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Transferable Resistance to Cefotaxime, Cefoxitin, Cefamandole and Cefuroxime in Clinical Isolates of Klebsiella pneumoniae and Serratia marcescens

Summary: In conjugational crosses, three *Klebsiella pneumoniae* strains and one *Serratia marcescens* strain have been demonstrated to transfer resistance determinants to newer types of cephalosporins. While *Klebsiella* strains donated cefotaxime, cefamandole and cefuroxime resistance to *Escherichia coli* K-12 recipients, the genetic analysis of exconjugants after the transfer of plasmids from *Serratia* strains to *Proteus* or *Salmonella* recipients showed that the cefoxitin resistance determinant was also co-transferred. In subsequent transfer cycles of this plasmid, cefotaxime and cefoxitin resistance determinants segregated in contrast to the relative stability of plasmids derived from *Klebsiella* strains in subsequent transfer cycles. From results obtained in this study, it may be concluded that in some strains of nosocomial *Enterobacteriaceae,* resistance to newer cephalosporins could be transmissible and thus plasmid-located.

Zusammenfassung: *Übertragbare Resistenz gegenüber Cefotaxim, Cefoxitin, Cefamandol und Cefuroxim in klinischen Isolaten von Klebsiella pneumoniae und Serratia marcescens.* Bei drei Stämmen von *Klebsiella pneumoniae* und einem Stamm von *Serratia marcescens* konnte demonstriert werden, daß sie bei Kreuzkonjugation Resistenzdeterminanten gegen neuere Cephalosporine übertragen. Während *Klebsiella*-Stämme Resistenz gegen Cefotaxim, Cefamandol und Cefuroxim auf *Escherichia coli* K-12-Empfängerstämme übertrugen, zeigte die genetische Analyse der Exkonjuganten nach Übertragung von Plasmiden von *Serratia*-Stämmen auf *Proteus*- oder *Salmonella*-Empfängerstämme, daß die Determinante für Cefoxitinresistenz ebenfalls mit übertragen wurde. Bei weiteren Übertragungszyklen mit diesem Plasmid spalteten sich die Determinanten für Resistenz gegen Cefotaxim und Cefoxitin ab; im Gegensatz dazu besaßen Plasmide von *Klebsiella*-Stämmen in weiteren Übertragungszyklen eine relativ hohe Stabilität. Aus den Ergebnissen dieser Studie kann geschlossen werden, daß bei manchen Stämmen von nosokomialen *Enterobacteriaceae* Resistenz gegen neuere Cephalosporine übertragbar und folglich auf Plasmiden lokalisiert sein könnte.

Introduction

Resistance to beta-lactam antibiotics is thought to be caused by:

1. Production by resistant strains of a beta-lactamase that is governed by chromosomal or plasmid-located genes;
2. Alterations in target site(s) that no longer bind the antibiotic;
3. "Trapping", by highly induced lactamase molecules, of the antibiotic that is not hydrolysed, thus preventing the transport and uptake of the drug (1, 2).

The last mechanism was found to apply to only few clinical bacterial strains resistant to new, non-hydrolysable cephalosporins (1) with an expanded activity spectrum (2).

In this paper we are presenting the first evidence that resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime is of a transmissible nature, i. e. plasmid-coded in some strains of nosocomial *Enterobacteriaceae.*

Materials and Methods

Donor strains: Klebsiella pneumoniae No. 2144, 2160 and 2169 were independent isolates from a University clinic. Biochemotyping and serotyping showed that all three strains are different clones. They were resistant to cefotaxime, cefamandole, cefuroxime, cephaloridine, azlocillin, gentamicin and other more classical antibiotics. They were, however, susceptible to cefoxitin. In this communication, we are presenting the results obtained with the R plasmid from strain 2144; the results for both other strains were virtually identical. This indicates that a single R plasmid was spread among the *Klebsiella* strains isolated.

Serratia marcescens No. 3 (non-pigmented) was also a clinical isolate from a patient in the same University clinic. It was cefoxitin-resistant.

Recipient strains: In transfer experiments, we used two *Escherichia coli* K-12 strains (No. 3110, resistant to rifampicin, and 185, resistant to nalidixin), one *Proteus mirabilis* strain (P-38, resistant to rifampicin) and one *Salmonella typhimurium* strain (LT-2, resistant to rifampicin).

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We received notification of this paper for Prof. *Siegenthaler's* birthday some months ago.

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* This manuscript is dedicated to Prof. *Walter Siegenthaler* on the occasion of his 60th birthday.

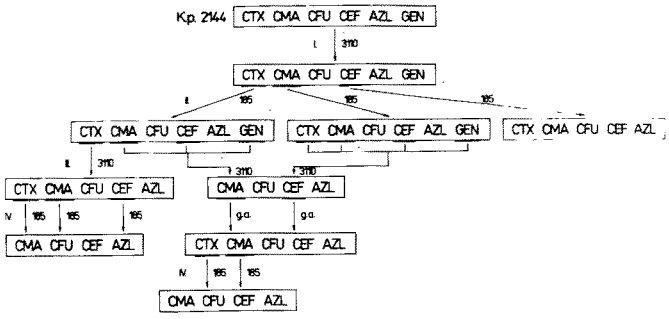

Figure 1: Schematic presentation of plasmid spectra derived in four cycles of transfer from an R plasmid transferred from *Klebsiella pneumoniae* strain No. 2144. Recipient strains were always substrains of *Escherichia coli* K-12. While strain No. 3110 is rifampicin-resistant, strain No. 185 is nalidixic acid-resistant. In this plasmid, cefamandole and cefuroxime resistance could not be genetically separated. The cefotaxime resistance determinant segregated only in the fourth cycle of transfer. The original donor strain is cefoxitin-susceptible. CTX = cefotaxime; COX = cefoxitin; CFU = cefuroxime; CMA = cefamandole; CEF = cephaloridine; AZL = azlocillin; GEN = gentamicin; CAR = carbenicillin.

Transfer experiments: From MacConkey Agar plates containing 20 mg/l of cefotaxime, we picked up one colony of each donor strain into Nutrient broth (DIFCO) and cultivated overnight. Overnight broth cultures of recipient strains were also prepared. 0.5 ml of both donor and recipient culture were then mixed and incubated for 6–8 h at 37° C. The mating mixture (0.05 ml) was then plated on a segment of MacConkey Agar (DIFCO) containing 100 mg/l of rifampicin or nalidixin plus 10 mg/l of cefotaxime or cefoxitin or 20 mg/l of other drugs, except gentamicin which was used in concentrations of 2.5 mg/l.

Genetic analysis: To ascertain which R determinants were co-transferred, the exconjugant colonies were re-inoculated on the same bi-antibiotic plate used for their selection. 25 to 50 individual colonies were then picked up into minitubes with 0.5 ml of broth, and inoculated by means of a multiloop applicator onto a series of mono-antibiotic plates containing drugs not used for the selection of particular exconjugants.

Macro-colonies that were grown from these (used for genetic analysis) were used in further cycles of transfers as donor clones, e. g. to ascertain whether fertility factor is associated and, if so, with which determinant(s).

Results

R plasmids from Klebsiella pneumoniae 2144, 2160 and 2169

Results of selected experiments with R plasmid from strain 2144 are presented in Figure 1. R determinants coding resistance to cefotaxime, cefamandole, cefuroxime, cephaloridine, azlocillin, gentamicin and other antibiotics not indicated here were readily transmissible to *Escherichia coli* K-12 (with a frequency of approx. 5×10^{-6}), but only fragments of the plasmid were transferred to P-38 or LT-2; the results obtained with them are not reported here. The plasmid transferred to K-12 No. 3110 was complex and relatively stable up to the fourth cycle of transfer with some segregation of gentamicin (second cycle) and azlocillin (third cycle) resistance. Although the cefotaxime determinant was not directly expressed after the fourth cycle of transfer, it appeared to be present in all genetic analyses of clones of various descent, regardless of the antibiotics on which the previous clones had been selected or tested.

R Plasmid from Serratia marcescens No. 3

This plasmid also coded resistance to cefoxitin and transferred it preferably to P-38 and LT-2 recipients; therefore, we are not presenting results obtained with K-12 exconjugants (Figure 2). The stability of this complex plasmid was, however, substantially lower than that of the previously described plasmid from strain 2144 of *K. pneumoniae*. From Figure 2 it can be seen that the cefoxitin determinant segregated in the second cycle of transfer, i. e. between strains K-12 3110 and K-12 185. It was a true segregation of this determinant and not a lack of phenotypic expression immediately after a transfer, since the cefoxitin determinant did not appear on any subsequent genetic analysis plates (regardless of the antibiotic used). Cefotaxime and cefuroxime resistance determinants also segregated after the second cycle of transfer, indicating that at least cefotaxime, cefuroxime and cefamandole resistances are coded by different genes. The complete segregation of cefoxitin and later of cefotaxime and cefuroxime determinants was fully confirmed in the fourth cycle of transfer and in its genetic analysis.

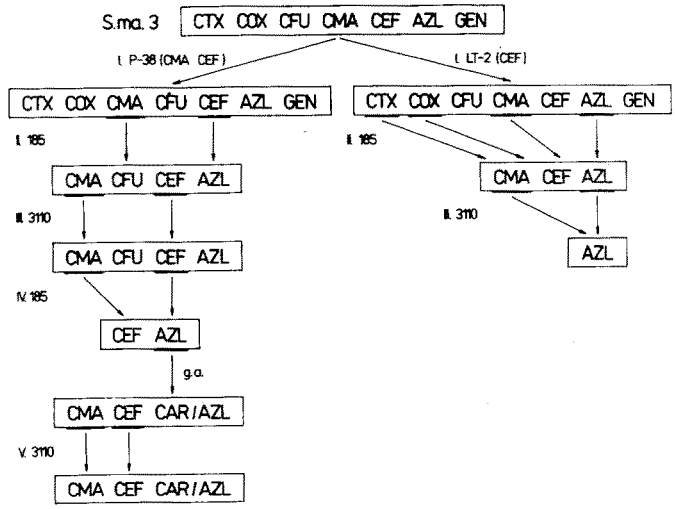

Figure 2: An R plasmid from a *Serratia marcescens* strain resistant to cefotaxime, cefoxitin and other antibiotics could be transferred *en bloc* to the recipient strains *Proteus mirabilis* P-38 or *Salmonella typhimurium* LT-2. In the second cycle of transfer, several R determinants could be transferred to *Escherichia coli* K-12 No. 185 nal^R, but both cefotaxime and cefoxitin resistance determinants segregated in this cycle. Cefamandole and cefuroxime determinants could be separated in transfer cycles of this plasmid. See Figure 1 for abbreviations.

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Discussion

Plasmids from *K. pneumoniae* and *S. marcescens* strains resistant to cefotaxime, cefuroxime and cefamandole, and eventually to cefoxitin, as reported here, have different genetic properties and thus presumably a different origin. They are transferred easily to *Enterobacteriaceae*, but different recipients showed different abilities to accept and harbor these plasmids. In repeated cycles of transfer, the plasmid that originated in *Klebsiella* retained its relative integrity, namely its cefotaxime and cefuroxime determinants, while the *Serratia* plasmid easily segregated cefoxitin as well as cefotaxime and cefuroxime determinants. Transferability of cefoxitin resistance has been demonstrated in anaerobic *Bacteroides* strains, and *Then* et al. (personal communication) found numerous lactamase species in a poly-resistant *Klebsiella* strain. Cefotaxime was found to inhibit enzymes which destruct classical cephalosporins (3). Whether resistance to cefotaxime, cefuroxime, cefamandole and cefoxitin is caused by new types of beta-lactamases or by an unknown mechanism remains open.

Literature

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