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Nosocomial klebsiellas
I. Colonization of hospitalized patients

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SUMMARY

The colonization of patients by *Klebsiella* and several other gram-negative bacteria was studied in a hospital urological ward over a period of six months. Before and during the survey there was no evidence of an outbreak of nosocomial infection and multi-drug resistant strains of *Klebsiella* were not isolated.

Klebsiella were biotyped by nine biochemical tests, which led to the detection of 66 biotypes spread uniformly throughout the survey period. This method of biotyping proved a useful epidemiological tool. The colonization rate of throats, hands, and faeces of patients increased after admission to the ward, especially when antibiotics were used. The effect of systemic antibiotics was greater than that of urinary antibiotics especially on throat and faeces carrier rates. Carrier rates for *Klebsiella* increased also after catheterization and operation – relationships which could well be multifactorial.

During the first two weeks after admission the proportion of antibiotic resistant strains of *Klebsiella* in carriers increased. The proportion of resistant strains amongst isolations from clinical infections was always greater than among strains isolated routinely from sites of carriage.

INTRODUCTION

Whilst the term 'nosocomial' is generally used to describe infections contracted during stays in hospital, in this paper its use is restricted to the epidemiology of colonization by *Klebsiella* in relation to hospitalization.

Since asepsis was introduced some 70 years ago to control infection within hospitals, no measure of similar effectiveness has been added to prevent hospital infection. The use of antibiotics for prophylaxis and treatment of infection has not significantly diminished the incidence of nosocomial infection and may even have increased the prevalence of plasmids in the hospital bacterial pool (Finland & McGowan, 1976).

Alexander (1973) estimated the incidence of hospital infection in the United States at 6·3 %, with the incidence reported varying strikingly between hospitals.

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Many reports on the prophylactic use of antibiotics in surgical patients have been published. Whilst some demonstrated beneficial effects, others did not. The infections that did occur usually involved antibiotic-resistant organisms and were more serious and more difficult to treat. The success or failure of prophylactic antibiotic therapy may well depend on local factors within hospitals, many of which are not always readily identifiable (Hunt *et al.* 1975).

As hospitals today present a reservoir of pathogens especially for patients susceptible to infection, it seems obvious that new or better antibiotics will not solve the problem of nosocomial infection. To find means for prevention of infection, the causes must be studied. Control of infection requires an understanding of the reservoirs and modes of transmission of organisms within hospitals rather than the blind use of chemoprophylaxis. This report surveys these factors in a hospital ward during a period when hospital infection was endemic and no epidemic was apparent.

METHODS

The ward

The ward for urological inpatients at the University Hospital, Dijkzigt, Rotterdam, where the survey was carried out, consists of two rows of rooms, mainly wards, separated by service rooms, opening on two parallel corridors. There are 3 four-bedded wards and 2 two-bedded wards along the East corridor and 4 four-bedded and 1 two-bedded wards on the West corridor. At the time of the survey the ward was not mechanically ventilated.

There is no through traffic on these corridors but patients who were not restricted to bed could move freely on their corridor to the bathroom or to meet each other. The nursing staff worked on either the East or West corridor and at central service rooms. Patients washed and ate in their wards. Sandwiches and beverages were prepared by nursing staff in the ward. Hot meals, prepared in a central kitchen for the whole hospital, were brought to the ward as individual meals for distribution by the nursing staff.

Survey design

The survey was carried out in two periods separated by an interval of 3 weeks to allow for Christmas to New Year's Day. The periods covered are 30 September to 22 December 1974 and 13 January to 27 April 1975. Patients were examined on admission to the ward or soon afterwards. During the survey on Mondays, patients, nursing staff and a number of objects were examined bacteriologically. From each patient, the throat, a hand (preferably the right hand) and a faecal sample were examined. Results from bacteriological examination requested by the patients' medical attendant were included only when one of the species surveyed was isolated. The result from strains repeatedly isolated from the same source on clinical request was included only once weekly.

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Bacteriological methods

Throats were swabbed with dry swabs which were spread on blood agar plates. Hands were moved in cups containing 0·9% NaCl in water for about half a minute. The normal saline was then filtered through a Millipore filter (0·8 μm) to collect the organisms from the hands, and the filter placed on a blood agar plate. Faecal samples collected by rectal swabbing were plated on MacConkey agar, a modified nitrogen-deficient medium and nalidixic acid cetrimide plates. All plates were incubated aerobically at 37 °C and read after 24 and 48 h.

Nalidixic acid cetrimide agar is selective for *Pseudomonas aeruginosa* by suppressing growth of enterobacteriaceae (Goto & Enomoto, 1970). Nitrogen-deficient medium for the isolation of *Klebsiella* and *Enterobacter* as described by Eller & Edwards (1968) was modified by the addition of 0·5% sodium taurocholate to improve its selectiveness for Enterobacteriaceae and 0·35 % (v/v) neutral red 2% (w/v) to contrast colonies of *Klebsiella* and *Enterobacter* against the colourless medium.

Bacteriological identification

The survey aimed at the isolation of *Klebsiella*, *Enterobacter*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Single colonies of these species were picked from plates for identification by the criteria of Edwards & Ewing (1972) and Cowan & Steel (1966).

The essential criteria for the identification of *Klebsiella* were absence of motility and ornithine decarboxylase. Other criteria used were: a negative phenylalanine test; the fermentation of mannitol, xylose, salicin, and inositol; urease production, and a positive Voges–Proskauer (VP) test. One of these tests could be negative without invalidating the identification of a strain as *Klebsiella* other than *K. rhinoscleromatis* in which both urease and VP tests are negative.

Klebsiellas were subdivided into biotypes by the fermentation of dulcitol, adonitol, sorbose, D-tartrate, mucate; citrate utilization, production of urease and indol, V.P. and methyl-red (MR) tests. If it is assumed that the result of these tests is mutually independent, which seems unlikely, 2^{10}, i.e. 1024 different biotypes of *Klebsiella* might be expected. Amongst the 72 strains of different serotypes of *K. aerogenes* collected by Ørskow (1955) 64 biotypes were found, whereas in this survey 530 strains of *Klebsiella* were divided into 66 biotypes of which 27 were not represented in Ørskow's collection.

In each series of biotyping tests, seven strains, including positive and negative results of each test, were included as controls. If the controls showed results inconsistent with previous tests, the series was discarded. If differences were noted in *Klebsiella* colonies on plates, one colony of each variety was picked for examination. In this way more than one biotype per sample could be identified.

Statistical analysis

A difference is considered statistically significant if the probability that chance alone could have caused it is less than 5% ($P = < 0{\cdot}05$). If a probability is

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Table 1. *Discrepancies in biotyping* in vitro

(Twenty strains biotyped 20 times.)

Tests	Results of biotyping tests: All positive	All negative	Showing discrepancies	Nos. of positive tests in strains showing discrepancies
Dulcitol	6	13	1	15
Adonitol	11	8	1	5
Sorbose	12	7	1	19
Indol	4	16	0	
Urease	14	3	3	19, 18, 15
V.P.	11	6	3	8, 8, 8
Methyl red	8	11	1	19
Tartrate	6	11	3	18, 15, 1
Citrate	3	11	6	18, 18, 18, 18, 15, 12
Mucate	12	1	7	17, 14, 8, 5, 5, 4, 2

Table 2. *Discrepancies in biotypes in the follow-up studies of patients*

(Biotypes of 40 isolates compared with 40 isolates of other biotypes from similar sources (1) and with 40 isolates of other biotypes from sources on other patient (2) in successive weeks.)

Tests	Nos. of comparisons with discrepancies: Similar source (1)	Different source (2)
Dulcitol	19	24
Adonitol	8	5
Sorbose	16	22
Indol	6	9
Urease	2	2
Voges-Proskauer	2	2
Methyl red	13	7
Tartrate	20	23
Citrate	23	20
Mucate	5	0
Totals	114	114

noted in this report by a single value, it has been calculated using Fisher's exact method. If it is indicated by a range, a method using χ^2-approximation has been used.

RESULTS

Consistency of biotyping

To study the consistency of biotyping, 20 *Klebsiella* strains were each biotyped 20 times. These strains had been freeze-dried for varying periods but during biotyping were maintained on nutrient agar at 4 °C. Strains were chosen to give both positive and negative results in each test and do not reflect the prevalence of biotypes found in the survey.

All tests except that for indole showed some inconsistency (Table 1). Inconsistent results occurred more than 5 out of 20 times in approximately half the strains, indicating an excess of discrepancies for some strains. In discrepant strains, the

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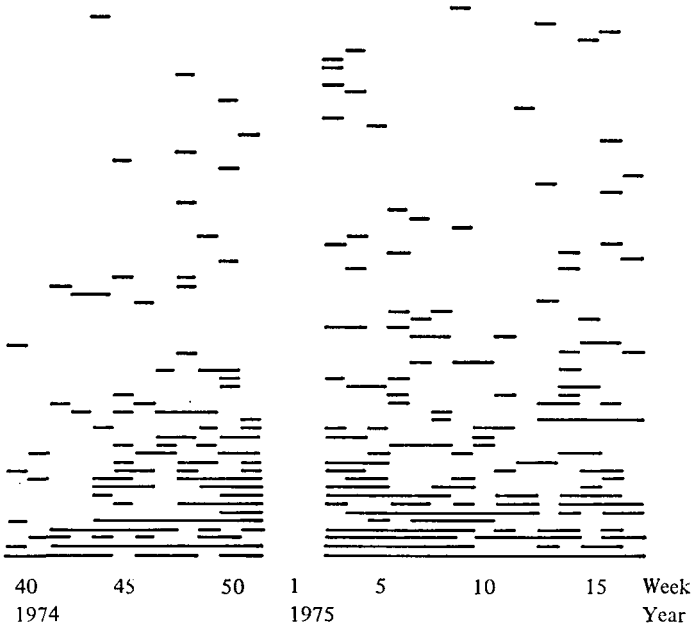

Fig. 1. Incidence of *Klebsiella* biotypes. Each line represents a particular Klebsiella-biotype.

results of several tests were often discrepant. Half the strains had discrepancies in two or more tests. The tendency towards discrepant results in biotyping is not uniform, either for strains or tests. This random distribution makes it impossible to estimate the effect of inconsistency on the epidemiological findings.

The possible effect of inconsistency on the data from the epidemiological survey can, however, best be assessed from the epidemiological data collected. As the biotype consists of a combination of results, one might expect fewer discrepant results to occur between correctly and incorrectly biotyped strains than between arbitrarily chosen strains of different biotype. For this purpose, two sets of data were selected from the survey. One group consisted of pairs of different biotypes isolated from the same patients in successive weeks; the other of pairs of different biotypes isolated from different patients in successive weeks. Incorrect typing can be expected to produce a greater number of similar results in the first group. Table 2 shows that the number of discrepant results was the same in both groups, thus providing no evidence for any effect of inconsistent biotyping on the data from the survey.

The patients

During the survey, 347 admissions of 310 patients were recorded. Six patients were admitted during both periods as well as during the interval between periods; their admission to hospital after the interval is considered as a new admission. Numbers of 'patients' in this report should be interpreted as number of admissions.

The 347 patients included 49 women, of mean age 50·7 years, which did not

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Table 3. *Prevalence of positive cultures during admissions of patients*

Admission period (weeks)	No. of examinations	Nos. of positive cultures and isolation rates (/1000)									
		*Klebsiella**		*E. coli*		*Pseudom.*		*Enterob.*		*P. mir.*	
		No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
					Throat						
1	325	16 (22)	49 (68)	14	43	3	9	4	12	2	6
2	136	21 (23)	154 (169)	13	96	5	37	7	51	0	—
3	71	15 (16)	211 (225)	6	85	3	42	5	70	0	—
4	37	6 (6)	162 (162)	4	108	2	54	1	27	0	—
					Hand						
1	326	23 (27)	71 (83)	10	31	3	9	7	21	1	3
2	137	21 (21)	153 (153)	8	58	2	15	6	44	4	29
3	73	19 (20)	260 (274)	1	14	3	41	2	27	5	68
4	38	6 (7)	158 (184)	3	79	1	26	1	26	1	26
					Faeces						
1	311	48 (52)	154 (167)	234	752	11	35	12	39	9	29
2	126	41 (48)	325 (381)	94	746	6	48	2	16	4	32
3	65	26 (28)	400 (431)	49	754	6	92	3	46	0	—
4	36	16 (18)	444 (500)	27	750	4	111	2	56	3	46

Statistical analysis of differences between the 4 weeks

Throat	χ^2/D.F.	23·7969/3	6·0730/2	6·2941/1	8·0714/1	—
	P	< 0·0005	0·050–0·025	0·025–0·010	0·005–0·001	
Hand	χ^2/D.F.	9·6193/3	1·9725/2	2·0510/1	1·1413/1	22·2640/1
	P	0·025–0·010	0·40–0·30	0·20–0·10	0·30–0·20	< 0·0005
Faeces	χ^2/D.F.	37·3974/3	0·0229/3	6·4013/3	0·2312/1	0·0164/1
	P	< 0·0005	> 0·99	0·10–0·05	0·70–0·60	0·90–0·80

* Isolated biotypes shown in parentheses.

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Table 4. *Colonization and antimicrobial treatment*

Admission period (weeks)	Before antibiotic treatment: No. examined	No. of isolations of *Kl.*	*Ec.*	*Ps.*	*Eb.*	*Pm.*	During or after antibiotic treatment: No. examined	No. of isolations of *Kl.*	*Ec.*	*Ps.*	*Eb.*	*Pm.*
					Throat							
1	252	13	9	2	3	2	73	9	5	1	1	0
2	49	3	3	0	2	1	87	20	13	4	6	0
3	20	1	0	1	1	0	51	15	6	3	5	0
4	6	0	0	0	0	0	31	6	3	2	1	0
					Hand							
1	252	22	6	1	5	1	74	5	4	2	2	0
2	49	7	4	1	1	2	88	14	5	1	5	2
3	21	5	0	0	0	2	52	15	3	2	2	3
4	6	0	0	0	0	0	32	7	3	1	1	1
					Faeces							
1	238	34	185	8	8	6	73	18	49	3	4	3
2	44	13	33	2	1	1	82	35	61	4	1	3
3	19	6	15	1	2	0	46	22	34	5	0	0
4	6	3	6	1	0	0	30	15	20	5	2	3

Statistical analysis: P (%)

	Klebsiella			*E. coli*			*Pseudomonas*			*Enterobacter*			*P. mirabilis*		
	T	H	F	T	H	F	T	H	F	T	H	F	T	H	F
1	6	64	5	32	24	9	99	13	72	99	66	49	99	99	69
2	2	69	18	17	72	61	30	99	69	71	42	99	36	62	83
3	3	78	28	17	55	76	74	99	66	67	99	8	—	62	—
4	56	33	—	99	99	16	99	99	99	99	99	99	—	99	99

T, throat; H, hand; F, faeces.

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Table 5. *Prevalence of* Klebsiella *and antimicrobial agents*

Admission period (weeks)	No antibiotic: No. of examinations	No antibiotic: Isolation of Klebsiella No.	No antibiotic: Isolation of Klebsiella Rate	Systemic antibiotic: No. of examinations	Systemic antibiotic: Isolation of Klebsiella No.	Systemic antibiotic: Isolation of Klebsiella Rate	Urinary antibiotic*: No. of examinations	Urinary antibiotic*: Isolation of Klebsiella No.	Urinary antibiotic*: Isolation of Klebsiella Rate
				Throat					
1	252	13	52	45	6	133	28	1	36
2	50	3	60	58	17	293	40	4	100
3	25	1	40	32	13	406	21	5	238
4	9	9	1000	19	6	316	11	1	91
				Hand					
1	252	22	87	46	4	87	28	1	36
2	51	7	137	58	10	172	26	2	77
3	26	5	192	32	10	313	40	5	125
4	9	0	0	20	5	250	22	7	318
				Faeces					
1	238	34	143	45	14	311	28	2	71
2	45	12	267	55	22	400	25	5	200
3	22	7	318	29	14	483	38	9	237
4	9	4	444	19	7	368	21	9	429

Statistical analysis: P (%)

	No *v.* systemic: Throat	No *v.* systemic: Hand	No *v.* systemic: Faeces	No *v.* urinary: Throat	No *v.* urinary: Hand	No *v.* urinary: Faeces	Systemic *v.* urinary: Throat	Systemic *v.* urinary: Hand	Systemic *v.* urinary: Faeces
1	9	99	1·5	78	49	39	24	64	2·0
2	0·2	79	20	70	49	58	2·3	33	13
3	0·2	37	27	8	50	55	25	8	4·2
4	14	15	99	99	8	99	22	74	99

* Excluded two patients who got a systemic antimicrobial in the same week also.

differ significantly from the mean age of male patients, 51·2 years. Although most patients remained in hospital less than a week and were examined bacteriologically only once or twice, the number of examinations is still considerable: 769 throat swabs, 711 hand samples and 723 faeces samples, and 173 samples requested on clinical grounds from other sources in patients are included as from these samples one or more of the bacterial species included in the survey was isolated.

Bacteriological

A total of 530 *Klebsiella* strains were isolated during the survey. This relatively high number includes a variety of strains isolated from the same sample. Sixty six biotypes were recognized: 28 (42 %) were isolated in 1 week only, and 8 (12 %) during more than half the survey periods (Fig. 1).

The incidence of cultures of *Klebsiella* or any of the other bacterial genera studied, isolated from patients, nursing staff and from food and objects during the survey did not show any significant fluctuation. The bacteria were endemic throughout without a significant rise in isolations that could have indicated an epidemic.

Colonization during admission

The rate of colonization by *Klebsiella* biotypes in patients increased at all sites, throat, hand, and faeces, during the first 3 or 4 weeks of residence in the ward (Table 3). For the other bacteria studied only throats showed the same trend. Insufficient strains of *Proteus mirabilis* were isolated from throats for analysis. The increase, however, in the rate of isolation of *Proteus mirabilis* from hands after a week in hospital is highly significant.

As most patients were discharged from the ward within 4 weeks, only 28 patients were examined weekly for 4 weeks. The trends in isolation rates from these patients are generally similar to the trends in all patients, but the number is too small for statistical analysis.

Antibiotic treatment

Isolation rates of *Klebsiella* were higher in patients who received antibiotic treatment in the week before bacteriological examination than in patients who did not (Table 4). The difference in rates was significant only for the isolation of *Klebsiella* from throats and faeces.

Most antibiotics were given for short periods and often in combination, which complicates analysis of the effect of individual antibiotics. The data, however, do allow the effect of groups of antibiotics to be analysed. All penicillins, cephalosporins, aminoglycosides, tetracyclines, chloramphenicol, rifamycins, and cotrimoxazole are grouped together as systemic antibiotics; sulphonamides, nitrofurantoin and nalidixic acid as urinary antibiotics. Systemic and urinary antibiotics were rarely given in the same week to the same patient. The highest rates for *Klebsiella* were always found in patients receiving systemic antibiotics (Table 5). The differences in rates between patients receiving urinary antibiotics and no antibiotics are smaller and not significant.

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Table 6. *Colonization and indwelling catheter in the bladder*

Admission period (weeks)	Before catheterization: No. examined	Before catheterization: No. of isolations of *Kl.*	*Ec.*	*Ps.*	*Eb.*	*Pm.*	During or after catheterization: No. examined	During or after catheterization: No. of isolations of *Kl.*	*Ec.*	*Ps.*	*Eb.*	*Pm.*
					Throat							
1	274	16	11	2	2	2	51	6	3	1	2	0
2	70	10	6	4	3	0	66	13	7	1	4	0
3	29	5	2	2	0	0	42	12	4	1	5	0
4	12	1	2	0	0	0	25	5	2	2	1	0
					Hand							
1	274	12	8	2	3	0	52	15	2	1	4	1
2	71	7	0	2	2	1	66	14	8	0	4	3
3	31	4	1	3	1	3	42	16	1	0	1	2
4	13	0	0	0	0	0	25	4	3	1	1	1
					Faeces							
1	262	39	199	7	6	8	49	13	35	4	6	1
2	65	18	44	2	1	1	61	29	50	4	1	3
3	27	7	18	3	0	0	38	21	31	3	3	0
4	13	4	11	0	0	0	23	14	16	4	2	3

Statistical analysis; P (%)

	Klebsiella			*E. coli*			*Pseudomonas*			*Enterobacter*			*P. mirabilis*		
	T	H	F	T	H	F	T	H	F	T	H	F	T	H	F
1	22	< 1	6	71	66	59	40	41	8	12	1	< 1	99	16	79
2	49	10	3	77	< 1	10	37	50	43	71	43	99	—	35	35
3	40	2	2	79	99	24	56	7	69	7	99	26	—	64	—
4	64	28	16	58	54	44	55	99	27	99	99	53	—	99	29

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Table 7. *Colonization and abdominal operation*

Admission period (weeks)	Before operation: No. examined	Before operation: No. of isolations of *Kl.*	*Ec.*	*Ps.*	*Eb.*	*Pm.*	After operation: No. examined	After operation: No. of isolations of *Kl.*	*Ec.*	*Ps.*	*Eb.*	*Pm.*
					Throat							
1	295	20	12	3	4	2	30	2	2	0	0	0
2	71	10	9	3	2	0	65	13	4	2	5	0
3	29	2	1	1	1	0	42	14	5	2	4	0
4	16	0	3	0	0	0	21	6	1	2	1	0
					Hand							
1	259	25	10	3	5	0	31	2	0	0	2	1
2	72	5	3	0	3	2	65	16	5	2	3	2
3	31	5	0	3	1	1	42	15	2	0	1	4
4	16	2	1	1	0	0	22	5	2	0	1	1
					Faeces							
1	281	45	213	11	10	7	30	7	21	0	2	2
2	67	21	49	3	1	0	59	27	45	3	1	4
3	26	9	24	3	2	0	39	19	25	2	0	0
4	14	6	11	1	1	0	22	12	16	3	2	3

Statistical analysis: P (%)

	Klebsiella			*E. coli*			*Pseudomonas*			*Enterobacter*			*P. mirabilis*		
	T	H	F	T	H	F	T	H	F	T	H	F	T	H	F
1	99	75	31	63	61	51	99	99	61	99	17	61	99	11	21
2	37	1	10	25	48	84	79	22	99	26	99	99	—	99	5
3	1	7	31	39	50	< 1	80	7	38	40	99	16	—	35	—
4	3	68	73	30	82	78	50	42	64	99	99	78	—	99	27

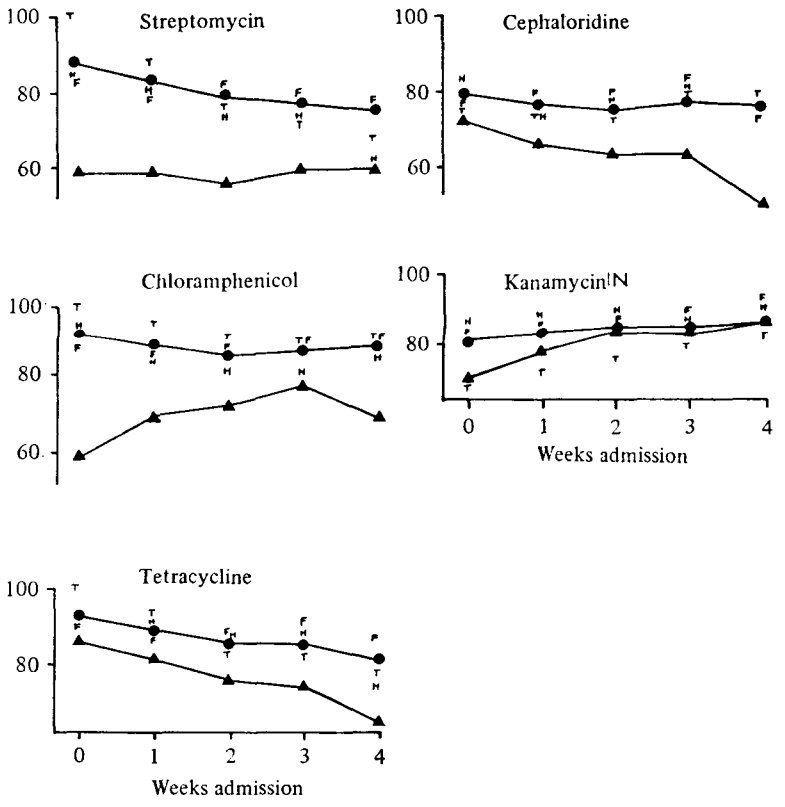

Fig. 2. Sensitivity of *Klebsiella* for antimicrobials, percentage of isolates being sensitive to the antimicrobials. For all except the first instant, 3-week moving means are indicated. ●, All routine isolates; ▲, all clinical isolates; T, throat; H, hand; F, faeces.

Total numbers of isolates

	Weeks admission				
	0	1	2	3	4
Routine	99	74	92	63	30
Clinical	7	8	11	10	5

While it would be attractive to analyse whether sensitivity of a strain to an antibiotic affects its isolation rate, such analysis is not feasible as either the antibiotic was given too rarely or the sensitivity was too uniform for comparable groups to be formed for analysis.

Catheterization and operation

In urological patients indwelling catheters of the urinary bladder are common. In Table 6 the rates in patients not (yet) catheterized are compared with those in patients catheterized at or before the time of bacteriological examination. Although the rates of isolation of *Klebsiella* during or after catheterization were higher than the rates before catheterization, often almost double, the differences

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were rarely statistically significant. The rates in relation to abdominal operation are presented in Table 7. While the overall picture is about the same as for catheterization, the differences in rates are smaller and less uniform. It was not possible to separate the effects of catheterization and operation as both occur together or soon after each other in most patients.

Antibiotic sensitivity

Antibiotic sensitivity is analysed only for *Klebsiella* strains isolated routinely in the survey and from clinical requests. Although the criteria for sensitivity of urinary strains differ from those for strains from other sources, all strains have been tested by the method used for non-urinary strains and only these data are presented.

The percentage sensitivity of strains to some antibiotics are presented in Fig. 2. As all strains isolated were resistant to penicillins and sensitive to gentamicin these antibiotics are not included. Three-week moving averages are shown except for isolations made in the first week of admission where the numbers of strains are only small. For some antibiotics the proportion of sensitive strains isolated from routine samples seems to diminish as the stay in hospital increases. This effect is statistically significant, however, for streptomycin and tetracycline during the first 2 weeks of admission only.

There is a striking difference in the proportion of sensitive strains isolated routinely and on clinical grounds. For almost all antibiotics the proportion of sensitive strains is lower in clinical isolations. This difference is statistically significant for chloramphenicol in the first 2 weeks after admission ($P < 0{\cdot}02$, and $P = 0{\cdot}04$ respectively) and almost significant for streptomycin ($P = 0{\cdot}06$, and $P = 0{\cdot}08$).

DISCUSSION

The prevention of nosocomial infection includes preserving or increasing the probability of successful treatment of infection by preserving the sensitivity to antibiotics of the bacteria involved. As nosocomial infection can be divided into the phases of colonization, penetration of the tissues, and infection, the effect of antibiotics is not always beneficial. Antibiotics administered for prophylaxis may prevent infection but may also decrease the probability of effective treatment by their effect on the bacterial population from which the infecting strains originate.

This survey deals mainly with klebsiellas which are of increasing importance in nosocomial infection (McGowan & Finland, 1974). By typing by capsular swelling 72 serotypes of *Klebsiella* have been recognized (Ørskow, 1955). By biochemical reactions, three or six biotypes are usually distinguished (Edwards & Ewing, 1972; Cruickshank *et al.* 1973). As more than one biotype is represented in certain serotypes and more than one serotype in certain biotypes (Cowan *et al.* 1960), Rennie & Duncan (1974) have proposed that biochemical and serological typing be combined. Serotyping by capsular swelling methods is a relatively tedious procedure but can be replaced by indirect fluorescent typing under standardized conditions (Riser, Noone & Poulton, 1976; Riser, Noone & Bonnet,

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1976). To increase the value of biotyping for epidemiological research, ten biochemical reactions were used to specify biotypes. Combinations of different results of these reactions distinguished the 66 biotypes found in this survey. The method of biotyping proved to be manageable and the results were useful for epidemiological analysis. No evidence of inconsistency in biotyping was found. The unreliability and lack of definition due to cross-reactions or to untypable strains which can seriously invalidate epidemiological analysis in surveys carried out by serotyping were avoided. The biotypes encountered during the survey are shown in Fig. 1. The distribution of biotypes is uniform in both periods of the survey and no predominance of a particular biotype or biotypes was noted. Even the most common biotype was not isolated continuously throughout the survey.

Most reports on nosocomial klebsiella infection are retrospective studies of outbreaks caused by multi drug-resistant strains and prospective studies in the periods following such outbreaks. As most hospitals have no continuous surveillance for the detection of nosocomial infection, attention is usually drawn to the problem by an outbreak of infection or by the clustering of multi drug-resistant infections in a ward, neither of which were encountered during this survey. There was no evidence of an outbreak of infection preceding or during the survey, nor were multi drug-resistant strains of *Klebsiella* isolated from the ward during the same periods.

Johanson, Pierse & Sanford (1969) found an increase in the throat carrier rate for Gram-negative bacteria during hospitalization but no significant effect of the administration of antibiotics on these rates. In this survey, the throat carrier rates for Gram-negative bacteria showed a similar trend but the effect of the administration of antibiotics on the *Klebsiella* carrier rate was significant. Enterobacteriaceae and *Pseudomonas aeruginosa* have been isolated from the throats of healthy individuals, outside hospital and not receiving antibiotics (Rosenthal & Tager, 1975). Although these bacteria which include *Klebsiella* belong to the normal pharyngeal flora, their carrier rate increases during hospitalization. This increase cannot be attributed solely to change in the population studied by the earlier discharge from hospital of non-carriers, as a weekly increase in throat carriage was found in patients remaining in hospital for 4 weeks.

The faeces carriage rates for *Klebsiella*, *Enterobacter*, *Pseudomonas*, and *Proteus mirabilis* at the date of admission to hospital are similar to those described by Rose & Schreier (1968) taking into account that the score for *Klebsiella* is increased by biotyping several colonies from each isolation. Rose & Schreier (1968) found an increase in carriage of *Klebsiella* in faeces from 10–34 %, only in patients receiving antibiotics. A similar relation was found in this survey, not only in faecal but also in throat and hand carriage. The increase in skin temperature and humidity in ill patients confined to bed might well have contributed to the increase in skin carriage after admission (McBride, Duncan & Knox, 1977).

The effect of antibiotics on carrier and infection rates of Gram-negative bacteria has been often described (Rose & Schreier, 1968; Rose & Babcock, 1975; Selden *et al.* 1971; Finland & McGowan, 1976). Not only do carrier rates increase but the

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proportion of antibiotic-resistant strains among clinical isolations also increases with the administration of antibiotics (Price & Sleigh, 1970; Noriega *et al.* 1975; Mouton, Glerun & van Loenen, 1976). Similar trends were noted in this survey. The effect of antibiotics on *Klebsiella* carriage was most evident in throat and faecal carriage. This might be attributed to the presence of some of the antibiotics administered in pharyngeal and intestinal secretions. The greater effect of antibiotics used in systemic infection than that of antibiotics used exclusively for urinary tract infections could be explained by the same argument. The number of isolations and the distribution of sensitive strains did not, however, allow detailed analysis for individual antibiotics and carriage rates. The increase in carriage rates of *Klebsiella* in sites other than urine in patients with an indwelling catheter is hard to explain as suitable controls were not available. The presence of an indwelling catheter is, however, not the only factor involved. Post-operative patients with indwelling catheters, and confied to bed, are more likely to be severely ill, exposed to the risk of bed sores, and are more likely to receive antibiotics. The increase in carrier rates associated with catheterization is more evident for faecal and hand carriage, probably indicating that confinement to bed and more frequent manual contact with skin has a positive effect on this relationship. Information on the transfer of *Klebsiella* between sites of carriage is presented in Part II of this report.

Difficulty is also met in interpreting the effect of operation on rates of carriage. The positive relation noted between operation and increased carriage rates of *Klebsiella*, especially for throat and hand, may be indirect, associated with confinement to bed, antibiotic treatment, occasional inhalation therapy, or other procedures.

The use of antibiotics stimulates the appearance of drug-resistant bacteria in hospitals (Price & Sleigh, 1970; Mouton *et al.* 1976). While strains resistant to the particular antibiotic used are more common, in addition strains resistant to other antibiotics, especially those determined by the same R factors, become prevalent. R factors are isolated more often from intestinal bacteria from patients receiving antibiotics (Sturtevant *et al.* 1971) and most of the multi drug-resistant strains involved in hospital outbreaks described to date were carriers of R factor (Martin *et al.* 1971; Eisenach *et al.* 1972; Noriega *et al.* 1975; Richmond *et al.* 1975; Forbes *et al.* 1977). The use of antibiotics selects populations of R factor bacteria which, in the absence of antibiotics, revert to predominantly R factor-negative strains (Lacey, 1975).

In the individual the proportion of intestinal bacteria carrying R factor may increase during antibiotic treatment (Datta, 1971). In this study the proportion of *Klebsiella* resistant to some antibiotics increased during the first 4 weeks after admission. This was more evident for streptomycin, chloramphenicol, and tetracyline, antibiotics rarely used in the urological ward, but was not noted for cephaloridine and kanamycin which were used often. Many R factors have been shown to be responsible for resistance against streptomycin and tetracycline.

It is striking that the proportion of antibiotic-sensitive strains of *Klebsiella* was always higher in colonizing strains isolated for the purpose of this survey

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than in 'clinical' strains isolated often from urine or from pus at the request of physicians attending the patients. This indicates that the resistance pattern of infecting strains is not necessarily that of the colonizing strains. The difference in resistance patterns between colonizing and infecting strains which is most apparent in the earlier part of the admission period, suggests that it is especially the hospital-acquired colonizing strain that is likely to be involved in nosocomial infection.

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