JOURNAL OF CLINICAL MICROBIOLOGY, July 2005, p. 3074–3082 Vol. 43, No. 7
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.7.3074–3082.2005

Detection and Typing of Integrons in Epidemic Strains of *Acinetobacter baumannii* Found in the United Kingdom

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Received 26 November 2004/Returned for modification 10 January 2005/Accepted 3 March 2005

Integrons were sought in *Acinetobacter* isolates from hospitals in the United Kingdom by integrase gene PCR. Isolates were compared by pulsed-field gel electrophoresis, and most belonged to a small number of outbreak strains or clones of *A. baumannii*, which are highly successful in the United Kingdom. Class 1 integrons were found in all of the outbreak isolates but in none of the sporadic isolates. No class 2 integrons were found. Three integrons were identified among the main outbreak strains and clones. While a particular integron was usually associated with a strain or clone, some members carried a different integron. Some integrons were associated with more than one strain. The cassette arrays of two of the integrons were very similar, both containing gene *aacC1*, which confers resistance to gentamicin, two open reading frames coding for unknown products (orfX, orfX′), and gene *aadA1a*, which confers resistance to spectinomycin and streptomycin. The larger of these integrons had two copies of the first (orfX) of the gene cassettes coding for unknown products. The third integron, with a cassette array containing gene *aacA4*, which codes for amikacin, netilmicin, and tobramycin resistance; a chloramphenicol acetyltransferase, *catB8*; and gene *aadA1*, conferring resistance to spectinomycin and streptomycin, was associated with an OXA-23 carbapenemase-producing clone, which has spread rapidly in hospitals in the United Kingdom during 2003 and 2004. These integron cassette arrays have been found in other outbreak strains of *A. baumannii* from other countries. We conclude that integrons are useful markers for epidemic strains of *A. baumannii* and that integron typing provides valuable information for epidemiological studies.

Acinetobacter baumannii is an important nosocomial pathogen which particularly affects critically ill patients in intensive care units, in whom it is associated with significantly increased mortality (26, 29). Most clinical isolates are now resistant to a wide range of antibiotics. While carbapenems are the drugs of choice against *Acinetobacter* infections, carbapenem resistance is now becoming common (5, 6, 11, 17, 30), and few therapeutic options remain against such resistant organisms (4, 14).

Most *A. baumannii* infections are caused by outbreak strains, which can spread widely and rapidly between patients. Since these strains also exhibit multiple-antibiotic resistance, it has been suggested that epidemic potential among isolates of *A. baumannii* may be linked to the presence of integrons (15). These are DNA elements capable of capturing genes by a site-specific recombination mechanism that often carry gene cassettes containing antibiotic resistance genes (25). Various studies have found antibiotic resistance genes located on integrons in *Acinetobacter* species (10, 12, 18, 19, 21, 24, 30). Since integrons possess an integrase gene (a site-specific recombinase) at their 5′ end, Koeleman et al. (15) postulated that PCR detection of this could be used as a simple method of identifying epidemic strains of *A. baumannii*. Their results suggested that this was indeed the case, with three-quarters of the epidemic isolates tested giving positive results.

In the United Kingdom, it has become clear during recent years that most clinical isolates of *Acinetobacter* belong to a small number of strains or to groups of closely related isolates, referred to as clones (27; J. M. Coelho, N. Woodford, M. Warner, J. F. Turton, M. E. Kaufmann, T. L. Pitt, and D. M. Livermore, Abstr. 14th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P1871, 2004), of *A. baumannii*. In this context, the term "clone" is used to describe groups of isolates which, although similar, may not have any known epidemiological link between them, perhaps because they have been independently selected in different hospitals from a common ancestor (8). In particular, three clones predominate in the United Kingdom, all of which are multiresistant. Here, we report the results of integrase gene PCR carried out on a panel of United Kingdom outbreak and sporadic strains and on isolates received by our laboratories for typing and antibiotic susceptibility testing over a 6-week period in April to May 2004, with the aim of determining to what extent the detection of integrons by integrase gene PCR predicts epidemic potential. Furthermore, amplification of the integron gene cassettes of the integrase-positive isolates has allowed us to compare the integrons present. Such integron typing can be used to further characterize isolates and should prove to be very useful in epidemiological studies.

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MATERIALS AND METHODS

Isolates. Our laboratories receive over 500 isolates of *Acinetobacter* species a year from hospitals in the United Kingdom for comparison by pulsed-field gel

electrophoresis (PFGE) and for antibiotic susceptibility determinations. Their PFGE profiles are held in a large database. From these, a panel of isolates, which included representatives of all the main outbreak strains or clones (described below) identified by PFGE in recent years, was selected. The panel also included isolates with PFGE profiles that were unique within the database and were therefore classified as sporadic. These isolates, and all isolates received during a 6-week period in April to May 2004, were used. Isolates were labeled according to hospital (hospitals 1 to 29), with isolates from the same hospital being differentiated by a letter after the hospital number. Each isolate was from a different patient. Panel isolates additionally have the letter "P" in brackets after the hospital number and isolate letter. Following completion of this main part of the study, integron typing was carried out on a further set of isolates received in August 2004 from a hospital group (hospitals 30 to 32) in which all three of the main outbreak clones had previously been found.

Description of outbreak strains and clones. (i) Widespread clones. Most of the isolates received by the laboratories belong to one of three multiresistant clones, the South East clone (SE clone) (27), OXA-23 clone 1, or OXA-23 clone 2 (Coelho et al., Abstr. 14th Eur. Congr. Clin. Microbiol. Infect. Dis. 2004). These clones, particularly the SE clone and OXA-23 clone 1, have been found in numerous hospitals. For example, isolates of OXA-23 clone 1 were first received in November 2002, and by the end of June 2004, the laboratories had received over 150 isolates from 134 patients from 25 hospitals. Isolates of the SE clone date from April 2000, and those of OXA-23 clone 2 date from July 2003. Isolates of the OXA-23 clones produced OXA-23 carbapenamase and were all highly carbapenem resistant.

(ii) T strain. The T strain was first identified in May 2003. The laboratories have since received 50 isolates from a similar number of patients from hospitals in an area of the Midlands.

W strain. W-strain isolates are representatives of European clone 1, described previously by Dijkshoorn et al. (8), which has been responsible for numerous outbreaks in many European countries. The reference isolate RUH 2034 used in this study is a representative from an outbreak in The Netherlands (7). This genotype has been found in several hospitals in the United Kingdom in the last few years (but not in large numbers) and has been designated the W strain. Most of the isolates are from an area in the Midlands (isolates from 15 patients to date) where it has been found since at least December 2001.

(iii) Isolates of uncertain strain designation. Isolates of uncertain strain designation were all very similar or identical to one another. Named after the hospitals from which they came, their PFGE profiles clustered between those of the SE clone and OXA-23 clone 1, but all were negative for OXA-23 by PCR.

Identification. All the isolates included in this study gave a band (of 0.4 kb) in a PCR using the primers 5′-GAAGGTAGCTTGCTAC-3′ and 5′-ACTATCTC TAGGTATTAACTAAAGT-3′ (designed by T. De Baere, personal communication). This PCR is based on the16S rRNA gene, and among *Acinetobacter* species, only genomic species 2 (*A. baumannii*), 3, 13TU, and sometimes 4 have been found to give a band (unpublished results). The PCR was carried out using a *Taq* PCR Core kit (QIAGEN, Crawley, United Kingdom) with a final $MgCl_2$ concentration of 1.5 mM. Conditions used were an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 1 min 30 s and a final extension step at 72°C for 5 min. The isolates were then further identified by amplified rRNA gene restriction analysis (28). All representatives of the SE clone, OXA-23 clones 1 and 2, the T and W strains, and the isolates of uncertain strain designation were identified as *A. baumannii*. Representatives of the three main outbreak clones described here have also been identified as *A. baumannii* by tDNA fingerprinting (9) and, in the case of the South East clone, amplified fragment length polymorphism (27). Of the sporadic strains, only isolates 3A(P), 16A(P), 22A(P), and possibly 11B (which did not give a conclusive identification) were identified as *A. baumannii*. The remainder belonged to genomic species 3.

Pulsed-field gel electrophoresis. Preparation and lysis of agarose-embedded cells, followed by pulsed-field gel electrophoresis of the ApaI-digested genomic DNA, were carried out as described previously (27). Gel images were analyzed by BioNumerics (Applied Maths, Kortrijk, Belgium), and the percentage similarity of profiles was calculated by the Dice coefficient. The unweighted-pair group method with arithmetic averages was used for clustering.

DNA extraction for PCR amplification. Isolates were grown overnight at 37°C on nutrient agar. Two to three colonies were suspended in 100 μl PCR-quality water, vortexed for 10 s, and centrifuged at 10,000 × *g* for 2 min. The supernatant (up to 30 μl/100 μl) was used as a template in PCRs.

PCR amplification. Detection of bla_{OXA-23}-related sequences was carried out as described previously (1). Detection of class 1 and class 2 integrons by integrase PCR was performed using the method of Koeleman et al. (15). Amplification of the class 1 integron gene cassettes was carried out in 50-μl volumes with primers 5′-CS and 3′-CS, as described previously by Lévesque et al. (16). Amplicons were resolved by electrophoresis at 120 V in 2% (wt/vol) agarose in 0.5× Tris-borate-EDTA (TBE) or, in the case of the larger integron cassette amplicons, 1% (wt/vol) agarose in 0.5× TBE. Gels were stained with ethidium bromide and visualized under UV light.

Characterization of class 1 integron gene cassette amplicons. Amplicon size and restriction patterns obtained using MspI and HaeIII were used to differentiate amplicons produced by various isolates. To facilitate differentiation of large products of similar size (2 to 3 kb), long (20 cm) 1% agarose gels were used to size the amplicons. Restriction digests were carried out at 37°C in 20-μl (MspI) or 25-μl (HaeIII) volumes containing integron cassette PCR product (8 μl or 5 μl, respectively), appropriate restriction cocktail (final concentration 1 strength), and 5 U MspI or 2 U HaeIII (Roche, Lewes, United Kingdom). Restriction fragments (10 μl or 20 μl, respectively, of digest) were separated by electrophoresis at 110 V in 2.5% (wt/vol) agarose in 0.5× TBE.

Antibiotic susceptibility testing. MICs were determined on Iso-Sensitest agar according to British Society for Antimicrobial Chemotherapy (BSAC) guidelines (3).

Sequencing. Integron cassette PCR products were cleaned using Qiaquick purification columns (QIAGEN, Crawley, United Kingdom) according to the manufacturer's instructions and sequenced on a Beckman Coulter CEQ8000 sequencer (Beckman Coulter, High Wycombe, United Kingdom) using the Beckman Coulter CEQ Dye Terminator Cycle Sequencing with Quick Start kit. Integrons were sequenced from either end, using the 5′-CS and 3′-CS primers, and from the sequences obtained, additional primers were designed to allow sequencing further into the cassettes. Further primers, which included 5′-CAA CAAGAAAACCGATA-3′ (IntseqFSE2), were then designed to enable completion and confirmation of the sequences. In addition, the primers 5′-GAACT GCGCACGCTG-3′ (X′FSE) and 5′-CACAGGTTGCTAGTACGA-3′ (X′RSE) were used in PCR mapping experiments.

Sequences obtained were compared with those in the NCBI database using an updated version of the BLAST program (2).

Nucleotide sequence accession numbers. The sequences of the variable regions of the integrons of isolates 6B(P) (2.3-kb integron), 16B(P) (3.0-kb integron), and 31H (2.5-kb integron) were deposited in GenBank under the accession numbers AY922989, AY922990, and AY922991, respectively.

RESULTS

The PFGE profiles of the isolates included in this study are shown in Fig. 1. Most of the isolates that had not previously been compared by PFGE (i.e., the nonpanel isolates) belonged to one of the outbreak strains or clones described; the remainder had unique PFGE profiles within our database and are therefore classified as sporadic. Integrase PCR showed that all the sporadic strains were negative for integrons of classes 1 and 2. All the representatives of the SE clone, OXA-23 clones 1 and 2, and T and W strains were PCR positive using the *int*1 primers, yielding a PCR product of approximately 160 bp (Fig. 2), consistent with class 1 integrons. Only three of the five isolates of uncertain strain designation contained the class 1 integrase gene. All isolates were negative for class 2 integrons by integrase gene PCR.

Amplification of the integron gene cassettes of representatives of the integrase-positive isolates gave PCR products of various sizes (Fig. 2). Among the United Kingdom isolates, all representatives of one clone or strain tested gave amplicons of the same size. Therefore, all 10 representatives of the amplified SE clone gave PCR products of approximately 3 kb, and the nine isolates of the OXA-23 clone 1 tested all gave slightly smaller amplicons of approximately 2.3 kb. The T-strain isolates gave PCR products of the same size as those of the SE clone isolates (3 kb), while the W-strain integron cassette PCR products were much smaller (approximately 0.8 kb). Interestingly, however, the amplicon from the isolate from The Netherlands (RUH 2034) of this genotype was different from that of

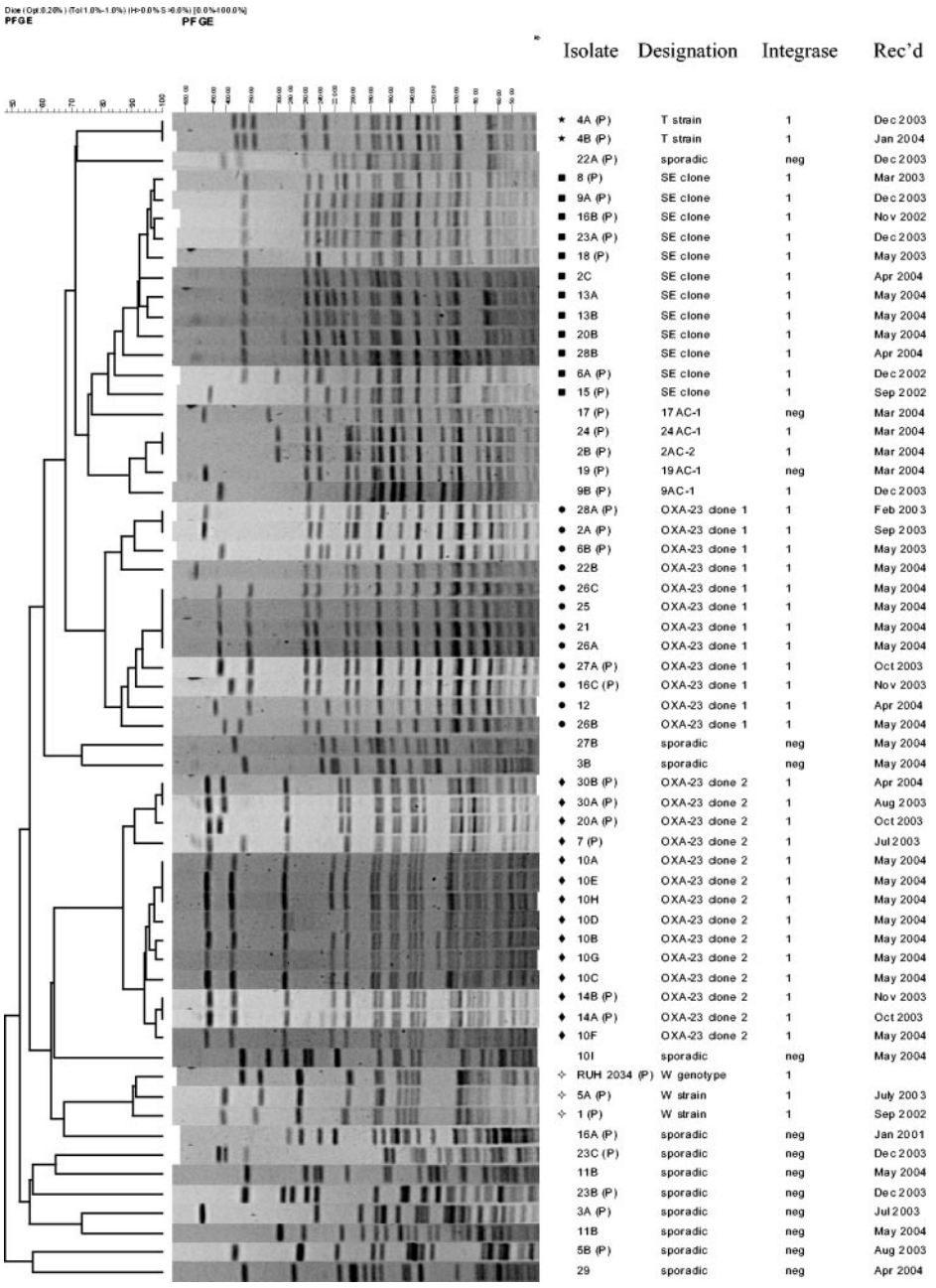

FIG. 1. PFGE profiles of ApaI-digested genomic DNA from *Acinetobacter* isolates. Isolates were labeled according to hospital (hospitals 1 to 29); isolates from the same hospital were differentiated by letters following the hospital number. Panel isolates are designated by the letter "P" in brackets. Results of integrase PCR (for class 1 and class 2 integrases) for each isolate are given in the third column. Symbols indicate the various outbreak strains, detailed in the figure. Isolates described as belonging to the W strain or to the W genotype are representatives of European clone 1 (8). neg, negative.

the United Kingdom isolates, being the same size as that of the SE clone and the T strain (3 kb). The gene cassettes of the OXA-23 clone 2 and uncertain strain designation isolates did not amplify well, giving at best only faint bands. These PCR products were obtained by reamplification of the original amplicon using a final concentration of 3 mM $MgCl_2$ (instead of 1.5 mM in the original PCR) in the PCR mix. These amplicons were all the same size (approximately 2.5 kb).

To further differentiate between the gene cassette amplification products, they were digested with HaeIII and MspI. The resulting restriction patterns are shown in Fig. 3. In agreement with the amplicon size results, these patterns suggested that among the United Kingdom isolates, the integrons of all the representatives of each clone or strain were the same and that the integrons of the T strain, SE clone, and the isolate from The Netherlands of the same genotype as the W strain were, as far as can be ascertained by this method, the same as one another. Despite being smaller in size, the integron cassettes of

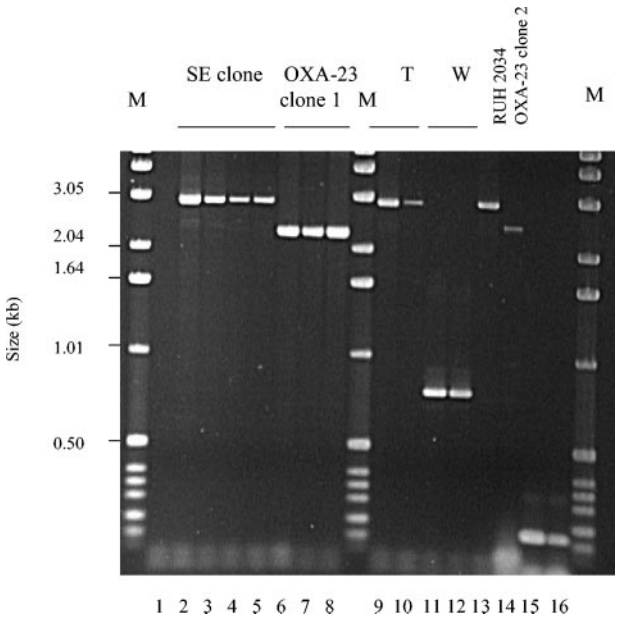

FIG. 2. Integron gene cassette PCR products from various isolates of the outbreak strains and clones and isolate RUH 2034 (which has the W genotype). Size standards (1-kb ladder) were run in the lanes labeled M. A negative (water) control was run in lane 1. Isolates were as follows: lane 2, 15(P); lane 3, 8(P); lane 4, 13A; lane 5, 18(P); lane 6, 28A(P); lane 7, 27A(P); lane 8, 22B; lane 9, 4A(P); lane 10, 4B(P); lane 11, 1(P); lane 12, 5A(P); lane 13, RUH 2034; lane 14, 7(P). Integrase gene PCR products from isolates 26A and 7A(P) are shown in lanes 15 and 16, respectively.

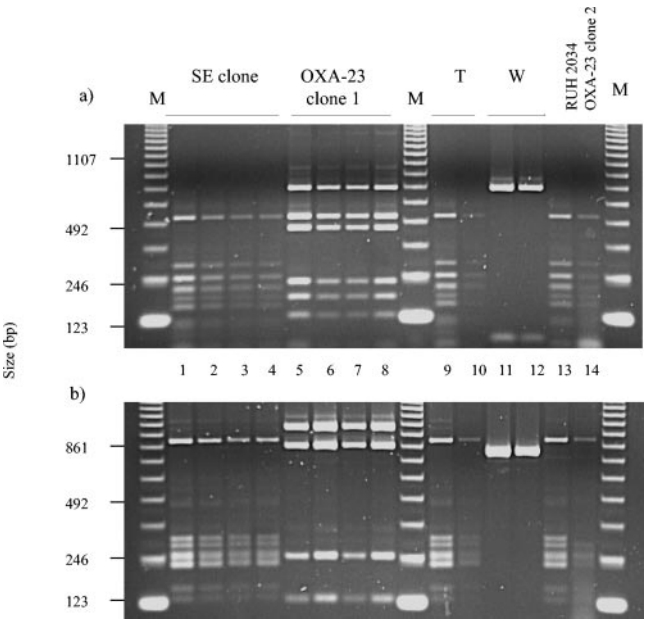

FIG. 3. Restriction patterns obtained following digestion of integron gene cassette amplicons with (a) HaeIII and (b) MspI. Size standards (123-bp ladder) were run in the lanes labeled M. Isolates used were as follows: lane 1, 15(P); lane 2, 8(P); lane 3, 13A; lane 4, 18(P), lane 5, 28A(P); lane 6, 26A; lane 7, 27A(P); lane 8, 22B; lane 9, 4A(P); lane 10, 4B(P); lane 11, 1(P); lane 12, 5A(P); lane 13, RUH 2034; lane 14, 7(P).

the OXA-23 clone 2 isolates gave restriction patterns that were very similar to those of the SE clone and T-strain integrons with both enzymes (Fig. 3).

Since these results suggested that integron typing could be helpful in differentiating between the major clones among United Kingdom isolates, integron typing was carried out on a set of isolates received from a hospital group in which all three clones (SE clone and OXA-23 clones 1 and 2) had previously been found. The PFGE profiles and integron types of these isolates are given in Fig. 4. The set included eight representatives of OXA-23 clone 1, all of which gave a 2.3-kb PCR product with the integron gene cassette primers, with restriction patterns identical to those found with the OXA-23 clone 1 isolates from the main study. However, of the 11 representatives of the SE clone, only three gave the 3-kb amplicon associated with this clone in the main study. The remainder, all from one hospital within this group, gave amplicons of the same size (approximately 2.5 kb) and with the same restriction patterns as those obtained with the OXA-23 clone 2 isolates from the main study. Unlike those in the main study, however, these were easily amplified. Unfortunately, the set of isolates did not include any representatives of OXA-23 clone 2. One isolate (31P) had a similar PFGE profile and contained the integron associated with OXA-23 clone 2 but was PCR negative for OXA-23 (and imipenem sensitive).

The antibiotic susceptibilities of many isolates of the outbreak strains and clones described here have been determined previously (27; M. Warner and R. Pike, unpublished data). Antibiotic susceptibilities of some representatives from this study are given in Table 1. Representatives of the SE clone were highly resistant to most antibiotics, and approximately three-quarters were carbapenem resistant. Amikacin sensitivity was variable. The vast majority of isolates were susceptible to colistin. Representatives of OXA-23 clone 1 were resistant to all antibiotics tested, with the exception of colistin, while representatives of OXA-23 clone 2 were also susceptible to amikacin and minocycline. Although isolates of OXA-23 clone 1 were found to be resistant to minocycline using BSAC guidelines, they would be considered susceptible using National Committee for Clinical Laboratory Standards guidelines. Isolates of the T and W strains were also highly resistant to most antibiotics but were susceptible to the carbapenems and colistin; representatives of the T strain were also susceptible to tobramycin and amikacin. The sporadic strains exhibited multiple-antibiotic resistance, with many isolates being resistant to ampicillin, augmentin, aztreonam, cefepime, cefotaxime, ceftazidime, cefoxotin, piperacillin, and piperacillin/tazobactam. Most of these isolates were, however, susceptible to the carbapenems, ciprofloxacin, colistin, tobramycin, amikacin, gentamicin, sulbactam, and minocycline.

The sequences of the variable regions of all four of the different integrons found in this study were determined, and the gene cassettes were identified. Comparison of the sequences using BLAST showed that they were the same as those found in integrons of other isolates of *A. baumannii* (Table 2) from Italy (12, 30), Ireland (GenBank accession number AJ784787), and Taiwan (GenBank accession number AY55739) and in pan-European clones I and II (19) (Table 2). The 0.8-kb integron associated with the W strain contained the

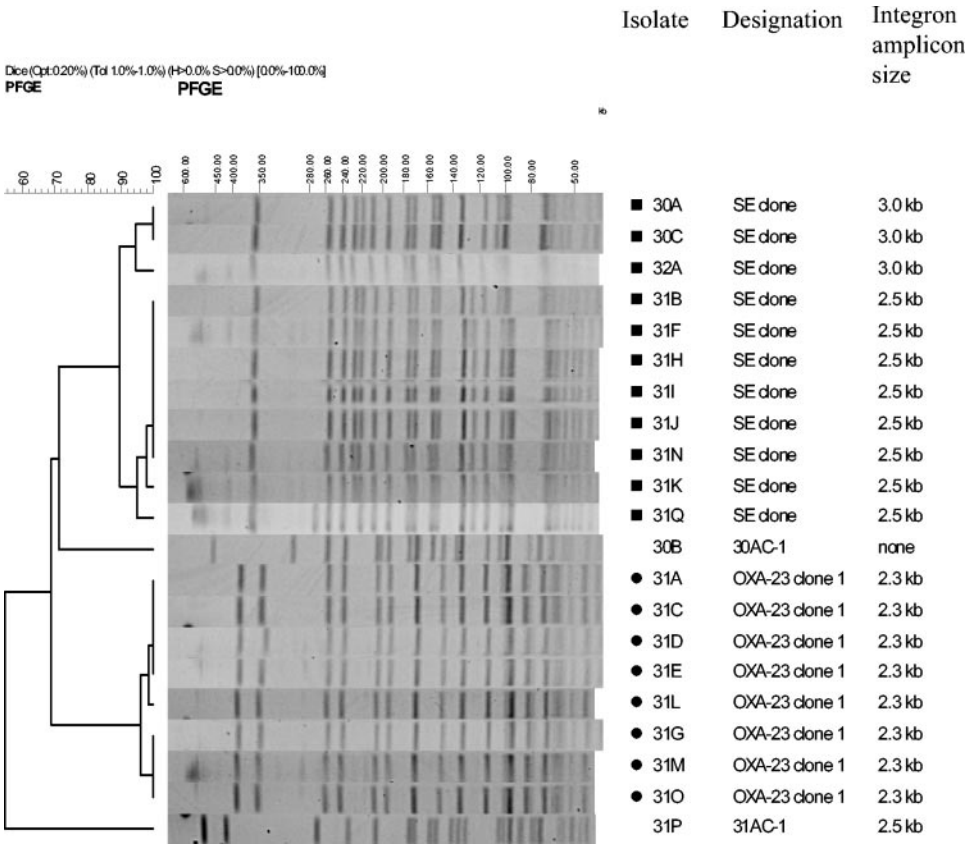

FIG. 4. PFGE profiles of ApaI-digested genomic DNA and integron types of a set of isolates received from a hospital group (hospitals 30 to 32). As in Fig. 1, isolates of the South East clone are indicated with a square symbol, and those of OXA-23 clone 1 are indicated with a circle.

aacA4 gene cassette which codes for amikacin, netilmicin, and tobramycin resistance. The 2.3-kb cassette array of the integron associated with OXA-23 clone 1 contained the *aacA4* gene, the chloramphenicol acetyltransferase gene *catB8*, and the *aadA1* gene, conferring resistance to spectinomycin and streptomycin. The 2.5-kb integron cassette array associated with OXA-23 clone 2 and some isolates of the SE clone contained an aminoglycoside acetyltransferase (*aacC1*) gene coding for gentamicin resistance, genes for hypothetical unknown proteins (orfX, orfX′), and an aminoglycoside adenyltransferase (*aadDA1*, also referred to as *aadA1a*) coding for spectinomycin and streptomycin resistance. The sequences obtained of the larger (3.0-kb) integron cassette arrays of The Netherlands isolate of the W genotype (RUH 2034), of representatives of the SE clone [16B(P) and 9A(P)], and of the T strain [4A(P)] all matched one another, confirming the integron typing results. The sequence is identical to that of the 2.5-kb integron associated with OXA-23 clone 2, except that it has two copies of the orfX gene cassette. The presence of two copies of the orfX cassette was confirmed by PCR using the IntseqFSE2 and X′RSE primers, which anneal at the beginning of the orfX cassette and at the end of the orfX′ cassette, respectively. Isolates containing the 2.5-kb integron gave a single PCR product of approximately 0.8 kb, as expected, while isolates containing the 3-kb integron gave two bands, one of 0.8 kb (the same as that given by the 2.5-kb integron isolates) and one of approximately 1.3 kb, as would be expected if the intseqFSE2 primer anneals to both copies of the orfX cassette (Fig. 5). The size of the orfX′ PCR product (0.3 kb), obtained using the X′FSE and X′RSE primers, was the same for both sets of isolates.

The antibiotic resistance genes found in these integrons are consistent with the observed resistance to these antibiotics in the relevant isolates.

DISCUSSION

Integrons are divided into classes on the basis of the sequence of their integrase gene. Although at least six classes of integron have been described (13, 20), class 1 integrons are by far the most common in clinical isolates of gram-negative bacteria (16), including acinetobacters (12, 15, 23). Class 2 integrons have been found in *Acinetobacter* species (15, 21) but are rare. In the present study, class 1 and class 2 integrons were sought by integrase gene PCRs, and only class 1 integrons were detected. The integrase gene PCR used has advantages over the integron cassette PCR in screening for integrons in that it is designed to give a small product (160 bp in the case of class 1 integrons) which is easily amplified. Integron cassette PCR can give a negative result even when integrons are present if the cassette array is difficult to amplify (which can be the case particularly if it is large) or if there are no cassettes present. The integrase gene PCR was simple, reliable, and easy to perform. Our results showed that all of our outbreak strain

TABLE 1. MICs of representatives of outbreak clones and strains and of sporadic isolates[a]

Isolate	PFGE	MIC (mg/liter)																	
		AMP	AUG	ATM	FEP	CTX	CAZ	FOX	PIP	TZP	IPM	MEM	CIP	COL	TOB	AMK	GEN	SUB	MIN
Bpt		8	8	8	2	1	2	4	16	16	4	4	1	4	1	4	1	8	0.5[b]
16A(P)	Sporadic	**16**	4	4	<=0.125	**2**	1	**16**	4	<=1.0	<=0.060	<=0.060	<=0.125	<=0.5	0.25	<=0.5	<=0.125	<=1	<=0.125
3A(P)	Sporadic	**16**	4	**32**	2	**8**	2	**64**	16	<=1.0	<=0.060	0.25	1	<=0.5	<=0.5	1	0.25	<=1.0	<=0.125
5B(P)	Sporadic	**32**	4	**16**	2	**8**	**4**	**64**	**32**	<=1.0	0.25	0.25	<=0.125	<=0.5	<=0.125	<=0.5	<=0.125	<=1.0	<=0.125
23B(P)	Sporadic	**64**	4	**32**	**4**	**16**	**4**	**64**	**32**	16	0.25	0.5	0.25	<=0.5	0.25	<=0.5	<=0.125	2	<=0.125
22A(P)	Sporadic	**>64**	**>64**	**>64**	**>64**	**>64**	**64**	**>64**	**>64**	**>64**	**8**	**16**	**>8.0**	<=0.5	**2**	1	**>32**	8	**8**
23C(P)	Sporadic	**>64**	**16**	**>64**	**16**	**32**	**16**	**>64**	**>64**	**64**	0.25	1	**>8.0**	<=0.5	0.25	1	0.25	2	<=0.125
27B	Sporadic	**>64**	8	**32**	**8**	**32**	**8**	**>64**	**>64**	16	0.12	0.25	0.5	1	0.25	<=0.5	0.25	2	<=0.125
3B	Sporadic	**>64**	**64**	**64**	**16**	**64**	**16**	**>64**	**>64**	**32**	**32**	2	1	2	0.5	<=0.5	0.25	2	<=0.125
2A(P)	OXA-23 clone 1	**>64**	**>64**	**64**	**32**	**>64**	**64**	**>64**	**>64**	**>64**	**32**	**16**	**>8.0**	<=0.5	**>32**	**>64**	**>32**	**16**	**1**
26B	OXA-23 clone 1	**>64**	**>64**	**64**	**64**	**>64**	**>64**	**>64**	**>64**	**>64**	**>32**	**>32**	**>8**	<=0.5	**>32**	**>32**	**>32**	**32**	**2**
7(P)	OXA-23 clone 2	**>64**	**>64**	**64**	**16**	**16**	**8**	**>64**	**>64**	**>64**	**8**	**8**	**>8.0**	<=0.5	**32**	2	**>32**	**>32**	0.5
14A(P)	OXA-23 clone 2	**>64**	**>64**	**>64**	**32**	**>64**	**>64**	**>64**	**>64**	**>64**	**>32**	**16**	**>8.0**	<=0.5	**2**	4	**>32**	**32**	0.5
20A(P)	OXA-23 clone 2	**>64**	**>64**	**>64**	**64**	**>64**	**>64**	**>64**	**>64**	**>64**	**16**	**16**	**>8.0**	<=0.5	0.5	2	**>32**	**32**	0.5
16B(P)	SE clone	**>64**	**32**	**>64**	**16**	**>64**	**64**	**>64**	**>64**	**>64**	1	1	**>8.0**	<=0.5	0.5	2	**32**	**16**	1
28B(P)	SE clone	**>64**	**>64**	**64**	**32**	**>64**	**64**	**>64**	**>64**	**>64**	**16**	**16**	**>8**	**>32**	**8**	**32**	**>32**	**32**	**8**
20B	SE clone	**>64**	**64**	**64**	**16**	**>64**	**64**	**>64**	**>64**	**>64**	**8**	**8**	**>8**	1	1	1	**32**	**>32**	**32**
4A(P)	T strain	**>64**	**>64**	**>64**	**16**	**>64**	**64**	**>64**	**>64**	**>64**	1	2	**>8.0**	<=0.5	1	1	**32**	**16**	**2**
4B(P)	T strain	**>64**	**64**	**64**	**32**	**>64**	**>64**	**>64**	**>64**	**>64**	1	2	**>8**	<=0.5	0.5	1	**>32**	**16**	**4**
1(P)	W strain	**16**				**64**	**16**		**>64**	**>64**	0.25	0.25	**>8.0**			4	**8**		
5A(P)	W strain	**>64**	**32**	**>64**	**16**	**>64**	**16**	**>64**	**>64**	**>64**	0.25	0.5	**>8.0**	1	**32**	**8**	**8**	4	0.25

[a] MICs were determined by agar dilution using BSAC methodology. Antibiotics used: AMP, ampicillin; AUG, augmentin; ATM, aztreonam; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; FOX, cefoxotin; PIP, piperacillin; TZP, piperacillin/tazobactam; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; TOB, tobramycin; AMK, amikacin; GEN, gentamicin; SUB, sulbactam; and MIN, minocycline. Resistance is indicated by boldface type. The BSAC breakpoint (bpt) is given under the antibiotic name.

[b] Under review.

TABLE 2. Details of integron cassette arrays sequenced and accession numbers of matching integrons found using BLAST[a]

Approx size (kb)	Sequenced size (5′-CS to 3′-CS [bp])	Isolate	PFGE type	GenBank accession no.	Gene cassette(s)	Matching integrons found on BLAST (99% similarity or greater)		
						Accession no. (reference)	Homology	%
0.8		1(P)	W strain	Identical to AJ313334	*aacA4*	AJ313334 (12) (*A. baumannii*)	759/759	100
2.3	2,381	6B(P)	OXA-23 clone 1	AY922989	*aacA4 catB8 aadA1*	AY557339 (*A. baumannii*)	2,355/2,358	99
2.5	2,542	31H	SE clone (OXA-23 clone 2)	AY922991	*aacC1* orfX orfX′, *aadA1a*	AJ784787, AY307113 (30), AJ310480 (12) (*A. baumannii*)	2,461/2,462	99
							2,461/2,462	99
							2,453/2,462	99
3.0	3,075	16B(P)	SE clone (T strain and RUH 2034)	AY922990	*aacC1* orfX orfX, orfX′ *aadA1a*	AY577724 (19) (*A. baumannii*)	2,983/2,988	99
						AF453999 (*Serratia marcescens*)	2,942/2,954	99

[a] Other PFGE types or isolates associated with each integron from this study are given in brackets in the "PFGE type"column. 5′-CS and 3′-CS are the primers used to amplify the integron cassette arrays. X and X′ indicate genes coding for unknown products.

isolates contained class 1 integrons, while none of our sporadic strain isolates contained integrons of either class 1 or class 2. Among the isolates of uncertain strain designation, three contained integrons and two did not. The correlation between the presence of integrons and epidemic strains was better in the present study than in the original study of Koeleman et al. (15), which used isolates dating from 1987 to 1997. This may reflect that epidemic strains containing integrons have become more successful than (and have therefore replaced) those that lack these elements. Our study differs from that of Koeleman et al. (15) in two aspects: (i) it concentrated, with one exception, on isolates from the United Kingdom rather than from many countries and (ii) our outbreak isolates fell within only a relatively small number of genotypes. In both studies, most of the isolates, including all of our outbreak isolates, were identified as *A. baumannii*. A smaller proportion of the sporadic strains were of this species, which highlights the fact that *A. baumannii* isolates are more likely to be associated with outbreaks. Since we receive very few sporadic isolates of *A. baumannii*, only a limited number could be included in the present study. However, we have since identified a further three sporadic isolates belonging to this species, none of which contained integrons, confirming the results presented here.

Antibiotic resistance is an important factor in nosocomial spread. It is generally considered that epidemic strains contain integrons because of the antibiotic resistance advantages that these confer. Certainly, the outbreak strains described here were multiresistant and were found to contain antibiotic resistance genes in their integrons. Many of the sporadic strains, which did not contain integrons (of classes 1 and 2 at least), were also multiresistant, but most were susceptible to gentamicin, amikacin, and tobramycin. These were the antibiotics for which resistance genes were found in the integrons of the epidemic strains. While the presence of these antibiotic resistance genes in the integrons is undoubtedly significant, the extent to which they would contribute to the success of an isolate is perhaps debatable. Possibly the most important advantage that some of the outbreak strains described here have is their resistance to carbapenems. In the case of the OXA-23 clones 1 and 2, this is due (at least in part) to the presence of OXA-23 carbapenemase, which is not located on an integron.

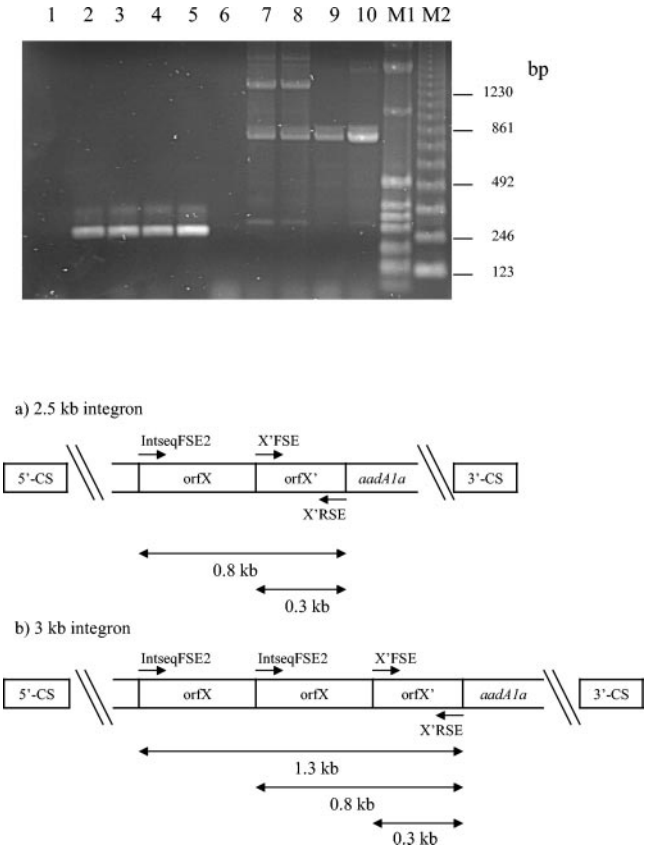

FIG. 5. Demonstration by PCR of the presence of two copies of the orfX gene cassette in the 3-kb integron. Primers used were X′FSE and X′RSE (lanes 1 to 5) and IntseqFSE2 and X′RSE (lanes 6 to 10). Negative (water) controls were included in lanes 1 and 6. Isolates were as follows: lanes 2 and 7, 16B(P); lanes 3 and 8, RUH 2034; lanes 4 and 9, 7(P); lanes 5 and 10, 31H. Molecular weight markers were run in lanes M1 (1-kb ladder) and M2 (123-bp ladder). Isolates 16B(P) and RUH 2034 both contain the 3-kb integron, while isolates 7(P) and 31H contain the 2.5-kb integron. These integrons are the same except that the 3-kb integron contains two copies of the orfX gene cassette (Table 2), and the fragments obtained from the PCRs are shown schematically under the gel image.

Some of the antibiotic resistance genes found on the integrons are against antibiotics that are no longer in use, such as streptomycin and spectinomycin, so they would not confer any advantage. Given the strong correlation between epidemic strains and integrons, it may be possible that integrons also carry genes that confer properties relevant to epidemicity, and the "unknown" genes, such as those found in the integrons associated with the SE clone and OXA-23 clone 2, could be possible candidates. The 3-kb integron described here has two copies of one of these genes. This is likely to increase expression, which may be relevant to the success of isolates carrying this integron. Epidemic strains will, of course, possess combinations of many characteristics that contribute, or have contributed in the past, to their success. Integrons may simply be common to most, or all, of these combinations in *A. baumannii* and for that reason serve as good markers for epidemic strains.

Among the United Kingdom isolates in the main study, all representatives of one clone or strain contained the same integron, as far as could be ascertained by integron cassette amplicon size and restriction fragment patterns. This was despite isolates being used that had been received from different hospitals or that had been received up to almost 2 years apart. It appeared that integron typing could therefore be used to distinguish between the United Kingdom clones and strains, unless, of course, the isolates of interest contained the same integron. However, the finding from the further study that the integron associated with OXA-23 clone 2 was also found in isolates of the SE clone suggested that integron typing is best used as a means of further characterizing isolates rather than for rapidly distinguishing between clones. The SE clone isolates with this smaller integron all came from one hospital, and this may indicate that they have a closer epidemiological link to each other than they do to the other isolates. The two integrons associated with the SE clone are, however, very similar to one another, differing only in that the larger integron had a second copy of the first of the genes coding for unknown proteins (orfX). Of note also is that the intermediate isolates, which clustered between the SE clone and OXA-23 clone 1 by PFGE, also contained the smaller integron.

It is clear from this study, and has been reported by others (12, 19, 22), that not only may isolates of the same genotype be associated with different integrons, but also unrelated isolates of different genotypes may contain the same integron. Information on both the genotype and integron type may be very useful in epidemiological studies. The finding that the majority of the SE clone isolates, the T strain, and The Netherlands isolate of the W genotype all contained the same integrons is very interesting. The SE clone is highly successful and is now widespread in the United Kingdom. The T strain is relatively new to the United Kingdom, dating back to approximately May 2003, but it has already become widespread in an area of the Midlands. It is, however, carbapenem sensitive, which may be a highly significant factor limiting its spread. In The Netherlands, the W genotype was responsible for an outbreak in Venlo (7), and strains of this genotype (which belong to European clone I) have caused many outbreaks in Europe (8, 19). However, in the United Kingdom, it has not recently affected large numbers of patients and appears to have been replaced by the T strain in the area in which it has most recently been found. The United Kingdom isolates contained a much smaller integron than the isolate from The Netherlands, the majority of the sequence of which codes for the *aacA4* antibiotic resistance gene. This suggests that some integrons, such as that found in most of the SE clone isolates, may be associated with greater epidemicity and that integron typing may therefore be useful in allowing some prediction of how successful an organism may become. The similar 2.5-kb and 3-kb integrons found in the present study have been widely found in isolates of European clones I and II from many countries (19). The 2.5-kb integron has also been found in a number of outbreak strains of *A. baumannii* of different genotypes in Italy (12, 30) and an outbreak clone of *A. baumannii* in Russia (A. Solomenny, personal communication) (and has also been described in an isolate from a horse in Ireland [GenBank accession number AJ784787]). In fact, all the cassette arrays found in this study have been described previously in association with successful outbreak strains of *A. baumannii*. It seems that outbreak strains are associated with only a limited number of integron cassette arrays, an observation also made previously by Gombac et al. (12). Detection of these arrays in an isolate may therefore indicate that it has a high potential to spread.

In conclusion, integrons appear to be a feature of epidemic strains or clones of *A. baumannii* currently found in the United Kingdom. Information on both the genotype and integron type is useful in epidemiological studies. The association of integrons with epidemic behavior merits further study.

ACKNOWLEDGMENTS

We are grateful to colleagues in hospital microbiology laboratories for sending the isolates used in this study and to Thierry De Baere for designing the identification PCR primers.

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