Table 1. Mutations in *gyrA* and *parC* genes and MIC values for ciprofloxacin-resistant mutants of *A. baumannii*

Strain	MIC (mg/L)							Amino acid substitutions		OMP 20 kDa band
	CIP	IPM	TET	TMP	GEN	CHL	CTX	*gyrA*	*parC*	
ATCC 19606[a]	1	0.06	2	4	1	128	8	no mutation	no mutation	+
C1	8	0.12	2	4	2	256	8	Ser-83→Leu	no mutation	–
C2	32	0.12	2	8	2	256	32	Ser-83→Leu	no mutation	–
C3	64	0.12	4	32	4	256	32	Ser-83→Leu	no mutation	–
C4	128	0.12	4	32	8	256	32	Ser-83→Leu	no mutation	–

OMP, outer membrane protein; CIP, ciprofloxacin; IPM, imipenem; TET, tetracycline; TMP, trimethoprim; GEN, gentamicin; CHL, chloramphenicol; CTX, cefotaxime, +, 20 kDa band conserved; –, 20 kDa band loss. Ser, serine; Leu, leucine; C1, ciprofloxacin first step mutant; C2, ciprofloxacin second step mutant; C3, ciprofloxacin third step mutant; C4, ciprofloxacin forth step mutant.
[a]Wild-type.

moxifloxacin-resistant mutants at detectable frequencies. In contrast, in the present study, ciprofloxacin-resistant mutants were stable when plated 10 times on ciprofloxacin-free agar and retained their resistance to ciprofloxacin. A *gyrA* mutation was found in all resistant mutants where serine is substituted by leucine at position 83. This mutation seems to be the most frequently found in clinical and laboratory quinolone-resistant isolates of many Gram-negative bacteria including *A. baumannii*.[1] Also, it appears to be a prerequisite for further mechanism(s) of resistance. However, no *parC* mutations at codons 80 or 84 were found in mutants with ciprofloxacin MICs ≥32 mg/L, a value at which such mutations may occur.[2] Moreover, in contrast to *gyrA*, these *parC* point mutations seem to be difficult to select *in vivo*, which begs the question of whether mutations within topoisomerase IV can only be selected *in vivo* and only under certain conditions. An *in vivo* experiment would be a good approach to solve this problem. As mutation within *gyrA* could not explain the MIC changes seen alone the role of permeability was investigated. The loss of a 20 kDa band in all isolates is an interesting observation, but this was observed at all four stages in the mutation process (Table 1). Investigation of the function of this protein may reveal its involvement in ciprofloxacin resistance. Hooper *et al.*[5] showed that alterations in the DNA *gyrA* subunit and the OmpF outer membrane porin protein were the main mechanisms of resistance in a norfloxacin-resistant mutant. The phenotypic results suggest that the activation of an efflux system may be responsible for the antibiotic resistance seen in the mutants (Table 1). The AdeB efflux pump has previously been linked to aminoglycoside resistance in *A. baumannii*.[6]

In conclusion, our findings highlight that ParC was not a secondary target for quinolones in *A. baumannii* laboratory mutants resistant to ciprofloxacin. In addition, with the loss of a 20 kDa band, it is possible that permeability is an alternative pathway of resistance to fluoroquinolones, at least in these mutants as no additional *gyrA* or *parC* mutations were present.

Acknowledgements

This study was supported by Altajir World of Islam Trust.

Transparency declarations

No declarations were made by the authors of this paper.

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Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dki408
Advance Access publication 10 November 2005

Occurrence of extended-spectrum β-lactamases and plasmid-mediated AmpC β-lactamases among Korean isolates of *Proteus mirabilis*

Yeon-Joon Park[1]*, Seungok Lee[2], Yang Ree Kim[3], Eun-Jee Oh[1], Gun-Jo Woo[4] and Kyungwon Lee[5]

[1]Department of Clinical Pathology, College of Medicine, The Catholic University of Korea, Kangnam St Mary's Hospital, 505 Banpo-dong, Seocho-ku, Seoul, 137-701, Korea; [2]Seoul Medical Science Institute, Seoul Clinical Laboratories, Seoul, Korea; [3]Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; [4]Korea Food and Drug Administration, Seoul, Korea; [5]Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea

Keywords: ESBLs, plasmids, *P. mirabilis*

*Corresponding author. Tel: +82-2-590-1604; Fax: +82-2-592-4190; E-mail: yjpk@catholic.ac.kr

Sir,

Proteus mirabilis is considered one of the most β-lactam-susceptible members of the Enterobacteriaceae because it does not express a chromosomally encoded AmpC cephalosporinase. Although extended-spectrum β-lactamases (ESBLs) have recently been identified in *P. mirabilis* isolates,[1] there are few reports of plasmid-mediated AmpC β-lactamase (PABL) in *P. mirabilis*, and the incidence was reported to be very low (0.4%) in a study carried out in the United States in the year 2000.[2] Here we report a survey on ESBL and PABL production among clinical isolates of *P. mirabilis* from 12 nationwide clinical laboratories in Korea.

In a nationwide survey conducted between April and June 2004, a total of 134 consecutive *P. mirabilis* isolates were collected from 12 clinical laboratories. They were identified using either a Vitek GNI card (bioMérieux, Marcy-l'Etoile, France) or a Microscan GN combo card (Dade Behring, West Sacramento, CA, USA.). The MICs of cefalotin, cefuroxime, ceftazidime, cefotaxime, aztreonam, cefepime, cefoxitin, amikacin, gentamicin, piperacillin, ciprofloxacin, meropenem and imipenem were determined using the agar dilution method.[3] ESBL detection was based on a double-disc-synergy test.[4] The modified Hodge test[5] was used to screen PABL.

For the ESBL screen-positive isolates, a search for the $bla_{\text{TEM-1}}$, $bla_{\text{SHV-1}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{PER-1}}$, $bla_{\text{GES-1}}$ and $bla_{\text{VEB-1}}$ genes was performed by PCR amplification with the following sets of primers: TEM-1F, 5′-AGCCATACCAAACGACGAG-3′ and TEM-1B, 5′-ATTGTTGCCGGGAAGCTAGA-3′ for $bla_{\text{TEM-1}}$; SHV-1F, 5′-TATCCCTGTTAGCCACCCTG-3′ and SHV-1B, 5′-CACTGCAGCAGCTGC(A/C)TT-3′ for $bla_{\text{SHV-1}}$; CTX-1F, 5′-GGTTTAAAAAATCACTGCGTC-3′ and CTX-1B, 5′-TTGGTGACGATTTTAGCCGC-3′ for $bla_{\text{CTX-M-1}}$; CTX-2F, 5′-ATGATGACTCAGAGCATTCG-3′ and CTX-2B, 5′-TGGGTTACGATTTTCGCCGC-3′ for $bla_{\text{CTX-M-2}}$; CTX-9F, 5′-CGCTTTATGCGCAGACGA-3′ and CTX-9B, 5′-GATTCTCGCCGCTGAAGC3′ for $bla_{\text{CTX-M-9}}$; PER-1F, 5′-ATGAATGTCATTATAAAAGC-3′ and PER-1B, 5′-AATTTGGGCTTAGGGCAGAG-3′ for $bla_{\text{PER-1}}$; GES-1F, 5′-ATGCGCTTCATTCACGCAC-3′ and GES-1B, 5′-CTATTTGTCCGTGCTCAGG-3′ for $bla_{\text{GES-1}}$; and VEB-1F, 5′-CGACTTCCATTTCCCGATGC-3′ and VEB-1B, 5′-GGACTCTGCAACAAATACGC-3′ for $bla_{\text{VEB-1}}$.

For the isolates with cefoxitin MICs of ≥16 mg/L, plasmids were isolated using a Wizard Plus SV Miniprep DNA purification system (Promega, MD, USA) and PABL amplification was carried out using multiplex PCR which can detect various types (MOX, CMY, LAT, DHA, ACC, MIR-1, ACT-1 and FOX) of PABLs.[6] All the sequencing reactions were performed using an automated sequencer (AI 377; Applied Biosystems, CA, USA). The genomic DNA for PFGE analysis was digested with *Sfi*I (New England Biolabs, Beverly, MA, USA).

Of the 134 *P. mirabilis* isolates, 39 (29.1%) were cefalotin resistant (MICs ≥32 mg/L) and 24 (17.9%) were found to be cefuroxime resistant (MICs ≥32 mg/L) according to the NCCLS guideline (M100-S15). Thirteen (9.7%) isolates showed some potentiation of the inhibitory zones of the β-lactams by clavulanic acid, suggesting the presence of ESBL activity. From these 13 putative ESBL-positive isolates, the structural genes for $bla_{\text{CTX-M-9-like}}$, bla_{TEM}, bla_{SHV} and $bla_{\text{PER-1}}$ were found either alone or in combination (Table 1). Sequencing analysis identified the ESBL genes $bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-52}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{PER-1}}$. $bla_{\text{CTX-M-14}}$ type, which is one of the CTX-M-9 group enzymes and has one amino acid change at position 231 from $bla_{\text{CTX-M-9}}$, was the most frequently encountered ESBL. Most of the ESBL producers also

Table 1. Profiles of the 16 ESBL- and/or PABL-producing *P. mirabilis* strains isolated in Korean hospitals

Isolate	β-Lactamases	Specimen	MIC (mg/L)												
			CEF	CXM	CAZ	CTX	ATM	FEP	FOX	PIP	AMK	GEN	CIP	IPM	MEM
YS10	CTX-M-14 + TEM-1	urine	≥64	≥64	≤1	8	≤1	16	≤4	128	≤8	≥32	≥8	4	≤0.5
SCL4	CTX-M-14 + TEM-1	urine	≥64	≥64	≤1	8	≤1	8	8	32	32	≥32	≥8	2	≤0.5
SCL7	CTX-M-14 + TEM-1	urine	≥64	≥64	≤1	4	≤1	4	≤4	32	≥128	≥32	4	≤0.5	≤0.5
SCL8	PER-1 + TEM-1	wound	≥64	≥64	≥64	16	16	16	≤4	16	≥128	≥32	4	4	≤0.5
SCL9	CTX-M-14 + TEM-1	sputum	≥64	≥64	≤1	8	≤1	4	≤4	64	≥128	≥32	2	2	≤0.5
SCL14	CTX-M-14 + TEM-1	urine	≥64	≥64	≤1	8	≤1	4	≤4	64	≥128	≥32	2	2	≤0.5
SCL18	TEM-52	wound	≥64	32	4	8	≤1	2	≤4	64	≤8	≥32	2	2	≤0.5
SCL25	CTX-M-14 + TEM-1	urine	≥64	≥64	≤1	8	≤1	4	8	32	≥128	≥32	≥8	≤0.5	≤0.5
SCL27	CTX-M-14 + TEM-1	urine	≥64	≥64	≤1	8	≤1	4	8	32	≥128	≥32	4	2	≤0.5
WJ2	CTX-M-14	urine	≥64	≥64	≤1	4	≤1	4	≤4	16	≤8	≤2	1	2	≤0.5
WJ3	SHV-12 + TEM-1	wound	≥64	≥64	32	16	16	8	32	≥256	≤8	≥32	≥8	1	≤0.5
WJ5	CTX-M-9 + TEM-1	wound	≥64	≥64	≤1	32	≤1	16	8	64	≥128	≥32	≥8	2	≤0.5
BD5	DHA-1	urine	≥64	≥64	≤1	≤1	≤1	≤0.5	16	≤8	≤8	≤2	≤0.5	2	≤0.5
SCL1	CMY-2	wound	≥64	16	4	8	≤1	≤0.5	32	32	≤8	≥32	≤0.5	1	≤0.5
SCL5	CMY-10	wound	≥64	≥64	≤1	16	≤1	≤0.5	≥64	≥64	≤8	≥32	4	≤0.5	≤0.5
SCL23	CMY-10 + PER-1 + TEM-52	wound	≥64	≥64	≥64	16	8	4	≥64	32	≥128	≥32	2	≤0.5	≤0.5

CEF, cefalotin; CXM, cefuroxime; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; FEP, cefepime; FOX, cefoxitin; PIP, piperacillin; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; IPM, imipenem; MEM, meropenem.

harboured bla_{TEM-1}. All but one of the ESBL-producing strains was resistant to gentamicin, and 8 (61.5%) of the 13 ESBL producers were resistant to amikacin. Of the ESBL-negative isolates, gentamicin resistance was observed in 26/121 (21.5%) and amikacin resistance was observed in only 2/121 (1.7%). The rate of ciprofloxacin resistance was also higher in the ESBL producers (8/13, 61.5%) than in the ESBL non-producers (21/121, 17.4%). However, all the ESBL producers were susceptible to carbapenems. The eight $bla_{CTX-M-14}$-positive isolates showed seven different PFGE patterns, indicating the presence of many clones. Moreover, the two bla_{PER-1} producers also showed different PFGE patterns (data not shown).

Of the 134 *P. mirabilis* isolates, 15 isolates were not susceptible to cefoxitin and 11 of them were not susceptible to cefuroxime. Five isolates had a positive reaction according to the modified Hodge test; all were not susceptible to cefoxitin and cefuroxime. PCR and sequencing analysis revealed that four of them harboured PABL genes (two contained CMY-10, one CMY-2 and one DHA-1), but in one isolate no PABL gene was detected (Table 1). Of them, one of the CMY-10 harbouring isolates also harboured bla_{PER-1} and bla_{TEM-52}. In contrast to general knowledge regarding the susceptibility pattern of the PABL producers, which are known to be resistant to cephalosporins in the oxyimino and 7-α-methoxy groups, this study found the DHA-1 producer to be susceptible to ceftazidime and cefotaxime (both MICs ≤1 mg/L), and the two isolates that produced only CMY-2 or CMY-10 to be susceptible to ceftazidime (MICs of 4 and ≤1 mg/L, respectively). This is in line with the finding that several AmpC-producing *Escherichia coli* showed variable MICs of ceftazidime and cefotaxime (0.5–128 mg/L and 0.5–16 mg/L, respectively).[2] Therefore, the potential exists for PABL production to be missed in screens of *P. mirabilis* or *E. coli* using a criterion of MIC ≥2 mg/L for the oxyimino-group of cephalosporins. In this study, although the number of PABL producers was very small, of the 11 isolates that were not susceptible to both cefuroxime and cefoxitin, 4 isolates were PABL producers.

In conclusion, this study found that the prevalence of ESBLs and PABLs in *P. mirabilis* was 9.7 and 3.0%, respectively. Further study is needed to investigate the screening criteria of PABL production in *P. mirabilis.*

Acknowledgements

We thank all the contributing laboratories that provided isolates for this study. We also thank Jin Kyung Yu for his excellent technical assistance and Hyun Jeong for the testing of isolates by PFGE. This work was supported by a grant from the Korea Food and Drug Administration in 2004 (FD100-04062).

Transparency declarations

None to declare.

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Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dki427
Advance Access publication 24 November 2005

Spread of an unusual penicillin- and imipenem-resistant but ampicillin-susceptible phenotype among *Enterococcus faecalis* clinical isolates

Evgenia Metzidie[1], Evangelos N. Manolis[2], Spyros Pournaras[3], Danai Sofianou[1] and Athanassios Tsakris[2]*

[1]*Department of Clinical Microbiology, Hippokration University Hospital, Thessaloniki, Greece;* [2]*Department of Basic Sciences, School of Health Sciences, University of Athens, 123 Papadiamantopoulou Street, 11527 Athens, Greece;* [3]*Department of Microbiology, Medical School, University of Thessalia, Larissa, Greece*

Keywords: enterococci, penicillin, ampicillin, imipenem, Etest, resistance

*Corresponding author. Tel: +30-210-746-1483; Fax: +30-210-746-1489; E-mail: atsakris@med.uoa.gr

Sir,

Enterococci have emerged as pathogens related to serious nosocomial infections, particularly in hospitals where cephalosporins are extensively used. The treatment of enterococcal infections is difficult and usually requires the administration of a penicillin compound alone or, for severe infections, in synergic combination with an aminoglycoside, usually gentamicin in high concentrations. *Enterococcus faecalis* usually demonstrates susceptibility to penicillin, ampicillin, amoxicillin/clavulanate and imipenem, whereas *Enterococcus faecium* is commonly resistant to the above-mentioned β-lactam antibiotics. Enterococci exhibiting susceptibility to ampicillin are regularly cross-susceptible to penicillin and MICs of both antibiotics may be equal or more often MICs of ampicillin are one to two dilutions lower than those of penicillin although the NCCLS/CLSI breakpoints for both antibiotics versus enterococci are identical.[1,2] In our hospital, recent data from the identification and susceptibility automated system have shown that a large percentage of our clinical *E. faecalis* isolates exhibit an unusual phenotype being penicillin-resistant but ampicillin-susceptible. The