Enterobacter species as a Major Reservoir for Extended-spectrum β-lactamase Encoding and Related Genes in the SENTRY Asia-Pacific Region 2006

C2-3917
48th ICAAC, 2008, Washington

J.M. Bell,[1] J.D. Turnidge,[1] and R.N. Jones[2]

[1]Women's and Children's Hospital, Adelaide, Australia; [2]JMI Laboratories, North Liberty, Iowa, USA

email: jbell@wcharl.net

INTRODUCTION

Enterobacter species have emerged as increasingly important nosocomial pathogens, especially in intensive care units (ICUs). Cefepime is an extended-spectrum cephalosporin that is hydrolysed only marginally by AmpC, but treatment failure with cefepime for *Enterobacter* infection has been reported, and this has been explained by reduced production of porins in the bacterial cell wall. However, clinically and epidemiologically, a more important mode of resistance to extended-spectrum cephalosporins, including cefepime, is the production of extended-spectrum β-lactamases (ESBLs).

Production of ESBLs has been observed in virtually all Enterobacteriaceae of clinical importance, where they may mediate in-vivo resistance to all cephalosporins. However, detection of ESBLs in a background of Bush class 1 β-lactamase production is difficult.

There have been sporadic reports of extended-spectrum β-lactamases (ESBLs) in *Enterobacter* spp. over the last few years. More recently, CTX-M-type ESBLs have emerged, especially in *Escherichia coli*. In this present study, we examined *Enterobacter* spp. isolated from patients in the Asia-Pacific region for the presence of ESBL genes including those of the CTX-M groups.

METHODS

Isolates

Enterobacter spp. from infected hospitalized patients in 10 countries (39 medical centres) collected during 2006. Isolates came from patients with bacteraemia, pneumonia, complicated skin and skin structure infections, and other infections. All strains were referred to the Women's and Children's Hospital, Adelaide, Australia for testing.

Susceptibility testing

Isolates were tested using custom made dry-form broth microdilution (BMD) panels (TREK Diagnostic Systems) against a wide range of antimicrobials according to CLSI standards. Breakpoints for resistance to other antimicrobial agents were those recommended by the CLSI.

Quality control strains utilized included *E. coli* ATCC 25922 and 35218, *P. aeruginosa* ATCC 27853; all MIC results were within CLSI specified ranges.

ESBL Phenotype

Enterobacter spp. were defined as ESBL phenotype if cefepime MIC was ≥ 0.25 mg/L; these isolates were subjected to molecular screening.

Molecular Methods

Isolates with cefepime MIC ≥ 0.25 mg/L were screened for the presence of the bla_{TEM}, and bla_{SHV} genes using previously reported oligonucleotide primers. A Multiplex real-time TaqMan PCR was used to detect CTX-M-type genes (Birkett et al J Med Micro (2007) 56; 52).

A selection of strains were also probed for plasmid-borne AmpC enzymes using the method described by Pérez-Pérez et al.(J Clin Microbiol.(2002) 40:2153-2162)

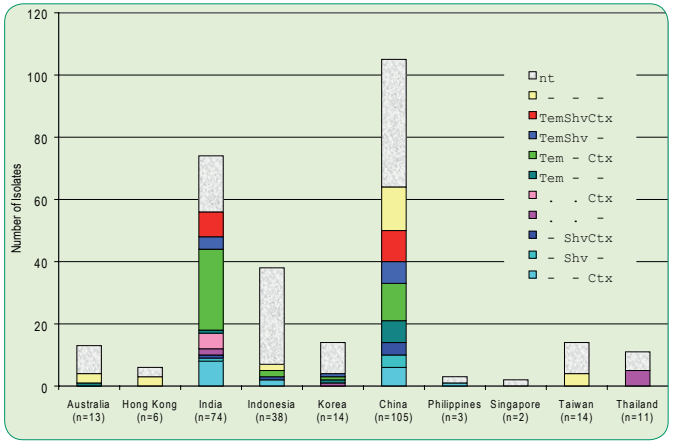
ESBL Genotype

Enterobacter spp. were defined as ESBL genotype if bla_{TEM}, bla_{SHV}, and/or bla_{CTX-M} were detected by molecular methods.

RESULTS

- A total of 360 *Enterobacter* species were collected from the APAC region during 2006. *E. cloacae* (n=280), and *E. aerogenes* (n=65) were the most common isolates; other species comprised *E. sakazakii* (n=4), *E. hormaechei* (n=3), *E. gergoviae* (n=2), *E. amnigenus* (n=2), *E. aburiae* (n=1), unspeciated (n=3).
- Isolates originated from patients with bloodstream (n=57), respiratory (n=134), and skin and soft tissue infections (n=89).
- The ESBL phenotypes among *Enterobacter* spp. was common in our region; India (74%), mainland China (55%), Korea (39%), Thailand (36%), Hong Kong China (29%), Philippines (25%), Indonesia (21%), Australia (18%), and Taiwan (7%).
- No ESBL phenotypes were found in Singapore. All *Enterobacter* spp., except 3 strains (one each from Australia, China, and Thailand), with cefepime MIC ≥16 mg/L contained TEM, SHV, or CTX-M type enzymes.
- Genotypes found among *E. cloacae* is shown in Fig 1.
- Multiple enzymes was common. ESBLs were found in 6 different *Enterobacter* spp.
- Genotypes found among isolates from each country is shown in Table 1.
- There was a strong correlation with the ESBL types detected in *E. cloacae* with that seen in *E. coli* for each country. In India, TEM+CTX-M types accounted for over 60% of the profile obtained, a figure similar to that found among *E. coli* from that country.
- CTX-M-1 group enzymes were dominant throughout the APAC region (Fig 2). CTX-M-9 group enzymes were found in China and Indonesia. Five strains (2 *E. cloacae* and 2 *E. aerogenes* from China; and 1 *E. cloacae* from Indonesia) contained both CTX-M-1 and CTX-M-9 types.
- The rate of ESBL gene detection increased significantly with increasing cefepime MIC (Fig 3).
- Plasmid-borne AmpC enzymes were found in 21 (37%) of 56 *Enterobacter* spp. tested to date from China. DHA was the most common (n=15).

Figure 1. *Enterobacter cloacae* ESBL genotypes



nt, PCR not performed on isolates with cefepime MIC ≤0.12 mg/L

Figure 2. CTX-M-group enzymes

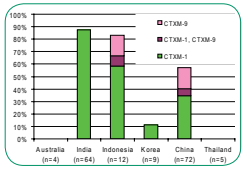


Figure 3. Rate of ESBL gene detection by cefepime MIC

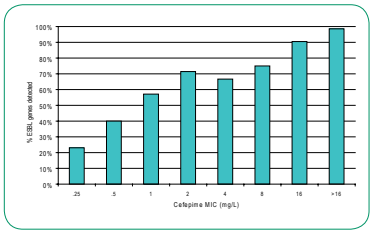


Table 1. Genotypes found among *Enterobacter* spp. from the APAC region

Country (No. tested)	Species	Total	ESBL[a] N	ESBL[a] %	CTX-M types N	CTX-M types %
Australia (n=22)	*E. cloacae*	13	1	7.7		
	E. aerogenes	8	0			
	Other species	1	0			
Hong Kong China (n=7)	*E. cloacae*	6	0			
	E. aerogenes	1	0			
India (n=86)	*E. cloacae*	74	54	73.0	50	67.6
	E. aerogenes	3	2	66.7	2	66.7
	Other species	9	6	66.7	6	66.7
Indonesia (n=57)	*E. cloacae*	38	5	13.2	5	13.2
	E. aerogenes	16	4	25.0	4	25.0
	Other species	3	1	33.3	1	33.3
Korea (n=23)	*E. cloacae*	14	3	21.4	1	7.1
	E. aerogenes	9	1	11.1		
China mainland (n=130)	*E. cloacae*	105	50	47.6	32	30.5
	E. aerogenes	23	10	43.5	8	34.8
	Other species	2	2	100	1	50.0
Philippines (n=4)	*E. cloacae*	3	1	33.3	1	33.3
	E. aerogenes	1	0			
Singapore (n=3)	*E. cloacae*	2	0			
	E. aerogenes	1	0			
Taiwan (n=14)	*E. cloacae*	14	0			
Thailand (n=14)	*E. cloacae*	11	5			
	E. aerogenes	3	0			
Asia-Pacific Region	*E. cloacae*	280	114	40.7	89	31.7
	E. aerogenes	65	17	26.2	14	21.5
	Other species	15	9	60.0	8	53.3

[a] ESBL genes dected

Conclusions

- ESBLs are very commonly harboured in *Enterobacter* spp. in the Asia-Pacific region.
- They can act as a reservoir capable of transferring their ESBLs into the *E. coli* and *Klebsiella* species and
- ESBLS in *Enterobacter* spp. present a major detection problem for routine laboratories and for infection control.