Diversity of plasmids responsible for multiple resistance in *klebsiella* serotype K2

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SUMMARY

Klebsiella of capsular type K2 were investigated to find out whether a single epidemic clone was the source of many outbreaks of infection in different hospitals, in different areas over a period of five years. The *klebsiellas* studied were found to be very similar; they were of the same biotype, had similar klebecin sensitivity patterns and carried multiple drug-resistance plasmids; however, characterization of these plasmids showed that they were heterogeneous. Thus there was not a single epidemic bacterial clone.

INTRODUCTION

Multiple-resistant *klebsiella* have become an increasing problem in the past years because they are often the cause of outbreaks of hospital infection (Casewell *et al.* 1977; Curie *et al.* 1978; Hughes & Datta, unpublished).

The organism responsible for a large outbreak of infection in hospitals in the Bristol area (Curie *et al.* 1978) was identified as *Klebsiella aerogenes*, capsular serotype K2. This serotype was also responsible for outbreaks of infection in hospitals in the Medway Health District at the end of 1979 (Dr A. M. Gordon, personal communication) and in clusters of infection in this hospital during February and April of this year and again in June and July. Serotype K2 was found by Casewell & Talsania (1979) to be the second most common type of *K. aerogenes*, comprising 16.5% of 357 strains isolated in the United Kingdom. We wanted to find out why this serotype is common in hospital infections, in particular if it is the same strain which is successfully spreading from hospital to hospital, and whether its success could be in any way attributed to the plasmids that it carries.

METHODS

Bacterial strains

Multiply resistant *klebsiella* strains were isolated from clinical specimens from in-patients of Hammersmith Hospital and biotyped by methods in use in the diagnostic laboratory in this department (Cowan & Steel, 1974). They were serotyped at the Public Health Laboratory, Coventry. Gentamicin-resistant *klebsiella* of serotype K2 were also received from other hospitals both in and out of the London area.

Klebecin typing was as described by Edmondson & Cooke (1979) using klebecin-producer and indicator strains provided by Professor E. M. Cooke. Sensitivity testing was as described by Datta *et al.* (1980).

Laboratory strains used in plasmid studies were *Escherichia coli* K12 J53 *lac*⁺ *pro met* and J62 *lac pro his trp* and mutants of each of these strains resistant to nalidixic acid, *nal* or rifampicin, *rif*.

Characterization of R plasmids

The R plasmids were transferred by conjugation from the klebsiella to *E. coli* K12 J62 *rif*, selecting for gentamicin resistance and using rifampicin to inhibit growth of the donor klebsiella. The concentration of gentamicin used was 2 µg/ml and that of rifampicin 50 µg/ml. Methods were as described by Datta *et al.* (1980).

The klebsiella and *E. coli* K12 transconjugants, carrying R plasmids from the klebsiella, were examined by agarose gel electrophoresis using single-colony lysates. Standard plasmids of known molecular weights were run in the same gels. After electrophoresis, DNA molecules in the gels were stained with ethidium bromide and photographed with u.v. light. The electrophoretic mobility of plasmid molecules being dependent on their molecular weight, the number of plasmid species and their sizes were thus recognized. The method was as described by Eckhardt (1978).

After transfer to *E. coli* K12, the R plasmids were classified into groups on the basis of compatibility tests. Each was tested for compatibility with a set of standard plasmids of known groups. These plasmids were in *E. coli* K12 J53, a strain of *E. coli* K12 differing in its auxotrophic markers from J62. Incompatible plasmids (that fail to coexist stably in a cell line) are classed together. The method was as described by Datta *et al.* (1980).

RESULTS

The table shows the sources of the *K. aerogenes* K2 strains examined, their characters and the plasmids that they carried.

Between December 1979 and April 1980 there was an outbreak of infection with multiply resistant klebsiella in Hammersmith Hospital. The main epidemic strain was of serotype K21, but small clusters of infection with K2 and K16 serotypes were also identified. The klebsiella serotype K2 strains were resistant to many antibacterial drugs, including nalidixic acid, and each carried two large plasmids and one small one. The smaller of the two large plasmids was transmissible to *E. coli* K12 and belonged to incompatibility group C (IncC). This plasmid carried most of the antibiotic resistances found in the klebsiella.

In November 1979 multiply resistant klebsiella of capsular type K2 were the cause of outbreaks of hospital infection in the Medway Health District in different wards of the same hospital and in different hospitals (A. M. Gordon, personal communication; PHLS unpublished, CDR 80/5). We received two representative strains from the Medway Health District and they were indistinguishable from the klebsiella serotype K2 strains isolated in Hammersmith Hospital in biotype,

Table 1. *Klebsiella aerogenes* serotype K2

Number of isolates examined	Date of isolation	Klebsiella isolates			Plasmid M.wts (Md)	Plasmids transferred to <i>E. coli</i> K12	
		Source ¹	Klebecin sensitivity ²	Resistance pattern ³		Resistance pattern	Incom-patibility group (Md)
5	Feb. 1980	HH	None	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSmTcCmKmSuGmTmTpHg	85
1	Apr. 1980	HH	None	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSmTcCmKmSuGmTmTpHg	85
9	June and July 1980	HH	None	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSmTcCmKmSuGmTmTpHg	85
2	Nov. 1979	Medway	None	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSmTcCmKmSuGmTmTpHg	85
3	1979/ 1980	NMH	± 13	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSuGmTmTp	85
			± 13	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSuGmTmTp	85
			± 13	ApSmTcCmKmSuGmTmTpHg-Nal	3.7, 85, 100	ApSmKmSuGmTmTp	85
1	1977	BRI	± 13	ApSmTcCmKmSuGmTmTpHg	nd	SmTp	Iα 62
						ApTcCmKmSuGmTmHg	nd 4 + 90
1	Mar. 1978	SHB	± 13	ApSmTcCmKmSuGmTmTpHg	nd	ApCmKmSuGmTmHg	nd 90
						SmTp	Iα 62
2	Sept. and Oct. 1978	QMH	None	ApSmTcCmKmSuGmTmTpHg-Nal	60, 70	ApSmTcCmKmSuGmTmHg	nd 95
1	Mar. 1978	KCH	+ 13	ApSmTcCmKmSuGmTmTpHg	56, 90	ApSmTcCmKmSuGmTm	FII 60
						CmTpHg	FII 54
						ApSmTcCmKmSuGmTmTpHg	FII 56

¹ HH, Hammersmith Hospital; Medway, Medway Health District; NMH, North Middlesex Hospital; BRI, Bristol Royal Infirmary; SHB, Southmead Hospital, Bristol; QMH, Queen Mary's Hospital for Children, Carshalton; KCH, King's College Hospital.

² 'None' indicates that the strain was sensitive to none of the 15 klebecins, ± 13 means that there was slight inhibition by klebecin 13. In the interpretation of klebecin typing minor reactions may have discriminatory value, but they are less reproducible than major differences in reaction (Edmondson and Cooke, 1979). The reproducibility of the ± 13 reaction was imperfect, sometimes no reaction was seen. Where the entry is 'none', no reaction was seen in repeated tests.

³ Ap, ampicillin; Sm, streptomycin; Tc, tetracycline; Cm, chloramphenicol; Km, kanamycin; Su, sulphonamide; Gm, gentamicin; Tm, tobramycin; Tp, trimethoprim; Hg, Mercuric chloride; Nal, nalidixic acid.

nd = not determined.

klebecin type, resistance pattern and plasmid content (Table 1). This suggests that the same klebsiella was the cause of infections in Hammersmith Hospital and in the Medway Health District hospitals.

Further examples of klebsiella capsular type K2 were received from the North Middlesex Hospital and again the klebsiella were indistinguishable, by the criteria used above, from the Hammersmith and the Medway isolates (Table 1). The plasmids transferred to *E. coli* K12 were of the same molecular weight and incompatibility group but differed from the other IncC plasmids in determining resistance to smaller numbers of antibacterial agents. These findings suggest that the serotype K2 isolates from the North Middlesex Hospital belonged to the same clone as the Hammersmith and Medway ones, with the same plasmid content, but that molecular rearrangements of the DNA of the 85 Md IncC plasmid had occurred, leading to non-expression of some resistance genes. We have previously reported plasmid variations of this kind (Datta *et al.* 1979).

We examined serotype K2 klebsiellas that had been isolated in Bristol in 1977 and 1978. That from the Bristol Royal Infirmary was part of the epidemic reported by Curie *et al.* (1978) and was very similar to the isolate from the Southmead Hospital, Bristol. Plasmids in these klebsiella could not be properly visualized in the gels after electrophoresis of single-colony lysates because there was background smearing, but resistance plasmids were transferred to *E. coli* K12, where they were characterized. Multiple resistance, including resistance to gentamicin and tobramycin, was determined by large conjugative plasmids that did not belong to incompatibility groups C or FII. In the strain from Bristol Royal Infirmary, tetracycline resistance was not carried on the multiple R plasmid but on a separate, 4 Md, nonconjugative one that was mobilized by the larger plasmid and thus transferred to *E. coli* K12. The Southmead strain differed in that its tetracycline resistance was linked to the multiple R plasmid. In both Bristol strains, trimethoprim resistance was on a distinct, conjugative plasmid of IncI α .

Serotype K2 klebsiellas received from Queen Mary's Hospital for Children, Carshalton were similar in biotype, klebecin type and nalidixic acid resistance to the Hammersmith and Medway strains, but their plasmids were quite different. These strains each carried two plasmids, one of which was transmissible to *E. coli* K12. It belonged to incompatibility group FII (Table 1).

One further example of a gentamicin-resistant serotype K2 klebsiella, from King's College Hospital, was not part of an outbreak of infection. The strain was similar in resistance pattern to the other serotype K2 klebsiella strains we had analysed but its plasmid content was again quite different (Table 1). The plasmid transferred to *E. coli* K12 was a multiple-resistant IncFII plasmid which frequently gave rise to variants of slightly lesser molecular weight that expressed resistance to only three antibacterial agents (Table 1).

DISCUSSION

Multiple-resistant klebsiella are frequently the cause of outbreaks of hospital infection, the most susceptible patients being immunologically 'compromised' or

elderly and debilitated. *Klebsiella* of serotype K2 seem to be particularly successful at colonizing and infecting patients.

The serotype K2 *klebsiellas* that caused infections in Hammersmith Hospital, Medway Health District hospitals and the North Middlesex Hospital appeared all to be examples of the same strain; the plasmid content was the same and so were the bacterial characteristics, including resistance to nalidixic acid, a resistance determined by chromosomal mutation rather than by plasmids. This strain has established itself in different hospitals which, although separated by up to 50 km, are in London or near enough to London for transfer of patients, directly or indirectly, to be a probable means of transmission of bacteria. Similarly, in the *klebsiella* serotype K2 outbreak in Bristol, one strain caused infections in several hospitals over a long period (Curie *et al.* 1978 and our own findings). However, the Bristol strain was different from the London-Medway strain in plasmid content, and in being sensitive to nalidixic acid. The multiple resistance phenotype of the *klebsiella* strains appeared similar but was determined in different ways. In the Bristol strains trimethoprim and gentamicin resistance were carried on separate plasmids, while in the London-Medway isolates these resistances were linked on one multiple-resistance plasmid, unrelated to those of the Bristol strain.

The serotype K2 strains from Queen Mary's Hospital for Children, Carshalton and from King's College Hospital were different from each other and from the Bristol and the London-Medway ones. Therefore, a single epidemic *klebsiella* clone has not been the cause of outbreaks of infection in hospitals over the past five years. There have been two successful combinations of a K2 *klebsiella* host plus multiple resistance plasmids. The first was responsible for the Bristol epidemic, was identified in February 1976 and colonized or infected over 240 patients (Curie *et al.* 1978 and this paper). The second combination, the London-Medway one was of a K2 *klebsiella* with a quite different multiple resistance plasmid. This strain has been identified repeatedly in Hammersmith Hospital during 1980, and has given rise to small clusters of infection involving a total of 15 patients, but no major outbreak. In Medway, too, there were clusters of cases in some wards or units. This strain has not affected as many patients as the Bristol one.

Multiple-resistant *klebsiella* are a hospital problem, and any one infection has the potential of evolving into an outbreak. Strain recognition allows clusters of infections to be identified as such; measures to prevent further spread can then be instituted in good time.

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