* spp., *E. coli*, and *S. aureus* as the most frequently isolated organisms from SSIs. From order *Enterobacterales*, *Serratia* spp. takes the sixth place (15). In concordance with this result, during the studied eight-year period, we found *S. marcescens* among the ten most frequently isolated organisms from clinical samples of patients hospitalized in the St. Marina University Hospital, Varna.

This study presents information about the resistance rates to major antimicrobial groups, used in the clinical practice and the genetic mechanisms to 3rd generation cephalosporins in 131 *S. marcescens* isolates, associated with SSIs and community-acquired wound infections diagnosed during the period 2016–2023 in our hospital. In this study we identified relatively preserved activity to ceftriaxone and cefepime with resistance rates below 15%, followed by fluoroquinolones and trimetoprim/sulfametoxazole with resistance below 13%. Resistance rates below 10% were observed only for the group of aminoglycosides. The activity of carbapenems was fully preserved in the studied isolates. This is in contrast with our previous study on invasive *S. marcescens* isolates from bloodstream infections, when we detected significantly higher resistance rates: >50% for 3rd generation cephalosporins and trimetoprim/sulfametoxazole; >40% for fluoroquinolones; aminoglycoside resistance ranged between 15% for amikacin to above 45% for gentamicin (16). The carbapenems were with relatively preserved activity and resistance below 5% (16).

In a collection of 158 isolates of *S. marcescens*, authors from Turkey reported higher rates of 3rd generation cephalosporin resistance (22.7% for ceftri-

axone and 19.6% for ceftazidime), as well as 13.2% resistance to carbapenems (17). Similar to our results, another study from Turkey, reported relatively low resistance rates for amikacin (4.3%), gentamicin (17.8%), and ciprofloxacin (8.5%), but higher resistance to carbapenems (8.5%) (18). Simsek et al. reported resistance rates for ceftriaxone and ceftazidime similar to ours (22.7% and 19.6%, respectively) and significantly lower resistance to aminoglycosides (0.6%) (19). In a collection of 393 isolates, Cosimato et al. reported relatively preserved activity of ceftazidime (20.2% resistance), cefepime (9%), fluoroquinolones (20.7%), and aminoglycosides (amikacin 4.4%; gentamicin 6.8%) (20).

The enzymatic mechanism associated with production of ESBLs and carbapenemases from different classes [A (CTX-M, SHV, KPC, SME); B (IMP, NDM, VIM); D (OXA-48)] is the major mechanism, mediating the resistance to 3rd generation cephalosporins and carbapenems in *S. marcescens* (21,22,23). The PCR experiments from this study demonstrate that the 3rd generation cephalosporin resistance is related to CTX-M type ESBL production. Among the tested isolates, $bla_{\text{CTX-M}}$ was found in 72.7% (8/11). Among these, 50% (4/8) were also bla_{TEM} positive. These results are in concordance with previous Bulgarian studies on *S. marcescens* isolates, reporting CTX-M ESBL as the leading enzymatic mechanism of 3rd generation cephalosporin resistance (24,25,26). The isolates with positive DDST, but negative PCR in this study, may harbor rare or new beta-lactamases, or another non-enzymatic mechanism. Similar to our results, a co-production of CTX-M and TEM beta-lactamases was also identified in *S. marcescens* isolates in a study performed by Mlynarczyk in Poland (27). A study from Algeria also reported CTX-M (59.2%) and TEM enzymes (50%) as leading types of beta-lactamases in *S. marcescens* clinical isolates (28).

The carbapenems are still the preferred treatment option for infections associated with *S. marcescens* isolates that are ESBLs and/or derepressed AmpC cephalosporinase hyperproducers (29). However, these agents should be wisely used, considering the emergence of carbapenemase-mediated resistance in *S. marcescens* (30). In the present study the carbapenems meropenem and imipenem are the agents with the highest activity against *S. marcescens*, demonstrating fully preserved activity. Unfortunately, in our previous study on invasive *S. marcescens,* associated with blood stream infections, we reported the first Bulgarian KPC-producing carbapenem-resistant isolate (26).

CONCLUSION

The present study demonstrates the role of *S. marcescens* as an important nosocomial pathogen. *S. marcescens*-associated wound infections were predominantly hospital-acquired, with the highest prevalence in the vascular surgery. We reported relatively preserved susceptibility of *S. marcescens* to the main antimicrobials: below 15% for ceftriaxone, cefepime, fluoroquinolones, and trimetoprim/sulfametoxazole, and below 10% for aminoglycosides. Carbapenems remained the group with the highest antibacterial activity. Our study found the leading role of $bla_{\text{CTX-M}}$ gene for 3rd generation cephalosporin resistance in *S. marcescens*, confirming the global trends.

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