

Integron-Associated Antibiotic Resistance in Enteroaggregative and Enteroinvasive *Escherichia coli*

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ABSTRACT

Ten enteroinvasive (EIEC) and 25 enteroaggregative (EaggEC) *E. coli* strains isolated from Senegalese patients were analyzed for their integron content. All strains were resistant to at least two antibiotics. Four EIEC and 15 EaggEC were found to carry a class 1 integron. An identical integron carrying a single *dfrA5* cassette, conferring resistance to trimethoprim, was identified in all four EIEC strains. Five EaggEC strains harbored an integron with a single cassette, *dfrA7*, while the remaining 10 strains carried two integrons, one with a single cassette, *aadA1a* conferring resistance to streptomycin and spectinomycin, and the second one bearing two cassettes, *dfrA13* and *oxa5*, the later being a β -lactam resistance cassette. The presence of these integrons is worrying, because trimethoprim is largely used for diarrheal disease therapy in Africa. Thus, the presence of integrons in diarrheagenic strains is of public health importance because a limited number of antibiotics are available in developing countries.

INTRODUCTION

DIARRHEAL DISEASES are responsible for high morbidity and mortality in developing countries, and sub-Saharan Africa is one of the regions with the highest morbidity and mortality rates.¹ The causes of diarrhea include viruses, bacteria, and parasites. *Escherichia coli* is one of the bacteria involved in endemic diarrheal disease in developing countries. Six main categories of diarrheagenic *E. coli* have been consistently associated with endemic forms of diarrheal disease: enteropathogenic, enterohemorrhagic, enterotoxigenic, enteroaggregative (EaggEC), enteroinvasive (EIEC), and diffusely adherent.

Resistance to antibiotics is increasing in diarrheagenic *E. coli*, and a high rate of resistance to ampicillin, tetracycline, chloramphenicol, sulfonamides, and trimethoprim has been reported.^{15,17} However, only few data are available on antimicrobial susceptibility of diarrheagenic bacteria. Integrons constitute a system for gene capture and expression composed of an *intI* gene encoding an integrase, a recombination site *attI*, and a promoter. Several classes of integrons have been described according to the sequence of the *intI* gene. Three of them are well characterized and are involved in antibiotic re-

sistance. The integrase is able to integrate or excise genes-cassettes, usually antibiotic-resistance genes, by a site-specific system of recombination. Cassette mobility results in a very efficient system for dissemination of resistance genes, and more than 70 cassettes have been described.¹³ The aim of this study was to evaluate the contribution of integrons in antibiotic resistance in EaggEC and EIEC strains isolated in Dakar, Senegal in sub-Saharan Africa.

MATERIALS AND METHODS

Bacterial strains

Twenty-five EaggEC and 10 EIEC strains were examined in this study. The strains were isolated from 1997 to 1999 in a university teaching hospital and in an urban hospital in Dakar, Senegal. The strains originated from diarrheal adult patients with negative stool culture for *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio*. *E. coli* was considered as the etiologic agent of the diarrhea when it was obtained as pure culture in nonselective bromocresol purple solid medium. *E. coli* pathogens were characterized by PCR, as described previously.⁴

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Antimicrobial susceptibility

Susceptibility testing was performed by the disk diffusion method on Mueller-Hinton agar (Becton Dickinson, Cockeysville, MD) according to the recommendations of the National Committee for Clinical Laboratory Standards.⁸

PCR mapping

Strains were screened for the presence of class 1, 2, and 3 integrons by PCR using three sets of primers specific for the *int11*, *int12*, and *int13* genes as described previously.¹² Cassette assortment in class 1 integrons was determined by amplification with primers annealing to the 5' and 3' ends (5'-CS and 3'-CS).⁵ The PCR products were sequenced directly by using the ABI PRISM dRhodamine terminator protocol following the recommendations of the manufacturer (Perkin-Elmer Applied Biosystems, Les Ulis, France). Products were analyzed with the ABI PRISM 310 automated DNA sequencing apparatus (Perkin-Elmer Applied Biosystems). Nucleotide sequence analysis was obtained at the National Center of Biotechnology Information Web site (<http://www.ncbi.nlm.nih.gov>).

Conjugation experiments

Transfer of antibiotic resistance from EIEC or EaggEC strains containing an integron to a nalidixic acid-resistant (Nal^R) *E. coli* C1a recipient strain was achieved on selective medium containing 50 µg/ml nalidixic acid plus either 5 µg/ml of trimethoprim or 25 µg/ml of streptomycin. Tests for the susceptibility pattern of the transconjugants and the presence and content of integrons were done as described above. Plasmid DNA preparations were as described previously.¹⁴

Typing by random amplified polymorphism DNA

The random amplified polymorphism DNA (RAPD) analysis was performed as described previously.¹²

RESULTS

Antibiotic resistance

All strains were resistant to at least two of the following antibiotics: ampicillin, chloramphenicol, spectinomycin, streptomycin, sulfamethoxazole, tetracycline, ticarcillin, and trimethoprim (Table 1). Four of 10 EIEC and 16 of 25 EaggEC were multiresistant to at least five antibiotics.

Mapping of integrons

Class 1 integrons were detected in 4 out of the 10 EIEC strains and 15 out of the 25 EaggEC strains. Class 2 or 3 integrons were not detected (Table 1). The cassette assortments in all the class 1 integron-containing strains were characterized by PCR with the 5'-CS and 3'-CS primers and by sequencing of the amplification products. The four class 1 integron-containing EIEC strains contained an identical integron harboring a single cassette, *dfrA5*, encoding resistance to trimethoprim. Among the EaggEC strains, five strains contained one integron with the single *dfrA7* cassette (Table 1). For the 10 remaining strains, amplification with 5'-CS and 3'-CS primers yielded two fragments of 1 kb and 1.5 kb containing respectively the single *aadA1a* cassette coding for resistance to streptomycin and spectinomycin and the two cassettes, *dfrA13* and the β-lactam resistance cassette *oxa5*. These two class 1 integrons were always found together, suggesting that they are physically linked.

All of the class 1 integron-containing strains were resistant to at least five antibiotics (Table 1). However, a single EaggEC strain which showed resistance to seven antibiotics did not contain integrons (Table 1).

Transfer of antibiotic resistance

Resistance to ampicillin, streptomycin, sulfamethoxazole, tetracycline, ticarcillin, and trimethoprim was transferred *en bloc* from each of the EIEC strains harboring the integron con-

TABLE 1. PROPERTIES AND CASSETTE CONTENT OF EIEC AND EAGGEC ISOLATES

	N ^a	RAPD type	Antibiotic resistance ^b	intI gene	Size of PCR product 5'-CS to 3'-CS (kb)	Gene cassette
EIEC	4	A	Sm ^R Sp ^R Te ^R	None		
	2	A	Sm ^R Sp ^R	None		
	4	B	Am ^R Sm ^R Su ^R Tc ^R Te ^R Tp ^R	<i>int11</i>	0.7	<i>dfrA5</i>
EaggEC	8	I	Am ^R Te ^R Tc ^R	None		—
	2	I	Am ^R Cm ^R Sm ^R Sp ^R Su ^R Tc ^R Te ^R Tp ^R	<i>int11</i>	1	<i>aadA1a</i>
					1.5	<i>dfrA13-oxa5</i>
	8	II	Am ^R Cm ^R Sm ^R Sp ^R Su ^R Tc ^R Te ^R Tp ^R	<i>int11</i>	1	<i>aadA1a</i>
					1.5	<i>dfrA13-oxa5</i>
	1	II	Am ^R Tc ^R Te ^R	None		—
	5	III	Am ^R Cm ^R Sm ^R Su ^R Tc ^R Te ^R Tp ^R	<i>int11</i>	0.7	<i>dfrA7</i>
	1	IV	Am ^R Cm ^R Sm ^R Su ^R Tc ^R Te ^R Tp ^R	None		—

^aNumber of strains.

^bAbbreviations: Am, ampicillin; Cm, chloramphenicol; Sm, streptomycin; Sp, spectinomycin; Su, sulfamethoxazole; Tc, ticarcillin; Te, tetracycline; Tp, trimethoprim.

taining the *dfrA5* cassette to *E. coli* C1a, suggesting that all the resistance determinants are physically linked. For EaggEC strains, all of the antibiotic resistances, except chloramphenicol resistance, were transferred *en bloc* to *E. coli* C1a from each of the strains harboring class 1 integrons. The PCR analysis of the plasmid DNA of all the transconjugants confirmed the transfer of the class 1 integrons.

Distribution of integrons among the RAPD types

For EIEC strains, two RAPD patterns were obtained (Table 1). The four strains containing the class 1 integron belonged to the same pattern B. Four RAPD patterns were obtained for EaggEC strains (Table 1). Class 1 integrons were found in strains belonging to profiles I, II, and III. The five strains of pattern III contained the integron with the single *dfrA7* cassette. The two integrons containing, respectively, the cassettes *aadA1a*, *dfrA13*, and *oxa5* were found in patterns I and II, suggesting a horizontal transfer of these integrons via plasmids and/or transposons. By contrast, nine other strains belonging to profiles I or II did not contain integrons.

DISCUSSION

We found integrons in all strains resistant to at least five antibiotics except the EaggEC strain belonging to the RAPD pattern IV. In this strain, the resistance determinants could be carried by another genetic element like plasmids able to replicate in *E. coli*. Integrons have been previously detected in other Gram-negative bacteria isolated in Africa, *Pseudomonas aeruginosa*,¹¹ *Vibrio cholerae*,^{2,3} and *Shigella*.⁹ Class 1 or 2 integrons were previously found in diarrheagenic *E. coli*, enteroaggregative and cell-detaching strains from Nigeria, but the cassettes were not characterized.¹⁰ Class 1 integrons were also detected in Shiga toxin-producing *E. coli* strains and in enterohemorrhagic strains from humans, foods and animals.^{7,18} All of these integrons contained a streptomycin-spectinomycin resistance determinant, mostly *aadA1* alone or associated with a *dfrA* cassette (*dfrA12* or *dfrA1*). In this study, most cassettes identified were determinants for trimethoprim resistance (*dfrA5*, *dfrA7*, *dfrA13*) or spectinomycin and streptomycin resistance (*aadA1*). Trimethoprim, spectinomycin, and streptomycin are antibiotics frequently used in Senegal. Indeed, the combination of trimethoprim and sulfamethoxazole is one of the commonly prescribed treatments of enteric pathogens in Africa (<http://www.who.int>), these two antibiotics being inexpensive. Moreover, the selective pressure with streptomycin and spectinomycin is still effective, spectinomycin being used to treat gonococci infections and streptomycin in tuberculosis. Otherwise, in Senegal, as in many countries in Africa, antibiotics are over-the-counter available drugs leading to a great self-medication, increasing the selective pressure. However, the *dfr* and *aadA* cassettes were previously shown to be also very common in integrons in Enterobacteriaceae isolated from patients from developed countries.^{6,16}

The analysis of RAPD patterns and conjugation experiments suggests that trimethoprim and streptomycin-spectinomycin resistance in EaggEC in Senegal is likely due to class 1 integron-containing plasmids, which may transfer between strains of dif-

ferent clonal types. Furthermore, in both EIEC and EaggEC, the spread of few multiresistant clones is probably also involved.

The endemicity of diarrheal disease in Africa allows the persistence of EaggEC and EIEC strains in the gut. As it was proposed for other diarrheagenic *E. coli*, multiresistant EIEC or EaggEC strains could become predominant in the gastrointestinal tract under selective pressure.^{10,18} This may increase the risk of horizontal transfer of resistance determinants between commensals or other pathogens and these multiresistant diarrheagenic strains, with the presence of integrons enhancing the capacity of capture and dissemination of new resistance determinants.

To our knowledge, this is the first report of the distribution of class 1 integrons among EIEC and EaggEC in sub-Saharan Africa. Our study showed that resistance determinants to trimethoprim are frequent in these enteropathogenic *E. coli*, suggesting a necessary alternative for diarrheal disease treatment in Africa. Moreover, most strains were resistant to multiple antibiotics including ampicillin and tetracycline, which are antibiotics commonly used in the treatment of diarrheal diseases. The remaining susceptible antibiotics are third-generation cephalosporins or quinolones. However, resistance to quinolones in diarrheagenic *E. coli* has emerged.¹⁵ Moreover, recently the GES-2 cassette encoding resistance to third-generation cephalosporins was found in an integron in a *Pseudomonas aeruginosa* strain in South Africa.¹¹ Therefore, strains containing integrons might easily become resistant to broad-spectrum cephalosporins. This acquisition of resistance might happen by capture of gene cassettes like GES-2 in one of the integrons described in this study. Otherwise, the acquisition of another plasmid able to replicate in *E. coli* could result in broad-spectrum cephalosporins resistance.

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