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Linkage of ciprofloxacin resistance with a single genotypic cluster of *Klebsiella pneumoniae*

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Abstract

The objective of this study was to examine the epidemiology of ciprofloxacin-resistant, extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* strains. Sixty-nine unique patient isolates of *K. pneumoniae* isolated from a variety of clinical specimens submitted to the clinical bacteriology laboratories of The Royal Infirmary of Edinburgh and associated General Practices were identified and susceptibility testing was performed with the Vitek system. Strains flagged as ESBL-positive by the Vitek system were subjected to isoelectric focusing. The results suggested that all 69 isolates harboured at least one ESBL, which was later confirmed by polymerase chain reaction (PCR) with bla_{TEM} and/or bla_{SHV} primers. The purified PCR product was subjected to automated sequencing and the results were compared with the BLAST online search engine. Of the 69 isolates, 32 (46.4%) were found to be resistant to ciprofloxacin, 11 (15.9%) were intermediate and 26 (37.7%) were sensitive. To investigate the epidemiological relationship between the ciprofloxacin-resistant ESBL-positive strains, pulsed-field gel electrophoresis (PFGE) was performed. Rapidest software was used to calculate the genetic distance by the Nei distance method. PFGE analysis indicated that the clinical isolates belonged to four distinct genotype clusters (Groups A, B, C and D); each group or cluster was homogeneous or compact with respect to certain characteristics. Group A consisted of 25 isolates, group B of 3 isolates and Groups C and D of 2 isolates each. These results indicate that the spread of resistance is largely as a result of the dissemination of a single clonal strain. PCR was used to amplify the *gyrA* and *parC* genes from genomic DNA of the ciprofloxacin-resistant isolates. The amplified product was sent for analysis by automated DNA sequencing and the resulting DNA sequences were compared with the *gyrA* gene of *K. pneumoniae*. The sequencing results demonstrated that alteration of the GyrA subunit of DNA gyrase at amino acid 83 and/or amino acid 87 plays a central role in conferring high-level quinolone resistance in *K. pneumoniae* possessing ESBLs.

Keywords: *Klebsiella pneumoniae*; Ciprofloxacin resistance; Epidemiology

1. Introduction

When ciprofloxacin was first introduced, resistance in *Klebsiella pneumoniae* and other Enterobacteriaceae, such as *Escherichia coli*, was virtually unknown. However, in the last 10 years, cases of bacteraemia with ciprofloxacin-resistant *E. coli* have increased in number, together with upward trends in the use of quinolones in the community and in hospitals [1]. The occurrence of ciprofloxacin resistance in *K. pneumoniae* is now known and, indeed, exceeds 5% in many centres in North America, Europe and Asia [2–5]. At the same time that resistance to ciprofloxacin emerged, resistance to β-lactam antibiotics became prominent. This resistance was largely as a result of extended-spectrum β-lactamases (ESBLs), which mediate resistance to newer β-lactam agents possessing an oxyamino group, such as ceftazidime, ceftriaxone, cefotaxime and aztreonam. In addition, plasmids that carry and contain genes encoding ESBLs also harbour genes that encode mechanisms of resistance to other classes of antimicrobials. In many regions of the world where antibiotic use is high, ESBLs are present in ca. 25% of all *K. pneumoniae* isolates from intensive care units, and patient-to-patient transfer of resistant organisms frequently occurs [6].

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The epidemiology of infection with ciprofloxacin-resistant *K. pneumoniae* has not previously been described in Edinburgh. This study was performed to determine the relationship between ESBL production and ciprofloxacin resistance in *K. pneumoniae* isolated from the Royal Infirmary of Edinburgh and associated General Practices.

2. Materials and methods

Sixty-nine unique patient clinical isolates of *K. pneumoniae* were collected from various clinical samples submitted to the clinical bacteriology laboratories of the Royal Infirmary of Edinburgh and surrounding district. These were collected between May 1999 and March 2000. The isolated strains were stored on nutrient agar slopes and transported to Kuwait for further analysis.

2.1. Identification and susceptibility testing

The identity of all 69 isolates was confirmed with Vitek GNI+ cards (BioMérieux Ltd., Basingstoke, UK) and ESBL production was screened for with the integrated ESBL screen on the Vitek GNS-532 card. ESBL production was further confirmed using cefotaxime and ceftazidime Etest ESBL strips (AB BIODISK, Solna, Sweden).

Minimum inhibitory concentrations (MICs) for the strains of *K. pneumoniae* to ciprofloxacin were confirmed by the agar dilution method following the guidelines of the National Committee for Clinical Laboratory Standards [7]. Ciprofloxacin powder was provided by Bayer AG (Newbury, UK). Ciprofloxacin resistance was defined as a MIC ≥ 4 μg/mL, intermediate as MIC = 2 μg/mL and susceptibility as MIC ≤ 1 μg/mL [7].

2.2. Isoelectric focusing (IEF)

IEF was performed by the method of Mathew et al. [8].

2.3. Polymerase chain reaction (PCR)

Primers used to amplify the *gyrA* gene had the following nucleotide sequences: *KpnGyr*A1, 5′-AAT ATG TTC CAT CAG CCC-3′; and *KpnGyr*A2, 5′-TGC GAG AGA AAT TAC ACC-3′. Primers used to amplify the *parC* gene had the following nucleotide sequences: *Par*C1, 5′-CTG AAT GCC AGC GCC AAA TT-3′; and *par*C2, 5′-TGC GGT GGA ATA TCG GTC GC-3′.

2.4. Restriction fragment length polymorphism (RFLP)

RFLP using a *Hinf*I restriction endonuclease assay (Promega Ltd., Southampton, UK) was used to screen for the specific mutation resulting in the substitution of amino acid threonine-83 in the quinolone resistance-determining region (QRDR) of the *gyrA* gene.

2.5. DNA sequencing

The amplified PCR product for *gyrA* was sent for analysis with an automated DNA sequencing system. The resulting DNA sequences were compared with the *Klebsiella gyrA* gene from GenBank at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast).

2.6. Pulsed-field gel electrophoresis (PFGE)

PFGE was performed using a Bio-Rad CHEF-DR®II apparatus (Bio-Rad, Hercules, CA). *Xba*1 (Promega Ltd.) was used as the restriction enzyme.

2.7. Outer membrane proteins (OMPs)

Electrophoretic analysis of OMPs by SDS-PAGE was performed in 11% acrylamide–0.35% bisacrylamide–0.1% SDS using Laemmli's buffer.

3. Results

Thirty-two of the 69 ESBL-producing *K. pneumoniae* (46.4%) were found to be resistant to ciprofloxacin, 11 (15.9%) were intermediate and 26 (37.7%) were sensitive. PFGE analysis indicated that the ciprofloxacin-resistant isolates belonged to four distinct genotype clusters (Groups A, B, C and D); each group or cluster was homogeneous or very closely related with respect to each other. Group A consisted of 25 isolates, Group B of 3 isolates and Groups C and D of 2 isolates each. Table 1 shows the results of β-lactamase analysis and the different PFGE groups for the 32 ciprofloxacin-resistant isolates.

3.1. IEF

The β-lactamase complement of the 32 ciprofloxacin-resistant ESBL-positive isolates was investigated. All isolates harboured a β-lactamase with an isoelectric point (pI) of 7.6, which was indicative of either the parental enzyme SHV-1 or its ESBL derivative, SHV-2. Twenty-five of the 32 strains also demonstrated a TEM-derivative β-lactamase with a pI of 5.4 (indicative of the parental enzyme TEM-1), and 4 of these 25 isolates also harboured another β-lactamase with a

Table 1
Results of isoelectric focusing and pulsed-field gel electrophoresis (PFGE)

PFGE group	pI values	Extended-spectrum β-lactamase	Total no.
A	5.4 and 7.6	TEM-1 and SHV-2	21
A	5.4, 7.6 and 8.2	TEM-1, SHV-2 and SHV-5	4
B	7.6	SHV-2	3
C	7.6	SHV-2	2
D	7.6	SHV-2	2

pI, isoelectric point.