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Identification of Clinical Isolates of Indole-Positive *Klebsiella* spp., Including *Klebsiella planticola*, and a Genetic and Molecular Analysis of Their β-Lactamases

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In a collection of 43 indole-positive *Klebsiella* clinical isolates, which were initially identified as *Klebsiella oxytoca*, there were 18 isolates which exhibited a pattern characteristic of extended-spectrum β-lactamase (ESBL) resistance. This study aimed to confirm their identity by biochemical tests and by PCR and to determine the genetic basis for their resistance to the β-lactams and broad-spectrum cephalosporins. Chromosomal β-lactamase genes were analyzed by PCR, and plasmid-mediated β-lactamase genes were analyzed by conjugation and transformation. There were 39 isolates which grew on melezitose but failed to grow on 3-hydroxybutyrate, confirming them as *K. oxytoca*. PCR analysis of their β-lactamase genes divided these isolates into two groups, the bla_{OXY-1} group and the bla_{OXY-2} group. Each group had β-lactamases with different isoelectric points; the bla_{OXY-1} group had β-lactamases with isoelectric points at 7.2, 7.8, 8.2, and 8.8, and the more common bla_{OXY-2} group had β-lactamases with pIs at 5.2, 5.4 (TEM-1), 5.7, 5.9, 6.4, and 6.8. A pI of 5.2 was the most frequently detected and accounted for 59% of all the bla_{OXY-2} β-lactamases. Hyperproduction of clavulanate-inhibited chromosomal β-lactamases was detected in 17 *K. oxytoca* isolates, resulting in an ESBL phenotype. *K. oxytoca* isolates having a plasmid-mediated genetic basis for their ESBL phenotype were not found, confirming that, in *K. oxytoca*, plasmids are rarely involved in conferring resistance to the newer cephalosporins. Four isolates proved to be isolates of *K. planticola* in which the β-lactamase genes failed to react with the primers used in the PCR. One *K. planticola* isolate contained a transferable plasmid harboring the SHV-5 β-lactamase gene and showed an ESBL phenotype, while the other non-ESBL *K. planticola* isolates contained chromosomal β-lactamases with isoelectric points at 7.2, 7.7, and 7.9 plus 7.2.

The genus *Klebsiella* is comprised of four species, *Klebsiella pneumoniae*, *K. oxytoca*, *K. planticola* (formerly *K. trevisanii*), and *K. terrigena* (6, 20). Modern identification systems used in many hospital diagnostic laboratories usually identify indole-negative *Klebsiella* isolates as *K. pneumoniae* and indole-positive *Klebsiella* isolates as *K. oxytoca*, unless supplementary tests are performed. However, up to 19% of *Klebsiella* isolates initially identified as *K. oxytoca* have been identified subsequently as *K. planticola* (9, 14). *K. planticola* strains were originally isolated from plant and aquatic environments (2, 6, 17) and have been associated with various human infections, including septicemia (9).

Growth on media containing melezitose, pectate, gentisic acid, and ethanolamine and no growth on media containing 3-hydroxybutyrate or histamine differentiate *K. oxytoca* from *K. planticola* (14). Monnet and Freney (14) suggest that *K. planticola* can be reliably distinguished from *K. oxytoca* by histamine and ethanolamine carbon substrate assimilation tests alone.

Apart from these substrate assimilation tests, PCR analysis of the β-lactamase gene on the chromosome of indole-positive *Klebsiella* isolates can be used to assist in the identification of the clinical isolates to the species level (8). The chromosome of *Klebsiella* spp. contains a class A β-lactamase gene, which confers resistance to penicillins but not cephalosporins (11, 24). The β-lactamase gene of *K. oxytoca* belongs to the *oxy* family, and DNA primers have been reported that recognize either the bla_{OXY-1} gene (668 bp) or the bla_{OXY-2} gene (723 bp), one of which is present in all *K. oxytoca* isolates (8). Each *K. oxytoca* isolate produces a positive PCR fragment for either the bla_{OXY-1} or the bla_{OXY-2} set of primers. Therefore, an indole-positive *Klebsiella* isolate not producing a PCR product should belong to *K. planticola*. The purpose of the present study was to use PCR methods to determine if an analysis of β-lactamase genes could be used to differentiate *K. planticola* from other isolates of indole-positive *Klebsiella* spp. The location of the β-lactamase genes in those isolates with extended-spectrum β-lactamase was also examined.

MATERIALS AND METHODS

Bacteria. A total of 43 indole-positive *Klebsiella* isolates from Australian hospitals, including Sir Charles Gairdner Hospital and Fremantle Hospital (both in Western Australia), The Canberra Hospital (Australian Capital Territory), Alfred Hospital and Monash Medical Centre (both in Victoria), and Princess Alexandra Hospital (Queensland), were investigated. All strains had been identified as *K. oxytoca* with the Vitek AutoMicrobic System (Biomerieux Vitek, Sydney, Australia). A plasmid-free nalidixic acid-resistant strain, *Escherichia coli* JP990, was used as the recipient in plasmid transfer experiments. *E. coli* strains harboring TEM-1, SHV-1, and SHV-5 β-lactamases were used as reference strains for isoelectric focusing (IEF) experiments.

Carbon substrate assimilation tests. The minimal medium, used to determine growth of indole-positive *Klebsiella* isolates on various carbon substrates, was made with the following heat-sterilized stock solutions in a final volume of 100 ml: 0.5 ml of solution A (70 g of K_2HPO_4 and 30 g of KH_2PO_4 in 100 ml of H_2O), 0.5 ml of solution B (1 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, and 0.15 g of $MgSO_4 \cdot 7H_2O$ in 100 ml of H_2O), 0.05 ml of solution C (0.15 g of citric acid and 0.15 g of $[NH_4]_2SO_4 \cdot FeSO_4 \cdot 6H_2O$ in 100 ml of H_2O), and 1 ml of solution D (20 g of $[NH_4]_2SO_4$ in 100 ml of H_2O). The carbon substrates examined, ethanolamine, gentisic acid, histamine, 3-hydroxybutyrate, melezitose, and pectate, were prepared as filter-sterilized stock solutions and used at a 0.2% (wt/vol) final concentration. For assimilation tests, isolates were grown overnight in broth,

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TABLE 1. Resistance of 39 strains of *K. oxytoca* to various β-lactams

β-Lactam[a]	Concn (mg/liter)	No. of ESBL strains (%) (n = 17)	No. of non-ESBL strains (%) (n = 22)
Amp	8	17 (100)	22 (100)
Amc	8 (4)[b]	17 (100)	6 (27)
Oth	8	17 (100)	8 (36)
Man	8	17 (100)	8 (36)
Fox	8	0 (0)	0 (0)
Azt	8	17 (100)	0 (0)
Ctx	8	0 (0)	0 (0)
Ctx	1	16 (94)	0 (0)
Imp	4	0 (0)	0 (0)
Ctz	8	0 (0)	0 (0)
Ctz	1	0 (0)	0 (0)

[a] Amp, ampicillin; Amc, co-amoxyclav; Oth, cephalothin; Man, cefamandole; Fox, cefoxitin; Azt, aztreonam; Ctx, ceftriaxone; Imp, imipenem; Ctz, ceftazidime.
[b] Amoxicillin, 8 mg/liter; clavulanic acid, 4 mg/liter.

washed in saline, and diluted to the opacity of a 0.5 McFarland opacity standard and 0.1 ml of diluted culture was added to 5 ml of assimilation medium. Growth was recorded after incubation for 24 and 48 h at 30 and 37°C, respectively.

Antibiotic susceptibility testing. The resistance phenotype of each strain was initially determined by agar dilution (18) and disk diffusion methods (19) with National Committee for Clinical Laboratory Standards interpretative criteria.

Clavulanate inhibition by double-disc diffusion. Strains resistant to broad-spectrum cephalosporins were tested for clavulanate inhibition of β-lactamase by the double-disc diffusion method described by Mulgrave and Attwood (16), which is a modification of the Casals and Pringler method (4).

PCR analysis for β-lactamase detection. Primer C, 5′-(GCG TAG CGC TGA TTA ACA CG)-3′, and primer D, 5′-(CCT GCT GCG GCT GGG TAA AA)-3′, were used to detect the bla_{OXY-1} gene, which codes for β-lactamases with pIs in the range of 7.8 to 8.8, and primer L, 5′-(CAG ATC TCG AGA AGC GTT CA)-3′, and primer M, 5′-(ACC TCT TTG CGG TTT TTC GC)-3′, were used to detect the bla_{OXY-2} gene, which codes for β-lactamases with pIs in the range of 5.2 to 6.8 (8). PCR conditions included 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 60°C (for bla_{OXY-1}) or 55°C (for bla_{OXY-2}), and extension at 72°C for 1 min. The PCR products were electrophoresed on 1.5% agarose gels, stained with ethidium bromide, and photographed with UV illumination. The PCR fragments were sized with SPP1/*Eco*RI size standards.

β-Lactamase extraction. Cells were grown overnight at 37°C in 10 ml of brain heart infusion broth. Suspensions were pelleted, washed, resuspended in 10 mM phosphate buffer (pH 7), and chilled on ice prior to sonication (MSE, London, United Kingdom). Sonicates were cleared by centrifugation at 14,000 × *g* for 10 min, and the supernatants containing the crude β-lactamase extract were stored at −80°C.

Analytical IEF. Crude β-lactamase extracts were diluted with 10 mM phosphate buffer (pH 7) until the durations of the reactions with 50 mg of Nitrocefin (Oxoid, Basingstoke, United Kingdom) per liter were within the range of 15 to 30 s. IEF was carried out with a Multiphor II unit (LKB, Bromma, Sweden) according to the manufacturer's recommendations. β-Lactamases with known pIs in the pH range of 3.5 to 9.5 (TEM-1, 5.4; SHV-1, 7.7; SHV-5, 8.2) were included in each focusing run. The gels were overlaid with 200 mg of nitrocefin per ml in 10 mM phosphate buffer (pH 7) to visualize the β-lactamases.

Resistance transfer. Conjugation experiments were conducted with broth both at room temperature overnight and at 37°C for 4 h by mixing fresh overnight broth cultures in the ratio of 1 ml of donor to 10 ml of recipient (23).

Electrotransformation was conducted with a high-efficiency electrotransformation kit (Electroporation; Bio-Rad Laboratories), using the pulse controller at 25 mF, 5.5 kV, and 200 Ω. The range of the time constant was 4 to 5 ms. Plasmid pUC18 was used as donor DNA in control experiments. Transformation was also conducted by osmotic shock (13). Transconjugants were selected on MacConkey agar containing nalidixic acid (100 mg/liter) and ampicillin (100 mg/liter), and transformants were selected on MacConkey agar containing ampicillin (100 mg/liter).

Preparation and electrophoresis of plasmid DNA. Plasmid DNA was extracted from *E. coli* by the alkaline lysis method (3) and from *Klebsiella* species according to the procedure of Domenico et al. (5). DNA was electrophoresed in 0.8% agarose gels for 2 h at 5 V cm^{-1} and sized against four reference plasmids with molecular sizes of 170, 71, 40, and 21 kb.

RESULTS

Of the 43 clinical isolates initially identified as *K. oxytoca*, 18 were resistant to 8 mg of aztreonam per ml and 1 mg of ceftriaxone per ml and were positive in the double-disc diffusion test. These properties are consistent with the characteristics of ESBL producers (7, 25). The remaining 25 isolates were susceptible to those cephalosporins and negative in the double-disc diffusion test and were classified as non-ESBL isolates. All isolates were examined for growth on ethanolamine, gentisic acid, histamine, 3-hydroxybutyrate, melezitose, and pectate. Two substrates, melezitose and 3-hydroxybutyrate, more reliably identified a subgroup among the indole-positive *Klebsiella* isolates. By using carbon substrate assimilation tests, four isolates were identified as *K. planticola*, while the remaining 39, which utilized melezitose and failed to utilize 3-hydroxybutyrate, were confirmed as *K. oxytoca.*

Antimicrobial susceptibility. All 39 *K. oxytoca* isolates were resistant to ampicillin. Seventeen isolates showed reduced susceptibility or resistance to narrow- and expanded-spectrum cephalosporins and aztreonam, and 16 exhibited resistance to ceftriaxone at 1 mg/liter. These were classified as ESBL *K. oxytoca* isolates, and the remaining 22 strains were classified as non-ESBL *K. oxytoca* isolates (Table 1). Clavulanate inhibition of β-lactamase activity was significantly greater in the non-ESBL group, as reflected in the percentage of strains resistant to co-amoxyclav (27% of non-ESBL isolates compared to 100% of ESBL isolates). In the non-ESBL group, eight (36%) strains showed resistance to cephalothin and cefamandole. Aztreonam and ceftriaxone resistance was absent from the non-ESBL *K. oxytoca* isolates. Cefoxitin resistance, which is commonly associated with chromosomally mediated β-lactamase production, was not detected in any *K. oxytoca* isolate. Also, resistance to imipenem and ceftazidime was not detected in any indole-positive *Klebsiella* isolate.

The antimicrobial resistance patterns of the four *K. planticola* isolates are presented in Table 2.

Transfer of antimicrobial resistance. In mating experiments with indole-positive *Klebsiella* donors and *E. coli* JP990, three donors produced ampicillin-resistant transconjugants. One *K. planticola* donor had a transferable plasmid with an SHV-5 gene, and it transferred resistance to ampicillin, cefotaxime, ceftazidime, ceftriaxone, aztreonam, and aminoglycosides. This *K. planticola* donor is an ESBL strain. Two non-ESBL *K. oxytoca* donors had transferable plasmids with the TEM-1 gene. Both isolates transferred ampicillin, trimethoprim, and aminoglycoside resistance. All three transferable plasmids were extracted from the *E. coli* transconjugants and appeared to be greater than 100 kb in size.

Plasmid DNA could not be detected in agarose gels from any ESBL *Klebsiella* strain. In transformation experiments, three preparations of plasmid DNA extracted from 17 ESBL

TABLE 2. β-Lactamase isoelectric points and antibiotic resistance patterns for *K. planticola* strains

Strain no.	Resistance pattern[a]	pI
1	Amp, Nit, Oth, Man, Azt, Cft, Ctx, Gm, Tb	8.2[b]
2	Amp, Nit, Oth, Man	7.9 + 7.2
3	Amp, Nit	7.7
4	Amp, Oth, Man, Fox	7.2

[a] Gm, gentamicin; Tb, tobramycin; Nit, nitrofurantoin; Amp, ampicillin; Oth, cephalothin; Man, cefamandole; Fox, cefoxitin; Azt, aztreonam; Cft, cefotaxime; Ctx, ceftriaxone.
[b] pI of SHV-5.

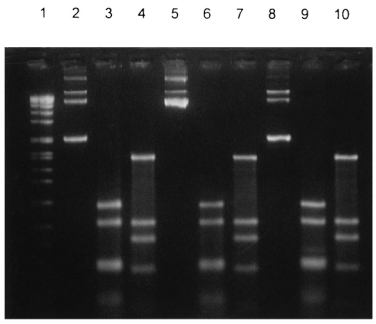

FIG. 1. Shown is a 1.5% agarose gel of plasmid DNA recovered from *E. coli* transformants selected on ampicillin. Lanes 2 to 4, plasmid DNA from LM169; lanes 5 to 7, plasmid DNA from LM206; lanes 8 to 10, plasmid DNA from 29086. Lane 1, SPP1/*Eco*RI DNA molecular weight markers; lanes 2, 5, and 8, undigested covalently closed circular DNA; lanes 3, 6, and 9, *Alu*I digests of the plasmids; lanes 4, 7, and 10, *Hin*fI digests of the plasmids. All three plasmids have identical restriction band patterns and the *Hin*fI and other restriction enzyme patterns (data not shown) indicate a plasmid size of 2.6 kb.

K. oxytoca isolates produced ampicillin-resistant transformants in *E. coli* JP990 by either electrotransformation or osmotic shock. All transformants contained an identical 2.6-kb plasmid, as determined by restriction enzyme mapping (Fig. 1). The donors, whose β-lactamase had a pI of 5.2, were resistant to ampicillin, narrow- and expanded-spectrum cephalosporins, and aztreonam and showed intermediate susceptibility to ceftriaxone (MICs, >1 to <8 mg/liter). By comparison, the transformants were susceptible to aztreonam and ceftriaxone and resistant to ampicillin and narrow- and expanded-spectrum cephalosporins and had a β-lactamase with a pI of 5.2.

PCR tests. All 43 indole-positive *Klebsiella* isolates were analyzed by PCR with both OXY-1 gene and OXY-2 gene primers. A total of 27 strains (13 ESBL and 14 non-ESBL) were OXY-2 gene positive, while 12 strains (four ESBL and eight non-ESBL) were OXY-1 gene positive. Four strains (one ESBL and three non-ESBL) contained neither OXY-1 nor OXY-2 genes (Fig. 2). Based on carbon substrate assimilation tests, these four PCR-negative strains were identified as *K. planticola.*

Three ampicillin-resistant *E. coli* transformants containing the 2.6-kb plasmid were negative for both OXY-1 and OXY-2 genes, while the donor *Klebsiella* isolates exhibited a positive reaction when tested with the OXY-2 gene primers.

Determination of the β-lactamase isoelectric points. The isoelectric points of the β-lactamase enzymes produced by each of the four *K. planticola* isolates (Table 2) and the 39 *K. oxytoca* isolates (Table 3) were determined. Chromosomal β-lactamase was produced in abnormally high amounts by the 17 ESBL *K. oxytoca* strains, based on short nitrocefin reaction times. The 22 non-ESBL *K. oxytoca* strains produced detectable amounts of β-lactamase. However, the crude extracts from the non-ESBL isolates with resistance to the cephalosporins required dilution to reduce activity for successful IEF.

Six β-lactamases, with pIs of 5.2, 5.4, 5.7, 5.9, 6.5, and 6.8, comprised the bla_{OXY-2} group. The bla_{OXY-2} β-lactamase with a pI of 5.2 was the predominant enzyme across all strains (59 and 47% in ESBL and non-ESBL strains, respectively). β-Lactamases with alkaline pIs of 7.8, 8.0, 8.2, and 8.8 comprised the bla_{OXY-1} group. Co-amoxyclav susceptibility and prolonged nitrocefin reaction times in the crude β-lactamase extracts suggested that enzyme hyperproduction was absent in all four *K. planticola* isolates. The isoelectric points and resistance patterns of *K. planticola* strains are listed in Table 2.

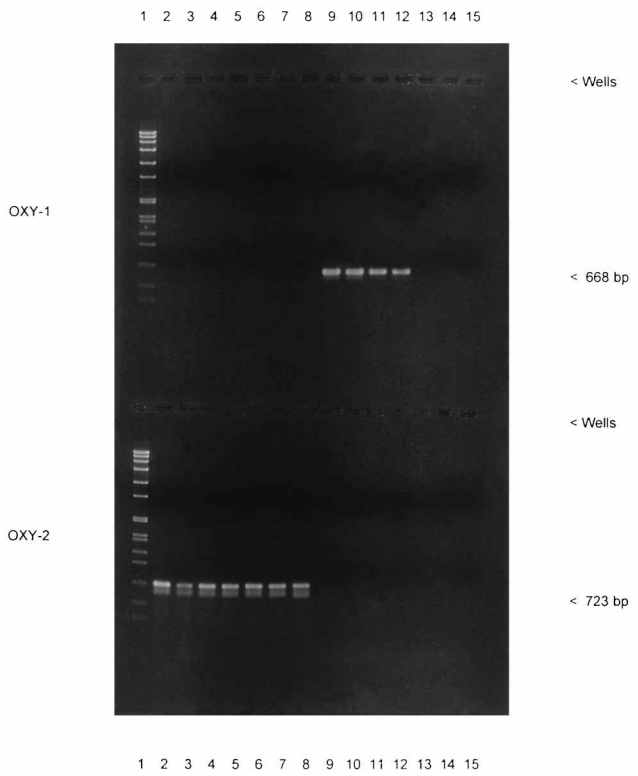

FIG. 2. Shown is a 1.5% agarose gel of PCR products produced by the OXY-1 gene primer pair and the OXY-2 gene primer pair. Lane 1, SPP1/*Eco*RI DNA molecular weight markers; lanes 2 to 5, ESBL isolates LM206, LM202, 20191A, and LM233, respectively; lanes 6 to 8, the routine clinical isolates KOR1, KOR2, and KOR6, respectively. All seven isolates tested negative with the OXY-1 primers and positive with the OXY-2 primers producing a 723-bp fragment. The ESBL isolates LM135 (lane 9) and LM183 (lane 10) and the routine clinical isolates KOR5 (lane 11) and KOR7 (lane 12) tested positive with the OXY-1 primers producing a 668-bp fragment and negative with the OXY-2 primers. KOR12 (lane 13) and the ESBL isolate LM114 (lane 14) are negative with both the OXY-1 primers and the OXY-2 primers and are *K. planticola* isolates. Lane 15 is a negative control.

DISCUSSION

Recently, the frequency of indole-positive *Klebsiella* strains identified as *K. oxytoca* has increased (15). Our study has shown, however, that a significant proportion (9%) of clinical isolates of indole-positive *Klebsiella* spp. could be identified as

TABLE 3. Isoelectric points of β-lactamases from *K. oxytoca* according to β-lactamase gene type

β-Lactamase gene	pI	No. of strains		
		ESBL	non-ESBL	Total (%)
bla_{OXY-2}	5.2	10	6	16 (41)
	5.4[a]	0	2	2 (5)
	5.7	0	1	1 (3)
	5.9	1	3	4 (10)
	6.4	0	1	1 (3)
	6.8	2	1	3 (8)
bla_{OXY-1}	7.8	1	2	3 (8)
	8.0	1	4	5 (12)
	8.2	1	2	3 (8)
	8.8	1	0	1 (3)

[a] pI of TEM-1.

K. planticola, confirming the findings of others (9, 14). We have shown also that there was no DNA sequence homology between the β-lactamase genes of the *K. planticola* and *K. oxytoca* isolates (1). It may be important to differentiate *K. planticola* from other indole-positive *Klebsiella* species in order to ascertain their epidemiological and clinical significance.

K. planticola can be differentiated from other indole-positive *Klebsiella* species by carbon substrate assimilation tests, but these are not readily available to diagnostic laboratories. By using PCR, 4 of 43 indole-positive *Klebsiella* clinical isolates proved to be isolates of *K. planticola*, confirming the results of carbon substrate assimilation tests. The PCR method is not entirely suitable for distinguishing *K. planticola* from other indole-positive *Klebsiella* spp. because the conclusion depends on a negative result, but it is specific, simple, and sensitive. Such an approach could be developed by cloning the *K. planticola* β-lactamase gene, sequencing the clone, and devising oligonucleotides specific for the cloned gene.

A plasmid-mediated ESBL (SHV-5) gene was found in one *K. planticola* strain, and the ESBL phenotype was transferable by conjugation to *E. coli*. There were three non-ESBL *K. planticola* isolates which were resistant to ampicillin and had chromosomal β-lactamases with pIs of 7.2, 7.7, and 7.9 plus 7.2. Two strains were resistant to cephalothin and cefamandole, and one was resistant to cefoxitin. It was concluded, based on co-amoxyclav susceptibility and nitrocefin reaction times, that the chromosomal β-lactamase was not hyperproduced in any *K. planticola* isolate.

K. oxytoca bacteria are generally moderately resistant to amoxicillin due to the activity of a chromosomally encoded β-lactamase (24). Recently, the emergence of resistance to extended-spectrum β-lactams, including aztreonam and broad-spectrum cephalosporins, has been described (16, 22). The genes responsible for extended-spectrum β-lactamase production are often located on transferable plasmids. Most of these enzymes are related to TEM or SHV β-lactamases and are characteristically inhibited by clavulanate. In addition, hyperproduction of chromosomally mediated β-lactamase has been described (1, 10, 12, 21), and the phenotype has a characteristic resistance pattern including aztreonam but not ceftazidime resistance. Hyperproduction can result from a mutation in the promoter of the β-lactamase gene (7). In *K. oxytoca* strains, hyperproduced chromosomally mediated β-lactamases are quite common, and 17 of the isolates in this study had this property.

The chromosomal β-lactamase genes of *K. oxytoca* are divided into two main groups, $bla_{\text{OXY-1}}$ and $bla_{\text{OXY-2}}$, as determined by PCR. The distribution of isoelectric points in our study was only slightly different from that reported by Fournier et al. (8). We found 27 $bla_{\text{OXY-2}}$ and 12 $bla_{\text{OXY-1}}$ β-lactamase genes, while Fournier et al. reported 35 and 20, respectively. The isoelectric points of OXY-1 β-lactamases in our collection ranged from 7.8 to 8.8, but the pI of 7.5, which accounted for 30 (42%) of the OXY-1 chromosomal β-lactamases in Fournier's study, and the pI of 7.1, which was represented by one isolate, were not found. The isoelectric points of OXY-2 chromosomal β-lactamases ranged from 5.2 to 6.8 and were more frequently detected than those of the OXY-1 chromosomal β-lactamases. The pI 5.2 form of β-lactamase was much more frequent than any other in both collections. Originally, a plasmid-mediated *K. oxytoca* β-lactamase with a pI of 5.2 was identified as KH (25) and is similar to the chromosomal K1 β-lactamase.

A transferable plasmid-mediated TEM-1 β-lactamase was identified in two donor strains which were OXY-2 gene positive by PCR. However, DNA from the TEM-1 transconjugants was OXY-2 gene negative. Thus, it seems likely that these donors had a plasmid-mediated TEM-1 β-lactamase and a chromosomally encoded OXY-2 gene, but the β-lactamase produced was undetectable in the isoelectric gels due in part to dilution of crude extracts.

Plasmid DNA could not be detected in any ESBL *K. oxytoca* isolate, nor could β-lactamase resistance be transferred by conjugation from any donor. Nevertheless, three transformants were obtained with donor DNA from 3 of the 17 ESBL strains. In the *E. coli* transformants, a plasmid of 2.6 kb was detected together with a β-lactamase (pI 5.2). However, the transformants were OXY-2 gene negative by PCR, even though the *K. oxytoca* donors contained the OXY-2 β-lactamase gene. This indicates that *K. oxytoca* might also have a chromosomally mediated ESBL (pI 5.2) which conferred resistance to aztreonam and reduced susceptibility to ceftriaxone. An explanation of the failure to detect plasmid DNA in the donors might be that the copy number of the nonconjugative plasmid in the *K. oxytoca* hosts was too low for detection in agarose gels. Capsular polysaccharides and lipopolysaccharides may also have interfered with plasmid extraction. In the transformants, the copy number of plasmid DNA may have increased or the plasmid may have been more efficiently extracted from the new host. However, since resistance to aztreonam was not expressed in *E. coli*, an increase in plasmid copy number is unlikely. The absence of plasmid-mediated ESBL resistance in *K. oxytoca* supports a previous report by Reig et al. (21), who found that less than 10% of *K. oxytoca* isolates had such resistance in contrast to 92% of *K. pneumoniae* isolates, which relied on plasmids for their ESBL phenotype.

The data indicate significant phenotypic and genotypic differences in resistance mechanisms between *K. oxytoca* and *K. planticola*. The future development of mechanisms for rapid routine taxonomic differentiation of these species in diagnostic laboratories will be necessary to clarify whether or not significant epidemiological or pathogenetic differences exist between the indole-positive *Klebsiella* species.

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