A TRANSMISSIBLE PLASMID DETERMINING LACTOSE FERMENTATION AND MULTIPLE ANTIBIOTIC RESISTANCE IN A STRAIN OF *KLEBSIELLA PNEUMONIAE*

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IN 1974 a strain of *Klebsiella pneumoniae*, of capsular type 43 and referred to as L1, was isolated from the River Taff in Wales. In mixed culture it co-transferred its chloramphenicol, ampicillin, tetracycline, streptomycin, spectinomycin and sulphonamide resistance and its ability to ferment lactose to *Escherichia coli* K12. Because of the possible medical significance of this observation, strain L1 was studied more fully; the results are recorded in this paper.

MATERIALS AND METHODS

Plasmid transmission. Donor and recipient strains were grown together in nutrient broth (Oxoid no. 2) at 37°C for 24 h, and then cultured on plates of solid medium containing an antibiotic, usually sodium nalidixate, to which the recipient was mutationally resistant. For detecting transfer of antibiotic resistance, the medium was MacConkey's agar containing, in addition to sodium nalidixate, an antibiotic to which the donor was resistant. The medium for detecting transfer of lactose fermentation contained (g per litre) K₂HPO₄ 7, KH₂PO₄ 3, (NH₄)₂SO₄ 1, MgSO₄·7H₂O 0·1, NaCl 5, lactose 5, agar 15, supplemented, where necessary, with growth factors, 50 µg per ml. All antibiotics were incorporated in culture media at concentrations of 20 µg per ml.

Plasmid elimination. This was attempted by the acridine orange method of Hirota (1960), the sodium lauryl sulphate method of Tomoeda *et al.* (1968) and by passage in broth at 44°C.

Plasmid transfer in chicks. Groups of chicks in their first day of life were given, by mouth, 0·3 ml of a broth culture of the L1 strain of *K. pneumoniae* in which transfer-factor I (I *drd* 16; Hardy *et al.*, 1973) or A2 (Smith and Heller, 1973) had been implanted. On the following day the chicks were given a similar dose of a broth culture of a mutant of either *Salmonella typhimurium* of phage-type 36 or *Salmonella gallinarum* that was resistant to sodium nalidixate (*nal*^r) and rifampicin (*rif*^r). Some of the groups were then fed ordinary diets and others were given food containing 500 mg per kg of chloramphenicol or ampicillin. Cloacal swabs were taken from the chicks at intervals and inoculated on to plates of Brilliant Green Agar (Oxoid, CM263) containing 20 µg per ml of each of sodium nalidixate and rifampicin. After disks containing 50 µg of chloramphenicol were placed on their surface, the plates were incubated at 37°C for 24 h and read. Only the *nal*^r *rif*^r salmonella organisms grew on this medium; any that had acquired the ability to ferment lactose (*Lac*⁺) grew as pale colonies and any that had acquired chloramphenicol resistance (*Cmp*⁺) grew in the immediate vicinity of the chloramphenicol disks.

Antibiotic-sensitivity tests. These were performed by the disk method described by Smith (1970).

RESULTS

Strain L1 transferred its resistance to chloramphenicol, ampicillin, tetracycline, streptomycin, spectinomycin and sulphafurazole to a *lac*⁻ strain of *E. coli* K12 at the very low rate of approximately 3×10^{-5} , i.e., three of every 100 000 K12 organisms acquired antibiotic resistance. Each of the antibiotics to which L1 was resistant, except streptomycin, was used separately in the selection plates of MacConkey's agar. All the colonies of K12 that grew

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on these plates were lactose fermenting and two colonies picked from each of the five different kinds of media had acquired the complete antibiotic-resistance pattern of strain L1. When 64 colonies picked from selection plates of the minimal medium, i.e., *Lac*⁺ colonies, were tested for susceptibility to chloramphenicol, 62 were resistant; one of the remaining two was resistant to ampicillin, tetracycline, streptomycin and spectinomycin and the other to ampicillin and tetracycline. The close relationship between the determinants for antibiotic resistance and lactose fermentation in strain L1 was also noted in other transfer experiments in which K12 strains were used both as donors and recipients and in all the experiments on genetic transfer and removal described below; for this reason the determinants will be referred to collectively as the L1 plasmid.

Transfer of the L1 plasmid to the *lac*⁻ K12 strain occurred at a lower rate at 28°C than at 37°C and no transfer was detected at 22°C. Transfer was not detected to 10 of 11 epidemiologically unrelated wild strains of *E. coli*, three strains of *Salmonella typhimurium* belonging to different phage types, and one strain each of *Salm. choleraesuis*, *Salm. dublin*, *Salm. thompson*, *Salm. gallinarum*, *Shigella flexneri*, *Sh. sonnei* and *Proteus mirabilis*. It was detected to a Vi strain of *Salm. typhi* at a rate of 10^{-7} .

Plasmid-deficient organisms were easily obtained from the L1 strain by passage in broth at 44°C but not by sodium lauryl sulphate or acridine orange treatment. Their colonies on MacConkey's agar were pink in colour whereas those of strain L1 were red. The colonies of the two kinds of organism were more sharply differentiated on the minimal medium, those of the plasmid-deficient organisms being much smaller than those of strain L1. The plasmid was transmitted at the relatively high rate of 10^{-2} from strain L1 to a culture of the plasmid-deficient organisms, all the colonies of the recipient organisms being red and composed of antibiotic-resistant organisms.

Ten of 23 epidemiologically unrelated strains of *K. pneumoniae* grew as red colonies on MacConkey's agar and 13 as pink ones. None of five red-colony strains transferred their characteristic property to the plasmid-deficient form of strain L1 or to a *lac*⁻ K12 strain, but it was eliminated from four of them either by passage in broth at 44°C or by sodium lauryl sulphate treatment.

The transfer rate and recipient range of the L1 plasmid was increased to approximately the same extent by implanting either transfer-factor I or A2 in the L1 strain. This was achieved by implanting in the plasmid-deficient form of the strain first I or A2, with a tetracycline-resistance determinant as marker, and then, from a K12 strain, the L1 plasmid. The transfer rates of the L1 plasmid from the I⁺ and A2⁺ forms of the L1 strain to the *lac*⁻ K12 and *Salm. typhi* strains were approximately 2×10^{-2} and 2×10^{-4} respectively; the tetracycline-resistance determinant with I or A2 transferred at higher rates indicating that a linkage had not been established between them and the L1 plasmid. Transfer of the L1 plasmid was also detected from these two forms to nine of the eleven wild strains of *E. coli*, the three strains of *Salm. typhimurium* and the one strain each of *Salm. gallinarum*, *Sh. flexneri* and *Sh. sonnei* at rates of 10^{-3} to 10^{-6} ; transfer was not detected to the other three salmonella strains previously employed as recipient strains. It was also not detected to the plasmid-deficient form of strain L1. The numbers of day-old chicks in groups of 25 that died after they had been infected orally with 5×10^8 viable organisms of one of the *Salm. typhimurium* strains or with a similar dose of organisms of this strain in which the L1 plasmid had been implanted with transfer factor I or A2 were 7, 6 and 12 respectively. The corresponding figures for a similar experiment in which the *Salm. gallinarum* strain was employed instead of the *Salm. typhimurium* strain were 10, 6 and 13 respectively.

Transfer of the L1 plasmid in chicks

No evidence of transfer of the L1 plasmid from the I⁺ or A2⁺ forms of the L1 strain to *Salm. typhimurium* or *gallinarum* was detected in groups of 14 chicks given ordinary food or chloramphenicol-containing food after infection. A considerable degree of transfer, however, occurred in the groups given ampicillin-containing food, especially in those infected with the I⁺ form. For example, of the 14 chicks given this form and *Salm. typhimurium*, the number

whose faeces were found to contain *Salm. typhimurium* organisms that were lactose-fermenting and resistant to chloramphenicol, ampicillin, tetracycline, streptomycin, spectinomycin and sulphonamide 4, 8, 11 and 14 days after infection were 3, 11, 13 and 14 respectively; at least half of the *Salm. typhimurium* organisms isolated from most of the 41 positive specimens belonged to this category. In the similar experiment employing *Salm. gallinarum* instead of *Salm. typhimurium*, *Salm. gallinarum* organisms possessing the L1 plasmid were found in the faeces of six chicks 3 days after infection. They were not found in the faeces of the four chicks that were still alive after 11 days or in the liver of those that had died by that time. None of the chicks that had been infected with *Salm. typhimurium* died.

DISCUSSION

Reeve and Braithwaite (1973) demonstrated that organisms of the genus *Klebsiella* fall into two classes—those with a strong and those with a weak lactose-positive phenotype. The former owe this phenotype to the presence of a *Lac* plasmid which could not promote its own transfer but which could be transferred by implanting transfer factors in cells harbouring it. The strains we designated red and pink-colonied undoubtedly correspond to the strong and weak lactose-fermenting phenotypes. The fact that we were able to obtain weak-fermenting organisms from strong-fermenting strains by passage in broth at 44°C and by sodium lauryl sulphate treatment confirmed the plasmid-determined nature of the strong-fermenting property. The L1 strain, however, was exceptional in that its *Lac* plasmid also determined antibiotic resistance and was transmissible. The transfer factor responsible for its transmissibility did not appear to be located in the plasmid because when I or A2 displaced it, as evidenced by the failure of the I⁺ and A2⁺ forms of strain L1 to transmit the plasmid to the plasmid-deficient form of L1, they clearly did not become integrated in the plasmid. The L1 transfer factor transmitted the plasmid to *lac*⁻ pathogens at very low rates or not at all indicating that strain L1 was of little medical significance in this particular context. However, the fact that its plasmid was transmitted by transfer factors I and A2 to salmonella organisms in the alimentary tract of chicks fed diets containing ampicillin, suggested that wild forms of strain L1 could be medically significant if they became infected with transfer factors like I and A2.

SUMMARY

In a wild strain of *Klebsiella pneumoniae* the plasmid that determined lactose fermentation also determined resistance to chloramphenicol, ampicillin, tetracyclines, streptomycin, spectinomycin, and sulphonamides. The plasmid transferred at a very low rate to *Escherichia coli* K12 and *Salmonella typhi*. By implanting other transfer factors in the strain the rate of transfer and the recipient range were increased. Plasmid transfer from the modified strain to *Salm. typhimurium* and *Salm. gallinarum* was detected in the alimentary tract of experimentally infected chicks fed diets containing ampicillin.

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