Epidem. Inf. (1987), **99**, 625–634
Printed in Great Britain

Comparison of biochemical and serological typing results and antimicrobial susceptibility patterns in the epidemiological investigation of *Klebsiella* spp.

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(*Accepted 22 July 1987*)

SUMMARY

An analysis of the serological and biochemical typing results of 925 clinical isolates of klebsiella revealed that biotyping and serotyping of klebsiella could replace each other for epidemiological purposes. The combination of both typing methods provided even more epidemiological information in analysing clusters of particular serotypes and biotypes in time. Clustering serotypes, mainly of neonatal origin, were nearly uniformly more resistant to the antibiotics in common use than other serotypes. Biotyping as well as serotyping of klebsiella isolates recovered from environmental surveys in the neonatal ward showed that epidemic and non-epidemic klebsiella isolates could occasionally be cultured from the environment and from the staff.

INTRODUCTION

Klebsiella species have been shown to be of prominence in nosocomial infections and consequently a matter for concern in almost any hospital. Many outbreaks, some with multiply resistant strains, have been described in the past decades (Price & Sleigh, 1970; Hable *et al.* 1972; Hill, Hunt & Matsen, 1974; Casewell *et al.* 1977; Curie *et al.* 1978; Morgan, Hart & Cooke, 1984). The control of these epidemics has sometimes necessitated very radical corrective measures, such as closure of departments, e.g. special care units.

In the investigation and control of such outbreaks the value of epidemiological typing is well established. These epidemiological investigations with respect to *Klebsiella* spp. have been largely dependent on the development of serological typing by means of capsular swelling (Julianelle, 1926). In the absence of other simple and reliable typing methods for *Klebsiella* spp. the capsular serotyping techniques have become the traditional ones, although the need for 77 capsular antisera has restricted this typing method to reference laboratories. Nevertheless it has been used successfully in the epidemiological investigations of infections with *Klebsiella* spp. (Casewell & Phillips, 1978; Casewell & Talsania, 1979; Cooke *et al.* 1979; Riser, Noone & Howard, 1980).

In a previous study (Simoons-Smit *et al* 1985) we found that a biochemical

typing system of clinical isolates of *Klebsiella* spp. showed sufficient different biochemical patterns to be potentially useful for epidemiological investigations. As biochemical typing can be performed in almost every routine laboratory, we compared our biochemical and serological typing methods in the investigation of an outbreak of infections with gentamicin-resistant klebsiella isolates in the neonatal intensive care unit (NICU) of our university hospital.

This study deals with the results of a computer-mediated analysis of the typing results of the klebsiella isolates from this outbreak, and of clinical isolates of klebsiella from other departments in the same hospital, in order to be able to compare the potential value of the biochemical with the serological typing system for epidemiological investigations.

MATERIALS AND METHODS

Bacterial strains

The biochemical and serological properties of 925 clinical isolates of *Klebsiella* spp. from 434 patients were available for this study (Simoons-Smit *et al.* 1985). These isolates included 355 klebsiella isolates from routine specimens from the neonatal ward (including the NICU) from July 1978 to April 1980, and 47 gentamicin-resistant klebsiella isolates collected between August 1980 and July 1981 from the same neonatal ward. The remaining isolates had been recovered from patients from all other wards in the hospital, in the period from June 1979 to 15 February 1980 (507 isolates); a few isolates (16) had been recovered after this period until March 1982.

In the NICU, specimens had been taken from ear, nose, umbilicus and rectum of each baby on admission. Thereafter, specimens had been taken if there was an indication of infection at any particular site; in periods in which many gentamicin-resistant klebsiella isolates had been cultured the admission cultures had been repeated weekly for each baby. In the other wards specimens only had been taken on suspicion of infection. The organisms had been identified to species level by the routine clinical microbiology laboratory.

The biotyping, based on 35 biochemical reactions, and the serotyping of these strains have been described previously (Simoons-Smit *et al.* 1985). Sensitivity to antibiotics was tested at the time of isolation by disk diffusion tests (AB Biodisks, Sweden).

Environmental surveys

During the epidemic in the NICU, surveys had been made in which the inanimate environment, the personnel, the babies in the ward and their parents were sampled, and settle plates were put down in the department in an attempt to identify the source(s) and/or route(s) of transmission of the epidemic gentamicin-resistant klebsiella isolates in the ward. The gentamicin-resistant klebsiella isolates which had been recovered from these surveys were biotyped and serotyped as described above.

Data management

The data of the collected isolates such as isolate number, isolation date, isolation site, antimicrobial susceptibility pattern, biotype and capsular serotype

Table 1. *Frequency of biotypes among 670 clinical isolates of klebsiella*

Neonatal isolates			'Other' isolates		
Biotype	Number of isolates	(%)	Biotype	Number of isolates	(%)
5215773-56363	48	(18)	5215773-56363	36	(9)
5255773-56767	47	(17)	5215773-56341	29	(7)
5255773-56747	42	(15)	5255773-56767	27	(7)
5215773-56341	33	(12)	5215773-56761	27	(7)
5215773-56741	33	(12)	5215773-56361	27	(7)
5215773-56761	14	(5)	5215773-52343	26	(6)
Others (24 types) (1–10 each)	57	(21)	5215773-56343	24	(6)
			5215773-56741	19	(5)
			5215773-56762	15	(4)
			5215773-56763	15	(4)
			Others (75 types) (1–14 each)	151	(38)
Total	274		Total	396	

were entered in a data management and retrieval system (Department of Medical Information Technology, directed by A. Hasman). The epidemiological research of our isolates was performed with the various data manipulation commands of this system.

RESULTS

Origin of the strains

For the analysis of the 925 available klebsiella isolates (402 neonatal isolates and 523 isolates from other patients), duplicate isolates of organisms of the same capsular serotype from the same source in the same patient were deleted.

Organisms of different serotypes from the same source in the same patient were retained. This led to a reduction of the total number of isolates from 925 to 670, of which 274 isolates were of neonatal and 396 isolates of other origin.

Frequency of sero- and biotypes

The distribution of the different biotypes and serotypes among the 274 neonatal isolates and the 396 'other' isolates is shown in Tables 1 and 2. Categories represented by 14 or more isolates are listed individually. In total, 93 different biotypes and 63 different serotypes could be distinguished. In the group of neonatal isolates the five most common biotypes accounted for 74% and the three most common serotypes (K55, K18 and K69) for 44% of all isolates. In the group of 'other' isolates there was a more homogeneous distribution of the isolates into the various categories of biotypes and serotypes, although a slight preponderance of a few serotypes (K33, K21, K24) could be observed.

Distribution in time

Figures 1 and 2 show the distribution in time of the commonest serotypes and biotypes. From these figures there is clear evidence for clustering of some isolates.

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Table 2. *Frequency of serotypes among 670 clinical isolates of klebsiella*

Neonatal isolates			'Other' isolates		
Serotype (K)	Number of isolates	(%)	Serotype (K)	Number of isolates	(%)
55	43	(16)	33	34	(9)
18	40	(15)	21	22	(6)
69	36	(13)	24	17	(4)
8	22	(8)	2, 55 (11–15 each)	26	(7)
63	15	(5)	3, 4, 6, 8, 18,27		
7, 24 (6–10 each)	14	(5)	54, 62, 69, 61/63* (6–10 each)	88	(22)
Others (20 types) (1–5 each)	55	(20)	Others (42 types) (1–5 each)	101	(25)
Non-typable	49	(18)	Non-typable	108	(27)
Total	274		Total	396	

* Serotypes not separated.

Some serotypes or biotypes predominated for a few months and were then replaced by others. Clustering of serotypes as well as biotypes was particularly seen among the neonatal isolates. Regarding the clusters outlined in Figs. 1 and 2 it is clear that these coincide with the clusters of biotypes. Serotype clustering occurred with types K8, K18, K55 (two clusters), K63 and K69, and was virtually confined to neonatal isolates. Isolates designated as 'other' in Figs. 1 and 2 in the period until June 1979 all appeared to be isolates from neonates who had been transferred to another ward after a stay in the neonatal department.

The relation of the serotypes and biotypes in the clusters is shown in Table 3. Nearly all of the strains in a particular serotype cluster had the same biotype. K55 was an exception in being more heterogeneous. In the first cluster of K55 two biotypes were predominant, which differed only in the D-tartrate reaction. One strain of the K69 cluster had a different biotype, due to a negative gelatinase reaction. With the exception of K69 strains, nearly all the strains with a known serotype not included in the outlined cluster of the same serotype had a biotype different from the strains in the cluster. The non-neonatal isolates showed a rather homogeneous distribution in time in the period of collection of these isolates. In this group, isolates with a serotype or biotype with a somewhat higher incidence were not considered to be clustered because of the variety of serotypes in a biotype group and of biotypes in a serotype group, and because they were recovered from patients in many different wards.

Antimicrobial susceptibility

Table 4 summarizes the results of the *in vitro* susceptibility tests to eight antibiotics for the total of neonatal and 'other' isolates and for the commonest neonatal individual serotypes (with the exception of urinary isolates). Comparison of the susceptibilities of all neonatal isolates with all 'other' isolates showed that the neonatal isolates were to a greater degree resistant to gentamicin than the 'other' isolates. A high grade of susceptibility to cotrimoxazole (80–100%) was

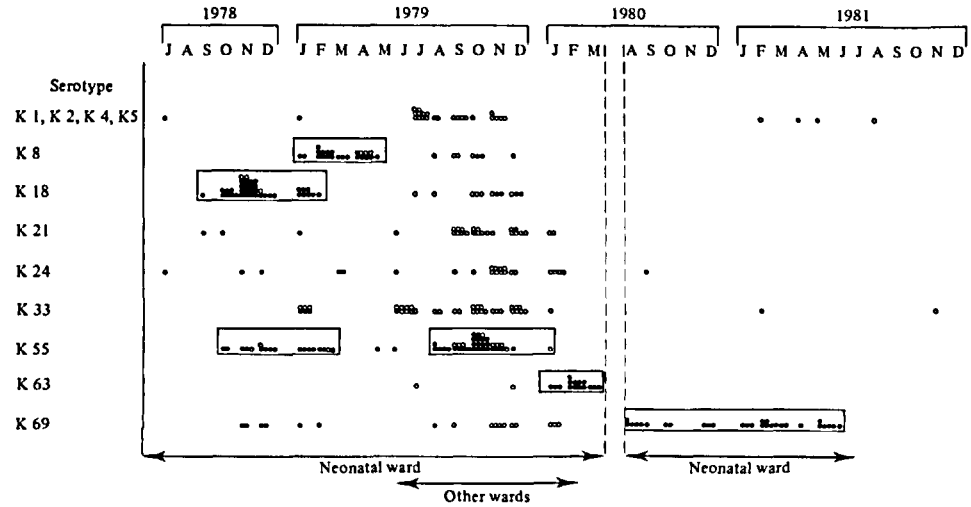

Fig. 1. Distribution in time of the commonest serotypes among 670 clinical isolates of klebsiella. The periods in which the isolates had been recovered from the neonatal ward and from the other wards are indicated. ●, Neonatal isolates, ○, other isolates.

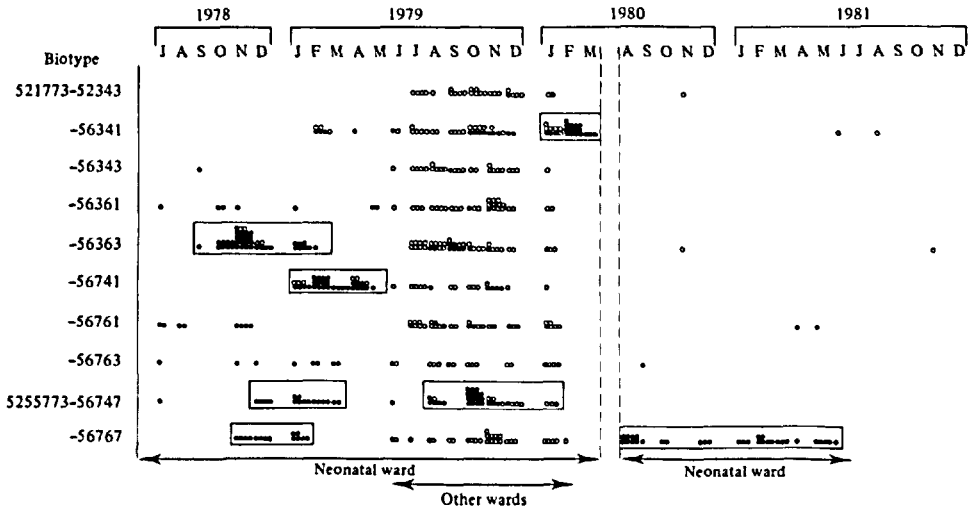

Fig. 2. Distribution in time of the commonest biotypes among 670 clinical isolates of klebsiella. The periods in which the isolates had been recovered from the neonatal ward and from the other wards are indicated. ●, Neonatal isolates, ○, other isolates.

found in all isolates. In the group of 'other' isolates no marked difference was found between the antimicrobial susceptibilities of the individual serotypes and the total of isolates in this group. Of the individual serotypes in the group of neonatal isolates, K 18 strains were the least susceptible to the antibiotics tested, and together with K 69 strains resistant to gentamicin in particular. Although the number is small, K 63 strains showed mainly intermediate susceptibility to gentamicin and were uniformly resistant to kanamycin and chloramphenicol. Strains of serotype K 8, however, showed a susceptibility comparable with that of

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Table 3. *Distribution of biotypes within clusters of a known serotype*

Serotype cluster	Major corresponding biotype	Number of strains with particular biotype/ total number of strains in the cluster
K8	5215773-56741	23/23
K18	5215773-56363	39/39
K55*	5255773-56767	6/18
	56747	5/18
K55†	5255773-56747	21/36
K63	5215773-56341	15/15
K69	5255773-56767	28/29

* First cluster of K55 in time (see Fig. 1).
† Second cluster of K55 in time (see Fig. 1).

the group of 'other' isolates. Comparison of the antimicrobial susceptibility of the strains in the first and in the second cluster of K55 strains revealed that the strains in the second cluster were to a greater degree resistant to gentamicin (45% vs 17%), to kanamycin (69% vs 36%) and to chloramphenicol (70% vs 29%).

Environmental surveys

The environmental surveys revealed that some of the gentamicin-resistant klebsiella isolates which had been cultured from the inanimate sources in the ward, such as soap-holders, wash-stands, cribs, window seats and air (slit sampler) had the same serotype and biotype as epidemic or non-epidemic gentamicin-resistant klebsiella isolates which had been routinely cultured from the neonates at that time. This held also with respect to the klebsiella isolates which had been cultured from the hands or faeces of the personnel. From these surveys, however, no clear conclusion could be drawn on the source or possible route of transmission of the epidemic klebsiella strains in the NICU.

DISCUSSION

The results of this study show clearly that serotyping and biotyping of klebsiella isolates are both very useful tools in the epidemiology of these bacteria. Biotyping has already been shown to be at least equivalent to serotyping in discriminating power, typability and reproducibility (Simoons-Smit *et al.* 1985). This study proves that an epidemiological investigation of a hospital epidemic performed with both typing methods led to exactly the same conclusions. The finding that a special biotype cluster consisted of only one serotype, and vice versa, indicates that biotyping and serotyping are equally valuable in epidemiological investigations of *Klebsiella* spp. In a few cases a serotype cluster could be divided into two clusters by biotype analysis. Combination of the two typing systems thus provides even more discrimination. For efficient typing the long incubation time of some biochemical tests might be a disadvantage. However, the gelatinase test (incubation time 90 days) can be omitted from the typing system as a test with a relatively low discriminating power. The incubation time for the D-tartrate and mucate reactions can in our experience be shortened from 14 to 5 days.

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Table 4. *Antimicrobial susceptibility of klebsiella isolates (with the exception of urinary isolates)**

'OTHER' ISOLATES

Antibiotic tested	No. of isolates	All types (%) S	I	R†
Ampicillin	265	1	2	97
Carbenicillin	266	1	3	96
Cephalothin	268	69	25	6
Tetracycline	264	80	10	10
Cotrimoxazole	267	91	—	9
Gentamicin	268	85	12	3
Kanamycin	—	—	—	—
Chloramphenicol	—	—	—	—

NEONATAL ISOLATES

Antibiotic tested	No. of isolates	All types (%) S	I	R	No. of isolates	K8 (%) S	I	R	No. of isolates	K18 (%) S	I	R
Ampicillin	248	—	2	98	20	—	—	100	38	—	—	100
Carbenicillin	248	0·5	0·5	99	20	—	—	100	38	3	—	97
Cephalothin	246	41	50	9	20	65	30	5	38	23	66	11
Tetracycline	249	74	19	7	20	60	35	5	38	63	26	11
Cotrimoxazole	240	94	1	5	20	90	—	10	35	80	—	20
Gentamicin	247	**45**	**25**	**30**	20	80	—	20	37	**2**	**41**	**57**
Kanamycin	223	45	7	48	18	94	—	6	36	**3**	—	**97**
Chloramphenicol	226	15	33	52	18	6	88	6	36	—	**3**	**97**

Antibiotic tested	No. of isolates	K55 (%) S	I	R	No. of isolates	K63 (%) S	I	R	No. of isolates	K69 (%) S	I	R
Ampicillin	39	—	—	100	15	—	—	100	31	—	—	100
Carbenicillin	39	—	—	100	15	—	—	100	31	—	—	100
Cephalothin	39	21	64	15	15	100	—	—	29	21	72	7
Tetracycline	39	95	5	—	15	100	—	—	32	88	12	—
Cotrimoxazole	38	100	—	—	15	100	—	—	30	93	7	—
Gentamicin	39	**41**	**15**	**44**	15	—	**93**	**7**	31	**23**	**6**	**71**
Kanamycin	37	43	—	57	15	—	—	**100**	26	15	39	46
Chloramphenicol	38	24	21	55	15	—	—	**100**	29	10	14	76

* Deviating susceptibility percentages are printed in bold type.
† S, sensitive; I, intermediate; R, resistant.

Although antimicrobial susceptibility patterns are often used in epidemiological investigations for comparing strains with each other, this study clearly shows that these susceptibility patterns are not reliable as an epidemiological marker. But for serotyping and biotyping, the switch in time of one sero/biotype to another would have gone unnoticed in our NICU, since the sensitivity patterns of our gentamicin-resistant klebsiella isolates were similar. Although nearly all of the known capsular types and many more biotypes were represented in the isolates tested, particular capsular types or biotypes occurred more commonly than others, especially in the neonatal ward. This clustering occurred in the neonatal ward with serotypes or biotypes which were not found to be particularly common in other wards of the hospital. This predominance of a known klebsiella type for a short period of time and its replacement by another klebsiella type is in agreement with the findings of Casewell & Phillips (1978). K18, K55 and K69 were the commonest serotypes in the neonatal ward during this study. As far as we know, these types have not or, only rarely, been described as the predominant ones in intensive care units or as a cause of outbreaks of infection. No epidemic increase of a particular serotype or biotype in the non-neonatal isolates was observed. Some investigators have shown that one or two serotypes could be isolated more frequently than others (Ørskov, 1952, 1954; Steinhauer *et al.* 1966; Eickhoff, Steinhauer & Finland, 1966; Martin, Yu & Washington, 1971; Thomas *et al.* 1977; Casewell & Talsania, 1979), whereas others could not find to a significant degree a predominance of particular serotypes (Davis & Matsen, 1974; Cooke *et al.* 1979).

In our study there was a remarkable association between the clusters of serotypes in the neonatal ward and the antimicrobial resistance. With the exception of the K8 strains, all serotype clusters were more resistant to the antibiotics in common use than the other serotypes or isolates from patients in other wards, which suggests the emergence of epidemics in relation to the antibiotic policy in specialized departments, as has been reported by others (Eickhoff, Steinhauer & Finland, 1966; Steinhauer *et al.* 1966). This supposition is confirmed in our study in that all of our neonatal strains showed a high susceptibility to cotrimoxazole and tetracycline, antibiotics not in common use in our neonatal department.

Although the selection of serotypes K18, K55 and K69, based on their resistance to commonly used antibiotics, might be a reason for their high incidence in our neonatal unit, the existence of multiple foci of infection cannot be ruled out.

The environmental surveys indicated that at the time when a particular sero/biotype was prevalent, the same sero/biotype could occasionally be isolated from surfaces in the environment, from the air, from infant feeds or from faeces or hands of the staff. Other sero/biotypes of klebsiella, not predominant at that time, could also occasionally be isolated from the environment and from the staff. The question remains, whether the environmental klebsiella isolates served as a source of infection or colonization of the neonates, or whether environmental klebsiella isolates simply reflected the types cultured from the neonates. The latter seems to be the more probable explanation. The occasional finding of a predominant sero/biotype on the hands of the staff suggests a role of the hands in transmission of these strains from a colonized or infected baby to the environment or to other

babies, as has been shown by other authors (Salzman, Clark & Klemm, 1967; Knittle, Eitzman & Baer, 1975; Casewell & Phillips, 1977; Shinebaum, Cooke & Brayson, 1979; Riser, Noone & Howard, 1980).

This research was supported by a grant from the Praeventiefonds, 's Gravenhage, number 28-712.

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