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Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds

P. Martinez-Freijo, A. C. Fluit*, F.-J. Schmitz, V. S. C. Grek†, J. Verhoef and M. E. Jones††

Eijkman-Winkler Institute for Clinical Microbiology, Infectious Diseases and Inflammation, University Hospital Utrecht, Utrecht, The Netherlands

Class I integrons are associated with carriage of genes encoding resistance to antibiotics. Expression of inserted resistance genes within these structures can be poor and, as such, the clinical relevance in terms of the effect of integron carriage on susceptibility has not been investigated. Of 163 unrelated Gram-negative isolates randomly selected from the intensive care and surgical units of 14 different hospitals in nine European countries, 43.0% (70/163) of isolates were shown to be integron-positive, with inserted gene cassettes of various sizes. Integrons were detected in isolates from all hospitals with no particular geographical variations. Integron-positive isolates were statistically more likely to be resistant to aminoglycoside, quinolone and β-lactam compounds, including third-generation cephalosporins and monobactams, than integron-negative isolates. Integron-positive isolates were also more likely to be multi-resistant than integron-negative isolates. This association implicates integrons in multi-drug resistance either directly through carriage of specific resistance genes, or indirectly by virtue of linkage to other resistance determinants such as extended-spectrum β-lactamase genes. As such their widespread presence is a cause for concern. There was no association between the presence of integrons and susceptibility to cefepime, amikacin and the carbapenems, to which at least 97% of isolates were fully susceptible.

Introduction

Horizontal gene transfer among bacteria, directed by a strong antibiotic selective pressure, has resulted in a widespread distribution of multiple antibiotic resistance genes on plasmids and transposons among many Gram-negative isolates.[1] Recent studies have shown that a conserved DNA sequence, the integron, is carried on such episomal genetic structures. A previous study demonstrated that these structures were widely disseminated throughout our hospital.[2] Integrons contain *intI*, encoding an integrase that mobilizes and inserts gene cassettes by a site-specific recombinational mechanism.[3,4] While three types of integrons, each with different *int* genes, have been identified to date, most of those found in clinical isolates are Class I integrons (Figure 1). Within these integrons, *intI* is contained between the 5′ conserved segment (CS) and *qacEΔ1* and *sulI* in the 3′CS region. Single or multiple gene cassettes, each followed by a recombination site (the so-called 59 bp element) in their 3′ extreme, can be inserted between the 5′CS and 3′CS regions. So far, all inserted genes characterized encode antibiotic resistance, including over 40 distinct genes encoding resistance to aminoglycosides, β-lactams, chloramphenicol, erythromycin, sulphonamides, antiseptics and disinfectants.[5] Most inserted gene cassettes have no promoter and are expressed via one common promoter, P1. Higher levels of expression can be achieved if a second promoter, P2, is included adjacent to the first (Figure 1).[6,7] Higher levels of expression can be obtained when more than one copy of a

*Corresponding author. Eijkman-Winkler Institute, University Hospital Utrecht, Room G04. 614, PO Box 85500, 3508 GA Utrecht, The Netherlands. Tel: +31-30-250-7630; Fax: +31-30-254-1770; E-mail: A. C. F@lab.azu.nl

†Present address: SmithKline Beecham Pharmeceuticals, rue de l'Institut, 89 B-1330 Rixensart, Belgium.

††Present address: MRL Pharmaceutical Services, Den Brieistraat 11, 3554XD Utrecht, The Nertherlands.

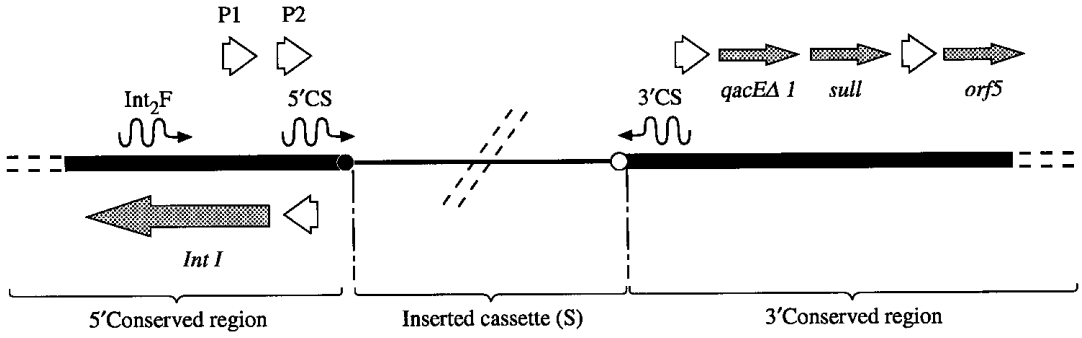

Figure 1. Diagrammatic representation of the general structure of class I integrons. Wiggly arrows show oligonucleotide primers and their approximate binding position for PCRs used in this study to detect integrons. Promoter sites are represented by white arrowheads. P1 and P2 are promoters controlling expression of inserted genes. Grey arrows represent the encoding regions of genes contained within the 3′ and 5′ conserved regions. Of these only *intI*, encoding an integrase, is essential for the insertion and excision of genes. Inserted regions of DNA may be of variable length, as denoted by the dashed line. The black and white circles on either side of the region of inserted DNA represent structures involved in site-specific recombination events. Adapted from Levesque *et al.*[6]

particular resistance gene is inserted within an integron.[8,9] However, since transcription of gene cassettes inserted within the integron is initiated from a common promoter, all inserted genes within an integron are expressed via a common mRNA transcript, resulting in a relatively lower efficacy of transcription of more distal genes.[10] As a result the gene may be poorly expressed, and consequently have little effect on susceptibility to the relevant antibiotics.[11–13] Previous studies, although demonstrating the widespread carriage of these structures, have not addressed the question of integron carriage with respect to their clinical impact, in terms of the effect on antibiotic susceptibility of organisms.

In this study we screened 171 clinical Gram-negative isolates for the presence of Class I integrons and determined the size of inserted DNA, which gives some indication of the number of inserted genes. Isolates were derived from 14 different hospitals in nine European countries. The association of integrons with reduced susceptibility to a range of different antibiotics was investigated.

Materials and methods

Bacterial strains

A total of 171 clinically significant Gram-negative isolates were collected. Bacteria were isolated during 1996–7 from intensive care and surgical units of hospitals in nine European countries. These included Austria (Krankenhaus des Elisabethinen, Linz), Belgium (Hôpital Sart Tilman, Liege), France (Hôpital St Joseph, Paris; Hôpital Eduard Herriot, Lyon), Germany (Heinrich-Heine Universität, Düsseldorf; University Hospital Freiburg, Freiburg), Italy (University of Genoa, Genoa), Poland (Sera and Vaccines Research Laboratory, Warsaw; Polish Mothers Health Centre, Lodz), Portugal (University Hospital of Coimbra, Coimbra), Spain (Hospital Ramon y Cajal, Madrid; University Hospital of Seville, Seville) and the UK (Southmead Hospital, Bristol; St Thomas's Hospital Medical School, London). The species included in the study are shown in Table I. Most organisms were collected as part of the SENTRY Antimicrobial Surveillance programme sponsored by Bristol-Myers Squibb Pharmaceuticals (Princeton, NJ, USA).

Antimicrobial agents and susceptibility testing

On receipt the identity of all isolates was confirmed and the MICs of a range of antibiotics were determined with a broth microdilution method (Table II). Cation adjusted Mueller–Hinton broth (Dade International, Amersfoort, The Netherlands) was used throughout. The final inoculum was approximately 5×10^5 cfu/mL. Trays were incubated for 20–24 h at 35°C in ambient air before reading MICs. NCCLS breakpoints were used to interpret MIC data.[14]

DNA extraction for use as PCR template

DNA was prepared from fresh overnight cultures grown in 5 mL of Luria broth (Oxoid, Basingstoke, UK) as described previously.[15] In short, 1.5 mL of cells were resuspended in 567 μL of TE buffer, 30 μL of 10% sodium dodecyl sulphate (SDS) and 3 μL of 20 mg/L proteinase K. Polysaccharide and protein complexes were removed following incubation for 60 min at 37°C in 100 μL of 5 M NaCl and 80 μL of 10% cetyl trimethyl ammonium bromide (CTAB) in 0.7 M NaCl solution and protein extraction with phenol/chloroform/isoamyl-alcohol (25:24:1). Purified nucleic acids were precipitated with isopropanol and resuspended in TE buffer.

PCR procedure to detect integron structures

The primers 5′CS (5′-GGCATCCAAGCAGCAAG-3′) and 3′CS (5′-AAGCAGACTTGACCTGA-3′) pre-

viously described[16] are complementary to CS regions flanking the inserted DNA and were used in order to identify the presence of an integron and to determine the size of any inserted gene cassettes. Primer Int_2F (5′-TCTCGGGTAACATCAAGG-3′), specific for the 3′ region of the integrase gene (approximately 600 bp upstream from the 5′CS primer site), was used in combination with the 3′CS primer to show the proximity of inserted gene cassettes to *intI* and to confirm the general structure of the integron (Figure 1). The 3′CS and Int_2F primer combination also assisted in detecting empty integrons containing no inserted gene cassettes. Primers 16S806R (5′-GGACTACCAGGGTATCTAATCC-3′) and 16S8F (5′-AGAGTTTGATCCTGGCTCAG-3′), specific for the 16S rRNA gene, were used as positive PCR controls ensuring the integrity of all sample DNA used to detect integrons. DNA was re-extracted from all samples giving no amplification product with the 16S-specific primer set, and amplification procedures repeated in order to validate the negative result.

PCR amplifications were performed in 25 μL volumes containing approximately 50 ng of template DNA, 20 μM of each primer stock solution, 250 μM of each dNTP, 2.5 μL of 10 × PCR buffer, 0.1 U of SuperTaq DNA polymerase (HT Biotechnology, Cambridge, UK) and 17.45 μL of sterile distilled water. PCR amplification was performed with the GeneAmp PCR system 9600 thermal cycler (Perkin-Elmer, Gouda, The Netherlands). An initial denaturation step of 4 min at 94°C was followed by 35 cycles of amplification with a three-step profile consisting of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C. Amplifications were completed using a final extension step of 10 min at 72°C. Amplified products were resolved by electrophoresis at 100 V for 1 h on 1% agarose gel with 0.5 × TBE running buffer containing ethidium bromide. Gels were visualized under ultraviolet light.

Typing with random amplified polymorphic DNA (RAPD)

RAPD was used as a rapid screening method to distinguish sample strains. In combination with antibiograms and epidemiological data, RAPD was used to exclude potential repeat isolates. For each PCR, 25 ng template DNA were added to a reaction mix containing 50 pmol of primer(s), 250 mM of each dNTP, 2.5 mL of 10 × SuperTaq reaction buffer and 0.1 U of SuperTaq DNA polymerase made up to a total volume of 25 μL using sterile distilled water. A 50 μL overlay of mineral oil was added to each tube. Target sequences were amplified by PCR consisting of a 4 min denaturation step at 94°C, followed by an initial amplification of four cycles of 1 min at 94°C, 1 min at 26°C and 1 min at 72°C. A second round of amplification, consisting of 40 cycles of 45 s at 94°C, 45 s at 40°C and 2 min at 72°C, was used. A negative control with 10 μL of sterile water instead of DNA template was included in every PCR. Amplified fragments were processed as described above. Samples were distinguished by considering data from two independent PCRs, the first with both primers Eric1R (5′-GCGAGTGGGGTCAGTGAATGAA-3′) and Eric2 (5′-ATGTAAGCTCCTGGGGATTCAC-3’), and the second with only primer AP1290 (5′-GTGGATGCGA-3′). Isolates were considered non-identical if RAPD patterns differed by at least two bands when banding patterns from each primer set were combined. This was considered sufficiently discriminatory for the purposes of this study, as previously described.[17] As isolates were derived from very different geographical locations those considered identical were excluded only when the same species originated from the same referring hospital.

Results

Frequency and detection of integrons and inserted gene cassettes

Integrons with various insert sizes were found in isolates from all centres included in the study. No distinct geographical epidemiological patterns of distribution could be detected. Of the 172 bacteria tested, 163 different types were distinguished, of which 70 (43.0%) were shown to carry detectable integron structures (Table I). Only those 163 isolates considered unrelated were considered further in this study. *Klebsiella oxytoca* was the species with the highest proportion of integron-positive isolates, with 71.4% (5/7) giving at least one amplification product with the 5′CS and 3′CS primer set. Sixty-two per cent (44/71) of *Escherichia coli* isolates, 44.4% (4/9) of *Enterobacter aerogenes* isolates and 30.8% (8/26) of *Klebsiella pneumoniae* isolates were integron-positive. In other species the incidence of integron carriage ranged from 11.1% (1/9) in *Pseudomonas aeruginosa* to 33.3% (1/3) in *Citrobacter freundii*. The range of inserted gene cassette sizes detected varied in size from 450 bp (found in one isolate of *P. aeruginosa*) to 3000 bp (in isolates of *E. coli*, *K. oxytoca*, *K. pneumoniae* and *Serratia marcescens*). In several species multiple insert sizes were recorded, demonstrating the heterogeneity of inserted sequence sizes.

Eight isolates (four *E. coli* and four *E. aerogenes*) were shown to carry two regions of inserted DNA of different sizes and one *E. aerogenes* isolate carried three inserted regions of different sizes, each ranging in size from 800 bp to 2000 bp. PCR with primers Int_2F and 3′CS showed each of these inserted regions of DNA to be adjacent to an integrase-encoding gene by virtue of the fact that PCR with this primer combination resulted in an amplicon of an expected 600 bp larger than the amplicon derived with primers 5′CS and 3′CS (Figure 1). There are two possible explanations for these data: either the isolates have structurally distinct integrons, or one integron possesses two adjacent 3′CS regions, each downstream of a region of

Table I. RAPD types and the sizes of inserted gene cassettes for different species of Gram-negative isolates. Inserted gene cassettes of 0 bp denote 'empty' integrons containing no inserted DNA

Bacterial species	No. of isolates (strain types[a])	No of integron-positive isolates (strain types[a])	Size of inserted gene cassettes (bp)
E. coli	75 (71)	45 (44)	3000, 2000, 1800, 1600, 1500, 1400, 1000, 800, 650, 0
K. pneumoniae	27 (26)	8 (8)	3000, 1800, 1700, 1600, 750
K. oxytoca	7 (7)	5 (5)	3000, 1600, 1000, 750
E. aerogenes	11 (9)	5 (4)	1500, 1000, 800
E. cloacae	14 (13)	3 (2)	1000, 800
S. marcescens	8 (8)	2 (2)	3000
Serratia liquefaciens	4 (4)	1 (1)	2700
Proteus mirabilis	8 (7)	2 (2)	1000
C. freundii	3 (3)	1 (1)	0
P. aeruginosa	9 (9)	1 (1)	450
Other[b]	6 (6)	0	

[a]Strain types are defined as isolates with different RAPD types and antibiograms, derived from different geographical sources.
[b]One *Proteus penneri*, one *Enterobacter intermedium*, one *Escherichia hermannii* and three other non-Enterobacteriaceae isolates.

inserted DNA and sharing the same integrase gene, as has been previously reported.[18]

PCR revealed some sequence heterogeneity among the 5′CS regions in several isolates. Ten isolates (seven *E. coli*, one *K. oxytoca*, one *S. marcescens* and one *C. freundii*) gave a product with the Int_2F and 3′CS primer set but gave no amplification product with the 5′CS and 3′CS primer set. Three of these (two *E. coli* and one *C. freundii*) possessed 'empty' integron structures with no inserted regions of DNA. In addition, seven isolates (two *E. coli*, one *S. marcescens*, one *K. oxytoca* and three *K. pneumoniae*) gave a product with the 5′CS and 3′CS primers but no product with the Int_2F and 3′CS primer set.

Relationship between MIC and integron carriage

In order to assess the effect of integron carriage on susceptibility profile, we compared MIC data of integron-positive organisms with those obtained from integron-negative organisms. Data from all Enterobacteriaceae were pooled; pseudomonads and other species were excluded because of their different susceptibility profiles. A comparison between integron-positive and integron-negative isolates, with respect to MIC range, the MIC_{90} value and the percentage sensitive, intermediate and resistant to each of the drugs tested, is shown in Table II. Similar analyses done for each species individually showed that there was no significant difference between species analysed individually or as pooled data for Enterobacteriaceae.

For aminoglycoside drugs, the range of MIC values for the two groups remained identical except for amikacin, for which the range for the integron-positive strains extended beyond the resistant breakpoint to >32 mg/L. In contrast, the MIC_{90}s were significantly different between the two groups, with integron-positive strains having a two- or four-fold higher MIC_{90} than the integron-negative group, and MIC_{90} values for integron-positive strains were in the resistant range for amikacin and gentamicin. The percentage of isolates susceptible to gentamicin and tobramycin among integron-positive strains was significantly lower than that among integron-negative isolates. The reduction in the number of isolates in the susceptible range was accompanied by an increase in the number in the intermediate group for both gentamicin and tobramycin, rather than by an increase in the number of resistant isolates. No significant differences were found between the groups with respect to susceptibility to amikacin.

While the ranges of MIC values remained identical, significant differences between the integron-positive and integron-negative groups were apparent for the fluoroquinolone compounds, with MIC_{90}s of each of the drugs tested being in the sensitive range for integron-negative isolates and in the resistant range for the integron-positive isolates. Only 76.7–80.8% of integron-positive isolates were susceptible to any of the fluoroquinolones tested, while 92.3–96.7% of the integron-negative isolates were susceptible. Most non-susceptible integron-positive isolates were fully resistant.

Two-fold differences in MIC_{90} values between integron-negative and integron-positive organisms were seen for aztreonam, cefepime and ceftazidime and four-fold differences for ceftriaxone and piperacillin–tazobactam. No differences in MIC_{90}s were noted between integron-negative and integron-positive strains for other penicillin

Table II. Antibiotic susceptibility of integron-positive and integron-negative unrelated Enterobacteriaceae isolates

	Integron-positive isolates (n = 69)					Integron-negative isolates (n = 85)					
Antibiotic	MIC_{90} (mg/L)	MIC range (mg/L)	% S	% I	% R	MIC_{90} (mg/L)	MIC range (mg/L)	% S	% I	% R	P value[a]
Gentamicin	16	>16–0.25	83.6	8.2	8.2	4	>16–0.25	94.6	2.2	3.2	<0. 01
Amikacin	8	>32–0.5	98.6	0	1.4	2	8–0.5	100	0	0	NS
Tobramycin	16	>16–0.25	74.0	20.6	5.5	8	>16–0.25	90.1	4.4	5.5	<0.01
Ofloxacin	>4	>4–<0.03	80.8	2.7	16.4	1	>4–<0.03	96.7	2.2	1.1	<0.01
Ciprofloxacin	>2	>2–<0.015	80.8	2.7	16.4	0.5	>2–<0.015	96.7	1.1	2.2	<0.01
Trovafloxacin	>4	>4–<0.03	76.7	2.7	20.6	1	>4–<0.03	92.3	3.3	4.4	<0.01
Sparfloxacin	>2	>2–<0.25	79.5	1.4	19.2	1	>2–<0.25	94.5	2.2	3.3	<0.01
Ticarcillin	>128	>128–<1	20.6	2.7	76.7	>128	>128–<1	39.6	25.3	35.2	<0.01
Ticarcillin–clavulanate	64	>128–<1	69.9	20.6	9.6	64	>128–<1	79.1	16.5	4.4	NS
Piperacillin	>128	>128–<1	28.8	27.4	43.8	>128	>128–<1	68.1	6.6	25.3	<0.01
Piperacillin–tazobactam	64	>64–<0.5	84.9	6.9	8.2	16	>64–<0.5	95.6	4.4	0	<0.03
Ampicillin	>16	>16–1.0	17.8	1.4	80.8	>16	>16–<0.25	33.0	8.8	58.2	<0.01
Co-amoxiclav	>16	>16–1.0	60.3	16.4	23.3	>16	>16–0.5	60.4	12.1	27.5	NS
Cefazolin	>16	>16–<2	54.8	2.7	42.5	>16	>16–<2	57.1	2.2	40.7	NS
Cefuroxime	>16	>16–<0.12	65.8	4.1	30.1	>16	>16–<0.12	70.3	2.2	27.5	NS
Ceftazidime	>16	>16–<012	80.8	1.4	17.8	16	>16–<0.12	92.3	3.3	4.4	<0.03
Ceftriaxone	32	32–<0.25	83.6	8.2	4.1	8	32–<0.25	94.5	5.5	0	NS
Cefepime	2	16–<0.12	97	1.5	1.5	1	8–<0.12	100	0	0	NS
Aztreonam	16	>16–<0.12	79.5	9.6	11.0	8	>16–<0.12	94.5	1.1	4.4	<0.03
Imipenem	0.5	1–0.12	100	0	0	0.5	1–0.12	100	0	0	NS
Meropenem	0.12	1–<0.06	100	0	0	0.12	2–<0.06	100	0	0	NS

Abbreviations: S, susceptible; I, intermediate resistant; R, resistant (according to NCCLS breakpoints[14]); NS, not significant.
[a]The statistical significance P, between the susceptibility in terms of S, I, and R percentage values of integron-positive isolates and integron-negative isolates was calculated using a Pearson χ^2 test.

and cephalosporin compounds. The percentage of strains resistant to ureido- and carboxy-penicillins tested was higher in the integron-positive group, with 76.7% and 43.8% resistant to ticarcillin and piperacillin respectively, compared with 35.2% and 25.3% respectively resistant in the integron-negative group. A similar significant difference was seen between the groups with ampicillin. These differences remained significantly different between the two groups when piperacillin was used in combination with tazobactam, while the inhibition of β-lactamases by clavulanic acid was not significantly affected by the presence of an integron. The presence of an integron had no significant effect on susceptibility percentages for the first- and second-generation cephalosporins, cefazolin and cefuroxime, the third-generation cephalosporin, ceftriaxone, the fourth-generation cephalosporin, cefepime, or the carbapenems, meropenem and imipenem. However, significant variation between the integron-negative and integron-positive isolates was evident for the third-generation compound, ceftazidime (4.4% and 17.8% resistant, respectively) and the monobactam, aztreonam (4.4% and 11.0% resistant, respectively).

Integron carriage and multiple resistance

Integron-positive isolates showed an increasing tendency towards multi-drug resistance with isolates resistant to up to 11 of the 12 antibiotics tested (Figure 2). While some integron-negative isolates were resistant to several of the drugs tested, none was resistant to more than seven. More than twice as many integron-negative strains were susceptible to all antibiotics tested as compared with integron-positive strains.

Discussion

The frequency of integrons amongst clinically significant Gram-negative isolates demonstrates that these genetic structures are widespread among isolates from independent

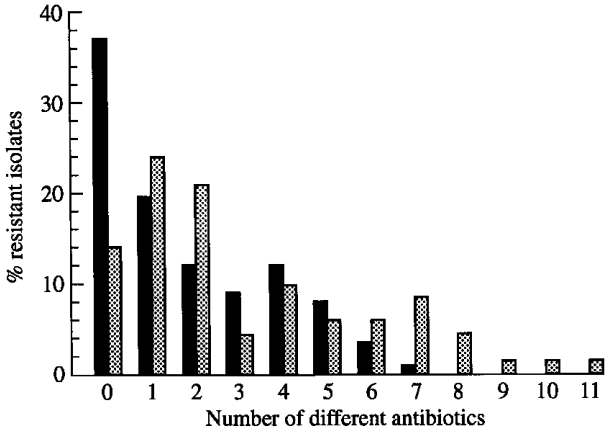

Figure 2. Resistance to increasing numbers of different antibiotics in integron-positive (▩) and integron-negative (■) isolates.

sources in different European countries and in diverse species. While we have demonstrated that integrons are widely distributed amongst Gram-negative nosocomial isolates from a single Dutch hospital,[2] this is the first report to demonstrate the ubiquity of these genetic structures within the hospital environment in Europe. The different sized cassettes inserted between CS regions found amongst the strains studied demonstrates the variable nature of these structures, presumably reflecting differences in the number and type of inserted gene cassettes. Additionally, many inserted regions of DNA, indistinguishable with respect to size, were detected in isolates from different species or from isolates of the same species shown to be unrelated by genotyping, which is suggestive of horizontal gene transfer. This is further supported by preliminary sequencing studies (data not shown) which show that 1000 bp inserted gene cassettes from *E. coli*, *K. oxytoca* and *Enterobacter cloacae* derived from Germany, Spain and Italy are identical. Similarly, 1600 bp inserted gene cassettes from *E. coli* and *K. oxytoca* were identical in isolates derived from Poland, Germany, Spain, France and the UK (data not shown).

The 3′CS region of an integron is known to be variable with respect to size and genetic structure,[9] and we did not attempt to investigate this. PCR aimed at determining the proximity of inserted gene cassettes to an integrase gene demonstrated that, in contrast to a previous report,[9] several of the integrons that we detected in different species were variable in the composition of the 5′CS region.

The presence of an integron significantly affected the susceptibility to the aminoglycoside compounds tested with the exception of amikacin. This is not surprising, given that many aminoglycoside resistance genes have been reported within integron structures, including *aadA*, *aadB*, *aadA7*, *aacA4* and *aacA1*. However, the aminoglycoside-modifying enzymes *aacA4* and *aacA1*, which can use amikacin as a substrate, may be uncommon in this study population, although they are probably present to some degree as demonstrated by the shift in the range and MIC_{90} of integron-positive isolates. While the number of strains fully resistant to the aminoglycosides did not alter much, intermediate resistance to gentamicin and tobramycin was more common in isolates carrying integrons. This suggests that aminoglycoside resistance genes associated with integron carriage mostly provide reduced susceptibility to these compounds rather than full resistance, probably owing to low-level gene expression.

Integrons are associated with a greatly reduced susceptibility to quinolone compounds, which is perhaps surprising as resistance to quinolone compounds is derived through chromosomal point mutations rather than being carried on any mobile genetic elements. Previous reports have related fluoroquinolone resistance to high levels of resistance to other classes of antibiotics,[19–21] a phenomenon often difficult to explain. There is a clear association of integron structures with mutator plasmids, such as R46, in many Enterobacteriaceae.[22,23] Such plasmids can confer an increased base-line mutation rate on the host cell.[24] This may lead to an increase in the frequency of point mutations that give rise to fluoroquinolone-resistant phenotypes. Alternatively, integrons or associated plasmids may carry genes that affect the permeability of cells or the efflux of the drug, thereby decreasing susceptibility.[25,26]

The lower rate of susceptibity to several classes of β-lactam compound in the integron-positive strains is probably attributable to an association of β-lactamase genes within integrons or integron-carrying plasmids. Several β-lactamase genes have been reported within integrons, including Oxa-type (Class D) β-lactamases,[5] which provide resistance to most penicillins and penicillin–β-lactamase inhibitor compounds, and BlaP (Class A) β-lactamase,[5] which can also provide extended-spectrum resistance including resistance to some newer cephalosporins. Oxa10 (PSE 2)[27] and Oxa15[28] encode extended-spectrum resistance to newer cephalosporins and monobactams but so far these genes have been reported only in *P. aeruginosa*. Resistance to ceftazidime, ceftriaxone and aztreonam in Enterobacteriaceae is most often derived from expression of extended-spectrum β-lactamases (ESBLs).[28] SHV- and TEM-derived ESBLs have not yet been reported as inserted gene cassettes in integrons. However, the significant increase in resistance to ceftazidime and aztreonam in integron-positive isolates provides circumstantial evidence for an association between integron and ESBL carriage, probably via a common host plasmid. Although bla_{IMP}, encoding a carbapenamase, has been reported to be carried in integrons and sometimes to decrease susceptibility to carbapenems,[11] no significant differences in MICs of the carbapenems were detected between the integron-positive and -negative isolates. Similarly, cefepime susceptibility remained unaffected by the presence of an integron; indeed, cefepime, amikacin and the two carbapenems

were the most active compounds tested, with 97%, 98.6% and 100% of isolates respectively, being susceptible to these compounds.

This study demonstrates the wide distribution of integron-like structures in Enterobacteriaceae within the hospital environment throughout Europe. Irrespective of whether or not resistance genes are contained within an integron structure, we have demonstrated a significant association between integron carriage and a reduced susceptibility to some aminoglycosides, quinolones and β-lactam compounds. In addition, multiple drug resistance was more common in integron-positive strains. Considering their widespread occurrence and association with reduced susceptibility to a range of first-line antibiotics, integrons offer cause for concern and may indirectly assist in our understanding of the dynamics and molecular basis of multi-drug resistance in Gram-negative bacteria.

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