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RESEARCH ARTICLE

Antibiotic Susceptibility and Plasmid Profile of Multidrug resistant Uropathogenic *Serratia marcescens*

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ABSTRACT

Objectives: Genetic elements such as plasmids play a role in spreading multi-antibiotic resistance, a global threat. Therefore, the purpose of this study was to investigate the antibiotic susceptibility and plasmid profiles of multidrug-resistant (MDR) uropathogenic *Serratia marcescens*.

Methods: Fifty *Serratia marcescens* isolates were obtained from urine samples of patients presenting with urinary tract infection (UTI) in Southeastern Nigeria. Bacteria samples were identified via their characteristic red pigmentation and other biochemical tests. The disc diffusion method was used for antibiotic susceptibility testing. Plasmids were extracted using the alkaline lysis method, and electrophoresis was done on a 1% agarose gel. Extracted DNA plasmids were visualized using the ultraviolet light illuminator and a photo documentation system. Plasmid curing was carried out using acridine orange.

Results: Antibiotic susceptibility testing of isolates revealed 41 (82%) were resistant to one or more antibiotics tested, and 8 (16%) isolates exhibited resistance to three or more antibiotics (MDR). Of the eight MDR isolates, five (63%) had plasmids with molecular weights ranging from 33 kb to 58 kb. One isolate (S9) was cured and became sensitive to ceftriaxone, but not cefuroxime or ceftazidime. Notably, one isolate (S23), initially sensitive to ciprofloxacin, became resistant post-plasmid curing.

Conclusion: The findings show that most resistant bacteria containing plasmids were immune to curing as they remained resistant to antibiotics after curing. This result confirms a concern about the growing presence of MDR plasmids of *S. marcescens* in healthcare facilities in Southeastern Nigeria. *J Microbiol Infect Dis 2022; 12(1):12-18*.

Keywords: *Antibiotic resistance, Serratia marcescens, Plasmid profiling, Urinary tract infection*

INTRODUCTION

Antimicrobial resistance (AMR) is a major global healthcare issue. As a result, infectious disease specialists are faced with a more significant problem as AMR bacteria strains have limited or jeopardized the available last resort medicines. Newer, more potent antibiotics are not immune to resistance emergence. [1]. Newer, more potent antibiotics are not immune to resistance emergence. Resistance against antibiotics increases due to the extensive and indiscriminate use of antibiotics in communities. Therefore, understanding how antibiotic resistance develops can help devise strategies for slowing or even stopping its spread [3].

According to estimates, 40–50% of healthy adult women have had at least one episode of urinary tract infection (UTI) [2]. It is the most common nosocomial infection and is the second leading cause of bacteremia in hospitalized patients [4]. Urinary pathogen occurrences vary with age, gender, catheterization, hospitalization, and prior

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antibiotic exposure, with symptoms including burning sensation during urination, frequent or intense urges, back or lower abdomen pain, fever, or chills. *Escherichia coli, Staphylococcus aureus, Proteus spp, Klebsiella spp, Pseudomonas aeruginosa*, and *Serratia spp*. are the common causes of acute and uncomplicated UTI in ambulatory patients [5].

Serratia marcescens is an opportunistic, gram-negative nosocomial pathogen of the Enterobacteriaceae family. It is responsible for only 1–2% of nosocomial infections, mainly in the digestive tract, urinary tract, respiratory tract, soft tissues, and surgical wounds. These organs are significant reservoirs for epidemics in newborns and adults. The microorganism can quickly spread in the nosocomial environment and contaminate medical equipment, instruments, commercial fluids, disinfectants, and dispensers [6]. Intensive care units are frequently implicated in *Serratia marcescens* colonization and infection epidemics [7]. Meningitis, caused by *Serratia marcescens*, has been reported in pediatric wards. It causes endocarditis and osteomyelitis in heroin addicts.

Similarly, 30–35% of patients are asymptomatic in urinary tract infections [7]. The mortality rate is significant in nosocomial bloodstream infections, meningitis, and endocarditis caused by Serratia infections. Hospitalization, intravenous, intraperitoneal, and urinary catheters, and prior instrumentation of the respiratory system are major risk factors for sepsis caused by Serratia spp [7]. *Serratia marcescens* uniquely can produce a β-lactamase or acquire plasmid-mediated extended-spectrum-β-lactamases (ESBLs), which confers resistance to broad-spectrum β-lactamase antibiotics [8,9]. Patients with *S. marcescens* infections commonly get empirical antimicrobials before antimicrobial susceptibility testing is available [10,11]. As empirical antimicrobials, third and fourth-generation cephalosporins and carbapenems are commonly used [12,13].

Chromosomal and extra-chromosomal DNA are two bacterial genetic mechanisms that convey antibiotic resistance [14]. These genes are transferred via mutation, recombination, and horizontal gene transfer, resulting in bacterial genomic diversity. Thus, multidrug-resistant plasmids help spread resistance via horizontal gene transfer. Plasmid-encoded antibiotic resistance includes resistance to many of today's most vanguard antibiotics. Because antibiotics cannot keep up with the rate at which bacteria-resistant strains evolve, biotechnology can help slow down the spread of resistant genes. Kamruzzaman et al. [15] demonstrated that plasmid interference might be utilized to create plasmid incompatibility and hence cure antibiotic resistance genes.

Furthermore, they put forward that plasmid research can aid patients who have been colonized. Van Hal et al. [15] previously demonstrated that removing plasmids from patients' gut flora could avoid consequences like sepsis induced by antibiotic resistance. Therefore, it is vital to continuously monitor antimicrobial resistance in bacteria isolates and characterize the genes responsible for such reported resistance.

Despite increasing studies on *Serratia marcescens* antibiotic resistance, their prevalence and resistance mechanisms against broad-spectrum antibiotics in Nigerian healthcare institutions are still sparsely studied. The findings of this study could assist in establishing if the resistance observed in the isolates is mediated chromosomally or extra-chromosomally. The objective of our study was to determine the antibiotic resistance profile and plasmid profile of MDR *S. marcescens* strains isolated from urine samples of inpatients in Southeastern Nigeria.

METHODS

Culture media and antibiotic disks

Mueller Hinton Agar, Mueller Hinton Broth, McConkey Agar, Nutrient Broth, and Nutrient Agar (Oxoid, UK) were all prepared and used according to the manufacturers' recommendations. The antibiotic disk used in this study was Optudisc (Optun Laboratory Nigeria Ltd). The antibiotics used were Azithromycin 5 µg, Ceftriaxone 30 µg, Ceftazidine 20 µg, Ciprofloxacin 5 µg, and Cefuroxime 5 µg.

Ethical consideration

The *S. marcescens* used for the study were obtained as part of routine patient care; therefore, no patient consent was necessary. However, the research protocol was approved by the Research and Ethics Committee, University of Nigeria, Nsukka, Nigeria.

Bacterial collection and identification

Between August 2018 and October 2018, consecutive non-duplicate isolates of *S. marcescens* were routinely collected from the microbiology laboratories of two public healthcare facilities (Bishop Shanahan Hospital and Enugu State University of Technology Teaching Hospital) in Enugu, Southeastern Nigeria. These isolates were only derived from urine samples of inpatients with urinary tract infections. Bacteria isolates were cultured within one hour of collection onto peptone glycerol agar at 30 ^{0}C (24–48 h). Putative *S. marcescens* isolates were identified and confirmed by Gram staining, and specific biochemical tests were carried out using standard procedures described by Cheesbrough [17]. All isolates were kept at a temperature of -4 ^{0}C.

Antibiotic susceptibility testing

Antibiotic susceptibility was determined using commercially available antimicrobial discs (Oxoid, UK). Susceptibility to five different antibiotics belonging to different classes was tested: Azithromycin (5 µg), a macrolide; Ciprofloxacin (5 µg), a fluoroquinolone; Cefuroxime (30 µg), a 2nd generation cephalosporin; and Ceftriaxone (30 µg) and Ceftazidime (20 µg), 3rd generation cephalosporins. According to the Kirby-Bauer disc diffusion method, a single colony was isolated and sub-cultured for each isolate prior to screening [18]. Recommendation standards by the Committee for Clinical Laboratory Standards for the interpretation of zone of inhibition were used for the data interpretation [19]. *S. marcescens* strain (ATCC 13880) was used as a control.

Plasmid isolation and profiling

Pure isolates of *S. marcescens* strains cultured on fresh agar plates were inoculated into 8 ml of fresh nutrient broth and then incubated at 30℃ for 72 h. Resistant plasmid DNA was extracted using the alkaline lysis method [20]. The broth culture of each isolate was shaken at 200 rpm and then centrifuged at 13,000 rpm for 2 min to pellet the cells. The supernatant was decanted and vortexed at high speed. The washed pellets were suspended in 150 µl of cold alkaline solution I (50 mM Tris pH 8.0 with HCl, ten mM EDTA, 100 µg/ml RNase), lysed with 300 µl of alkaline solution II (200 mM NaOH, 1% SDS) and completely lysed with 150 µl of alkaline solution III (3M Sodium acetate, pH 5.2), vortexed for 2-5 secs and incubated on ice for 5 min. The bacterial lysate was separated via centrifuge at 13,000 rpm for 10 min. Finally, plasmids were precipitated using ice-cold 70% ethanol and dried in a vacuum.

Plasmids were electrophoresed on a 1% agarose gel (1 g of agarose dissolved in 100 ml of 1x Tris-Boric Acid-EDTA buffer, TBE) stained with ethidium bromide. After electrophoresis, the obtained plasmid DNA bands were visualized using an ultraviolet light illuminator, and a photo documentation system was used for analysis [21]. The mobility (mm) and plasmid size (kb) were determined relative to the standard DNA loaded at the 1 kb marker [22].

Plasmid curing and antibiotic susceptibility post-plasmid curing

The curing of the resistant plasmids of the *S. marcescens* isolates was carried out using the method previously described by Salisbury et al. [23] with slight modifications. A small inoculum (100 to 200 cfu/ml) was added to a serial dilution of acridine orange nutrient broth (pH 7.6) and incubated at 30 ^{0}C for 24 h. Cultures with clear, visible growth and the highest acridine orange concentration were further tested for antibiotic susceptibility.

RESULTS

Antibiotic susceptibility testing

Fifty *S. marcescens* isolates were collected from urine samples of patients with UTI. The results presented in Figure 1 revealed that *S. marcescens* isolates were resistant in the following order: cefuroxime (64%), ceftazidime (50%), azithromycin (32%), ceftriaxone (20%), and ciprofloxacin (18%). The cephalosporins, cefuroxime, and ceftazidime, showed the highest resistance, at 64% and 50%, respectively. Based on patient prescribing information, cefuroxime (second-generation cephalosporin), one of the most commonly prescribed antibiotics in health facilities, had the highest resistance level. In contrast, ciprofloxacin (quinolone) had the lowest level of resistance (Figure 1). Of the 50 isolates, eight isolates showed resistance to three or more antibiotics and were regarded as MDR. Table 2 shows the antibiotic resistance pattern of the MDR isolates.

Plasmid extraction

The gel electrophoresis result of the visualized DNA plasmid from the eight MDR *S. marcescens* isolates showed that five of the isolates (63%) had plasmids extracted while three (37%) were without plasmids (Table 1). Figure 1 shows that all the isolates had the same number of bands but with different molecular weights of the plasmid. All five *S. marcescens* isolates possessed plasmids with a molecular weight of 30 kb and above. The molecular weights ranged between 33 kb and 58 kb (Table 2).

Post plasmid curing

The antibiotic susceptibility results of MDR *S. marcescens* isolates post-plasmid curing are shown in Table 1. Results showed that most of the isolates remained resistant to tested antibiotics. One isolate (S9) was cured and became sensitive to ceftriaxone, but not cefuroxime or ceftazidime. Other isolates were resistant to the cephalosporins, except S39, which retained its sensitivity to ceftazidime. Though these isolates remained resistant to cephalosporins, they showed an increased zone of inhibition after plasmid curing. For ciprofloxacin, all the isolates remained resistant. However, isolates S39 and S45 showed increased inhibition zones after curing, S14 showed a slight decrease in inhibition zones, and S9 remained the same (Table 1). A unique situation was encountered for isolate S23, initially sensitive to ciprofloxacin but resistant after plasmid curing. For azithromycin, S39 and S23 were sensitive, while others remained resistant. For the isolates resistant to azithromycin, there was an increase in zones of inhibition (Table 1). Plasmid molecular weights and antibiotic resistance patterns of the MDR *S. marcescens* isolates are represented in Table 2.

DISCUSSION

Previous studies in recent years have established the pathogenic nature of *Serratia marcescens* in causing urinary tract infections (UTIs), hence the importance of studying the antibiotic sensitivity pattern and resistance mechanism of the clinical isolates of *S. marcescens* [21–24]. The overall findings from the study indicate high antibiotic resistance. This resistance was found in all isolates and across all antibiotic classes tested. Findings from the present study indicate that *S. marcescens* isolates were more susceptible to ciprofloxacin. Yah et al. [25] reported an 80% susceptibility of clinical isolates of *S. marcescens* from different clinical samples to ciprofloxacin, which is in tandem with the present study. In addition, *S. marcescens* isolates from our study showed significant resistance to cefuroxime and ceftazidime. Several reports have indicated the relatively significant potential of pathogenic *S. marcescens* for developing resistance, especially to the first line broad-spectrum antibiotics [26,27].

Since *S. marcescens* strains can produce ESBL, which helps them develop resistance to many β-lactamase antibiotics, it is worth noting that the isolates in the present study can also be classified as ESBL-producing bacteria based on the antibiotic susceptibility test results. Previous studies have documented high antibiotic resistance by *S. marcescens* [25,26], with Yah et al. [25] revealing the antibiotic susceptibility of ESBL-producing *S. marcescens* in Nigeria. All the eight *S. marcescens* isolates with plasmids could be characterized as ESBL-producing since they were resistant to the cephalosporins tested. Since the resistance of ESBL producers is plasmid dependent, it would have been assumed that plasmid cure would result in increased susceptibility to the antibiotics to which they were previously resistant [28].

The increase in antimicrobial susceptibility of some of the isolates after plasmid curing indicates plasmid-mediated resistance, of which the degree of susceptibility varied among the isolates. However, only two isolates (S39 and S45) were found to have lost their resistance to two antibiotics. Isolate S39 lost its resistance to ceftazidime and azithromycin, and isolate S45 lost its resistance to ceftriaxone and azithromycin. This result could be due to various contributing factors. First, plasmid curing is claimed to occur spontaneously in various ways, one of which is the treatment of cells with chemicals [28]. Again, the effectiveness of curing agents varies significantly, and this variation could be due to the efficacy of the curing agent and the organism being cured [29].

The molecular weight of most of the molecular isolates was between 33 kb and 58 kb. These findings are similar to previous studies from other countries. For example, Pérez-Viso et al.

[30] reported that MDR profiles of clinical isolates of *S. marcescens* in Madrid, Spain, possessed several plasmids with an average molecular size of about 60 kb.

Table 1. Antibiotic susceptibility of MDR *S. marcescens* isolates before and after plasmid curing

Isolates	Antibiotic inhibition zone diameter (IZD) (mm)														
	Ceftriaxone			Cefuroxime			Ceftazidime			Ciprofloxacin			Azithromycin		
	BF	AT	I	BF	AT	I	BF	AT	I	BF	AT	I	BF	AT	I
S9	3	27*	S	5	9	R	0	13†	R	13	13	R	13	15	R
S39	20	20	R	9	12	R	18	19	S	5	13†	R	12	25†	S
S14	11	28†	S	0	0	R	2	13*	R	14	12**	R	10	15	R
S23	5	8	R	2	6	R	0	10†	R	21	8**	R	12	25†	S
S45	24	28	S	10	13	R	12	12	R	0	17*	R	0	13	R
S17¶	27	-	-	6	-	-	2	-		35	-	-	5	-	-
S40¶	12	-	-	8	-	-	7	-		34	-	-	27	-	-
S36¶	30	-	-	5	-	-	11	-		40	-	-	13	-	-

BF: Before plasmid curing; AT: After plasmid curing; I: Interpretation of results; *Plasmid cured; **More resistant; †More sensitive after curing; ¶Isolates without plasmids

Table 2. Plasmid molecular weights and antibiotic resistance pattern of MDR *S. marcescens* isolates

Isolates	Estimated molecular weight of plasmid (kb)	Resistance pattern before plasmid curing	Resistance pattern post plasmid curing
S9	33.0	CRO; CXM; CAZ; CIP; AZD	CXM; CAZ; CIP; AZD
S39	40.0	CRO; CXM; CIP; AZD	CRO; CXM; CIP
S14	52.0	CRO; CXM; CAZ; CIP; AZD	CXM; CAZ; CIP; AZD
S23	48.0	CRO; CXM; CAZ; CIP; AZD	CRO; CXM; CAZ; CIP
S45	58.0	CRO; CXM; CAZ; CIP; AZD	CXM; CAZ; CIP; AZD

CRO; Ceftriaxone; CXM: Cefuroxime; CAZ: Ceftazidine; CIP: Ciprofloxacin; AZD: Azithromycin

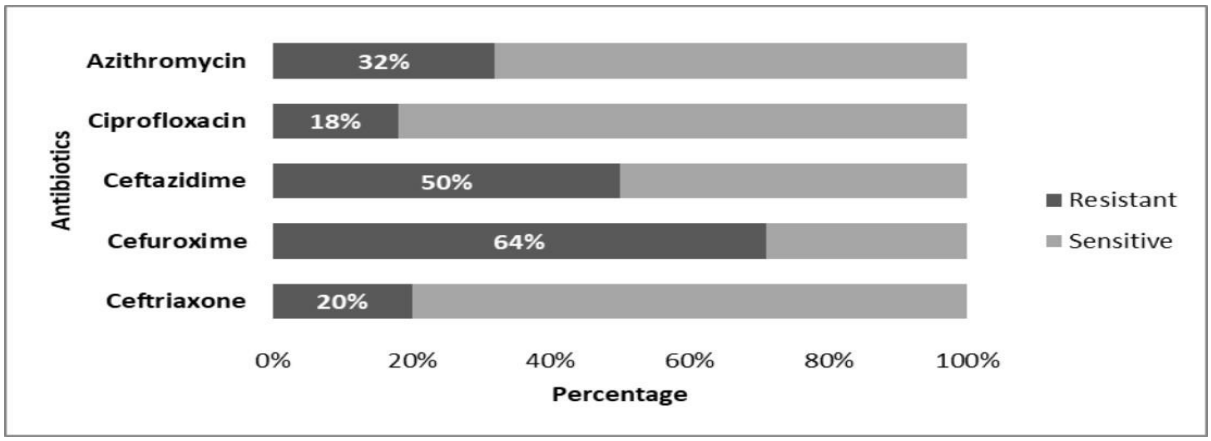

Figure 1. Susceptibility of *S. marcescens* isolates against tested antibiotics.

The present study's high resistance found among isolates could be related to 30 kb and above plasmid weights. Of the isolates treated to plasmid curing, only three (S9, S14, and S45) were cured and became susceptible to ceftriaxone, ceftazidime, and ciprofloxacin, respectively, while remaining resistant to other antibiotics. Several previous studies have shown that even after curing plasmids, isolates in their investigation remained resistant, implying that resistance was chromosomally mediated [28,31]. Remarkably, one isolate (S23), initially sensitive to ciprofloxacin, lost its sensitivity after plasmid curing. Although perplexing, this result can be attributed to various factors. One of which might be the different methods of plasmid curing, which are suggested to be capable of inducing mutations in the host DNA.

This study has some limitations. First, a more significant number of samples of plasmids were not profiled. Since only five plasmids out of fifty isolates were profiled, the data generated from the plasmid profile analysis does not represent all of the *S. marcescens* isolates used in this study.

CONCLUSION

The present study revealed the high resistance of clinical isolates of *S. marcescens* obtained from UTI origins. Most of the identified MDR isolates had plasmids and demonstrated high antibiotic resistance before and even after curing, indicating that they harbored several resistance genes. This also signals danger, as these genetic elements can be transferred to *S. marcescens* strains and other pathogenic bacteria. Given the presence of *S. marcescens* carrying multiple resistant genes, this study further highlights the significance of constant, rigorous surveillance intended to inform policy on treatment failures and the emergence of resistant bacteria within the region of the present study.

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