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Research Note

The integron prevalence of extended-spectrum beta-lactamase producing enterobacterial isolates in a Malaysian teaching hospital

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Abstract. The increased frequency of antibiotic resistance is known to be associated with the dissemination of integrons in the *Enterobacteriaceae*. This study determined the prevalence and type of integrons amongst 160 extended-spectrum beta-lactamase producing enterobacterial isolates kept in our culture collection. Integrons were detected in 98(61.3%) isolates, including 28(62.2%) *Escherichia coli*, 34(64.2%) *Klebsiella* spp., 27(61.4%), *Enterobacter* spp. and 9(50.0%) *Citrobacter* spp. investigated in this study. Restriction analysis of the integron gene fragments revealed that class I integron was the principal integron detected in 92(57.5%) of our isolates. Class II integron was detected in 6(3.8%) of our isolates, while no class III integron was detected in this study. The high rates of integron prevalence particularly of the class I integron in the *E. coli* and *Klebsiella* spp. concur with previous studies in other geographical regions. The higher (≥50%) integron prevalence of *Citrobacter* and *Enterobacter* isolates comparing to previous studies suggests the potential of these isolates as sources for dissemination of resistance determinants. The finding in this study serves as a basis for further study on the antibiotic resistance mechanisms of enterobacterial species in this teaching hospital.

In recent years, the incidence of multidrug resistance in the *Enterobacteriaceae* is arising in the hospital settings (Bush, 2010). The resistance in these isolates has been linked with the carriage of integrons, genetic elements which allow the integration of antimicrobial drug resistance genes through site-specific recombination events. Integrons are capable of recognizing, capturing and expressing multiple resistance genes in cassette structures, and hence, are assumed to play important roles in the dissemination of antimicrobial resistance (White *et al.*, 2001). High prevalence of integrons among clinical isolates of *Enterobacteriaceae* particularly *Escherichia coli* and *Klebsiella* spp. has been reported worldwide including Asian countries (Martinez-Freijo *et al.*, 1998; Chang *et al.*, 2000; Schmitz *et al.*, 2001; Rao *et al.*, 2006; Su *et al.*, 2006; Yao *et al.*, 2007; Bhattacharjee *et al.*, 2010). However, there has been little information on the integron prevalence of *Citrobacter* and *Enterobacter* spp. As information on the integron of *Enterobacteriaceae* in Malaysia is scarce, this study is carried out to determine the prevalence, distribution and types of integrons in our clinical isolates.

A total of 160 extended-spectrum beta-lactamase (ESBL) producing enterobacterial isolates obtained from the Microbiology Diagnostic Laboratory, University of Malaya

Medical Center (UMMC) were used in this study. The isolates included *Escherichia coli* (n=45), *Klebsiella* species (n=53), *Enterobacter* species (n=44) and *Citrobacter* species (n= 18) which were isolated from 2006 to 2008 from various clinical specimens. Antibiotic susceptibility of these isolates was determined by Kirby-Bauer agar diffusion method. The bacteria were classified as sensitive, intermediate, or resistant according to CLSI guidelines (2009).

DNA was extracted from bacteria using standard techniques and integrons were detected by polymerase chain reaction assays using degenerate primers, hep35 (5' TGCGGGTYAARGATBTKGATTT 3') and hep36 (5' CARCACATGCGTRTARAT 3'), which amplified the conserved regions of integron-encoded integrase genes, *intI1*, *intI2*, and *intI3*. The class of each integron was determined by performing restriction fragment length polymorphism (RFLP) analysis on the amplified product using *Hinf*I restriction enzyme as described previously (Gu *et al.*, 2007).

The resistance profiles of the isolates against other antibiotics were shown in Table 1. Resistance to trimethroprim-sulfamethoxazole, piperacillin-tazobactam, gentamicin and ciprofloxacin was observed in 91.9%, 55.0%, 68.1% and 68.1% of the isolates, respectively. In general, the resistance rate against amikacin was low, as demonstrated in less than 20% of the isolates (Table 1). None of the isolates were resistant to imipenem.

In this study, intermediate and resistant isolates were pooled as nonsusceptible for the convenience of analysis. Integrons were detected in 98(61.3%) isolates, including 28(62.2%) of 45 *E. coli*, 34(64.2%) of 53 *Klebsiella* spp., 27(61.4 %) of 44 *Enterobacter* spp. and 9(50.0 %) of 18 *Citrobacter* spp. Table 2 shows that class I integron was the principal integron class in 92(57.5%) of the isolates investigated in this study. Class II integron was detected in 6(3.7%) isolates, including two *E. coli* and four *Enterobacter* isolates. There was no class III integron detected in this study.

Table 1. Antibiotic resistance profiles of 160 bacterial isolates investigated in this study

Antibiotic	No. (%) resistant isolates				Total
	Citrobacter spp. (n=18)	*Escherichia coli* (n=45)	*Enterobacter* spp. (n=44)	*Klebsiella* spp. (n=53)	
Trimethoprim-sulfamethoxazole	15 (83.3)	44 (97.8)	41 (93.2)	47 (88.7)	147 (91.9)
Gentamicin	13 (72.2)	25 (55.6)	33 (75.0)	38 (71.7)	109 (68.1)
Amikacin	2 (11.1)	5 (11.1)	10 (22.7)	13 (24.5)	30 (18.8)
Ciprofloxacin	14 (77.8)	32 (71.1)	23 (52.3)	40 (75.5)	109 (68.1)
Imipenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Piperacillin-tazobactam	7 (38.9)	22 (48.9)	26 (59.1)	33 (62.3)	88 (55.0)

Table 2. Integron analysis of bacterial isolates in this study

Integron	No. (%) isolates				Total No. (%)
	Citrobacter spp.	*Escherichia coli*	*Enterobacter* spp.	*Klebsiella* spp.	
Class I	9 (50.0)	26 (57.8)	23 (52.3)	34 (64.2)	92 (57.5)
Class II	0 (0.0)	2 (4.4)	4 (9.1)	0 (0.0)	6 (3.8)
No integron	9 (50.0)	17 (37.8)	17 (38.6)	19 (35.8)	62 (38.8)
Total	**18 (100.0)**	**45 (100.0)**	**44 (100.0)**	**53 (100.0)**	**160 (100.0)**

Multidrug resistance in the *Enterobacteriaceae* has been linked with the carriage of integrons, in particular, aminoglycoside and anti-folate resistances are significantly associated with integron carriage in the *Enterobacteriaceae* (White *et al.*, 2001). The high rates (approx. 60%) of integron prevalence particularly of the class I integron in the *E. coli* and *Klebsiella* spp. concur with previous studies in other geographical regions including Europe, Northern America and Asia (Martinez-Freijo *et al.*, 1998; Chang *et al.*, 2000; Schmitz *et al.*, 2001; Rao *et al.*, 2006; Su *et al.*, 2006; Yao *et al.*, 2007; Bhattacharjee *et al.*, 2010). In all these studies, integrons are significantly associated with the resistance to multiple classes of antibacterial compounds. The prevalence of class I integron in clinical *E. coli* strains ranged from 43 to 49% in countries from Europe and Northern America (Martinez-Freijo *et al.*, 1998, Rao *et al.*, 2006). In studies conducted in the Asia Pacific region, class I integron was detected in 85.6% of *E. coli* isolates in Guangzhou City, China (Su *et al.*, 2006) and 52% of the isolates in Kaohsiung, Taiwan (Chang *et al.*, 2000). The prevalence of integrons was 70% in *Klebsiella* isolates in a USA study (Rao *et al.*, 2006); however, two studies in Asia reported even higher frequency of occurrence of integrons among ESBL-positive *Klebsiella pneumoniae* (>90%) in China (Yao *et al.*, 2007) and India (Bhattacharjee *et al.*, 2010).

Integrons of enterobacterial bacteria such as *E. coli* and the *Klebsiella* spp. are capable of encoding every class of beta-lactamase including AmpC-type cephalosporinases, metallo-beta lactamases and extended-spectrum beta-lactamases (Bush, 2010). Since carbapenem resistance was not found in our isolates, the association of integron carriage and metallo-β-lactamases could not be determined in this study.

The reports on the integron prevalence of *Citrobacter* spp. and *Enterobacter* spp. are limited to a few publications. In an earlier study, only one of three *Citrobacter* isolates and four of nine *Enterobacter* isolates in a European study (Martinez-Freijo *et al.*, 1998) were integron positive. In another study, the integron prevalence of *Enterobacter cloacae/ E. aerogenes* has been reported to increase from about 10% in 1993 to 20% in 1996 and finally to approximately 30-40% in 1999 (Schmitz *et al.*, 2001). A later study by Pepperell *et al.* (2002) reported that only 13(36%) of 36 *Citrobacter* isolates in their study were integron positive. The integron prevalence as reported for *Citrobacter* spp. and *Enterobacter* spp. in this study (50% and 61.4%, respectively) was considered the highest compared to previous studies (Martinez-Freijo *et al.*, 1998; Schmitz *et al.*, 2001; Pepperell *et al.*, 2002). The potential of these two enterobacterial species, i.e., *Citrobacter* spp. and *Enterobacter* spp. as hidden sources for dissemination of resistance determinants merits further investigation.

The prevalence of class II and III integron of the isolates in the present study shows similar trend as those reported previously, whereby class II integron has low rate of prevalence, and class III integron is always undetectable in clinical isolates. The finding in this study serves as a basis for further study on the antibiotic resistance mechanisms of our isolates. Rapid detection of antibiotic resistance genes in these clinical isolates and understanding of the mechanism for their spread is critical.

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