RESEARCH NOTE

Molecular characterisation of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* spp. isolates at a tertiary-care centre in Lebanon

S. S. Kanj[1], J. E. Corkill[2], Z. A. Kanafani[1], G. F. Araj[3], C. A. Hart[2], R. Jaafar[4] and G. M. Matar[4]

[1]Division of Infectious Diseases, American University of Beirut Medical Center, Beirut, Lebanon, [2]Department of Medical Microbiology, University of Liverpool, Liverpool, UK, [3]Department of Pathology & Laboratory Medicine and [4]Department of Microbiology and Immunology, American University of Beirut Medical Center, Beirut, Lebanon

ABSTRACT

The prevalence of $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} genes among extended-spectrum β-lactamase (ESBL)-producing clinical isolates of *Escherichia coli* (n = 50) and *Klebsiella* spp. (n = 50) from Lebanon was 96%, 57% and 67%, and 40%, 82% and 84%, respectively. Genotyping revealed that the clonal diversity was unrelated to the presence of *bla* genes. Sequence analysis of 16 selected isolates identified the $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$ and six bla_{SHV} genes, as well as the gene encoding the quinolone-modifying enzyme AAC(6′)-Ib-cr. The genes encoding CTX-M-15 and AAC(6′)-Ib-cr were carried on a 90-kb plasmid of the pC15-1a or pCTX-15 type, which transferred both ESBL production and quinolone resistance from donors to transconjugants.

Keywords AAC(6′)-Ib-cr, *Escherichia coli*, extended spectrum β-lactamase, *Klebsiella* spp., plasmid, quinolone resistance

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Corresponding author and reprint requests: S. S. Kanj, American University of Beirut Medical Center, Cairo Street PO Box 11-0236, Riad El Solh 1107 2020, Beirut, Lebanon
E-mail: sk11@aub.edu.lb

Although geographical variations exist, Gram-negative bacteria producing extended-spectrum β-lactamases (ESBLs) have become an increasing problem worldwide [1]. In Lebanon, the prevalence of ESBL-producing isolates reached 8% among *Escherichia coli* and 20% among *Klebsiella* spp. in 2005 [2]. Until recently, TEM- and SHV-derived enzymes have been the ESBLs encountered most commonly in *E. coli* and *Klebsiella* spp. However, CTX-M ESBLs have become more frequent, particularly in *E. coli*, during the past decade [3]. Given the scarcity of data from Lebanon [4,5], the aim of the present study was to characterise ESBL-producing isolates of *E. coli* and *Klebsiella* spp. at the molecular level in a non-outbreak setting.

Clinical isolates of ESBL-producing *E. coli* (n = 50) and *Klebsiella* spp. (n = 50) were collected at the American University of Beirut Medical Center during 1997–2002 and 2004–2005. ESBL production was confirmed using ceftazidime/clavulanate and cefotaxime/clavulanate disks according to CLSI recommendations [6]. DNA was extracted using the GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's guidelines. Each isolate was genotyped by random amplified polymorphic DNA (RAPD) analysis using a Ready-To-Go RAPD Analysis Kit (Amersham Pharmacia Biotech) according to the manufacturer's recommendations.

All isolates were screened by PCR for bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$ genes using primers CTX-M-1-F and CTX-M-15-R for $bla_{\text{CTX-M}}$ genes (873 bp) [4], OT-3 and OT-4 for bla_{TEM} genes (858 bp) [7], and SHV-F and SHV-R for bla_{SHV} genes (795 bp) [5]. In addition, on the basis of the *bla* genes detected, 16 isolates (eight *E. coli* and eight *Klebsiella* spp.) were selected for screening and subsequent sequencing analysis of *qnrA* (primers QP1 and QP2) [8], *qnrB* (primers FQ1 and FQ2 [9]), *qnrS* (primers QnrS-A2 and QnrS-B2 [10]), class I integrons (5′-CS and 3′-CS [11]), class II integrons (Igr2-f and Igr2-r [12]), bla_{SHV} (primers SHV-FOR and SHV-REV [13]), *aac(6′)*-Ib-cr [14], $bla_{\text{OXA-1}}$ [15], bla_{TEM} (primers LCM1 and LCM2 [16]), $bla_{\text{CTX-M}}$ (primers CTX-MA1 and CTX-MA2 [17]) and IS*Ecp1B* (primers CTX-MA2 [17] and PROM+ [18]), as well as plasmid incompatibility typing (Inc FII, primers CA 1 and OR 1 [19]). All amplifications were performed as described previously [5]. Amplicons were detected follow-

ing electrophoresis on agarose gels, and were photographed using an Olympus 3.0 camera (Olympus, Tokyo, Japan) and the Doc-It program (Ultra-Violet Products, Cambridge, UK). The amplicons of the 16 selected isolates were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and were then sequenced in both directions using Big-Dye 3.1 chemistry (PE Biosystems, Foster City, CA, USA) and an ABI 31000 automated sequencer (Applied Biosystems, Warrington, UK). Sequence data were analysed using Seqman software (DNAStar Inc., Madison, WI, USA) [20].

Conjugation experiments were performed with the 16 selected isolates using *E. coli* J53 (sodium azide-resistant) as the recipient on MacConkey agar containing sodium azide 100 mg/L and cefotaxime 2 mg/L. Plasmids were extracted from donor isolates and transconjugants using a Qiagen Midi Plasmid Kit, (Qiagen), visualised following electrophoresis on agarose 1% w/v gels, and photographed as described above. Antimicrobial susceptibility tests on *E. coli* J53 transconjugants and PCR analysis of the genes carried by the extracted plasmids were performed to confirm plasmid transfer from the donors to the recipients [20].

The majority (96.1%) of *E. coli* isolates harboured a $bla_{\text{CTX-M}}$ gene; bla_{SHV} and bla_{TEM} genes were detected less frequently (66.7% and 56.9%, respectively). All three types of β-lactamase gene were found concomitantly in 45.1% of *E. coli* isolates. A bla_{SHV} gene was most common in *Klebsiella* spp. (84.1%), while bla_{TEM} and $bla_{\text{CTX-M}}$ genes occurred in 82.0% and 40.0% of isolates, respectively; only 24.0% of the *Klebsiella* spp. isolates harboured all three resistance genes. These results are in accord with previous studies describing the increasing global prevalence of CTX-M enzymes [3], but a previous study in Lebanon [4] failed to detect any $bla_{\text{CTX-M}}$ genes, probably because of geographical variations in the distribution of plasmid-encoded enzymes.

Table 1 shows the molecular characteristics of the 16 selected isolates. All except K6 and K38 harboured the $bla_{\text{CTX-M-15}}$ gene, the $bla_{\text{OXA-1}}$ gene, and the gene encoding the quinolone-modifying enzyme AAC(6′)-Ib-cr. The $bla_{\text{TEM-1}}$ gene was detected in 12 isolates and the bla_{SHV} gene in nine isolates (one *E. coli* and eight *Klebsiella* spp.), with six different bla_{SHV} types.

Conjugation experiments revealed that $bla_{\text{CTX-M-15}}$ and the mobilising insertion sequence IS*Ecp1* were transferred to all the transconjugants, rendering them resistant to third-generation cephalosporins (Table 1). The quinolone-modifying enzyme AAC(6′)-Ib-cr was also transferable, increasing the resistance of transconjugants to ciprofloxacin and norfloxacin, but not to nalidixic acid. Class I integrons were detected in transcon-

Table 1. Molecular characteristics of 16 selected isolates of *Escherichia coli* and *Klebsiella* spp.

Isolate	*qnrA/B*	*qnrS*	AAC(6′)-Ib	AAC(6′)-Ib-cr	CTX-M-15/ IS*Ecp1*	AAC(3)-II	OXA-1	SHV type	TEM-1	Inc FII plasmid	*Hpa*I digest profile[a]	Resistance in transconjugants
E11	–	–	–	+	+	+	+	–	+	–	–	ND
E12	–	–	–	+	+	+	+	–	–	+	+ pC15-1a	AMX/CPD/ATM/CAZ/GEN/KAN
E13	–	–	–	+	+	–	+	–	+	+	–	ND
E17	–	–	–	+	+	+	+	–	+	+	+ pCTX-15	AMX/CPD/ATM/GEN/KAN/TRI
E18	–	–	–	+	+	–	+	–	+	+	+ pCTX-15	AMX/CPD/ATM/CAZ/KAN
E23	–	–	–	+	+	–	+	–	+	+	–	ND
E26	–	–	–	+	+	–	+	–	+	+	+ pCTX-15	AMX/CPD/ATM/CAZ/KAN
E42	–	–	–	–	–	–	–	12	–	–	–	ND
K3	–	–	–	+	+	+	+	5	+	+	+ Not readable	AMX/CPD/ATM/CAZ/GEN/KAN
K6	–	–	+	–	–	–	–	12	+	–	–	ND
K13	–	–	–	+	+	+	+	1	+	+	+ pC15-1a	AMX/CPD/ATM/CAZ/GEN/KAN
K16	–	–	–	+	+	+	+	28	–	–	+ Other[b]	ND
K38	–	–	+	–	–	–	–	12	+	–	–	ND
K7	–	–	–	–	+	–	–	77	+	–	–	ND
K10	–	–	–	–	+	–	–	11	+	–	–	ND
K14	–	–	–	–	–	–	–	12	–	–	–	ND

[a]Transconjugant *Hpa*I digest profile as defined by Boyd *et al.* [22].
[b]Not pC15-1a or pCTX-15.
AMX, amoxycillin; CPD, cefpodoxime; ATM, aztreonam; CAZ, ceftazidime; GEN, gentamicin; KAN, kanamycin; TRI, trimethoprim; ND, not determined.

jugants derived from E17 and K16, but the bla_{TEM-1} and bla_{SHV} genes were not transferable by conjugation to *E. coli* J53, indicating that they are probably chromosomally encoded. Analysis of transconjugants confirmed that the genetic determinants of AAC(6′)-Ib-cr, class I integrons, CTX-M-15 and IS*Ecp1* were encoded on a large 90-kb plasmid. Restriction digest analysis (using *Hpa*I) of the transferred plasmids indicated that most were indistinguishable from the pC15-1a or pCTX-15 plasmids described previously. The antimicrobial resistances transferred to five transconjugants are shown in Table 1.

RAPD analysis revealed a range of banding profiles among both the *E. coli* (18 profiles) and the *Klebsiella* spp. (15 profiles) isolates. No association was found between the RAPD profiles and the β-lactamase genes carried by the isolates, indicating that antimicrobial resistance is probably acquired through horizontal plasmid transmission. These findings are commensurate with those of previous studies [21]. The finding of the transferable quinolone resistance gene AAC(6′)-Ib-cr, together with ESBL genes, on plasmids from ten of 13 isolates is of particular concern, since use of either quinolones or extended-spectrum β-lactams would provide a selective advantage for these bacteria.

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RESEARCH NOTE

Identification of *Bordetella* spp. in respiratory specimens from individuals with cystic fibrosis

T. Spilker, A. A. Liwienski and J. J. LiPuma

Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA

ABSTRACT

Bordetella spp. are not normally included when considering the opportunistic bacterial species that are typically involved in respiratory tract infections in individuals with cystic fibrosis (CF). By using a combination of bacterial genotyping and 16S rDNA sequencing, *Bordetella* spp. were identified in cultures obtained from 43 individuals with CF. Most (n = 23) patients were infected with *Bordetella bronchiseptica/parapertussis*; five were infected with *Bordetella hinzii*, four with *Bordetella petrii*, three with *Bordetella avium*, and eight with unidentified *Bordetella* spp. Consideration should be given to the presence of these organisms in the evaluation of CF sputum cultures.

Keywords 16S rDNA sequencing, *Bordetella* spp., cystic fibrosis, rep-PCR, respiratory tract infection, sputum

Corresponding author and reprint requests: J. J. LiPuma, Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, 1150 W. Medical Center Dr, 8323 MSRB III, SPC5646, Ann Arbor, MI 48109, USA
E-mail: jlipuma@umich.edu

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Cystic fibrosis (CF) is an autosomal recessive disease, characterised by defective chloride ion channels that result in multi-organ dysfunction, most notably affecting the respiratory tract. The alteration in the pulmonary environment is associated with increased susceptibility to bacterial infection. Recent advances in bacterial taxonomy and improved microbial identification systems have led to an increasing recognition of the diversity of bacterial species involved in CF lung infection. Many such species are opportunistic human pathogens, some of which are rarely found in other human infections [1]. Processing of CF respiratory cultures therefore employs selective media and focuses on detection of uncommon human pathogens. In recent years, the use of molecular methods (e.g., species-specific PCRs and assays based on identifying restriction fragment length polymorphisms) has complemented the diagnostic capacity of routine phenotypic analyses. The present study describes the use of molecular methods to identify *Bordetella* spp. in respiratory tract cultures from 43 patients with CF.

The 43 bacterial isolates included in the study were recovered between June 2001 and June 2007 from sputum cultures obtained from 43 CF patients receiving care in 19 treatment centres in the USA. All isolates were sent to the *Burkholderia cepacia* Research Laboratory and Repository (BcRLR; University of Michigan, Ann Arbor, Michigan, USA) for microbiological evaluation. Reference strains comprised *Achromobacter xylosoxidans* ATCC 9220, *Alcaligenes faecalis* ATCC 337, *Alcaligenes faecalis* subsp. *faecalis* LMG 1229^T, *Alcaligenes* sp. ATCC 21030, *Bordetella avium* LMG 1852^T, *Bordetella bronchiseptica* LMG 1232^T, *Bordetella hinzii* LMG 13500, *Bordetella holmesii* LMG 15945^T, *Bordetella parapertussis* LMG 14449^T, *Bordetella pertussis* LMG 14455^T and *Bordetella trematum* LMG 13506^T. *B. pertussis* was grown on Regan–Lowe charcoal agar (BBL, Franklin Lakes, NJ, USA). All other bacterial isolates were grown aerobically on plates containing Mueller–Hinton broth (Becton Dickinson, Cockeysville, MD, USA)