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Isolation of ciprofloxacin-resistant *Legionella pneumophila* in a patient with severe pneumonia

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Sir,

Legionella species are responsible for 1%–5% of cases of community-acquired pneumonia. *Legionella pneumophila* serogroup 1 (SG1) accounts for >90% of Legionnaires' disease (LD) in North America and Europe[1] and is the cause of significant mortality. The mortality rate among patients with *L. pneumophila* infections continues to be high, up to 26%.[2] The antimicrobial agents most commonly used for treatment of LD are fluoroquinolones (e.g. ciprofloxacin or levofloxacin) and macrolides.[3] In recent studies, we established wild-type distributions and determined the epidemiological cut-off values (ECOFFs) in clinical *L. pneumophila* SG1 isolates for 10 antimicrobials commonly used for the treatment of *Legionella* infections.[4]

A patient sought care at his general practitioner after several days of falling and body pains. On examination, the patient appeared ill and was sent to the emergency department of a nearby hospital. The initial chest radiograph demonstrated an infiltrate of the left lower lung field. The patient was admitted to the intensive care unit. Blood cultures were taken and antibiotic treatment was started with cefazolin and gentamicin. Urine was examined for the presence of *Legionella* antigens and when this test was reported positive, treatment was switched to 400 mg of ciprofloxacin intravenously twice daily. After initial improvement the clinical condition of the patient deteriorated, leading to intubation and mechanical ventilation. A new chest radiograph revealed a diffuse interstitial pneumonia. Bronchoalveolar lavage (BAL) was performed 4 days after treatment with ciprofloxacin was started and the patient slowly recovered. Eventually, culture of the BAL grew *L. pneumophila* SG1 after 4 days of ciprofloxacin treatment. After 10 days, the patient could be transferred to the ward. Therapy was then switched to 500 mg of clarithromycin orally twice daily. The patient's further recovery was uncomplicated.

The *L. pneumophila* SG1 strain was sent, as part of a national *Legionella* outbreak detection programme, to the reference laboratory for *Legionella* in Haarlem, The Netherlands.[5] Susceptibility testing for ciprofloxacin was performed with Etest and an MIC value of ciprofloxacin of 2 mg/L was found. This value is outside the wild-type distribution range ECOFF = 1 mg/L as previously described and therefore potentially resistant.[4]

For sequencing of *gyrA* and *gyrB* (DNA gyrase) and *parC* and *parE* (topoisomerase IV) genes, extraction of *L. pneumophila* DNA was performed. The DNA extraction was performed by use of Qiagen's BioRobot® EZ1 (Hilden, Germany) according to the manufacturer's instructions. The sequencing reaction was performed twice by using primer systems previously described for the *L. pneumophila* SG1 strain Paris.[6] A comparative analysis of the obtained sequences was done using the published *L. pneumophila* genomes and data from the literature describing mutations in the quinolone resistance-determining region (QRDR) of type II topoisomerase of *L. pneumophila* by using DNAStar (WI, USA) software and the NCBI database.[6,7] For control experiments, the wild-type strain MTZ OLDA and a spontaneous quinolone-resistant mutant of this strain were used. MTZ OLDA is an environmental isolate (*L. pneumophila* SG1), isolated from the water supply of a large building.[8] A point mutation in the QRDR of the *gyrA* gene was identified and this mutation led to an amino acid exchange at position 83 (*Escherichia coli* numbering system). The result of this amino acid exchange is a change in ciprofloxacin susceptibility. Mutation at the same position (amino acid 83) has also been reported for other spontaneous quinolone-resistant mutants (Table 1).

It is known that, in general, pathogens can become resistant during the course of a patient's therapy and also induction of resistance upon exposure to antibiotics has been described.[6,7] The origin of resistance in the clinical isolate is as yet unclear. There are two possibilities. The first is that the patient contracted an *L. pneumophila* SG1 strain with this point mutation from the environment. Alternatively, the mutation occurred during the

Table 1. Amino acid position 83 in the QRDR of the *gyrA* gene from *L. pneumophila* SG1 (*E. coli* numbering system)

Strain	Amino acid codon	Amino acid	MIC (mg/L)			Method used	Reference/accession number
			ciprofloxacin	levofloxacin	moxifloxacin		
Philadelphia 1	ACA	Thr	0.012	ND	ND	microdilution	8
Paris	ACA	Thr	ND	ND	0.0625	microdilution	6
Lens	ACA	Thr	ND	ND	ND		CR628337[a]
Corby	ACA	Thr	0.014	ND	ND	microdilution	8
Alcoy	ACA	Thr	ND	ND	ND	—	CP001828[a]
HL06041035	ACA	Thr	ND	ND	ND	—	FQ958211[a]
Lorraine	ACA	Thr	ND	ND	ND	—	FQ958210[a]
Strain 1							
wild-type	ACA	Thr	0.015	ND	ND	microdilution	7
in vitro mutant	AAA	Lys	0.25	ND	ND		
Strain 2							
wild-type	ACA	Thr	0.015	ND	ND	microdilution	7
in vitro mutant	ATA	Ile	0.25	ND	ND		
LP4							
wild-type	ACA	Thr	ND	ND	0.0625	microdilution	6
in vitro mutant	ATA	Ile	ND	ND	32		
MTZ OLDA							
wild-type	ACA	Thr	0.38	0.094	0.38	Etest	this study
in vitro mutant[b]	ATA	Ile	2	0.5	1.5		
Wild-type isolate #123	GCA	Ala	2[c]	0.75[d]	0.75[d]	Etest	this study

ND, not determined.
[a]NBCI accession number.
[b]Mutants selected by ciprofloxacin.
[c]Determined in both laboratories.
[d]Bruin *et al.*[4]

course of the patient's ciprofloxacin therapy. The start of antimicrobial therapy with ciprofloxacin was 4 days before collecting BAL material for culturing of *Legionella* and it could well be that the mutation did occur during that period. However, the initial response of the patient to therapy was poor and it was only with sustaining therapy in intensive care that recovery occurred. Although *Legionella* infections often result in severe disease, the elevated MIC of ciprofloxacin for this isolate may have contributed here. Our finding emphasizes the need for culture of clinical samples for *Legionella* and susceptibility testing of *Legionella* isolates.

This is, to our knowledge, the first report of a ciprofloxacin-resistant *L. pneumophila* isolated from a clinical specimen. Clinicians should be aware that a lack of clinical response during treatment with quinolones could be due to resistance. There is a clear need to perform susceptibility testing for clinical *L. pneumophila* isolates.

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Transparency declarations

None to declare.

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Molecular surveillance and prevalence of carbapenem-resistant Enterobacteriaceae in Northern Taiwan

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Sir,

Carbapenem resistance among Enterobacteriaceae has recently become an urgent problem in terms of healthcare-associated infections worldwide. The Taiwan Nosocomial Infection Surveillance System documented an annual increase in the proportion of carbapenem-resistant Enterobacteriaceae (CRE) from 1% in 2003 to 10% in 2012. This study provides updated data on the types and prevalence of CRE isolated in Northern Taiwanese hospitals.

All clinical CRE isolates (MICs of ertapenem ≥1 mg/L) were collected from a medical centre (comprising two branches) in Northern Taiwan from January 2009 to December 2012. The identification was performed using the VITEK2 system. MICs were interpreted according to the CLSI criteria (2012). The β-lactamase-encoding genes were analysed by performing PCR with plasmid DNA templates and gene sequencing. All primers used, such as those used for bla_{DHA}-type, bla_{CMY}-type, bla_{TEM}-type, bla_{SHV}-type, bla_{CTX-M}-type, bla_{IMP}-type and bla_{KPC}-type genes, have previously been described.[1–7] The genetic relationship of the isolates was investigated by PFGE[8] following the digestion of intact genomic DNA with XbaI.

In total, 210 CRE isolates were collected—100 *Klebsiella pneumoniae*, 53 *Escherichia coli*, 41 *Enterobacter cloacae* and 16 other isolates (1 *Klebsiella oxytoca*, 1 *Citrobacter freundii*, 2 *Providencia rettgeri*, 8 *Serratia* spp. and 4 *Enterobacter aerogenes*). These isolates predominantly originated from urine, blood and respiratory materials (37.6%, 32.4% and 10.0%, respectively).

Nearly all CRE isolates were also resistant to ceftazidime and cefotaxime, and were in part susceptible to meropenem, imipenem and doripenem (Table S1, available as Supplementary data at *JAC* Online). Carbapenem-resistant *K. pneumoniae* isolates were frequently co-resistant to cefpirome and ciprofloxacin, and were in part susceptible to amikacin and gentamicin, but remained susceptible to colistin and tigecycline. The carbapenem-resistant *E. coli* isolates were also frequently co-resistant to cefpirome and ciprofloxacin, and in part susceptible to gentamicin, but were still susceptible to amikacin, colistin and tigecycline. The carbapenem-resistant *E. cloacae* isolates were in part susceptible to cefpirome, ciprofloxacin, gentamicin and tigecycline, but were still susceptible to amikacin.

One hundred and thirty CRE isolates (61.9%; 130/210) exhibited an AmpC β-lactamase phenotype; bla_{DHA-1} was most prevalent in carbapenem-resistant *K. pneumoniae* (85.0%; 85/100), whereas bla_{CMY-2} was predominant in carbapenem-resistant *E. coli* (79.2%; 42/53) (Table 1). Among the 134 CRE isolates (63.8%; 134/210) harbouring ESBL genes, $bla_{CTX-M-14}$ was predominant in carbapenem-resistant *K. pneumoniae* (88.0%, 88/100), and 77.0% (77/100) of carbapenem-resistant *K. pneumoniae* carried bla_{DHA-1} and $bla_{CTX-M-14}$ simultaneously. Twenty-two carbapenem-resistant *E. coli* isolates (41.5%; 22/53) carried the ESBL genes $bla_{CTX-M-15}$, $bla_{CTX-M-55}$, $bla_{CTX-M-14}$, $bla_{CTX-M-27}$, bla_{SHV-12} and $bla_{TEM-176}$. Nineteen carbapenem-resistant *E. cloacae* isolates (46.3%; 19/41) carried bla_{SHV-12}. In addition, one *K. oxytoca* isolate carried bla_{SHV-12}, one *C. freundii* isolate carried bla_{DHA-1}, one *P. rettgeri* isolate carried bla_{CMY-2} and one *P. rettgeri* isolate carried $bla_{CTX-M-14}$. Huang *et al*.[4] reported that the bla_{DHA-1} gene was found in 58.3% of carbapenem-resistant *K. pneumoniae* isolates and that the bla_{CMY-2} gene was found in 93.8% of carbapenem-resistant *E. coli* isolates. The bla_{SHV-5} gene was found in 75.0% of carbapenem-resistant *K. pneumoniae* isolates. We propose that the types and prevalence of β-lactamases among CRE isolates exhibit substantial geographical differences.

Carbapenemase genes were detected in 3.8% (8/210) of isolates. Six of these isolates (one *K. pneumoniae* and five *E. cloacae*) expressed bla_{IMP-8}, and two carbapenem-resistant *K. pneumoniae* isolates expressed the gene encoding KPC-2. However, bla_{VIM}, bla_{OXA} and bla_{NDM-1} were not detected in this study. The five bla_{IMP-8}-expressing *E. cloacae* isolates all came from one hospital; they simultaneously produced SHV-12 and appeared to be resistant to all carbapenems and tigecycline, but were still susceptible to amikacin, gentamicin and colistin. Two dominant pulsotypes were observed in these isolates, suggesting that a clonal spread had occurred within the hospital. Furthermore, one bla_{IMP-8}-expressing isolate of carbapenem-resistant *K. pneumoniae* also expressed DHA-1, TEM-1, SHV-11 and CTX-M-14, and was resistant to meropenem, ertapenem, amikacin and tigecycline, but susceptible to imipenem, doripenem and colistin.

Two carbapenem-resistant *K. pneumoniae* isolates that carried bla_{KPC-2} had been identified by the end of 2012, and these were