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*Review*

# Mechanisms of antimicrobial resistance in *Serratia marcescens*

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***Serratia marcescens*, one of the Enterobacteriaceae, was considered originally to be non-pathogenic. But in recent decades, nosocomial infections caused by this organism are often hard to treat because of both the intrinsic resistance of this species and its abilities to acquire further resistance to multiple groups of antimicrobial agents, including β-lactams, aminoglycosides and fluoroquinolones. *S. marcescens* have their own ways to become resistant in common with other Enterobacteriaceae: production of β-lactamases including extended-spectrum β-lactamases, AmpC-type cephalosporinase and carbapenemases; diminished outer membrane permeability; modification of the target site-PBPs; overexpression of active efflux systems; synthesis of aminoglycoside-modifying enzymes; and structural alterations of the GyrA protein. This review will show a brief history, the virulence factors and the known resistance mechanisms to the most frequently administrated antimicrobial agents: β-lactams, aminoglycosides and fluoroquinolones in *S. marcescens*.**

**Key words:** *Serratia marcescens*, virulence factors, antimicrobial resistance.

## INTRODUCTION

*Serratia marcescens*, a Gram-negative bacillus that belongs to the family Enterobacteriaceae, was considered originally to be non-pathogenic and relatively uncommon in the health care system. First described in 1819, *S. marcescens* was thought to be a nonpathogen for years, although sporadic reports in the medical literature implicated that the organism could cause opportunistic infections. Because many strains of *S. marcescens* have red pigment, and the organism was assumed to be nonpathogenic, it was used as a tracer organism in medical experiments and as a biological warfare test agent by bacteriologists for many years. It is a widely distributed saprophytic bacterium, and has been found in food, particularly in starchy variants which provide an excellent growth environment. While this organism was known formerly by a variety of names, including *Chromobacterium prodigiosum* (Sleigh, 1983), Gaughran (1968) used the name *S. marcescens* that had been assigned by Bizio in 1823. However, *S. marcescens* is now becoming an important cause of nosocomial infections, particularly in neonatal intensive care units. It has been reported that *S. marcescens* is associated with respiratory tract infections, urinary tract infections, septicemia, meningitis, conjunctivitis, endocarditis, and wound infections (Hejazi and Falkiner, 1997). *S. marcescens* infections induce inflammation and fever, but more serious conditions can develop in patients weakened by previous infections, surgery, and immunosuppression (Johnson et al., 1998). A serious problem in the treatment of infections due to *S. marcescens* is that this organism shows resistance to a wide variety of antimicrobial agents including ampicillin and both second and third generation cephalosporins, even carbapenems (Hejazi and Falkiner, 1997). *S. marcescens* was revealed to be a pathogen capable of causing a full spectrum of clinical disease, from urinary tract infections (UTIs) to pneumonia. *S. marcescens* is

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now an accepted clinical pathogen, and multiantibiotic-resistant isolates are prevalent.

Based on the actual literature, the purpose of this review is to give perspective on the history of *S. marcescens*, discuss the virulence factors of this bacteria, update infections that *S. marcescens* cause, particularly in humans, and describe the mechanisms of antimicrobial resistance in *S. marcescens*.

## BRIEF HISTORY OF *SERRATIA MARCESCENS*

*S. marcescens* is ubiquitous, and some strains are capable of producing pigment extracted by Kroft in 1902 and named “prodigiosin”. The intensity of pigment ranges from dark red to pink, depending on the age of the colonies (Bunting, 1942). Because of the sensitivity to light, the attempts to exploit it as a commercial dye failed. While the present appreciation of the pathogenicity of this organism, it is difficult to realize how benign it was once considered to be. Indeed, it was often used as a biological marker before its reputation as a notorious pathogen.

The recognition of *S. marcescens* as an opportunistic human pathogen could be dated from 1951. It was a report about the infections of 11 cases due to this organism over a 6-month period in 1951 at Stanford University Hospital (Wheat et al., 1951). Infections caused by *S. marcescens* have been reported with increasing frequency since 1960. McCormack and Kunin, (1966) reported a nursery epidemic involving 27 babies. Although only 15 cases of *S. marcescens* bacteremia had been recorded in the medical literature by 1968, Yu et al. (1979) reported 76 cases of bacteremia caused by *S. marcescens* in one hospital alone from 1968 to 1977. Infections and deaths caused by *S. marcescens* have not rarely been reported in papers nowadays. In 2006, 9 neonates were infected by *S. marcescens*, and among them 3 died because of septicemia in a neonatal intensive care unit in Turkey (Bayramoglu et al., 2011). From November 2007 to January 2008, there was a national outbreak of *S. marcescens* bacteremia with a total of 162 cases reported across 9 states in the United States (Su et al., 2009). Engel et al. (2009) reported 38 episodes of *Serratia* spp. bacteremia occurred over a 10-year period in Australia. Sikka et al. (2010) described 22 patients from a multistate outbreak of *S. marcescens* bacteremia that was linked to contaminated prefilled syringes of heparin and saline supplied by 1 manufacturer. *S. marcescens* can cause health care-associated infections. Iosifidis et al. (2012) reported the investigation and control of an outbreak of *S. marcescens* bloodstream infections in a general pediatric department. 4 patients developed bloodstream infections because of a *S. marcescens* strain demonstrating the same antimicrobial susceptibility pattern as well as the same molecular profile. Maltezou et al. (2012) described 3 consecutive outbreaks caused by genetically unrelated *S. marcescens* clones that occurred in a neonatal intensive care unit (NICU) over a 35-month period.

The ability of *S. marcescens* to cause infections was once thought to be limited, but it has now been implicated as an aetiological agent in every conceivable kind of infection, including respiratory tract infection, urinary tract infection, septicaemia, meningitis, conjunctivitis, and cellulitis (Samonis et al., 2011; Madani et al., 2011). And the most troublesome problem is their ability to confer resistance to a wide variety of antimicrobial agents. In the 20th century, the role of *S. marcescens* has undergone a dramatic change. As a biological marker, it has had a leading role in a number of classic experiments, which led to an improved understanding of the pathogenesis of infection. Now it has graduated to the full-fledged status of a pathogen that causes a steady increase in nosocomial infections that can be life-threatening.

## VIRULENCE FACTORS

The virulence factors secreted by *S. marcescens* are proteases, nuclease, and hemolysin (Hines et al., 1988). And it produces a wetting agent or surfactant called 'serrawettin' which helps in the colonization of surfaces (Matsuyama et al., 1992). A 56 kDa protease has been shown to promote keratitis by cleaving IgG, IgA and lysozyme. Hemolysin production is a common attribute of *S. marcescens* strains. The hemolysin causes hemolysis of human or animal erythrocytes and the release of inflammatory mediators (Kurz et al., 2003; Ralf Hertle and Schwarz, 2004). Its hemolysin is determined by 2 chromosomal genes termed *shlA* and *shlB*. The ShlA (162kDa) polypeptide is the hemolysin itself, whereas ShlB (61kDa) is required for activation and secretion of ShlA. Histone-like protein H1 (H-NS) may play an important role in the regulation of hemolysin expression in *S. marcescens*. Franzon and Santos (2004) claimed the hypothesis that H-NS acts at specific sites to influence DNA topology in order to enhance the transcription and expression of *shlA* and *shlB*, however, little is known about the mechanism.

Bacterial toxins acting on mammalian cell lines were currently detected in *S. marcescens*, as evidenced by changes in cell morphology (Carbonell et al., 1997). The studies of the virulence factors of the bacillus demonstrated that clinical isolates produce a cytotoxin affecting Vero cells in culture. This cytotoxin induces irreversible damage of the cells, provoking vacuolization with a subsequent lysis. The molecular mass of the cytotoxin was estimated to be about 50 kDa. This cytotoxic effect is not plasmid-mediated, excreted by the cell and heat labile, optimally produced by at temperatures ranging from 30 to 37°C. And the purified cytotoxin did not present any hemolytic activity, which is distinct from *S. marcescens* hemolysin.

## MECHANISMS OF ANTIMICROBIAL RESISTANCE

The discovery and synthesis of antimicrobial agents are extremely valuable for human beings in the treatment of infectious diseases. However, an extremely serious problem is the emergence and spread of multiresistant strains in the last decades. And *S. marcescens* is also not exceptional. Infections caused by these strains may be difficult to be treated because of resistance to multiple antimicrobial agents, including β-lactams, aminoglycosides, and fluoroquinolones.

## RESISTANCE TO β-LACTAMS

There are 4 principal mechanisms of resistance to β-lactam drugs in *S. marcescens*: 1) production of inactivating enzymes (β-lactamases); 2) the constitutive expression of efflux pumps; 3) low permeability of its outer membrane; and 4) alteration of penicillin-binding proteins (PBPs) targets. In *S. marcescens*, all possible mechanisms determining resistance to β-lactam antimicrobial agents may exist simultaneously or in various combinations in clinical strains. The resistance is either passed on genetically to daughter cells or is acquired by transfer from other bacteria, usually by plasmids.

Like other Enterobacteriaceae, the production of β-lactamases which inactivate certain β-lactam antimicrobial agents is the most common mechanism of resistance in *S. marcescens*. Penicilloyl-serine transferases (usually referred to as β-lactamases) rupture the amide bond of the β-lactam ring, thus the obtained products lack antibacterial activity (Sykes and Matthew, 1976). These β-lactamases include extended-spectrum β-lactamases (ESBLs), AmpC-type cephalosporinase and carbapenemases.

### ESBLs

The ESBLs are a heterogeneous group of enzymes that are encoded by plasmid-borned genes. They now number 532 distinct enzymes and convey varying degrees of resistance to cephalosporins, penicillins, β-lactamase inhibitors, and monobactams (Jacoby and Bush, 2005). ESBLs belong to molecular class A and functional group 2be (Bush et al., 1995), which lead to the resistance not only to carboxypenicillins and ureidopenicillins, but also to extended-spectrum cephalosporins (ceftazidime, cefepime, cefpirome) and aztreonam (Weldhagen et al., 2003). They show low affinity to carbapenems and can be inhibited by clavulanic acid and tazobactam *in vitro* (Nordmann and Guibert, 1998). ESBLs have been detected in *S. marcescens* isolates worldwide (Pagani et al., 1994; Gianneli et al., 1994; Luzzaro et al., 1995; Palucha et al., 1999). ESBL production was observed in 14 *S. marcescens*, including CTX-M and TEM (Nedjai et al., 2012). Kiratisin and Henprasert, (2011) reported six ESBL-positive *S. marcescens* isolates were clonally related and could be divided into three subgroups. There are varieties of ESBLs in clinical isolates of *S. marcescens*. The CTX-M and TEM types are well known in *S. marcescens*, other types have also been detected (Table 1). These types have low identity at the genetic level, and yet they have similar hydrolysis profiles.

The CTX-M-3 enzyme was widely observed among the *S. marcescens* isolates around the world. It was originally detected from 13 isolates of *S. marcescens* in the Kinki region of Japan (Yamasaki et al., 2003), and later in Korea, Taiwan, Bulgaria, and Poland (Yu et al., 2003; Naumiuk et al., 2004; Wu et al., 2004; Kim and Lim, 2005; Peng et al., 2007; Ivanova et al., 2008; Sabtcheva et al., 2008). Ivanova et al. (2008) reported a nosocomial outbreak of CTX-M-3 ESBL-producing *S. marcescens* in a Bulgarian university hospital. The epidemic strain was resistant to oxyimino beta-lactams, aztreonam, aminoglycosides, tetracycline, and chloramphenicol. The isolate was also found in the hospital environment and from a nurse's hands, suggesting transmission by staff handling. It was the first report from Bulgaria describing a hospital outbreak caused by CTX-M-3 ESBL-producing *S. marcescens.* The CTX-M-3 enzyme was the most common ESBL type in the *S. marcescens* isolates, followed by TEM-47 and SHV-5, in Poland (Naumiuk et al., 2004). In Taiwan, Wu et al. (2004) reported 1 strain of *S. marcescens* carried concurrent CTX-M-3 and SHV-5 conferring high-level MICs to both cefotaxime (128 µg/ml) and ceftazidime (64 µg/ml). Yu et al. (2003) detected 4 CTX-M-3-producing clinical strains of *S. marcescens* that exhibited resistance to cefotaxime (MICs, >256 µg/ml) and cefepime (MICs, ≥ 32 µg/ml), but were susceptible to imipenem and meropenem. All 4 isolates had significant MIC reductions of ≥ 3 log (2) dilutions for cefepime in the presence of clavulanic acid. Peng et al. (2007) reported class I integrons were conjugally transferred to recipients in 92.0% of *S. marcescens* harboring 2 different class I integrons containing the gene cassettes *aadA2* and *aadB-catB3*, respectively. The transfer of class I integron carrying the gene cassette *dfrA12-orfF-aadA2* was detected in 77.4% of *S. marcescens* isolates. The results showed that all CTX-M-3-producing isolates carried class I integrons on the conjugative plasmids. In Argentina, CTX-M-2 was originally detected in Metropolitan Public Hospitals of Buenos Aires City in 2000. CTX-M-2 was ubiquitous in all species, being the only ESBL detected in *S. marcescens* in that 1-month survey. The CTX-M-15 was firstly identified in Enterobacteriaceae isolates in India and demonstrated significant hydrolytic activity against ceftazidime. Two *S. marcescens* isolates, BB 1758 and BB 1763, which were cultured in 2000 from intubation tubes of 2 patients in the ICU of a hospital in Poland, were found to produce CTX-M-15. The isolates

**Table 1.** Epidemiology of molecular class A ESBLs produced by *S. marcescens.*

| Enzyme type | Initial isolation | | Other regions of isolation | References |
|---|---|---|---|---|
| | Region | Year | | |
| CTX-M-1 | Japan | 2000 | | Yamasaki et al. (2002) |
| CTX-M-2 | Argentina | 2000 | | Quinteros et al. (2003) |
| CTX-M-3 | Japan | 1998-2000 | Korea, Taiwan, Bulgaria, Poland | Yamasaki et al. (2003), Yu et al. (2003), Naumiuk et al. (2004), Wu et al. (2004), Kim et al. ( 2005), Peng et al. (2007), Ivanova et al. (2008), Sabtcheva et al. (2008) |
| CTX-M-15 | Poland | 2000 | | Baraniak et al. (2002) |
| TEM-AQ | Italy | 1993 | | Perilli et al. (1997) |
| TEM-12 | Italy | 2002 | | Sanguinetti et al. (2003) |
| TEM-47 | Poland | 1996-2000 | | Naumiuk et al. (2004) |
| TEM-52 | Korea | 2003 | | Kim et al. (2005) |
| TEM-149 | Italy | 1999-2003 | | Perilli et al. (2008) |
| SHV-2 | Hungary | 1998 | | Nagy et al. (1998) |
| SHV-5 | Greece | 1994 | Mexico, Poland, Taiwan | Gianneli et al. (1994), Wu et al. (2004), Naumiuk et al. (2004), Monteros et al. (2008) |
| SHV-12 | Japan | 1998-2000 | Korea | Yamasaki et al. (2003), Kim et al. (2005) |
| BES-1 | Brazil | 1996-1997 | | Bonnet et al. (2000) |

contained the IS*Ecp1* insertion sequence located upstream of the CTX-M-15 gene, which has been demonstrated to mobilize 3'-adjacent genes to transfer between DNA replicons (Baraniak et al., 2002).

TEM-AQ, a natural TEM variant β-lactamase was detected from an epidemic strain of *S. marcescens* in Italy in 1993 (Perilli et al., 1997). This isolate, named S5, was resistant to ceftazidim, cefotaxime, and aztreonam. Gene sequencing revealed multiple point mutations located in the 42-to-44 tripeptide and positions 145 to 146, 178, and 238. In addition, a glutamic acid 212 deletion was also found. The purified enzyme revealed the highest catalytic efficiency values for ceftazidime and aztreonam compared with the TEM-1 prototype enzyme. Between 2003 and 2008, *S. marcescens* strains producing the following TEM enzymes were consecutively isolated: TEM-12, TEM-47, TEM-52, and TEM-149 in Italy, Korea and Poland. The hydrolytic spectrum of TEM enzymes in *S. marcescens* is similar to that of the classical ESBLs in other Enterobacteriaceae and includes: narrow-spectrum penicillins, extended-spectrum cephalosporins, and aztreonam (Sanguinetti et al., 2003; Naumiuk et al., 2004; Kim and Lim, 2005; Perilli et al., 2008).

Fourteen *S. marcescens* isolated from a nosocomial outbreak in Hungary were not only produced in the Class C, inducible chromosomal β-lactamase, but also acquired a plasmid-mediated SHV-2-type ESBL in 1998 (Nagy et al., 1998). Between 1994 and 2000, SHV-5 and SHV-12 were detected in Greece and Poland in Europe, Mexico in North America and Japan, Korea and Chinese Taiwan in Asia (Gianneli et al., 1994; Wu et al., 2004; Naumiuk et al., 2004; Monteros et al., 2008; Yamasaki et al., 2003; Kim and Lim, 2005). SHV-5 was the most prevalent type among the SHV-type ESBL.

A novel ESBL designated BES-1 (Brazil extended-spectrum beta-lactamase) was detected in *S. marcescens* Rio-5 isolated in Brazil during 1996 and 1997. Rio-5 exhibited a high level of resistance to aztreonam (MIC, 512 µg/ml) and a distinctly higher level of resistance to cefotaxime (MIC, 64 µg/ml) than to ceftazidime (MIC, 8 µg/ml). In common with CTX-M enzymes, BES-1 exhibited high cefotaxime-hydrolyzing activity. However, BES-1 differed from CTX-M enzymes by its significant ceftazidime-hydrolyzing activity, high affinity for aztreonam and lower susceptibility to tazobactam than to clavulanate (Bonnet et al.,

2000). Dissemination of ESBL-encoding genes plays an important role in drug resistance, and may limit the choice of antimicrobial agents in the treatment of life-threatening infections caused by *S. marcescens*.

## AmpC β-lactamase

Like other species of the Enterobacteriaceae family, *S. marcescens* produces an inducible chromosome-encoded AmpC β-lactamase that belongs to molecular class C, based on Ambler and the first functional group according to Bush (Bush et al., 1995), which can make *S. marcescens* resistance to third generation cephalosporins. AmpC β-lactamase is encoded by the *ampC* gene (Lodge et al., 1993). The *ampC* of *S. marcescens* contains 1128 nucleotides encoding a protein of 355 amino acids preceded by 21 amino acids which probably constitutes the signal peptide. The mature protein has a predicted molecular mass of 38, 901 Da. About 40% of the amino acid sequence was identical among AmpC β-lactamases resided in *S. marcescens* (Nomura and Yoshida, 1990). *ampC* and *ampR* are transcribed divergently with the AmpR-binding site located within the *ampC/ampR* intergenic region (Honoré et al., 1986). AmpC expression is inducible by some β-lactam drugs, such as cefoxitin and imipenem, in the presence of AmpR (Gatus et al., 1988). In addition to AmpR, two other gene products are required for AmpC induction, a permease (AmpG) and an amidase (AmpD) (Korfmann and Sanders, 1989). Mechanisms regulating *ampC* expression in *S. marcescens* have been studied by Mahlen et al. (2003). They identified an extended 5' untranslated region (UTR) of 126 nucleotides in the *ampC* gene of *S. marcescens*, which formed a prominent stem-loop structure. The stem-loop structure was involved in transcript stability. These findings indicate that constitutive expression of *ampC* in *S. marcescens* was regulated by both transcriptional initiation and post-transcriptional events.

Amino acid substitution in the omega loop of chromosomal AmpC β-lactamase is important in the selection of broad-spectrum resistance in *S. marcescens*. Hidri et al. (2005) reported a novel S3 AmpC β-lactamase in France. S3 showed E57Q, Q129K and S220Y substitutions compared with the closest AmpC enzyme. The S220Y substitution was located in the omega loop. The catalytic efficiency of this mutated enzyme toward ceftazidime was increased by about 100-fold. Yu et al. (2008) reported a novel S4 gene detected from *S. marcescens* isolates in southern Taiwan. S4 gene was with 98% identity to SRT-1 gene. Isolates with S4 exhibited a phenotype of resistance to cefotaxime but not ceftazidime. A novel SRT-2 enzyme conferred resistance to cefotaxime was reported by Wu et al. (2004) in middle Taiwan. Mammeri et al. (2004) reported a AmpC gene from *S. marcescens* HD had a 12-nucleotide deletion, leading to a 4-amino-acid deletion located in the H-10 helix of the β-lactamase. This enzyme significantly hydrolyzed ceftazidime, cefepime, and cefpirome. It underlined that resistance to expanded-spectrum cephalosporins may be mediated by structurally modified AmpC-type β-lactamases.

Mata et al. (2010) reported a clinical *S. marcescens*, which coharboured DHA-1 and *qnrB* genes on the same IncL/M-MOB (P13) plasmid approximately 70 kb in size. It was the first report of an isolate of *S. marcescens* harbouring a plasmid-mediated AmpC β-lactamase (pACBLs). pACBLs confer resistance to all β-lactams, including cephamycins, except cefepime and carbapenems, and they are not inhibited by commercialized β-lactamase inhibitors. Plasmids with these genes often carry multiple other resistances. Bagattini et al. (2004) reported a nosocomial outbreak of AmpC-producing *S. marcescens.* More important problem is that this epidemic strain was multiply antibiotic resistant, carrying the trimethoprim-resistance gene and the adenyltransferase gene, which confers resistance to streptomycin and spectinomycin, within a class I integron.

Recently, Suh et al. (2010) reported that *S. marcescens* isolates were resistant to meropenem because of combination with the overproduction of the chromosomal AmpC enzyme and the loss of outer membrane protein OmpF.

## Carbapenemases

Carbapenemases are β-lactamases that hydrolyze most β-lactams including carbapenems. Carbapenemases involved in acquired resistance are of Ambler molecular classes A, B, and D. SME type, KPC type and IMP type have been detected in *S. marcescens* worldwide (Table 2). SME and KPC enzymes belong to class A. SME-1 had been first identified from the carbapenem-resistant *S. marcescens* strain S6, isolated in London in 1982 (NAAS et al., 1994), and subsequently detected in 1999 in the United States (Gales et al., 2001). It hydrolyzes penicillins, aztreonam, cephalosporins, and carbapenems and is inhibited by clavulanic acid. The $bla_{SME-1}$ gene was chromosome encoded in isolate S6. SME-1 had the greatest amino acid identity (70%) with the pI 6.9 carbapenem-hydrolyzing β-lactamase, NMC-A, from *Enterobacter cloacae* NOR-1 (NAAS et al., 1994). SME-producing *S. marcescens* have been detected sporadically throughout the world and in the USA. Fairfax et al. (2011) reported the detection of carbapenem resistant *S. marcescens* in 2 separate incidents, involving different hospitals, 7 months apart. Both isolates produced the SME-1 carbapenemase and were clonally related. SME-2 is an enzyme with a single amino acid change relative to SME-1, a substitution from valine to glutamine at position 207. Purified SME enzymes had β-lactam hydrolysis profiles similar to SME-1 (Queenan et al., 2000). SME-2 has also been detected in Canada and Switzerland recently (Poirel et al., 2007; Carrër et al., 2008).

**Table 2.** Epidemiology of Carbapenemases found in *S. marcescens.*

| Enzyme | Geographical distribution | Location of encoding gene | Hydrolysis on β-lactam antimicrobial agents | | | | Inhibition by | |
|---|---|---|---|---|---|---|---|---|
| | | | PCs | ATM | Cephalosporins | Carbapenems | CLV | TAZ |
| SME-type | | Chromosome | YES | YES | YES | YES | YES | YES |
| SME-1 | United Kingdom, United States | | | | | | | |
| SME-2 | United States, Canada, Switzerland | | | | | | | |
| SME-3 | United States | | | | | | | |
| SMB-1 | Japan | Chromosome | | | | | | |
| KPC-type | | Plasmid | YES | YES | YES | YES | YES | YES |
| KPC-2 | China, Greece, United States | | | | | | | |
| IMP-type | | Plasmid | YES | NO | YES | YES | NO | NO |
| IMP-1 | Japan | | | | | | | |
| IMP-6 | Japan | | | | | | | |
| IMP-10 | Japan | | | | | | | |
| VIM-type | | | | | | | | |
| VIM -2 | Korea | Class I integron | | | | | | |

PCs, penicillins; ATM, Aztreonam; CLV, clavulanic acid; TAZ, tazobactam.

SME-3 differed from SME-1 by a single amino acid substitution of tyrosine for histidine at position 105, and displayed similar hydrolytic profiles with SME-1 (Queenan et al., 2006).

The KPC class A carbapenemases are capable of hydrolysing carbapenems, penicillins, cephalosporins and aztreonam, and are inhibited by clavulanic acid and tazobactam. The first report of this β-lactamase, KPC-1, was from a carbapenem-resistant *Klebsiella pneumoniae* (Yigit et al., 2001). Sporadic occurrences of KPC-2 were reported in China. Zhang et al. (2007) described 3 clinical isolates of carbapenem-resistant *S. marcescens*, isolated in February 2006, that produce KPC-2. It was the first report of detection of a plasmid-encoded carbapenem-hydrolysing enzyme KPC-2 in *S. marcescens*. Almost simultaneously, Cai et al. (2008) identified KPC-2 in 3 *S. marcescens* isolates from the same city but a different hospital. *S. marcescens* isolates that exhibited reduced susceptibility to carbapenems were identified in a Greek hospital. All three patients infected had clonally indistinguishable isolates of *S. marcescens* that acquired a plasmid-mediated $bla_{KPC-2}$ gene during the hospitalization. This report provided evidence that *S. marcescens* has the ability to easily acquire KPC carbapenemases (Tsakris et al., 2010).

The class B enzymes are the most clinically significant carbapenemases. They are metalloenzymes of the IMP or VIM types. Metalloenzymes, whose genes are plasmid and integron located, hydrolyze virtually all beta-lactams except aztreonam. From 1994 until 2009 IMP variants of MBL, including IMP-1, IMP-6 and IMP-10 were just found in *S. marcescens* in Japan (Osano et al., 1994; Ito et al., 1995; Yano et al., 2001; Hu and Zhao, 2009). The IMP-6 gene differed from the gene encoding IMP-1 by one point mutation, leading to one amino acid substitution: 640-A in the base sequence of the IMP-1 gene was replaced by G, and Ser-196 was replaced by Gly in the mature enzyme. IMP-6 had extended substrate profiles against carbapenems. However, the activity of IMP-6 was very low against penicillin G and piperacillin (Yano et al., 2001). Hu and Zhao, (2009) identified $bla_{IMP-1}$ and $bla_{IMP-10}$ in clinical isolates of *S. marcescens* in Japan in 2000. The $bla_{IMP-1}$ and $bla_{IMP-10}$ gene cassettes were carried by a class I integron and followed by the ***aac(6')-IIc*** gene cassette. They found that strains harbouring $bla_{IMP-10}$ showed higher-level resistance to imipenem, meropenem and panipenem than the strains harbouring $bla_{IMP-1}$, although the nucleotide sequences of the class I integrons carrying $bla_{IMP-10}$ or $bla_{IMP-1}$ were identical except for a single point mutation. A $bla_{VIM-2}$ gene cassette on a class I integron was found in an imipenem-resistant *S. marcescens*

isolate from a urine specimen in Korea for the first time (Yum et al., 2002). Then $bla_{VIM-2}$ was also found in 0.5% (1/201) of *S. marcescens* isolate in Korea in 2002 (Lee et al., 2005).

Recently, a novel MBL, named SMB-1 (*Serratia* metallo-β-lactamase), was detected in a carbapenem-resistant *S. marcescens* strain isolated in a Japanese hospital in 2010. SMB-1 possessed a zinc binding motif, H(Q)XHXDH (residues 116 to 121), H196, and H263 and was categorized as a member of subclass B3 MBL. SMB-1 has 75% amino acid identity with the most closely related MBL, AMO1, of uncultured bacterium, recently identified through the metagenomic analysis of apple orchard soil. SMB-1 demonstrated high $K_{cat}$ values of $>500s^{-1}$ for carbapenems, resulting in the highest hydrolyzing efficiency ($K_{cat}/K_m$) among the agents tested. The hydrolyzing activity of SMB-1 was well inhibited by chelating agents. The $bla_{SMB-1}$ gene was located on the chromosome of *S. marcescens* strain and at the 3′ end of the IS*CR1* element in complex with a typical class I integron carrying *aac(6')-Ib* and *catB3* gene cassettes (Wachino et al., 2011).

## DUE TO ALTERED OUTER MEMBRANE PERMEABILITY

Sánchez et al. (1997) described 3 different porins from *S. marcescens*. They were named Omp1, Omp2, and Omp3 and their molecular weights were 42, 40 and 39 kDa, respectively. Omp2 and Omp3 showed osmoregulation and thermoregulation in a similar way to OmpC and OmpF of *Escherichia coli.* Weindorf et al. (1998) reported that low-level resistance to extended-spectrum β-lactam antibiotics was caused by overproduced β-lactamase alone in *S. marcescens*. High-level resistance was due to β-lactamase overproduction and defects of porin OmpF or OmpF and OmpC. Suh et al. (2010) reached the same conclusion. They reported an outbreak of meropenem-resistant *S. marcescens* comediated by chromosomal AmpC β-lactamase overproduction and outer membrane protein loss. This isolate showed a lack of the 42-kDa outer membrane protein OmpF. The loss of OmpF may have played a role in the acquisition of meropenem resistance.

## DUE TO ALTERED TARGET

The rarest mechanism of resistance to β-lactams in *S. marcescens* involves modification of the target site-PBPs. Alteration of PBPs has proved an effective way for Gram-positive bacteria to become resistant to β-lactams. Gunkel et al. (1991) reported that in *S. marcescens* which were high resistant to extended-spectrum β-lactam antibiotics, the amounts of PBPs 3 and 6 were, respectively, 1.5 and 2 times than those in sensitive reference strains. Only a single target, PBP3, was highly sensitive to cefotaxime, ceftazidime, and aztreonam. Three PBPs (2, 1A and 3) were highly sensitive to imipenem. They supposed that the combination of the PBP-2-specific mecillinam and PBP-3-specific β-lactams could kill highly resistant *S. marcescens*.

## RESISTANCE TO AMINOGLYCOSIDES

Bacteria acquire resistance to aminoglycosides by preventing the drug from reaching the target site in the ribosome in one of two ways: firstly, alterations in the cell envelope can prevent uptake of the drug; and secondly, the drug itself can be modified by inactivating enzymes that adenylate, acetylate, or phosphorylate the aminoglycoside hydroxyl or amino groups (Hejazi and Falkiner, 1997).

The AAC (6') enzymes are of particular interest. The AAC (6')-I class of enzymes encodes resistance to tobramycin, dibekacin, amikacin, netilmicin, *2'-N*-ethylnetilmicin and sisomicin. The DNA sequences of 3 genes encoding *6'-N*-acetyl-transferase type I enzymes have been determined: the *aacA1* gene from *Citrobacter diversus* [*aac*(*6'*)-*Ia*], and the *aacA4* gene from an IncM plasmid isolated from *S. marcescens* [*aac*(*6'*)-*Ib*]. The amino-terminal portion of the bifunctional AAC (6')+APH (2") enzyme from *Enterococcus faecalis* has additionally been shown to encode AAC (6') activity [*aac(6')-Ie*] (Tenover et al., 1988). A newly discovered bifunctional antibiotic resistance enzyme from *S. marcescens* catalyzes adenylation and acetylation of aminoglycoside antibiotics. The structure assignment of the enzymic products indicated that acetylation takes place on the 6'-amine of kanamycin A and the adenylation on 3''- and 9-hydroxyl groups of streptomycin and spectinomycin, respectively. The adenyltransferase domain appears to be highly specific to spectinomycin and streptomycin, while the acetyltransferase domain shows a broad substrate profile (Kim et al., 2006).

The AAC (6')-Ic protein was the third member of a family of AAC (6') proteins which included a coding region identified between the *aadB* and *aadA* genes of Tn4000. The -35 region of the *aac(6')-Ic* promoter overlapped a large palindromic sequence which may be involved in the regulation of the *aac(6')-Ic* gene. Hybridization experiments utilizing a restriction fragment from the *aac(6')-Ic* gene showed that all *S. marcescens* organisms carried this gene whether or not the AAC(6')-I resistance profile was expressed (Shaw et al., 1992). García et al. (1995) investigated a total of 127 amikacin-resistant *S. marcescens* isolates to study the molecular mechanisms of resistance involved. They found that the *aac(6')-Ic* gene was detected by dot-blot hybridisation in every *S. marcescens* isolate. A novel plasmid-mediated 16S rRNA methylase which conferred an extraordinarily high level of resistance to aminoglycosides was identified in a

*Pseudomonas aeruginosa* clinical strain in Japan (Yokoyama et al., 2003). In a study of detecting the 16S rRNA methylase genes in amikacin-resistant Enterobacteriaceae isolates that were collected in 1995 to 1998 and 2001 to 2006 at a university hospital in South Korea, the IncL/M conjugative plasmid carrying *armA* was detected in six *S. marcescens* isolates (Kang et al., 2008). Doi et al. (2004) identified a *S. marcescens* clinical strain producing a novel 16S rRNA methylase. This novel enzyme RmtB conferred high-level resistance to various aminoglycosides, including 4,6-disubstituted deoxytreptamine aminoglycosides such as kanamycin, tobramycin, amikacin, arbekacin, gentamicin, sisomicin and isepamicin. RmtB shared 82% identity with RmtA of *P. aeruginosa*, while its similarity with the 16S rRNA methylases of the genera *Streptomyces* and *Micromonospora* was relatively low (up to 33%). *rmtB* was carried on a large plasmid, which was nonconjugative but transferable to *E. coli* by electroporation. The 0.8-kb region downstream of *rmtB* shared significant identity with the corresponding region of *rmtA*, thus reenforcing the idea that the 2 genes may have come from similar bacterial species.

## RESISTANCE TO FLUOROQUINOLONES

Fluoroquinolone resistance has been shown to be the result of mutations in DNA gyrase and overexpression of multidrug resistance efflux pumps. DNA gyrase has been isolated from quinolone-resistant clinical strains of *S. marcescens* in Japan (Fujimaki et al., 1989), which suggested that DNA gyrase alterations are the basis of quinolone resistance. Masecar and Robillard, (1991) reported that the decreased susceptibility to quinolones in *S. marcescens* MP051 is due to a mutation in *gyrA*. The genetic characterization of MP051 as a *gyrA* mutant by gyrase A gene probe analysis was consistent with results obtained from gyrase inhibition assays in which resistance to ciprofloxacin was transferable with the A subunit from the resistant isolate. Kim et al. (1998) reported the observation that an amino acid substitution at position 83 or 87 of the GyrA protein was present in all *S. marcescens* clinical isolates in which the MICs to ciprofloxacin increased. It suggested that DNA gyrase is the primary target of quinolones in *S. marcescens* and that these 2 residues of the GyrA protein are especially important in the formation of the quinolone-gyrase-DNA complex. The mutational alteration in *gyrA* is a common mechanism of quinolone resistance in *S. marcescens*. Weigel et al. (1998) reported the fluoroquinolone-resistant clinical isolates of *S. marcescens* displayed the greatest diversity in GyrA mutations, including Gly-81 to Cys, Ser-83 to Ile or Arg, and Asp-87 to Asn. No double mutations were detected in the *gyrA* QRDRs. Three kinds of plasmid-mediated quinolone resistance (PMQR) determinants have been detected conferring low-level resistance to quinolones by different mechanisms: quinolone resistance proteins (Qnr), AAC(6')-Ib-cr, and QepA efflux. The qnr genes (*qnrA*, *qnrB*, *qnrS qnrC*, and *qnrD*) encode pentapeptide repeat proteins that block the action of ciprofloxacin on bacterial DNA gyrase and topoisomerase IV and, most importantly, facilitate the selection of chromosomal mutants in the presence of a quinolone. Park et al. (2007) reported the prevalence of *qnr* determinants was low in *S. marcescens* (2.4%). The *aac(6')-Ib-cr*, one of the approximately 30 variants of *aac(6')-Ib*, has 2 amino acid changes (Trp102Arg and Asp179Tyr) and can acetylate quinolones having unsubstituted piperazinyl nitrogen such as ciprofloxacin and norfloxacin. The *aac(6')-Ib-cr* was detected in 2 *S. marcescens* in a study from Korea. Although *aac(6')-Ib-cr* was uncommon in Korean, its association with various resistance genes and mobile elements would facilitate the dissemination of this variant (Kim et al., 2009). Park et al. (2009) reported the spread of *S. marcescens* coharboring *aac(6')-Ib-cr*, $bla_{CTX-M}$, *armA*, and $bla_{OXA-1}$ carried by conjugative IncL/M Type plasmid in Korean Hospitals. Velasco et al. (2010) reported a new pentapeptide repeat (PRP) protein, named SmaQnr, from the clinical *S. marcescens*. SmaQnr conferred a reduced susceptibility phenotype against fluoroquinolones. It was the first identification of a *qnr*-like gene in the chromosome of an enterobacterial species.

Multidrug resistance efflux pumps play a major role in the intrinsic and acquired resistance of *S. marcescens*. Kumar and Worobec, (2002) reported a proton gradient-dependent efflux of fluoroquinolone drugs in *S. marcescens*. One clinical isolate, T-861, and the mutant strain, UOC-67WL, were found to efflux ciprofloxacin and ofloxacin. Western immunoblot results confirmed overexpression of an AcrA-like protein in T-861 and UOC-67WL. Sequencing of the PCR product showed the presence of a *mexF*-like gene, which was overexpressed in *nfxC* mutants of *Pseudomonas aeruginosa.* Kumar and Worobec, (2005) also reported the presence of an RND family multidrug efflux pump, SdeAB that can pump out a diverse range of substrates that include fluoroquinolones, chloramphenicol, detergent, ethidium bromide, and organic solvents. Furthermore, their findings indicated this pump was overexpressed in *S. marcescens* strains that are multidrug resistant. Maseda et al. (2009) concluded that *S. marcescens* gains resistance to both biocides and antibiotics by expressing the SdeAB efflux pump upon exposure to cetylpyridinium chloride. It is also known that the flouroquinolones administrated with salicylic acid induce a reduced accumulation of ciprofloxacin, thus enhancing the resistance to the flouroquinolones (Berlanga and Vinas, 2000).

## CONCLUSION

It is concluded that *S. marcescens* is a growing problem

for public health, because of its high resistance and its increasing role in nosocomial infections (Johnson et al., 1998). Although the potential import of resistance mechanisms on mobile genetic elements is a continuing threat, perhaps the most difficult challenge we face with *S. marcescens* is its ability to rapidly develop resistance to multiple classes of antibiotics during the course of treating a patient. Numerous multidrug-resistant strains of *S. marcescens* have been isolated from both clinical and environmental settings, indicating that antibiotic treatment options for infections by *S. marcescens* has to be restricted and alternative methods for treatment have to be implemented.

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