Presence of Integron-Associated Resistance in the Community Is Widespread and Contributes to Multidrug Resistance in the Hospital

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Integrations are strongly associated with the multidrug resistance seen in gram-negative bacilli in the hospital environment. No data, however, are available on their prevalence in the community. This study is the first to show that integrons are widespread in *Enterobacteriaceae* in the community and that integron-associated resistance genes in the community constitute a substantial reservoir for multidrug resistance in the hospital.

Multidrug resistance among *Enterobacteriaceae* in the hospital setting is an increasing problem. The development of control strategies, therefore, is of major importance. The acquisition of an array of resistance genes by horizontal transfer, mediated by plasmids and transposons, is currently thought to play an increasing role in the development of this multiresistance. A substantial portion of the resistance genes present on plasmids and transposons is integrated into DNA elements called class 1 integrons (4, 9). These genetic elements comprise a site-specific recombination system capable of integrating and expressing those genes contained in cassette-like structures. The majority of the cassettes identified to date encode resistance to antimicrobial agents. In addition to these cassettes, integrons may contain the *sulI* gene, which confers resistance to sulfamethoxazole (8). Sulfamethoxazole resistance can also be encoded by the *sulII* gene, which is predominantly found on plasmids (3, 8). Class 1 integrons are strongly associated with the multiresistance seen in *Enterobacteriaceae* in the hospital environment (6). No data are available, however, on the prevalence of class 1 integrons in the community.

To address the problem of increasing multidrug resistance, we wondered whether strict infection control measures for patients carrying integrons would prevent the dissemination of multiresistance among *Enterobacteriaceae* in the hospital. To consider such a strategy, it is necessary to determine the influx of integron-carrying strains from the community into the hospital through patients. The aims of this study were to determine the prevalence of integrons in the community, to characterize their contents, and to determine whether resistance to sulfamethoxazole can be used as a screening criterion for integron detection.

Rectal swabs were taken from patients on the day of their admission to the neurodivision of our hospital after written informed consent for study participation was obtained. To

obtain strains representative for the community, patients who had been admitted to a hospital or long-term-care facility in the 3 months prior to admission were excluded. Swabs were cultured on MacConkey agar (McC) and on McC with sulfamethoxazole (512 mg/liter) (McCS). Identification and susceptibility testing were performed using the VITEK 1 System with AMS R09.1 software (Biomérieux, Marcy-L'Etoile, France). The VITEK 1 is an instrument that automatically performs rapid identification and antimicrobial susceptibility testing on a manually prepared inoculum. The disk diffusion method was used to test for sulfamethoxazole susceptibility. Integrons were detected by PCR amplification of the class 1 integrase-specific *Int1* gene (GenBank accession no. M73819). The primers were *Int1*-F (5'-TCTCGGGTAACATCAAGG-3') and *Int1*-R (5'-AGGAGATCCGAAGACCTC-3'). Integron contents were characterized by performing a conserved-segment PCR and subsequent sequencing of the amplicon, containing the inserted gene cassettes (5). PCR primers for *sul* gene amplification (GenBank accession no. *sulI* M73819, *sulII* M28829) were *sulI*-F (5'-GTGACGGTGTTCGGCATTCT-3'), *sulI*-R (5'-TTTACAGGAAGGCCAACGGT-3'), *sulII*-F (5'-GGCAGATGTGATCGACCTCG-3'), and *sulII*-R (5'-ATGCCGGGATCAAGGACAAG-3'). Eighty-four *Enterobacteriaceae* (72 *Escherichia coli* strains, 5 *Klebsiella pneumoniae* strains, 3 *Klebsiella oxytoca* strains, 3 *Enterobacter cloacae* strains, and 1 *Citrobacter freundii* strain) were cultured from 53 out of the 57 patients included. Twenty sulfamethoxazole-resistant strains, all recovered from McCs, were obtained from 18 patients. Eleven strains (9 *E. coli* strains, 1 *K. pneumoniae* strain, and 1 *K. oxytoca* strain), obtained from 11 patients were *intI*-PCR positive, indicating a 19% prevalence of integrons in the community (11 of 57 patients). For 5 of the 11 strains the conserved-segment PCR result was positive, and for 4 of these the amplicons were subsequently sequenced. *AadA1a*, which encodes resistance to streptomycin and spectinomycin, was found three times, and *dfrA1/AadA1a*, which encodes additional resistance to trimethoprim, was found once (Table 1). Both integrons have been detected previously in multiresistant strains isolated at the neurodivision of our hospital (7), in Canadian clinical isolates (5), and in food-producing animals worldwide (1, 2, 10).

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TABLE 1. The relationship between resistance phenotype and integron presence among community isolates

N-ab ^a	Antimicrobial agent(s) ^b	Int1-PCR positive (n = 11) ^f		Int1-PCR negative (n = 73) ^g	
		Species	No. of strains	Species	No. of strains
0	None		0	<i>E. coli</i>	45
1	Amp		0	<i>K. pneumoniae</i>	4
				<i>K. oxytoca</i>	2
				<i>E. coli</i>	2
				<i>C. freundii</i>	1
	Sulfa	<i>E. coli</i>	1 ^c	<i>E. coli</i>	2
2	Cxm		0	<i>E. coli</i>	1
	Gen		0	<i>E. coli</i>	1
	Amp, Amc		0	<i>E. coli</i>	4
	Sulfa, Tmp		0	<i>E. coli</i>	4
3	Sulfa, Amp	<i>K. pneumoniae</i>	1	<i>E. coli</i>	1
		<i>K. oxytoca</i>	1		
4	Amp, Amc, Cxm		0	<i>E. cloacae</i>	3
	Sulfa, Amp, Amc		0	<i>E. coli</i>	2
	Sulfa, Tmp, Amp	<i>E. coli</i>	4 ^d		0
	Sulfa, Tmp, Cxm	<i>E. coli</i>	1		0
	Sulfa, Tmp, Amp, Amc	<i>E. coli</i>	1 ^e	<i>E. coli</i>	1
5	Sulfa, Tmp, Amp, Amc, Cxm	<i>E. coli</i>	1		0

^a N-ab, number of antimicrobial agents for which resistance is expressed.

^b Abbreviations: Amc, amoxicillin-clavulanic acid; Amp, ampicillin; Cxm, cefuroxime; Gen, gentamicin; Sulfa, sulfamethoxazole; Tmp, trimethoprim.

^c Integron content *AadA1a*, which encodes resistance to streptomycin and spectinomycin.

^d Integron content of two of these strains was identified as *AadA1a*.

^e Integron content *dfpA1/AadA1a*, which encodes resistance to trimethoprim, streptomycin, and spectinomycin.

^f n, total number of strains testing positive.

^g n, total number of strains testing negative.

These findings show that integrons have a widespread presence in the community and suggest that their acquisition may occur via the food chain.

The presence of an integron was significantly associated with multidrug resistance, i.e., resistance to more than two antimicrobial agents (chi-square test; $P < 0.0001$). In addition to sulfamethoxazole, 10 of the 11 isolates were resistant to ampicillin, 8 were resistant to trimethoprim, 2 were resistant to amoxicillin-clavulanic acid, 2 were resistant to cefuroxime, and 1 was resistant to gentamicin (Table 1). No resistance was observed to third-generation cephalosporins, amikacin, carbapenems, or quinolones. Linkage between integrons and resistance to these last antimicrobials, therefore, seem to occur predominantly within the hospital environment. This may be the result of acquisition of additional resistance determinants by persistent integron-carrying community strains (a persis-

TABLE 2. Association between class 1 integrons and sulfamethoxazole resistance

Source	Sulfamethoxazole-resistant isolates		Sulfamethoxazole-susceptible isolates	
	No. tested	No. (%) Int1-PCR positive	No. tested	No. Int1-PCR positive
Community	20	11 (55)	64	0
Blood cultures	22	10 (45)	100	0
European hospitals	78	53 (68)	65	0
Multiresistant strains, UMCU ^a	47	45 (96)		
Total	167	119	229	0

^a UMCU, University Medical Center Utrecht.

tence facilitated by antimicrobial selective pressure within the hospital environment) or due to the transfer of integron-carrying genetic elements from community strains to already-resistant nosocomial strains. These two possibilities are not mutually exclusive.

In order to assess the relationship between sulfamethoxazole resistance and the *sulI*, *sulII*, and *intI* genes, the collection of strains was extended with 122 blood culture isolates collected in our hospital (1994 to 2000), 47 multiresistant clinical isolates collected in our hospital (1997 to 2000), and 143 clinical strains sent from various European hospitals. These 312 strains comprised 122 *E. coli* strains, 44 *K. pneumoniae* strains, 33 *K. oxytoca* strains, 29 *E. cloacae* strains, 22 *C. freundii* strains, 30 *Proteus mirabilis* strains, 24 *Enterobacter aerogenes* strains, and 8 *Serratia marcescens* strains. Of the complete test panel of 396 strains, 167 were sulfamethoxazole resistant, and for 119 strains, the *intI*-PCR result was positive. All 119 strains were sulfamethoxazole resistant, indicating a 100% correlation between sulfamethoxazole resistance and the *intI* gene (Table 2). Of these 119 strains, 108 (91%) contained the *sulI* gene, which was detected exclusively in *intI*-PCR-positive strains. These results comply with the findings of Radstrom et al., who performed colony hybridization assays on 156 sulfonamide-resistant *Enterobacteriaceae* from Stockholm, Sweden, Houston, Texas, and Lagos, Nigeria (8). The results of the *sulII*-PCR (performed only on community and blood culture isolates) showed that half of the integron-carrying strains harbored a *sulII* gene in addition to the *sulI* gene (Table 3). The *intI*-PCR-positive strains lacking *sulI* harbored the *sulII* gene. Therefore, resistance to sulfamethoxazole is a very sensitive screening criterion for the detection of integrons in *Enterobacteriaceae*.

TABLE 3. The distribution of the *sulI* gene, the *sulII* gene, and the *intI* gene in sulfamethoxazole-resistant *Enterobacteriaceae*^a

Genotype	No. of community isolates (n = 20)		No. of Blood culture isolates (n = 22)	
	Int 1-PCR positive	Int 1-PCR negative	Int 1-PCR positive	Int 1-PCR negative
<i>sulI</i> only	6	0	4	0
<i>SulII</i> only	2	7	0	12
<i>SulI</i> and <i>sulII</i>	3	0	6	0
No <i>sul</i> gene identified	0	2	0	0

^a n, number studied.

The finding that all integron-carrying isolates in this study were recovered from McC agar with sulfamethoxazole shows that this criterion may be employed successfully in the clinical laboratory.

In conclusion, integrons are widespread in *Enterobacteriaceae* in the community, and integron-carrying elements are responsible for a substantial portion of the resistance genes introduced into the hospital. This high prevalence renders our initial thought of instituting isolation precautions for all integron-carrying patients unfeasible. Further studies are needed to determine the extent and manner in which community-acquired integrons contribute to multiresistance among clinical isolates.

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