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Characterization of Ertapenem-Resistant *Enterobacter cloacae* in a Taiwanese University Hospital

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The emergence of carbapenem resistance in *Enterobacteriaceae* has become a great concern. The aim of this study was to characterize ertapenem-resistant *Enterobacter cloacae* isolates from a Taiwanese university hospital. A total of 355 nonduplicated *E. cloacae* isolates collected in 2007 were analyzed by antimicrobial susceptibility testing with and without an inhibitor of efflux pumps and AmpC β-lactamase. The phenotype of extended-spectrum β-lactamase (ESBL), profile of outer membrane proteins (OMPs), and clonal relatedness were investigated by the double-disk synergy test, urea/SDS-PAGE, and pulsed-field gel electrophoresis (PFGE), respectively. β-Lactamase genes were examined by PCR and sequencing, and the expression of efflux pump gene *acrB* was evaluated by reverse transcription-PCR. The contribution of porin deficiency to resistance was investigated by restoring functional porin genes on plasmids. We demonstrated that ertapenem resistance was prevalent (53/355; 14.9%) in *E. cloacae*. Among the strains, IMP-8, SHV-12, and TEM-1 β-lactamases were identified in 3 (5.7%), 40 (75.5%), and 46 (86.8%) isolates, respectively. PFGE showed clonal diversity among these isolates. Phenotypes of ESBL, AmpC β-lactamase overproduction, an active efflux pump, and change in the expression of OMPs were found in 18 (34%), 11 (20.8%), 51 (96.2%), and 23 (43.4%) of ertapenem-resistant strains, respectively. Ertapenem MICs were restored in strains with OmpC and OmpF expression plasmids. This study suggests that ESBL, AmpC β-lactamase overproduction, and decreased OMP expression combined with an active efflux pump contribute to the ertapenem resistance of *E. cloacae*. The presence of IMP-8 may also play a partial role in ertapenem resistance in Taiwan.

Enterobacter cloacae causes nosocomial infections involving the urinary tract, lower respiratory tract, skin and soft tissue, biliary tract, wounds, intravenous catheters, and central nervous system (20). It may become resistant to broad-spectrum cephalosporins by a chromosomally encoded AmpC β-lactamase, but a growing number of *E. cloacae* strains with an extended-spectrum β-lactamase (ESBL) have been observed worldwide (1, 11).

Carbapenems, such as ertapenem, imipenem, and meropenem, are considered the first choice for treating serious infections caused by ESBL- or AmpC-producing *Enterobacteriaceae* family members (13, 17, 19). However, the emergence of an increasing incidence of resistance to carbapenems has become a global issue (13, 19).

E. cloacae strains have acquired carbapenem-hydrolyzing β-lactamases (KPC, IMP, NDM, and VIM), a combination of AmpC β-lactamase or ESBL production and reduction of permeability of outer membrane proteins, or efflux pump expression, all of which are involved in carbapenem resistance (2, 5, 10–12, 21, 25).

The present study was conducted to investigate the prevalence and characteristics of ertapenem-resistant *E. cloacae* isolates. Our findings demonstrated that ertapenem resistance in *E. cloacae* is becoming a great concern. ESBL, AmpC β-lactamase overproduction, and decreased OMP expression combined with the active efflux pump were the factors resulting in ertapenem-resistant *E. cloacae* in Taiwan.

MATERIALS AND METHODS

Clinical isolates. Among 355 *E. cloacae* isolates recovered in 2007 in the Department of Pathology, National Cheng Kung University Hospital, Tainan, Taiwan, 53 (14.9%) nonduplicated *E. cloacae* isolates that were resistant to ertapenem based on disk diffusion method results (inhibition zone diameters ≤ 19 mm) were collected and examined. The isolation sites of the *E. cloacae* isolates collected were as follows: skin and soft tissue (13 isolates), sputum (12 isolates), urine (10 isolates), bloodstream (4 isolates), ascitic fluid (2 isolates), bile (1 isolate), drainage tube (1 isolate), pleural fluid (1 isolate), and others (9 isolates). *E. cloacae* was identified by colony morphology, Gram stain, biochemical tests, or the Vitek system (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing. Susceptibility to ertapenem for *E. cloacae* isolates was determined by the disk diffusion method on Mueller-Hinton agar based on the CLSI guidelines (7). MICs of amikacin, cefepime, ceftazidime, ciprofloxacin, and levofloxacin (Sigma Chemical Co., St. Louis, MO), cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ), ertapenem and imipenem (Merck & Co., Inc., West Point, PA), gentamicin (Amresco Inc., Solon, OH), and meropenem (Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan) were determined in duplicate by the agar dilution method according to the recommendations of the CLSI (8). Briefly, bacteria were suspended in saline to 1/10 the turbidity of the 0.5 McFarland standard and then inoculated directly onto antibiotic-containing Mueller-Hinton agar. After 16 to 20 h of incubation, the MIC of each antibiotic was determined. *Escherichia coli* ATCC 25922 was used as the quality control strain. The interpretation of resistance to these antimicrobial agents was determined according to the recommendations of the CLSI (9). In addition, MICs of ertapenem in strains

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containing OmpC and OmpF expression plasmids were evaluated by the Etest strip (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions.

Detection of efflux pump activity. To investigate the role of an efflux pump in ertapenem-resistant *E. cloacae* isolates, MICs of ertapenem in the presence of efflux pump inhibitor Phe-Arg β-naphthylamide dihydrochloride (PAβN) or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Sigma) were determined (14, 16, 21). PAβN at 25 μg/ml or 12.5 μM CCCP was incorporated in Mueller-Hinton agar, and ertapenem susceptibility testing was performed in parallel on agar plates with and without an efflux pump inhibitor (14, 16). *E. cloacae* ATCC 13047 was used as the control.

β-Lactamase characterization. The double-disk synergy test was performed to screen for ESBLs by placing disks of ceftazidime, cefotaxime, ceftriaxone, aztreonam, and cefepime (30 μg each) at distances of 30 and 20 mm (center to center) from a disk containing amoxicillin plus clavulanic acid (AMC; 20/10 μg) (23). AmpC overproduction was investigated using cloxacillin (200 μg/ml; Sigma)-containing plates, since cloxacillin inhibits AmpC β-lactamase activity and may restore susceptibility to ceftazidime (18). *E. cloacae* ATCC 13047, which contains AmpC class C β-lactamase, and ATCC 15337 were used as positive- and negative-control strains, respectively.

Isolates were screened for class A and B carbapenemases with the modified Hodge test and 2-mercaptopropionic acid double-disk method, respectively (8, 26).

Identification of carbapenemase activities in crude extracts of bacterial isolates was performed by a spectrophotometric assay using ertapenem as the substrate (26). Briefly, the bacterial cells from an overnight culture were diluted 1:20 into fresh medium and incubated for 2 h. The bacterial cells were suspended in a solution of 10 mM HEPES (pH 7.5) and disrupted by sonication. The supernatants were obtained by centrifugation at 5,000 × *g* for 10 to 15 min, and 0.1 mM ertapenem was added to measure carbapenemase activities by monitoring ertapenem hydrolysis with a Beckman Coulter DU 800 UV/visible spectrophotometer (Beckman Coulter, Inc., Brea, CA) at 300 nm.

The β-lactamase genes (bla_{CMY}, bla_{CTX-M}, bla_{DHA}, bla_{IMP}, bla_{SHV}, bla_{TEM}, and bla_{VIM}) were amplified by PCR as described previously (3, 25). The purified PCR products were directly sequenced using an automated ABI Prism 3730 DNA sequencer (Applied Biosystems, Foster City, CA).

Analysis of *acrB* and *rpoB* gene expression. Total RNA was extracted using the acid phenol-chloroform method and treated with RNase-free DNase and RNasin (Promega, Madison, WI). Reverse transcription-PCR (RT-PCR) was performed, and the expression of the housekeeping *rpoB* gene was used as the internal control for relative quantification. The oligonucleotide primers used to examine gene expression of *acrB* and *rpoB* were described previously (11). All amplifications were carried out in triplicate, and negative controls without reverse transcriptase were performed to detect DNA contamination in the purified RNA.

Examination of porin expression. Bacterial outer membrane proteins (OMPs) were isolated and purified by treatment of the cell envelopes with 2% sodium-*N*-lauryl sarcosinate (Sigma) (4). OMPs were quantitated by using a Bio-Rad protein assay (Bio-Rad Laboratories, Inc., Hercules, CA), and 20 μg of OMPs was loaded into each well. OMPs were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) through a 10% SDS-polyacrylamide gel containing 4 M urea and stained with 0.1% Coomassie brilliant blue (4). *E. cloacae* ATCC 13047 was used as the control. OMPs from selected isolates suspected of being OmpC, OmpF, or OmpA on SDS-polyacrylamide gels were submitted to the National Cheng Kung University Proteomics Research Core Laboratory for nanoelectrospray liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The data were analyzed by the database search software on the Mascot website (Matrix Science).

The coding sequences of *E. cloacae ompC* and *ompF*, including their respective promoter regions, were amplified and inserted into pACYC184 to generate plasmids pMW706 and pMW707, respectively. The primers used were described previously (11). OmpC and OmpF expression plasmids were then transformed into *E. cloacae* isolates.

PFGE. Pulsed-field gel electrophoresis (PFGE) of XbaI-digested genomic DNA samples of *E. cloacae* isolates was carried out with a CHEF Mapper XA apparatus (Bio-Rad Laboratories, Inc., Hercules, CA) according to the instruction manual. Electrophoresis was performed for 27 h at 14°C with pulse times ranging from 5 to 35 s at 6 V/cm. PFGE patterns were interpreted in accordance with the criteria of Tenover et al. (22). PFGE profiles were analyzed and compared using the GelCompar II software, version 2.0 (Unimed Healthcare Inc., Houston, TX).

TABLE 1 *In vitro* activity of 10 antimicrobial agents against 53 *E. cloacae* strains isolated in 2007 at National Cheng Kung University Hospital

Antibiotic	MIC (μg/ml)			% resistance
	Range	50%	90%	
Amikacin	0.5–16	2	16	0
Gentamicin	0.25–>256	>256	>256	84.9
Cefotaxime	32–>256	128	>256	100
Ceftazidime	4–>256	256	>256	98.1
Cefepime	0.25–256	16	128	47.2
Ciprofloxacin	0.03–256	8	128	83
Levofloxacin	0.03–256	16	128	83
Ertapenem	8–>256	16	32	100
Imipenem	0.25–32	1	2	5.7
Meropenem	0.06–4	0.25	0.5	1.9

RESULTS

Susceptibility testing. A total of 53 isolates were interpreted to be ertapenem-resistant *E. cloacae* on the basis of the disk diffusion test, having inhibition zone diameters of ≤19 mm. They represented 14.9% of the 355 nonduplicated isolates. The MICs of the 53 isolates to 10 antimicrobial agents are listed in Table 1. Among them, all isolates were resistant to ertapenem and cefotaxime but susceptible to amikacin, imipenem, and meropenem, according to the 2011 criteria of the CLSI. All 53 ertapenem-resistant isolates were defined to be multidrug-resistant strains because of resistance to 3 or more classes of antimicrobial agents.

β-Lactamase characterization and genetic diversity. Among the 53 ertapenem-resistant *E. cloacae* isolates, the ESBL phenotype was found in 18 (34%) isolates by the double-disk synergy test. In detection of AmpC overproduction, a greater than or equal to 2 2-fold decrease in the MIC for ceftazidime tested in combination with the AmpC inhibitor cloxacillin was shown in 11 (20.8%) of the ertapenem-resistant strains (Table 2). Genotyping of β-lactamases identified IMP-8, SHV-12, and TEM-1 type β-lactamases in 3 (5.7%), 40 (75.5%), and 46 (86.8%) isolates, respectively. No KPC-type enzymes were detected by the modified Hodge test. The hydrolysis assay showed that none of them had activity against ertapenem. Moreover, 23 pulsotypes were investigated, and 5 major PFGE pulsotypes accounted for 35 (66%) of the 53 *E. cloacae* isolates: type I (30.2%), II (9.4%), III (15.1%), IV (5.7%), and V (5.7%). The remaining 18 isolates showed distinct individual patterns.

Outer membrane protein analysis. No discernible changes in the expression of OmpC, OmpF, and OmpA were found for 30 (56.6%) of the 53 ertapenem-resistant isolates. Among the other isolates, 6 (11.3%), 13 (24.5%), and 4 (7.5%) isolates showed moderately decreased expression of OmpC, OmpF, or OmpC and OmpF, respectively.

TABLE 2 Phenotypes of ESBL, AmpC overproduction, active efflux pump, and outer membrane protein profile among 53 ertapenem-resistant *E. cloacae* clinical isolates[a]

ETP MIC (μg/ml)	No. of strains	Phenotype			
		ESBL	AmpC	Efflux pump	Loss or decreased OMPs
8–32	14	−	−	+	−
8–>256	10	−	−	+	+
16	2	−	+	+	−
8–64	9	−	+	+	+
8	2	+	−	−	−
8–32	4	+	−	+	+
8–64	12	+	−	+	−

[a] ESBL, extended-spectrum β-lactamase; ETP, ertapenem; OMPs, outer membrane proteins.

To investigate the contribution of porin loss to carbapenem resistance in our *E. cloacae* isolates, an attempt was made to transform OmpC and OmpF expression plasmids into 11 strains with decreased expression of OmpC or OmpF, respectively. Only 3 isolates (strains 130, 401, and 592) were successfully transformed: strain 130 containing OmpC/OmpF, strain 592 containing OmpC, and strain 401 containing the OmpF expression plasmid, confirmed for all strains by urea/SDS-PAGE. Strains 592 and 401 with OmpC and OmpF expression plasmids restored the ertapenem MICs from the original 64 and 16 μg/ml to 1 and 2 μg/ml, respectively. However, strain 130, containing OmpC and OmpF expression plasmids, did not decrease the ertapenem MIC. In fact, there was a modest enlargement of the ertapenem inhibition zone diameter, from 6 to 10 mm, when strain 130 contained the OmpF expression plasmid but not in strain 130 with the OmpC plasmid.

Efflux pump activity. A greater than or equal to 2 2-fold concentration decrease in MIC for ertapenem tested in combination with either efflux pump inhibitor, PAβN or CCCP, was shown in 16 (30.2%) and 51 (96.2%) of the ertapenem-resistant strains, respectively. This indicates that an active efflux pump contributes to the ertapenem resistance of our *E. cloacae* isolates. Moreover, analysis of *acrB* expression by RT-PCR consistently revealed no difference in ertapenem-resistant strains.

DISCUSSION

In this study, we demonstrated that ertapenem resistance was prevalent in *E. cloacae* isolates (14.9%) and that ESBL, AmpC β-lactamase overproduction, and decreased OMP expression combined with an active efflux pump contribute to ertapenem resistance in Taiwan. Although the prevalence of ertapenem nonsusceptibility in the *Enterobacteriaceae* family is relatively lower than that in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, the current trend of rising ertapenem resistance of the *Enterobacteriaceae* has become a great concern (13, 19, 27). Surveillance of antimicrobial susceptibility by the Study for Monitoring Antimicrobial Resistance Trends (SMART) reported that the rate of susceptibility of *E. cloacae* strains to ertapenem decreased from 100% (2002) to 89.7% (2009) in China (27). However, the rate of ertapenem susceptibility of *E. cloacae* isolates among 12 countries in the Asia-Pacific region during 2009 was reported by SMART to be 96% (6). It indicates that the prevalence of ertapenem nonsusceptibility in *E. cloacae* is dependent on geographic and drug usage differences.

Active efflux pumps transport drugs through the bacterial envelope and limit the intracellular accumulation of toxic compounds (14, 15). It is well established that multidrug resistance efflux pumps encoded by bacteria can confer clinically relevant resistance to antibiotics. Overexpression of the RND family pumps AcrAB-TolC in *E. coli* and *Salmonella enterica* serovar Typhimurium and MexAB-OprM in *Pseudomonas aeruginosa* has been reported (14, 15). Two efflux pump inhibitors, PAβN and CCCP, reduced carbapenem MICs in clinical isolates of *E. cloacae* and *P. aeruginosa* (16, 21). However, the transcription levels of the *acrB* gene were equivalent in ertapenem-susceptible and -resistant *E. cloacae* strains (21). In this study, either PAβN or CCCP decreased the ertapenem MICs in our ertapenem-resistant *E. cloacae* isolates, suggesting that active efflux pumps may confer resistance to ertapenem in *E. cloacae*. Analysis of *acrB* mRNA expression consistently revealed no difference in ertapenem-resistant strains, suggesting the presence of an additional unknown efflux pump influencing ertapenem resistance.

The efflux pump inhibitors PAβN and CCCP are active against RND pumps in Gram-negative bacteria, including *Campylobacter* spp., *Enterobacter aerogenes*, *E. coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *S. enterica* (14). A potential toxic effect of PAβN and CCCP on bacteria was assessed, but concentrations of 25 μg/ml of PAβN and 12.5 μM CCCP did not kill bacteria (14, 16, 21). Therefore, our results showing the MICs of ertapenem being reduced in the presence of PAβN and CCCP should be due to their direct effect on efflux pumps and not due to the efflux pump inhibitors influencing bacterial growth.

Loss or reduction of outer membrane porins is common in *Enterobacteriaceae* with carbapenem resistance (10, 11, 21). The contribution of OmpC, OmpD, and OmpF deficiency, as well as decreased RNA transcripts of the *ompF* and *ompD* genes, to the carbapenem resistance of *E. cloacae* was investigated (10, 11, 21). To investigate the contribution of porin loss to carbapenem resistance, OmpC and OmpF expression plasmids were transformed into 11 strains with decreased expression of OMPs. However, only 3 isolates (strains 130, 401, and 592) had restored OMP expression; for the remaining 8 isolates, we failed to obtain transformants, despite several attempts. Strains 592 and 401, with OmpC and OmpF expression plasmids, had decreased ertapenem MICs, indicating that loss of OmpC and OmpF contributed to carbapenem resistance in these strains. Although strain 130 with the OmpF expression plasmid showed enhancement of the ertapenem inhibition zone diameter, its ertapenem MIC was not restored. We suggest that the high ertapenem MIC value (>256 μg/ml) could be part of the reason, and some other mechanism(s) may also be involved in the carbapenem resistance of this isolate.

ESBL production is prevalent among isolates of the *Enterobacteriaceae* in Asia, including Taiwan, and has participated in resistance to broad-spectrum β-lactams and carbapenems (1, 3, 28). It has been shown that SHV-12 predominates among ESBL-producing *E. cloacae* isolates in Taiwan (28), and all of our SHV-type ESBL-producing isolates were identified to be SHV-12 producers. On the basis of the high prevalence (75.5%) of SHV-type ESBLs in ertapenem-resistant *E. cloacae* isolates, β-lactamases play a role in ertapenem resistance. Moreover, increased production of the AmpC chromosome-encoded cephalosporinase is known to contribute to carbapenem resistance in *E. cloacae* and *P.*

aeruginosa (11, 18, 24). In this study, overproduction of AmpC β-lactamases in our isolates also conferred carbapenem resistance.

In conclusion, ertapenem resistance in *E. cloacae* has become a great concern in Taiwan. ESBL, AmpC β-lactamase overproduction, and decreased OMP expression combined with an active efflux pump are involved in ertapenem resistance in *E. cloacae* isolates. The presence of IMP-8 may also play a partial role in ertapenem resistance. Since most ertapenem-resistant strains were susceptible to imipenem and meropenem but did not have a carbapenemase, these antimicrobial agents may still be suitable for treatment of infections caused by such ertapenem-resistant *E. cloacae* isolates in Taiwan.

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REFERENCES

1. **Bell JM, Turnidge JD, Jones RN, the Asia-Pacific Participants SENTRY.** 2003. Prevalence of extended-spectrum β-lactamase-producing *Enterobacter cloacae* in the Asia-Pacific region: results from the SENTRY Antimicrobial Surveillance Program, 1998 to 2001. Antimicrob. Agents Chemother. **47**:3989–3993.
2. **Bennett JW, Herrera ML, Lewis JS II, Wickes BW, Jorgensen JH.** 2009. KPC-2-producing *Enterobacter cloacae* and *Pseudomonas putida* coinfection in a liver transplant recipient. Antimicrob. Agents Chemother. **53**: 292–294.
3. **Biendo M, et al.** 2008. Molecular typing and characterization of extended-spectrum TEM, SHV and CTX-M beta-lactamases in clinical isolates of *Enterobacter cloacae*. Res. Microbiol. **159**:590–594.
4. **Biendo M, et al.** 2008. Successive emergence of extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacter aerogenes* isolates in a university hospital. J. Clin. Microbiol. **46**:1037–1044.
5. **Castanheira M, et al.** 2011. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrob. Agents Chemother. **55**:1274–1278.
6. **Chen YH, et al.** 2011. Antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region according to currently established susceptibility interpretive criteria. J. Infect. **62**:280–291.
7. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial disk susceptibility tests; M02-A10, approved standard, 10th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; M07-A8, approved standard, 8th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing, 21st informational supplement, M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Cornaglia G, Russell K, Satta G, Fontana R.** 1995. Relative importances of outer membrane permeability and group 1 beta-lactamase as determinants of meropenem and imipenem activities against *Enterobacter cloacae*. Antimicrob. Agents Chemother. **39**:350–355.
11. **Doumith M, Ellington MJ, Livermore DM, Woodford N.** 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J. Antimicrob. Chemother. **63**:659–667.
12. **Falcone M, et al.** 2009. Infections with VIM-1 metallo-β-lactamase-producing *Enterobacter cloacae* and their correlation with clinical outcome. J. Clin. Microbiol. **47**:3514–3519.
13. **Lee CC, et al.** 2010. Bacteremia due to extended-spectrum-beta-lactamase-producing *Enterobacter cloacae*: role of carbapenem therapy. Antimicrob. Agents Chemother. **54**:3551–3556.
14. **Pagès JM, Masi M, Barbe J.** 2005. Inhibitors of efflux pumps in Gram-negative bacteria. Trends Mol. Med. **11**:382–389.
15. **Piddock LJ.** 2006. Multidrug-resistance efflux pumps—not just for resistance. Nat. Rev. Microbiol. **4**:629–636.
16. **Pournaras S, et al.** 2005. Spread of efflux pump-overexpressing, non-metallo-beta-lactamase-producing, meropenem-resistant but ceftazidime-susceptible *Pseudomonas aeruginosa* in a region with bla_{VIM} endemicity. J. Antimicrob. Chemother. **56**:761–764.
17. **Queenan AM, Bush K.** 2007. Carbapenemases: the versatile beta-lactamases. Clin. Microbiol. Rev. **20**:440–458.
18. **Rodríguez-Martínez JM, Poirel L, Nordmann P.** 2009. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **53**:4783–4788.
19. **Rodriguez-Villalobos H, et al.** 2011. Trends in production of extended-spectrum beta-lactamases among *Enterobacteriaceae* of clinical interest: results of a nationwide survey in Belgian hospitals. J. Antimicrob. Chemother. **66**:37–47.
20. **Sanders WE, Jr, Sanders CC.** 1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. Clin. Microbiol. Rev. **10**:220–241.
21. **Szabó D, et al.** 2006. Outer membrane protein changes and efflux pump expression together may confer resistance to ertapenem in *Enterobacter cloacae*. Antimicrob. Agents Chemother. **50**:2833–2835.
22. **Tenover FC, et al.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
23. **Tzelepi E, et al.** 2000. Detection of extended-spectrum beta-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J. Clin. Microbiol. **38**:542–546.
24. **Woodford N, et al.** 2007. Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory. Int. J. Antimicrob. Agents **29**:456–459.
25. **Yan JJ, Ko WC, Chuang CL, Wu JJ.** 2002. Metallo-beta-lactamase-producing *Enterobacteriaceae* isolates in a university hospital in Taiwan: prevalence of IMP-8 in *Enterobacter cloacae* and first identification of VIM-2 in *Citrobacter freundii*. J. Antimicrob. Chemother. **50**:503–511.
26. **Yan JJ, Wu JJ, Tsai SH, Chuang CL.** 2004. Comparison of the double-disk, combined disk, and Etest methods for detecting metallo-beta-lactamases in gram-negative bacilli. Diagn. Microbiol. Infect. Dis. **49**:5–11.
27. **Yang Q, et al.** 2010. Surveillance of antimicrobial susceptibility of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in China: the 2002-2009 Study for Monitoring Antimicrobial Resistance Trends (SMART). Int. J. Antimicrob. Agents **36**: 507–512.
28. **Yu WL, et al.** 2006. Characterisation and molecular epidemiology of extended-spectrum β-lactamase-producing *Enterobacter cloacae* isolated from a district teaching hospital in Taiwan. Clin. Microbiol. Infect. **12**: 579–582.