First detection of a class 2 integron among clinical isolates of *Serratia marcescens*

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ABSTRACT

Serratia marcescens is a frequent nosocomial isolate and is associated with a variety of clinical sources, including blood, urine and sputum, and can cause significant infection. Infections can be difficult to treat due to its resistance to a variety of antimicrobial agents. An investigation of a population of 30 clinical strains revealed the presence of a class 2 integron among nine of the isolates, which represents the first isolation of this integron in *Serratia* species. This integron contained the gene cassettes *dfrA*1, *sat*1 and *aadA*1, conferring resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively. One of these isolates also carried a class 1 integron identified by sequence analysis as containing the open reading frames *aacC*1 (encoding gentamicin resistance), ORFX, ORFY and *aadA*1. Polymerase chain reaction analysis confirmed the presence of the *qacE*Δ1 and *sul*1 markers, which are common among class 1 integrons.

KEY WORDS: Integrons.
Serratia marcescens.

Introduction

Serratia marcescens is ubiquitous in the environment and is often isolated from soil and moist areas.[1] This organism is a well-known cause of hospital infections, particularly in intensive care units, and has been associated with respiratory, urinary, wound and bacteraemia infections.[2] Infections caused by *S. marcescens* may be difficult to treat due to their resistance to a variety of antibiotics, including β-lactams, aminoglycosides and fluoroquinolones.

Bacterial resistance to antimicrobial agents, which is increasing worldwide, is caused frequently by the acquisition of new genes, rather than by mutation.[3,4] One vehicle for the acquisition of such genes is the integron, a naturally-occurring element that facilitates the transfer of gene cassettes.[3,5] An integron is defined as having conserved features, namely the ability to incorporate gene cassettes such as the *int*1 gene (a specialised site-specific recombination enzyme, integrase), an *att*I site (a recombination site), and a promoter that drives the transcription of genes within the variable region.[6]

Integron classes 1 and 2 are the most frequently occurring among Gram-negative bacteria.[7] The structure of the class 1 integron includes 5′ and 3′ conserved regions and a variable region. The class 2 integron has an organisation similar to class 1, but is associated with the transposon *Tn*7 and is known to carry the three gene cassettes *dfrA*1, *sat*1 and *aadA*1, which confer resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively.[8]

The aim of this study is to investigate the presence of class 1 and 2 integrons among a population of clinically-significant *S. marcescens* isolates.

Materials and methods

Bacterial isolates

A total of 30 *Serratia* spp. isolates was included in the study, from 30 individual patients. All were isolated from the Health Services Executive Southern Area of Ireland. The strains were isolated between October 2003 and July 2006 from sputum samples (n=17), urine (n=11) and blood (n=2). Identification was performed by API 20E (bioMérieux, France). All isolates gave profiles corresponding to *S. marcescens*.

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Susceptibility testing

Susceptibility testing was performed by a disc-diffusion method, using Mueller-Hinton agar and the BIOMIC automated zone reader (Giles Scientific, NY), in accordance with CLSI (formerly NCCLS) guidelines.[9] The following antibiotics were tested: ampicillin (10 μg/disc), amoxicillin/clavulanic acid (30 μg/disc), cefuroxime (30 μg/disc), ciprofloxacin (5 μg/disc), gentamicin (10 μg/disc), trimethoprim (1.25 μg/disc) and aztreonam (30 μg/disc).

Bacterial storage and culture conditions

S. marcescens isolates were stored at –80°C in Protect bacterial preserver vials (Technical Services Consultants, UK) and were cultured routinely on nutrient agar prior to incubation overnight at 37°C.

DNA extraction

Chromosomal DNA was prepared from each isolate using the QIAamp DNA blood mini kit (Qiagen, UK), according to the manufacturer′s instructions.

Integron analysis

Integron analysis was performed by polymerase chain

reaction (PCR) to detect classes 1 and 2 integrons, using specific primer pairs shown in Table 1. To each reaction was added 100 ng template DNA, 1.5 mmol/L $MgCL_2$, each deoxynucleotide triphosphate at a concentration of 200 µmol/L, 5 µL 10x PCR buffer (50 mmol/L Tris-HCl [pH 8.0], 100 mmol/L NaCl, 0.1 mmol/L EDTA, 1mmol/L dithiothreitol, 50% glycerol and 1% Triton X-100), 25 pmol each primer and 2.5 units of *Thermus aquaticus* (*Taq*) DNA polymerase in a final reaction volume of 50 µL. Amplification was performed on a thermal cycler (9700: Perkin-Elmer, Foster City, CA, USA). Each set of reactions included a negative control. The amplified DNA products were analysed by conventional 1.5% (w/v) agarose gel electrophoresis in 1x TAE buffer. Gels were stained with ethidium bromide and visualised under ultraviolet (UV) light.

DNA sequencing

Amplified DNA products were purified using a PCR purification kit (Qiagen), according to the manufacturer's instructions. The purified amplicons were sequenced by automated methods (MWG-Biotech, Germany). Briefly, class 2 integron sequencing was performed using a forward and reverse read of the amplicon generated by the primer pairs hep74 and hep51. This generated two sequences of approximately 800 bp. The Sequencher version 4.7 (Gene Codes Corporation, Ann Arbor, MI) program was used to design a pair of primers that would allow amplification of the central portion of the 2143-bp amplicon to complete sequencing. The resultant DNA sequence was analysed using the BLAST suite of programs.[15]

Nucleotide sequence accession number

The sequence of the 2143-bp class 2 integron structure was submitted to GenBank and assigned the Accession No. DQ914527.

DNA amplification fingerprinting

Repetitive-sequence PCR (rep-PCR) typing using the RW3A primer sequences (Table 1) was performed according to the method of del Vecchio *et al.*,[16] incorporating 200 ng DNA template and 100 pmol RW3A. Amplification consisted of 35 cycles at 94°C for 1 min, 54°C for 1 min and 72°C for 2 min. A predenaturation step at 94°C for 5 min and a final extension step at 72°C were also included.

Results

Direct amplification fingerprinting analysis

RW3A fingerprinting generated between two and eight bands. The amplified DNA fragments obtained ranged in size from approximately 850 bp to 3 kbp. A total of nine distinct patterns were obtained from the collection of 30 isolates (data not shown). Groups A and B encompassed nine and 10 isolates, respectively, while groups C and D comprised two and four strains, respectively. The remaining DAF types each contained a single isolate.

Integron analysis

Nine of the isolates in this study harboured a class 2 integron. Carriage appeared to be related to DAF type, with all nine isolates comprising the DAF group A, suggesting a clonal spread of *Serratia* spp. strains. BLAST analysis revealed that this integron carried the three conserved resistance gene cassettes of the class 2 integron (*dfrA*1, *sat*1 and *aadA*1), which confers resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively. One of these isolates also carried a class 1 integron identified by sequence analysis as containing the open reading frames *aac*C1 (gentamicin resistance), ORFX, ORFY and *aadA*1 (spectinomycin and streptomycin resistance). PCR analysis confirmed the presence of the *qacE*Δ1 and *sul*1 markers, which are common among class 1 integrons.

Discussion

The 30 *Serratia* spp. isolates in this study were detected sporadically in a selection of hospital settings between October 2003 and July 2006. Class 2 integrons have been isolated from a wide variety of Gram-negative bacteria;[17–20] however, this study represents the first isolation of a class 2 integron from *Serratia* spp. A total of nine isolates harboured this integron, accounting for 30% of the collection of strains. A BLAST analysis of the class 2 integron detected in this study revealed complete sequence homology with class 2 integrons isolated from a diverse range of organisms, including *Shigella flexneri, S. sonnei, Klebsiella oxytoca, K. pneumoniae* and *Salmonella enterica*.

Integron carriage was restricted to DAF group A. This group of strains was found in a population isolated between October 2003 and April 2004, while the collection continued until July 2006. The strains were isolated from various locations within Cork University Hospital, including intensive care (n=4), in addition to two other hospitals in the region, between which transfer of patients is common. The largest group, DAF group B, consisted of 10 strains isolated between November 2003 and January 2006. Another group (consisting of four isolates) was isolated initially in 2003 and again in 2006, indicating the persistence of two groups within the hospital environment. The final group contained only two strains, which were isolated within a short period. Each of the remaining isolates showed a unique fingerprint. In addition to the clonal spread, these findings suggest heterogeneity among *Serratia* spp. isolates.

There were slight differences in antibiotic resistance profiles (R types) between the strains in each group. Severino and Magalhães[21] found that integron content among Gram-negative outbreak strains appeared to be more consistent with clustering than with the R type, which concurs with the findings of the present study. It is interesting to note that eight of the nine strains that contained the class 2 integron also were resistant to ciprofloxacin. In contrast, only four of the remaining 21 isolates in the collection were resistant to ciprofloxacin.

These findings correlate with those of Grape *et al.*,[22] who noted a high prevalence of ciprofloxacin resistance among isolates carrying integrons, despite the fact that ciprofloxacin resistance is conferred through chromosomal mutations and not via a mobile genetic element. This may be explained by the demonstrated association between integrons and mutator plasmids, which can increase the mutation rate within the bacterial cell, possibly including the point mutations that confer resistance to quinolones.[23] However, it may also be interesting to note that the inclusion of genes that encode resistance to spectinomycin and streptomycin in

Table 1. DNA sequences and corresponding references of the oligonucleotide primers used in the analysis of *S. marcescens* isolates.

Primer	Nucleotide sequence (5'–3')	Size (bp)	Reference
Int-1F (5'CS)	GGC ATC CAA GCA GCA AGC		10
Int-1R (3'CS)	AAG CAG ACT TGA CCT GAT		10
*qacE*Δ1 F	ATC GCA ATA GTT GGC GAA GT	225	11
*qacE*Δ1 B	CAA GCT TTT GCC CAT GAA GC	225	11
*Sul*1 F	CTT CGA TGA GAG CCG GCG GC	436	12
*Sul*1 B	GCA AGG CGG AAA CCC GCG CC	436	12
hep74 (5'CS)	CGG GAT CCC GGA CGG CAT GCA CGA TTT GTA		13
hep51(3'CS)	GAT GCC ATC GCA AGT ACG AG		13
Int1A (5'CS)	AAA ACC GCC ACT GCG CCG TTA	1200	14
Int1B (3'CS)	GAA GAC GGC TGC ACT GAA CG	1200	14
Int2A (5'CS)	ATG TCT AAC AGT CCA TTT TTA AAT TCT A	443	14
Int2B (3'CS)	AAA TCT TTA ACC CGC AAA CGC	443	14
RW3A	TCG CTC AAA ACA ACG ACA CC		16

an integron (as shown in the current study) does not appear to confer a selective advantage to an organism in the hospital setting, as these antimicrobial agents are rarely used clinically.

A single isolate in the collection contained a class 1 integron, which contrasts with the findings of a recent study of *S. marcescens* in Taiwan. This reported that 70% of the isolates contained a class 1 integron, comprising a variety of gene cassettes,[24] none of which harboured the *aac*C1 or *aad*A1 genes detected in the present Irish study.

S. marcescens isolates have been shown to survive on dry surfaces for months,[25] which may facilitate spread within the hospital environment. This species is among the most frequent isolates obtained from patients with nosocomial infections.[25] Furthermore, this survival may enhance its ability to acquire or disseminate antibiotic resistance genes, either horizontally or vertically. □

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