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The use of a fluorescence typing method in an epidemiological study of *Klebsiella* infection in a London hospital

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SUMMARY

The fluorescent antibody technique was used to investigate an epidemic of *Klebsiella* infection in a urological ward and to trace the probable source to a contaminated sink in the treatment room. It was also shown that cross infections by particular capsular types were very common within each ward. Certain types of *Klebsiella* occurred in cut-flower water but could not be associated with the types infecting the patients.

Antibiotic resistance patterns within one capsular type were found to vary whether the type was from different sources in one patient or from different patients in the same ward. One capsular type was observed to develop resistance to increasing numbers of antibiotics over a 3-year period. This was probably due to the acquisition of R-plasmids. There also appeared to be a relation between capsular type and the site of infection.

The frequency of *Klebsiella* infections in the urological wards dropped significantly after up-grading the treatment room, improving catheter storage and reducing ampicillin use.

INTRODUCTION

The standard fluorescent antibody technique was modified in a previous paper (Riser, Noone & Poulton, 1976) to enable it to be used for serotyping antigenic strains of *Klebsiella*. Once the new method had been established, it was then applied to an epidemiological study in the Middlesex Hospital in London.

The investigation centred around an outbreak of *Klebsiella* infections in a urological ward to determine whether one particular capsular type was involved. A survey of the environment was conducted to trace the source of infection. As various sites were investigated for the presence of *Klebsiella*, it was noticed that these organisms were appearing in the flower water of cut plants by the patients' bedside. Bacteria from flower water have been shown to be responsible for some infections in patients (Ansorg, Thomssen & Stubbe, 1974); therefore a thorough examination was made of all the flower water in the urological wards and several

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other wards to see if there was a correlation between the types appearing in the water and those types infecting the patients.

Laboratory strains of *Klebsiella* which had been collected over the previous 3 years were also typed from ten selected wards to see if there had been a spread of infection with particular capsular types during that time within one ward or within the entire hospital. A predominance of any one type would indicate an endemic hospital type.

This study examined the varying antibiotic resistance patterns of otherwise identical organisms isolated from one patient and from patients in a ward over a period of time. It was undertaken to see whether the variation in antibiotic resistance observed in *Klebsiella* isolates occurred within one capsular type. The presence of R-plasmids was also looked for as a possible means of acquisition of resistance.

Antibiotic resistance patterns were considered to be too unstable to act as reliable markers of similar types of *Klebsiella*, especially in an environment where the acquisition of additional antimicrobial resistance over a period of weeks or years is not uncommon.

A particular biotype however can include many serotypes and is not an indication of an epidemic. As the serotyping of 72 different capsular antigenic types is more specific than the classifying of the half dozen common species of *Klebsiella*, it is recommended that serotyping be employed to identify these organisms and determine whether a state of cross infection exists.

MATERIALS AND METHODS

Urological ward outbreak

Laboratory isolations of *Klebsiella* from infected patients in the male urological ward (Ward A) and the female urological ward (Ward B) were serotyped by capsular fluorescence. The environment was also examined by swabbing various surfaces and materials in the two wards and streaking the swabs immediately on MacConkey plates. Typical mucoid colonies were identified by standard methods (Cowan & Steel, 1966) and *Klebsiella* species were saved for capsular typing.

Investigation of flower water

Swabs of water from vases containing various cut flowers were plated directly on MacConkey plates and incubated at 37 °C. Cultures identified as *Klebsiella* were typed by fluorescence.

General hospital typing

Strains of *Klebsiella* had been routinely collected from infected patients over a 3-year period and stored at 4 °C. Those from ten wards showing the greatest incidence of *Klebsiella* infections were selected for the study and were typed by fluorescence.

Fluorescence typing method

The *Klebsiella* strains were typed by the indirect capsular fluorescence technique of Riser *et al.* (1976). This method consists of growing the organisms on Worfel–Ferguson Medium to promote capsule production; suspending the bacteria in phosphate buffered saline (PBS); making smears; incubating the bacteria with the diluted pool antisera at pH 9·0 and then with the fluorescein conjugate; reading the slides with the fluorescent microscope; choosing the pool or pools exhibiting the brightest fluorescence and then testing with the individual antisera of the pool by the same process.

Antibiotic resistance patterns

A study was made of the changing antibiotic resistance patterns of a particular type (type 21) isolated repeatedly from one ward over a long period. A changing pattern was also examined in type 21 isolated from one patient. In addition, a comparison was made of the resistance of a capsular type isolated from different sites in one patient and from different patients in the same ward. Those cultures having resistance to four or more antibiotics were typed to see if there was a predominant resistant strain.

Antibiotic sensitivity testing

At the time of the isolation of the *Klebsiella* strains in the Middlesex Hospital, the antibiotic sensitivity testing was carried out by using multodisks (Oxoid) with control strains of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 10418) to test each batch of medium and each set of disks daily. Plates of Diagnostic Sensitivity Test (DST) agar (Oxoid) with 10% lysed horse blood were used. The following antibiotics were routinely tested from 1971: ampicillin (A), carbenicillin (C), trimethoprim (TMP), tetracycline (T), sulphatriad (S), cephalosporin (CR), nalidixic acid (NA), nitrofurantoin (N), gentamicin (G) and polymyxin B (PB).

To look for the transmission of R-plasmids, the Stokes method of sensitivity testing was employed (Stokes, 1968), which introduced the control onto each plate with the organism to be tested. Individual antibiotic Oxoid or Mast disks were used.

R-plasmid studies

Seven strains were selected from the two urological wards, four of which were the same capsular type 21 from different sites in Ward A, two were a different type (type 44) from the same ward, and one was a type 9 from Ward B. These were tested for their ability to transfer their R-plasmids to another organism.

R-plasmid techniques

A modification of the method described by Datta (1969) was used. Plates containing antibiotics were made by adding an appropriate concentration of antibiotics to the autoclaved media, as follows

MacConkey agar + rifampicin (50 μg/ml) + tetracycline (15 μg/ml).

MacConkey agar + rifampicin (50 μg/ml) + ampicillin (25 μg/ml).

Iso-sensitest agar + rifampicin (50 μg/ml) + trimethoprim (50 μg/ml).

Iso-sensitest agar + rifampicin (50 μg/ml) + sulphadiazine (100 μg/ml).

The rifampicin was dissolved in dimethylsulphoxide (DMSO) (5 mg/0·5 ml) and the other antibiotics in a small amount of water.

The donor cultures were *Klebsiella* isolates showing resistance to several antibiotics and the recipient was an *Esch. coli* K12 (F^-) J62–2 (lac^- pro his tryp rif^r) which was kindly supplied by Dr N. Datta of the Department of Bacteriology at Hammersmith Hospital, London.

Both the donor and recipient strains were grown in still cultures overnight at 37 °C in Oxoid No. 2 (glucose free) nutrient broth; 0·2 ml of the donor culture was mixed in a T-tube with 1·8 ml recipient and 6 ml of fresh broth and left on a slow shaker in a 37 °C water bath overnight. Volumes of 0·1 ml undiluted mixture were spread evenly over half a selection plate and streaked thinly over the other half and incubated at 37 °C. For application to the sulphadiazine plates only, the cells were washed once with PBS and resuspended in PBS.

Colonies that appeared to be *Esch. coli* on the MacConkey or Sensitest plates were picked off and restreaked on plain MacConkey plates to obtain a single isolated colony. The pure cultures were inoculated into peptone water and spread on sensitivity plates with an Oxford Staphylococcus control and appropriate disks. The plates were incubated at 37 °C overnight and resistance determined from the zone sizes.

RESULTS

Type 21 was the primary capsular type found in the male urological ward (Ward A) with a secondary type of 44 (Table 1). Both types were identified biochemically as *Klebsiella aerogenes*, which was confirmed by Colindale (NCTC). Two patients died during this outbreak, each with a septicaemia caused by one of these types. Type 21 was also found in the treatment room sink of this ward and in a pool of water on the sink ledge in which a box of catheters was standing. The first patient from whom this type was observed had been in the bed nearest the treatment room. On one occasion, the same type was found in flower water by his bed. When this patient was moved to the Intensive Care Unit, type 21 was also isolated from flower water outside his cubicle.

These were the only correlations between the strains isolated from flower water and from patients. Other capsular types were identified in the flower water from five wards. Types 68 and 70, however, were more common than other types in flower water and they occurred in three and two different wards respectively, out of five investigated (Table 2).

In the adjacent Ward B (the female urological ward), six of the ten *Klebsiella* strains isolated during this period were type 9, indicating that this ward had its own particular strain causing infection (Table 3). A study of the environment revealed the presence of this organism only in the sluice sink and on the ledge at the side of the sluice sink. Type 9 had been previously isolated only once in Ward B 2 years before the outbreak. Type 9 was also identified and confirmed by the Central Public Health Laboratory as *K. aerogenes*.

Table 1. *Capsular types and antibiotic resistance patterns of* Klebsiella *strains isolated during the Ward A (male urological ward) outbreak*

Patient	Specimen	Date	Capsular type	Antibiotic resistance A	C	TMP	T	S	CR	NA	N	G
1	Urine	21. x. 71	21	+	.	.	.	.	.	.	.	.
2	Urine	1. xii. 71	11, 21	+	.	.	.	.	.	.	.	.
3	Urine	16. vii. 72	21	+	+	+	+	+	+	.	.	.
4	Wound	29. v. 72	21	+	+	+	+	+	+	nt	.	.
5	Urine	iii. 74	21	+	+	+	+	+	.	+	.	.
	Sputum	iii. 74	21									
	Wound	iii. 74	21									
	R. hand	iii. 74	21									
	Mouth	iii. 74	21									
6	Suprapubic catheter	iii. 74	21									
	Urethral catheter	iii. 74	21	+	+	+	+	+	+	+	.	.
Survey	Flowers	iv. 74 to v. 74	68									
	Flowers		23									
	Flowers (Pat. 5)		68									
	Flowers (Pat. 5)		21									
	Treatment room sink		21									
	Ward sink		38									
	Ward sink		5									
	Ward sink		7									
	Plughole, treatment room sink		21									
	Catheter box*		21									
8		20. v. 74	21	+	+	+	+	+	.	+	.	.
9	Urine	28. v. 74	21	+	+	+	+	+	+	+	.	.
10†	Urine	4. vi. 74	21	+	+	+	+	+	.	+	.	.
5	Urine	4. vi. 74	21	+	+	+	+	+	+	.	.	.
5	Urine	10. vi. 74	21	+	+	+	+	+	+	.	.	.
11	Urine	vi. 74	21	+	+	+	+	+	.	+	+	.
12		vi. 74	21	+	+	+	+	+	+	+	+	.
13		vi. 74	21	+	+	+	+	+	+	+	+	.
14	Urine	7. vi. 74	21	+	+	+	.	+	.	.	+	+
15	Urethral catheter	iii. 74	44	+	+	+	+	+	+	+	.	.
15	Wound	3. vi. 74	44	+	+	+	+	+	+	.	.	.
16		vi. 74	44	+	+	+	+	+	+	+	.	.
17†		vi. 74	44	+	+	+	+	+	+	+	.	.
18		26. vi. 72	10	+	+	.	+	+	.	.	.	.
19	Blood	12. vii. 73	10	+	+	+	+	+	+	.	.	.

A, Ampicillin; C, carbenicillin; TMP, trimethoprim; T, tetracycline; S, sulphonamide; CR, cephalosporin; NA, nalidixic acid; N, nitrofurantoin; G, gentamicin.

* Catheter box standing in a pool of water on the treatment room sink surround.

† Died of septicaemia.

nt, Not tested.

Table 2. *Capsular types of* Klebsiella *found in flower water in several wards*

Source	Ward	Capsular type
Patient 5's bedside:		
Daffodils	A (male urological ward)	68
Flowers outside isolation	Intensive therapy unit	21
Rose	A	23
Flowers	A	68
Tulips	A	21
Tulips	B (female urological ward)	3
Tulips and daffodils	B	70
Tulips and daffodils	K (general surgery)	64
Dirty vase in sluice room	K	28
Tulips	K	26, 30, 69
Flowers	K	1
Flowers	L (female surgical)	70
Flowers	M (female surgical)	68

Table 3. *Capsular types and antibiotic resistance patterns of* Klebsiella *isolated from Ward B (female urological ward)*

Patient	Specimen	Date	Capsular type	A	C	TMP	T	S	CR	NA	N	G
				Antibiotic resistance								
7	Urine	27. iii. 72	9	+	.	.	.	+	.	.	.	.
24		iii. 74	11	.	.	.	.	.	.	.	.	.
		v. 74	11	.	.	.	.	.	.	.	.	.
20		20. v. 74	9	+	+	+	+	+	.	+	.	.
Survey	Sluice sink	v. 74	9	+	+	+	+	+	+	+	.	.
Survey	Ledge at side of sluice sink	v. 74	9	.	.	.	.	.	.	.	.	.
Survey	Flowers	v. 74	3	.	.	.	.	.	.	.	.	.
Survey	Flowers	v. 74	70	.	.	.	.	.	.	.	.	.
21		vi. 74	9	+	+	+	+	+	.	+	.	.
22		12. vi. 74	9	+	+	+	+	+	+	+	.	.
23		vi. 74	9	+	+	+	+	+	+	+	+	.

Most of the *Klebsiella* strains that were collected from ten wards over the 3-year period yielded random capsular types. Table 4 lists the capsular types that occurred two or more times in a particular ward.

Ward C (maternity) had two cases of type 28 out of 12 *Klebsiella* isolated over a 9-month period, but the two were separated by 9 months.

Ward D (general surgery) had three cases of type 51 over a 6-week period.

Ward E (general medicine) showed three of type 30 over a 3-month period.

Ward F (male cardiothoracic medicine) showed two of type 46 within 2 days, one of which was isolated from a patient's blood culture and the other from an infected member of staff's sputum.

Ward G (radiotherapy) had two of type 28 in 8 days.

Ward H (Neurosurgery) had two type 31 within 7 weeks and two of type 21 within $8\frac{1}{2}$ months, the latest of which was almost a year before the outbreak in Ward A.

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Table 4. *General capsular typing of Middlesex Hospital* Klebsiella *strains* (*recurring types only*)

			Cap-sular	Antibiotic resistance								
atient	Specimen	Date	type	A	C	TMP	T	S	CR	NA	N	G
Ward C (maternity)												
25 aby)	Nose swab	11. ii. 72	28	+	+	.	.	.	.	.	.	.
27	Urine	2. xi. 72	28	+	.	.	.	.	.	.	.	.
Ward D (general surgery)												
28	Sputum	4. iv. 72	51	+	+	.	.	.	.	.	.	.
	Wound	10. iv. 72	51	+	+	.	.	.	+	.	.	.
29	Wound	17. iv. 72	51	+	+	.	.	.	.	.	.	.
30	Sputum	16. v. 72	51	+	+	.	.	.	.	.	.	.
Ward E (general medicine)												
31	Urine	29. x. 71	30	+	.	.	.	+	.	.	+	.
32	Sputum	9. xii. 71	30	+	+	.	.	.	.	.	.	.
33	Sputum	30. i. 72	30	+	+	.	.	.	.	.	.	.
Ward F (male cardiothoracic medicine)												
34	Blood	31. v. 72	46	+	+	.	.	.	.	.	.	.
35	Sputum (staff)	2. vi. 72	46	+	+	.	.	.	.	.	.	.
Ward G (radiotherapy)												
36	Urine	6. iii. 72	28	+	.	.	.	.	.	.	.	.
37	Sputum	14. iii. 72	28	+	.	.	.	.	.	.	.	.
Ward H (neurosurgery)												
38	Urine	5. ix. 72	21	+	+	.	.	.	.	.	.	.
39	Urine	11. ix. 72	31	+	+	.	.	.	.	.	.	.
40	Sputum	30. x. 72	31	+	+	.	.	.	.	.	.	.
41	Urine	21. v. 73	21	+	+	+	+	+	.	.	.	.
Ward I (cardiothoracic surgery)												
42	Wound	16. viii. 71	12	+	.	.	+	.	+	.	.	.
43	Sputum	16. viii. 71	12	+	.	.	.	.	+	.	.	.
44	Sputum	21. x. 71	3	+	.	.	.	.	.	.	.	.
45	Sputum	7. xi. 71	3	+	.	.	.	.	.	.	.	.
46	Wound	10. xi. 71	3	+	+	.	.	.	.	.	.	.
47	Wound	11. xi. 71	3	+	.	.	.	.	+	.	.	.
	Tracheal swab	11. xi. 71	3	+	.	.	.	.	.	.	.	.
50	Sputum	11. xii. 71	64	+	+	.	+	.	.	.	.	.
51	Sputum	17. i. 72	64, 22	+	+	.	.	.	.	.	.	.
52	Sputum	27. i. 72	64	+	+	.	.	.	.	.	.	.
53	Sputum	28. i. 72	64	+	+	.	.	.	.	.	.	.
54	Sputum	17. iv. 72	64	+	+	.	.	.	.	.	.	+
55	Sputum	5. i. 72	66	+	+	+	+	.	.	.	.	.
56	Sputum	20. i. 72	66	+	+	+	+	.	+	.	.	.
57	Sputum	11. i. 72	66	+	+	.	+	.	+	.	.	.
58	Catheter tip	25. i. 72	66	+	+	.	.	.	.	.	.	.
59	Sputum	1. ii. 72	66	+	+	.	.	.	.	.	.	.
60	Sputum	17. iv. 72	25	+	+	.	.	.	.	.	.	.
61	Sputum	17. iv. 72	25	+	+	.	.	.	.	.	.	.
54	Sputum	17. iv. 72	25	+	+	.	.	.	.	.	.	.
62	Sputum	2. v. 72	25	+	+	.	.	.	.	.	.	.
63	Sputum	3. v. 72	25	+	+	.	.	.	.	.	.	.

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Table 4 (*cont.*)

Patient	Specimen	Date	Capsular type	Antibiotic resistance A	C	TMP	T	S	CR	NA	N	G
64	Sputum	7. v. 72	25	+	+	.	.	.	.	.	.	.
65	Sputum	23. v. 72	25	+	+	.	.	.	+	.	.	.
66	Sputum	23. v. 72	25	+	+	.	.	.	.	.	.	.
67	Sputum	16. vi. 72	25	+	+	.	.	.	.	.	.	.
68	Sputum	23. v. 72	51	+	+	.	.	.	.	.	.	.
69	Sputum	23. v. 72	51	+	+	.	.	.	.	.	.	.
70	Sputum	30. v. 72	51	+	+	.	.	.	.	.	.	.
71	Sputum	29. vi. 72	51	+	+	.	.	.	.	.	.	.
72	Sputum	27. vi. 72	51	+	+	.	.	.	.	.	.	.
73	Sputum	21. vii. 72	51	+	+	.	.	.	.	.	.	.
74	Sputum	23. vi. 72	23	+	+	.	.	.	.	.	.	.
75	Sputum	4. vii. 72	23	+	+	.	.	.	.	.	.	.
76	Throat	6. v. 72	68	+	+	.	.	.	.	.	.	.
77	Sputum	20. vii. 72	68	+	.	.	.	.	.	.	.	.
78	Wound	22. viii. 72	68	+	.	.	.	.	.	.	.	.
79	Sputum	26. viii. 72	68	+	+	.	.	.	.	.	.	.
80	Urine	5. ix. 72	68	+	+	.	.	.	.	.	.	.
81	Sputum	11. ix. 72	68	+	+	.	.	.	.	.	.	.
82	Sputum	18. x. 72	68	+	+	.	.	.	.	.	.	.
83	Sputum	18. xi. 72	68	+	+	.	.	.	.	.	.	.
84	Sputum	4. x. 72	24	+	+	.	+	.	.	.	.	.
85	Wound	17. v. 72	24	+	+	+	+	.	+	.	.	.
		Ward J (female cardiothoracic medicine)										
86	Sputum	24. ix. 71	12, 3	+	.	.	.	.	.	.	.	.

Ward I (cardiothoracic surgery) had two patients with type 12 on the same day. Type 3 was present in four patients over 21 days. There were five examples of type 66 within a month and four of type 64 in 7 weeks, with one more occurring about 3 months later. Type 25 appeared in three patients at once followed by three more over 6 days and 2 weeks later, by two more on the same day and one more within a month with a total of eight over 2 months. This was followed by five cases of type 51 in 2 months and two cases of type 23, within 2 weeks: type 68 then occurred eight times in 6 months. This was followed by two widely spaced incidences of type 24.

Ward J (female cardiothoracic medicine) yielded random types but one patient had types 12 and 3 from a sputum specimen taken during the period that types 12 and 3 were occurring in Ward I.

Type 21 was found to have been present in Ward A over the 3-year period before the outbreak. Resistance to more antibiotics was acquired over that time and during the epidemic itself (Table 1). The original isolation was resistant to ampicillin in 1971. By 1972 it was resistant to ampicillin (A), carbenicillin (C), trimethoprim (TMP), tetracycline (T), sulphonamides (S) and cephalosporin (CR).

At the time of the outbreak in 1974, some strains had also acquired resistance to nalidixic acid (NA) and nitrofurantoin (N). Near the end of this period there was one case of resistance to gentamicin (G). This resistance pattern of A, C,

Table 5. *Transference of R-plasmids* from* Klebsiella *strains to* Escherichia coli

Patient	Specimen	Capsular type	Present resistance patterns						Transferred to *Escherichia coli*
5	Urine	21	A	C	TMP	T	NA		—
5	Sputum	21	A	C	TMP	T			—
5	R. hand	21	A	C	TMP	T		S	(ATS) (AT)
Survey	Plughole of treatment room sink	21	A	C	TMP	T	NA	S	(S)
15	Urethral catheter	44	A	C	TMP		NA	S	—
17		44	A	C	TMP	T	NA	S	(TS)
20		9	A	C	TMP	T	NA	S	(AT)

A, Ampicillin; C, carbenicillin; TMP, trimethoprim; T, tetracycline; S, sulphonamide; NA, nalidixic acid.

–, No transference.

* Transference of A, TMP, T and S tested for.

TMP, T, S, CR and NA was also found with type 44 in that ward at that time. A similar pattern was also observed in type 9 which was being spread, to a lesser extent, around the adjacent Ward B (Table 3).

The investigation of the R-plasmids indicated that four of the seven *Klebsiella* isolates tested had transferred R-plasmids to the *Esch. coli* recipient (Table 5). After mating with type 21 from Patient 5's right hand, *Esch. coli* was isolated that had acquired resistance to T, A and S on six separate occasions. On one occasion it was found to be resistant to T and A but not S.

When the *Esch. coli* was mated with a type 21 from the sink plughole on two occasions resistance was demonstrated to S only.

When the donor was a type 44 (Patient 17) there were four instances of *Esch. coli* being recovered that were resistant to T and S.

With a type 9 donor (Patient 20), there were five incidents of resistance to the T, A combination. The other specimens did not demonstrate transferability.

The organisms showing resistance to four or more antibiotics were generally of random capsular type. However, type 66 was the most common type to show multiple resistance, comprising eight of the 39 resistant isolates typed. These were present in six different wards in sputum (four cases), wound (one case), CSU (two cases) and HVS (one case) specimens; and they all occurred between 27. xi. 71 and 26. i. 72. There were also generally more multiply resistant *Klebsiella* strains of other types occurring throughout the hospital during this period than at any other time during the study (Fig. 1).

The same capsular type was usually found in any specimen taken from one patient, whatever the source; but the resistance patterns could vary (Table 6). The same type from different patients in one ward also displayed an inconsistent antibiotic resistance pattern (Tables 1, 3 and 4).

Table 7 shows that types 3, 25, 31, 51, 64 and 68 were more common in respiratory tract infections; while type 21 and possibly 28 and 30 were more frequent in urinary tract infections. Type 66 occurred equally in any site.

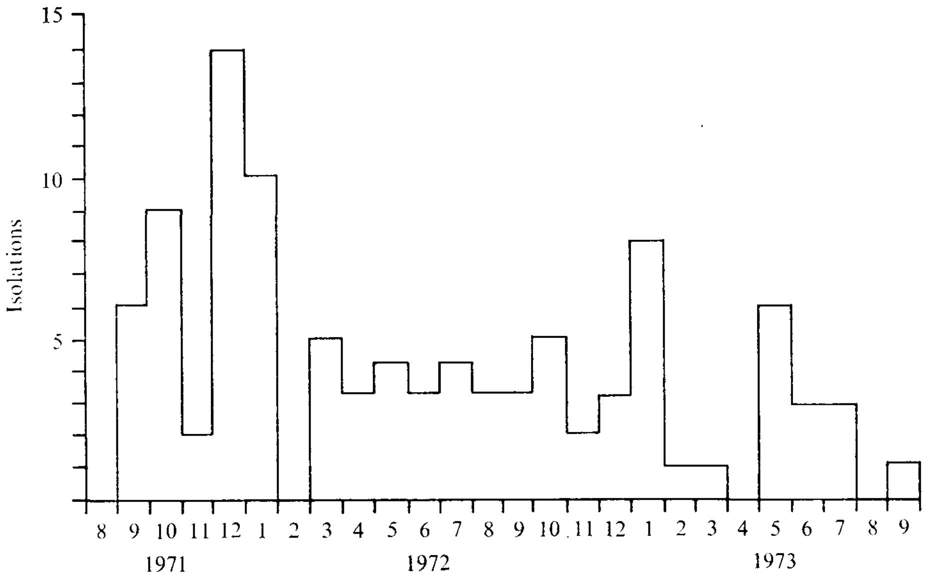

Fig. 1. Number of *Klebsiella* isolations showing resistance to four or more antibiotics.

DISCUSSION

The organism responsible for the major outbreak in the male urological ward (Ward A) was found to be a type 21 *Klebsiella aerogenes*. This organism was traced to the treatment room sink, which consisted of a stainless-steel basin sunk into a formica skinned wooden surround. The lip of the sink overlapped the surround, creating a trap for water. This area was always moist and was impossible to clean or disinfect. The *Klebsiella*, which requires a moist environment, was recovered from the sink as well as the surround where many preparations were made. It was isolated from a box of catheters standing in a pool of water on the surround. Catheters were widely used post-operatively in the ward and this may have been the source of infection.

It is difficult to know whether the organism was spread from the sink to the patient or vice versa, but it has been shown that this particular type had been present and causing sporadic infections in this ward for at least $2\frac{1}{2}$ years before the outbreak.

This underlines the importance of being able to type a strain accurately so that it is possible to know whether one particular type is responsible for the infections observed. Such a finding will indicate that a common source of infection or cross infection exists and the necessary steps can then be taken to stop transmission of the organism.

Patient 5, who showed the first type 21 in the major outbreak, had a urethroplasty followed by a prolonged stormy convalescence with a great deal of *Klebsiella* infection. Finally he was very strictly isolated in a portable island ward. Under these conditions his urinary tract cleared itself of *Klebsiella* within a week without any antibiotic or other specific anti-infection treatment.

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Table 6. *Capsular types and resistance patterns of multiple isolations from one patient*

				Antibiotic resistance								
Patient	Specimen	Date	Capsular type	A	C	TMP	T	S	CR	NA	N	G
5	Sputum	iii. 74	21	+	+	+	+	+	.	nt	.	.
	Urine	iii. 74	21	+	+	+	+	+	.	+	.	.
	Urine	4. vi. 74	21	+	+	+	.	+	+	.	.	.
	Urine	10. vi. 74	21	+	+	+	.	+	+	.	.	.
6	Suprapubic catheter	iii. 74	21	+	+	+	+	+	+	+	.	.
	Urethral catheter	iii. 74	21	+	+	+	+	+	+	+	.	.
15	Urethral catheter	iii. 74	44	+	+	+	+	+	+	+	.	.
	Wound	4. vi. 74	44	+	+	+	+	+	+	nt	.	.
28	Sputum	4. iv. 72	51	+	+	.	.	.	.	.	.	.
	Wound	10. iv. 72	51	+	+	.	.	.	+	.	.	.
47	Wound	11. xi. 71	3	+	.	.	.	.	+	.	.	.
	Tracheal swab	11. xi. 71	3	+	.	.	.	.	.	.	.	.
54	Sputum	17. iv. 72	64	+	+	.	.	.	.	.	.	+
	Sputum	17. iv. 72	25	+	+	.	.	.	.	.	.	.
3	Urine	16. vii. 72	21	+	+	+	+	+	+	.	.	.
	Sputum	17. vii. 72	21	+	+	+	+	nt	+	.	.	.
87	Urine		27	+	+	.	.	.	.	.	.	.
	Blood		27	+	+	.	.	.	.	.	.	.
	Wound		27	+	+	.	.	.	.	.	.	.
88	Wound	1. viii. 72	31	+	+	.	.	.	.	.	.	.
	Wound	9. viii. 72	31	+	+	.	.	.	.	.	.	.
89	Rectal swab	14. ii. 72	38	+	+	.	.	.	.	.	.	.
	Urine	15. ii. 72	38	+	.	.	.	+	.	.	.	.
	Urine	18. ii. 72	38	+	.	.	.	.	.	.	.	.
90	Wound	29. iii. 72	3	+	+	.	.	.	.	.	.	.
	Wound	5. iv. 72	3	+	+	.	.	.	.	.	.	.
91	Bile, Urine	10. i. 72	66	+	+	+	+	.	+	.	.	.
	Bile, Urine	10. i. 72	66	+	+	.	.	.	.	.	.	.

nt, Antibiotic not tested.

The treatment room in Ward A was closed, the old sink replaced by a modern one, and the room thoroughly cleaned. Also around this time, the antibiotic policy in this unit changed, in that ampicillin virtually ceased to be used, having previously been widely employed therapeutically and for prophylactic purposes. Whether stopping ampicillin, removing the sink reservoir, changing the location of the catheter store, or something else was responsible for the decline in infections, is not exactly clear. However, the incidence of *Klebsiella* isolations dropped dramatically from being very common to very rare. Between July 1975 and July 1976, *Klebsiella* was isolated from this ward on only nine occasions.

In two cases, type 21 was found in flower water by Patient 5's bedside; however, it may have been passed from the patient to the flowers as this type was also found on his hands and mouth.

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Table 7. *Capsular type versus site of infection*

Capsular type	No. of specimens	No. of wards involved	No. of infections of: Respiratory tract	Urinary tract	Others
3	7	3	5 (71)*	—	2 (29)
21†	16	5	1 (6)	14 (88)	1 (6)
25	11	3	10 (91)	1 (9)	—
28	10	8	3 (30)	5 (50)	2 (20)
30	7	5	2 (29)	4 (57)	1 (14)
31	6	3	4 (67)	1 (17)	1 (17)
51	15	7	11 (73)	2 (13)	2 (13)
64	9	3	8 (89)	1 (11)	—
66	9	7	4 (44)	3 (33)	2 (22)
68	11	4	8 (73)	1 (9)	2 (18)

* Figures in parentheses are percentages.
† Ward A had 12 of the 16 specimens, 10 of which were from the urinary tract.

In the survey of several wards, many organisms were found to be present in the flower water but only a few of them were *Klebsiella*. Of these, there were three incidences of type 68 and two of type 70, which were not isolated from the patients. These may prove to be types associated with plants. These organisms, therefore, do not seem to constitute a threat of infection to the patients in general wards; however, the flower water was not tested in the four wards where patients did have infections caused by type 68. Seven of these latter specimens were from sputum; two from wounds, and one from urine.

From the study of *Klebsiella* strains isolated over 3 years from ten different wards, it appears that most wards have periodic outbreaks of particular types. An organism may be harboured in the hospital and passed to the patient with a lowered resistance or it may be brought in by the patient himself, either as an active infection or as one that develops after use of antibiotics. It may spread rapidly or slowly from one patient to another and then disappear, sometimes to be replaced by another type, as observed in Ward I, or to reappear at a later date, as reflected in occasional large gaps between specimens of the same type.

These observations may imply a common contaminated material as a source of infection, such as hand cream, disinfectants, medicines, food, water, fomites, or, as in the case of Ward A, a contaminated sink. They may suggest careless procedures of the staff, such as the case in Ward F where a sputum specimen from a member of staff yielded the same type 46 two days after it had been isolated from a patient's blood culture; or, again in Ward A, where catheters were taken from a wet box to be used on the patients.

These conditions may also be caused by patient communication or colonization. If an organism can colonize a series of patients, it may only be expressed as an infection under the appropriate conditions. Thus a subliminal reservoir may be maintained over a long period of time.

All these factors may be involved in the spreading of a type of *Klebsiella*. The minor spreading is usually self-limiting; but when larger numbers of patients become involved, it is advisable to try to trace the particular cause.

There did not seem to be a predominant hospital type at the Middlesex Hospital. The most common types which were randomly scattered and whose numbers were not associated with the two major outbreaks were types 51 (9·4 %), 25 (6·1 %), 28 (5·6 %), and 68 (5·6 % of strains typed).

If the number of strains is to include those which were were found in Wards A and B, then type 21 comprised 10·9 %, and type 51 was then 8·4 % of the total strains typed.

There appeared to be a relation between the capsular type and the site of infection. There was an indication that types 3, 25, 31, 51, 64 and 68 had a greater affinity for respiratory tract infections; while types 21, 28 and 30 occurred predominantly in urinary tract infections. This may suggest a preference of certain types for the different substrates or conditions present in a particular site; although, given the opportunity, these types will also cause infections in other areas. Other types, such as type 66, may not be as affected by the environment. This may be related to the fact that it was the most frequently occurring resistant strain, although not every type 66 showed multiple resistance.

Four of the eight multiply resistant specimens of type 66 were from Ward I (cardiothoracic surgery) and Ward J (female cardiothoracic medicine). This implies a spread of this type in that area; but it was also present in four other unrelated wards during the same 2-month period. It was also noted that at this time there was a dramatic increase in the total number of resistant strains of *Klebsiella* of random types in the entire hospital.

This 6-month period of higher incidence of multiply resistant klebsiellas may have been associated with antibiotic use at the time. Unfortunately, records of this are not available.

The same capsular type was usually isolated from different sources in a given patient and could display the same or different antibiotic resistance patterns. Isolations from the same source in one patient can also have varying resistance. This shows that both sensitive and resistant strains can occur simultaneously in the same patient, even in the same infected site. Identical types isolated from different patients within one ward also showed this variation.

The capsular type 21 *Klebsiella aerogenes* which caused the outbreak in Ward A had been present in that ward for at least 2½ years. The only difference was a change in the antibiotic sensitivities over time. In 1971 this type was resistant only to ampicillin but by 1972 had acquired resistance to five other antibiotics. During the outbreak, resistance to three other antibiotics was also observed and the same general resistance pattern was also found in a type 44 which appeared in the ward at that time. A similar pattern was also found in type 9 in an adjacent ward which used the same staff. During this time an *Esch. coli* was also isolated from Ward A with the same resistance.

This suggests the presence of R-plasmids which may be responsible for the accumulation of the resistance observed.

When seven *Klebsiella* strains were tested for the presence of R-plasmids, four were found able to transfer resistance to *Esch. coli*.

By studying the combinations of resistance transferred, it appears that at least two and possibly three plasmids are responsible for the transmission of resistance to ampicillin, tetracycline and sulphonamide. Compatibility testing of the R-plasmids would have to be done to show whether the plasmids were identical in all the strains.

At this stage, it is possible to say only that transferable R-plasmids do exist in these *Klebsiella* strains and that the increase in resistance could have been due to their acquisition, and this resistance could have been passed on to other organisms.

The multiple resistance expressed in the *Klebsiella* antibiotic patterns is probably a direct reflexion of the antibiotics administered to the patients, many for prophylactic reasons. The more antibiotics that were used, the greater the resistance that was acquired and the more predominant these organisms became. This was associated with the death of two patients with bacteraemia in one ward. Reducing the use of antibiotics, and on this occasion particularly ampicillin, resulted in the disappearance of *Klebsiella* species as a serious cause of infection in the urological wards.

This is similar to the observation of Price & Sleigh (1970) who found that *Klebsiella aerogenes* infections in a neurosurgical unit could be controlled by withdrawal of all antibiotics, except those needed to treat proved infections.

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