ANTIBIOTIC-RESISTANT GRAM-NEGATIVE BACILLI IN THE FAECES OF NEONATES

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ANTIBIOTIC-RESISTANT Gram-negative bacilli commonly occur in the faeces of normal adults and infants (see, for example, Datta, 1969; Moorhouse, 1969; Linton *et al.*, 1972). These strains may be acquired from food (Shooter *et al.*, 1971) or as a result of cross-infection from infected patients or healthy carriers. Contaminated meat may be an important source (Anderson 1965, 1968*b*). It seems likely that neonates acquire antibiotic-resistant Gram-negative bacilli from their mothers during delivery. Resistant strains detected in the faeces may also be acquired from infant feeds (Ayliffe, Collins and Pettit, 1970) and by cross-infection in the nursery. In the present study, the acquisition of resistant Gram-negative bacilli and the frequency of transferable resistance in these strains has been investigated in neonates born in hospital. Surveys were made of faeces of infants in general maternity wards and Special Care Units of three hospitals in the Birmingham area. In the Bristol Maternity Hospital, daily samples of faeces from individual babies were examined to determine the time of acquisition of resistant Gram-negative bacilli; faeces samples from mothers were also examined.

MATERIALS AND METHODS

Source of samples

Faeces. A series of surveys was made in the Birmingham hospitals (A, B, C) in which samples of faeces were collected from each infant present in a ward or unit on one day. Samples were also obtained from a few mothers. Daily samples of faeces were collected from each baby in the Bristol hospital D until the baby was discharged from hospital. A sample of faeces or of enema fluid was also obtained from mothers in hospital D soon after admission and before delivery. Where possible, faeces were again obtained from the mothers about 1 week after delivery. The dates and types of delivery and details of any antibiotics given to mothers and babies were recorded.

Other samples were taken from a number of sites, e.g., incubators, sinks, creams, baths suction equipment and soaps in Hospital A on several occasions; and 83 samples of infant feeds from Hospitals A, B and C were examined bacteriologically. Samples other than faeces were cultured on blood agar and MacConkey medium and in nutrient broth. Subcultures were made from the nutrient broth to the modified cetrimide medium after incubation for 18 hours.

Bacteriology

Swabs of faeces were spread evenly over the surface of one MacConkey plate. An antibiotic ring or individual disks ("Mast") containing the following antibacterial agents were applied to the plate: streptomycin (25 µg), tetracycline (100 µg), ampicillin (25 µg),

Received 24 Jan. 1974; accepted 25 Apr. 1974.

chloramphenicol (50 µg), nalidixic acid (30 µg), nitrofurantoin (200 µg), colistin (200 µg), kanamycin (30 µg), carbenicillin (100 µg), gentamicin (10 µg) and cephaloridine (25 µg). A second MacConkey plate was seeded from the swab to obtain individual colonies, and the swab was then transferred to nutrient broth. Plates and broths were incubated for 18 hours at 37°C. Organisms growing adjacent to antibiotic disks were subcultured and transferred to agar slopes for further tests. Broth cultures were subcultured to a modified cetrimide agar (Brown and Lowbury, 1965) for isolation of *Pseudomonas aeruginosa*. Gram-negative bacilli were identified by the methods of Cowan and Steel (1965) and, apart from *Escherichia coli* and *Ps. aeruginosa*, are reported according to their genera. Sensitivity tests with the same antibiotics were repeated for all strains of *E. coli* and *Klebsiella* spp. subcultured from the primary plates; for these sensitivity tests, plates of Oxoid DST Medium were flood-seeded with a broth culture diluted 1 in 500 and the plates were dried. Resistant *E. coli* isolates were further tested for sensitivity to sulphonamide with a sulphadiazine (200 µg) disk on the Oxoid medium containing lysed blood. After incubation for 18 hours at 37°C, zone sizes (mean diameters) were compared with those obtained with a standard sensitive strain of *E. coli*. Organisms giving reduced zone sizes (2 mm less than control) were classified as resistant, and strains resistant to at least one of the antibacterial agents used for screening are referred to as "antibiotic-resistant strains" in the text.

Transfer of resistance was tested by the method of Datta (1969) on strains of *E. coli* and *Klebsiella* spp. with a nalidixic acid-resistant strain of *E. coli* K12 as a recipient. *Klebsiella* spp. were typed by a bacteriocine technique modified from that described by Hall (1971). Three membrane filters (Oxoid 4.7 cm, pore size 0.45 µm) were placed on the surface of a 6-in. plate containing MacConkey medium. The membrane filters were inoculated in different areas with a loopful of solid culture of each of the 10 standard bacteriocine-producing strains—nos. 3, 4, 5, 7, 23, 24, 27, 30, 77 and 9137. The plates were incubated at 37°C for 18 hours and the membrane filters were removed. A suspension was made from 3–4 colonies of the test strain emulsified in 5 ml of nutrient broth. The MacConkey plates were flooded with the suspension and dried; after incubation at 37°C for 6–8 hours, inhibition of growth was recorded. Control test strains sensitive to all bacteriocines were included in each batch tested. Strains of antibiotic-resistant *E. coli* were tested by slide-agglutination with a polyvalent antiserum against enteropathogenic serotypes of *E. coli*. Agglutination was obtained with one strain only, which was identified with a single antiserum as O86. Some strains of *Klebsiella* spp. were typed serologically by Dr I. Ørskov.

RESULTS

Isolation of antibiotic-resistant Gram-negative bacilli

Most of the samples isolated from the faeces of neonates yielded a predominant growth of *E. coli*, usually fully sensitive to antibiotics. Other Gram-negative bacilli, especially *Klebsiella* spp., were sometimes found as the predominant organisms, but were usually present in much smaller numbers. The frequency of isolation of different species of Gram-negative bacilli from samples is shown in table I.

Isolations of *Klebsiella* spp. were varied, ranging from 6% in the ordinary wards of Hospital C to 65% in the Special Care Unit of Hospital B. Gram-negative bacilli other than *Escherichia* and *Klebsiella* spp. were less frequently found in all hospitals. No *Salmonella* spp. or recognised intestinal pathogens other than one enteropathogenic strain of *E. coli* were isolated.

Acquisition of antibiotic-resistant Escherichia coli and Klebsiella spp.

The data given in detail in table II and summarised in figs. 1–4 show that some resistant strains of *E. coli* and *Klebsiella* were acquired by babies soon

after birth. Acquisition of resistant *E. coli* was slower in the special care units than in other wards during the first few days after birth, but increased rapidly after 5-6 days in special care units. The acquisition of *Klebsiella* spp. rose

TABLE I
Incidence of some Gram-negative bacilli isolated from neonates

Hospital and area*	Number of babies sampled	Number (and percentage) of babies from whom the following organisms were isolated					
		<i>Proteus</i> spp.	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella</i> spp.	<i>Escherichia coli</i>	<i>Serratia marcescens</i>	Other Gram-negative bacilli
A—SCU	214	18	5	122	169	0	9
A—wards	109	6	1	38	79	1	12
B—SCU	46	6	1	30	29	0	6
B—wards	70	4	0	16	39	0	9
C—SCU	21	5	3	13	13	0	3
C—wards	31	1	1	2	22	2	4
D—wards	93	4	2	14	71	0	17
All	584	44 (8)	13 (2)	235 (40)	422 (72)	3 (0.5)	60 (10)

* Wards or special care units (SCU) of hospitals in Birmingham (A, B, C) or Bristol (D) were included in the study.

TABLE II
Age of acquisition of resistant Escherichia coli and Klebsiella spp. in neonatal faeces

Age in days	Data for neonates in special care units			Data for neonates in other wards		
	Total samples taken	Number of resistant <i>E. coli</i> isolated	Number of resistant <i>Klebsiella</i> spp. isolated	Total samples taken	Number of resistant <i>E. coli</i> isolated	Number of resistant <i>Klebsiella</i> spp. isolated
1	10	0	0	141	20	4
2	34	4	3	85	22	9
3	23	3	7	87	18	15
4	14	1	3	74	16	12
5-6	37	12	18	104	25	27
7-8	24	9	15	81	24	21
9+	139	77	90	35	11	8
All	281	106	136	607	136	96

steadily to over 60% in special care units, contrasting with the lower rate of acquisition (less than 30%) in other wards.

The four hospitals showed marked differences in the proportion of neonates carrying antibiotic-resistant strains. This was partly due to the contribution of the special care baby units, since almost twice as many babies in the Special Care Unit were sampled than in the general wards of Hospital A, whereas no

babies were sampled in the Special Care Unit of Hospital D (see table I). Approximately 35% of babies in Hospital A carried antibiotic-resistant *E. coli* and 46% carried klebsiellae; in Hospital D, approximately 10% carried

FIG. 1.—Acquisition of *Escherichia coli* by neonates in special care units.

FIG. 2.—Acquisition of *E. coli* by neonates in ordinary wards.

FIG. 3.—Acquisition of klebsiellae by neonates in special care units.

FIG. 4.—Acquisition of klebsiellae by neonates in ordinary wards.

antibiotic-resistant *E. coli* and only 4% carried klebsiellae. In Hospital B, 46 of the 116 babies sampled were in the Special Care Unit and the proportion of these carrying antibiotic-resistant *E. coli* was only 10%.

Antibiotic-resistance patterns

The resistant strains of *E. coli* isolated in all four hospitals were mainly resistant to streptomycin, tetracycline, ampicillin and carbenicillin, and usually

to sulphonamides (tables III and IV). Kanamycin resistance, although much less common, was noted particularly in Hospitals A and C, and chloramphenicol resistance in Hospital C. Among the *klebsiellae* examined, tetracycline resist-

TABLE III

Antibiotic-resistance patterns of E. coli isolated from neonatal faeces from four hospitals

Hospital	Number of samples taken	Number containing resistant strains of <i>Klebsiella</i> spp. of <i>E. coli</i>	Number of <i>E. coli</i> isolates resistant to the stated agent*											
			St	T	A	C	NA	NF	Col	K	Car	Cep	G	Su
A	323	115	88	86	83	9	0	0	0	21	80	39	0	57
B	116	11	6	8	5	2	0	0	0	1	4	1	0	6
C	52	12	9	11	12	7	0	0	0	4	12	4	0	9
D	397	41	15	22	14	5	0	0	0	0	12	0	...	14
All	888	179	118	127	114	23	0	0	0	26	108	44	0	86

* St = streptomycin; T = tetracycline; A = ampicillin; C = chloramphenicol; NA = nalidixic acid; NF = nitrofurantoin; Col = colistin; K = kanamycin; Car = carbenicillin; Cep = cephaloridine; G = gentamicin; Su = sulphonamide.

TABLE IV

Antibiotic-resistance patterns of klebsiellae isolated from neonatal faeces from four hospitals

Hospital	Number of samples taken	Number containing resistant strains of <i>Klebsiella</i> spp.	Number of <i>Klebsiella</i> isolates resistant to the stated agent*												
			St	T	A	C	NA	NF	Col	K	Car	Cep	G	Su	
A	323	160	9	4	156	0	1	1	0	0	139	53	0	...	
B	116	46	2	10	44	1	0	1	0	0	24	7	0	...	
C	52	15	10	10	15	9	0	1	0	1	13	6	0	...	
D	397	15	1	1	14	0	0	0	0	0	12	0	...	2	
All	888	236	22	25	229	10	1	3	0	1	188	66	0	2	

* See footnote to table III.

ance was uncommon but most strains were resistant to ampicillin (and carbenicillin); strains in ordinary wards of Hospital C were also commonly resistant to chloramphenicol (7 of 23), and no gentamicin-resistant strains were isolated. Resistance to kanamycin was rare.

Transferability of resistance

Tables V and VI show the number of antibiotics to which the isolates of *E. coli* and *Klebsiella* spp. were resistant and the proportions of these in which transfer of resistance was demonstrated. Resistance was transferred in 49%

of all *E. coli* strains tested, ranging from 18% of strains resistant to one antibiotic to 87% of strains resistant to five antibiotics. Transfer was demonstrated less often in strains resistant to six or seven antibiotics, possibly because these included 13 isolates from a group of samples from one special care unit all of

TABLE V
Antibiotic resistance and resistance transfer in E. coli

Organism resistant to	Number of isolates from		Number (and percentage) of isolates in which resistance was transferred
	S.C.U.	wards	
One antibiotic	15	25	7 (18)
Two antibiotics	6	22	9 (32)
Three antibiotics	8	21	14 (48)
Four antibiotics	25	9	26 (76)
Five antibiotics	25	5	26 (87)
Six antibiotics	14	1	6 (40)
Seven antibiotics	18	4	9 (41)
Any number of antibiotics	111	87	97 (49)

TABLE VI
Antibiotic resistance and resistance transfer in Klebsiella spp.

Organism resistant to	Number of isolates from		Number (and percentage) of isolates in which resistance was transferred
	SCU	wards	
One antibiotic	18	9	0
Two antibiotics	82	59	1 (0.7)
Three antibiotics	44	8	1 (2)
Four antibiotics	2	4	0
Five antibiotics	8	0	7 (88)
Six antibiotics	6	0	6 (100)
Any number of antibiotics	160	80	15 (6.25)

which failed to transfer resistance. Transferable resistance was demonstrated much less frequently in klebsiellae (6.25%) than in *E. coli*. Resistance to ampicillin, carbenicillin and often to cephaloridine is inherent in most klebsiellae, but this resistance was usually not transferable.

Sources of resistant strains in babies' faeces

Mothers. A comparison of organisms isolated from the faeces of 111 mothers and 93 babies in Hospital D is shown in table VII. In general, the proportions of mothers and babies colonised by different species are similar, except that klebsiellae were more commonly found in babies than in mothers.

Of 55 paired mothers and babies, 21 neonates were colonised by strains not isolated from the mother and 14 neonates were colonised by strains apparently identical with the maternal strain in each case. In 27 pairs studied in Hospital A, the frequency of colonisation with resistant *E. coli* was 18.5% in babies and 48% in mothers, but the isolation of *Klebsiella* spp. was much higher in babies (44%) than in mothers (18.5%). In three cases unusual easily recognisable strains were present in mother and baby.

Environment. *Klebsiella* spp. and resistant *E. coli* were rarely isolated from the environment in Hospital A except from an occasional sink, soap-dish or

TABLE VII
Comparison of bacterial flora isolated from the faeces of 111 mothers and 93 babies in Hospital D

Category of the organism isolated	Percentage number of mothers and babies colonised by				
	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Pseudomonas aeruginosa</i>	Other Gram-negative bacilli
<i>Predominant strain in mothers babies</i>	87	2	2	0	10
	72	5	1	1	5
<i>Isolated in large or small numbers from mothers babies</i>	96	8	6	2	22
	76	15	4	2	18

drain. In the infant feeds examined klebsiellae were isolated in small numbers from 2 of 45 samples in Hospital A and none of 38 samples in Hospitals B and C.

Cross-infection and length of stay

The high incidence of *Klebsiella* spp in the faeces of babies and the rising carrier-rate of resistant *E. coli* and *Klebsiella* spp. with increasing length of stay in special care units, as shown in Figs. 1 and 2, suggest that cross-infection between babies was common. Table VIII shows the predominant bacteriocine patterns of *Klebsiella* spp. isolated from different wards in Hospital A. The endemic types in different wards of the same hospital often differed and varied over a period of months. The types endemic in individual hospitals differed from each other sometimes in antibiotic-resistance patterns and sometimes in bacteriocine patterns. On occasions when bacteriocine types in different hospitals were similar, serological types were usually different: e.g., a strain with the bacteriocine-typing pattern (4/7/24/27) found in Hospital A was of serological type 9 and a strain with the same pattern in Hospital B was of serological type 29. Isolation of an enteropathogenic strain of *E. coli* O86 was followed by a subclinical outbreak of cross-infection. A 12-day-old baby in the Special Care Unit of Hospital A was initially colonised by this strain. A month

later, 7 of 26 babies had acquired this organism and all but one were in the ward for at least 17 days. Nearly 2 months later, 9 of 20 babies carried this strain and the average age of these babies was more than 14 days.

TABLE VIII

Predominant bacteriocine patterns of Klebsiella spp. in several wards and units of Hospital A

Ward or unit	Date of isolation	Predominant pattern	Number of isolates showing this pattern	Number of isolates tested
SCU	1 Dec. 1970	27	7	15
	9 Feb. 1971	NT	9	13
	22 May 1971	3/4/5/7/23/24/27/30/77	7	15
	27 June 1971	3/4/5/7/23/24/27/30/77	5	14
D6	10 Nov. 1970	4/5/7/24/27	2	2
G2	27 Oct. 1970	4/7/24	2	2
D5	3 Nov. 1970	4/7/24/27	3	6

Factors affecting acquisition of resistant strains

Antibiotic therapy. Table IX shows the effect that the administration of antibiotics to mothers and babies had on their faecal colonisation with antibiotic-resistant organisms. The frequency of resistant *E. coli* and *Klebsiella*

TABLE IX

The effect of antibiotic administration on colonisation by Klebsiella spp. and E. coli in faeces of babies

Antibiotic given to	Number of samples tested	Number (and percentage) of samples yielding <i>Klebsiella</i> spp.	Number (and percentage) of samples yielding resistant <i>E. coli</i>
Baby	69	52 (75)	46 (67)
Mother	29	13 (45)	8 (28)
Baby and mother	19	17 (89)	6 (32)
Neither	610	126 (21)	97 (16)

spp. was significantly higher in the faeces of treated than of untreated babies ($P = <0.001$). Antibiotics given to mothers alone did not have a significant effect on the babies' faecal flora. Of the 88 babies treated, only five were from ordinary wards and four of these babies carried *Klebsiella* spp. and only one carried resistant *E. coli*.

Type of delivery. Table X shows organisms isolated from babies in Hospital D in relation to the type of delivery. It suggests that organisms other than *E. coli* were more likely to colonise infants after an abnormal than after a normal delivery.

The type of delivery also affected the rapidity with which babies became colonised by Gram-negative bacilli. The babies most rapidly colonised were delivered with the aid of forceps (average < 2 days); normal delivery was followed by colonisation after an average of $2\frac{1}{2}$ days and Caesarean section by colonisation after an average of 5 days. Colonisation with antibiotic-resistant organisms occurred in 86% of the babies born by Caesarean section, but in only 40% of babies born normally or with forceps.

Detailed timings of events immediately before delivery were available for 58 of the babies studied in Hospital D. These data showed that the longer the delay between rupture of membranes and delivery, the more rapidly did faecal colonisation of the babies by Gram-negative bacilli occur. For example, in the case of those babies for whom the delay between membrane rupture and

TABLE X
The effect of the type of delivery on colonisation of neonates by Gram-negative bacilli

Type of delivery	Number of babies sampled	Number of babies colonised by					
		no Gram-negative bacilli	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Ps. aeruginosa</i>	Other Gram-negative bacilli
Normal	70	9	58	7	5	0	12
Forceps	16	0	11	3	0	1	5
Caesarean	7	0	5	3	1	1	2

delivery was 5 hours or less, colonisation did not occur until the 3rd day or later, whereas babies born after a delay of 10 hours or more between membrane rupture and delivery were colonised by Gram-negative bacilli in their faeces within 24 hours of birth.

DISCUSSION

Antibiotic-resistant intestinal Gram-negative bacilli were rapidly acquired by neonates after birth. Carriage rates for *Proteus* spp. (7.5%) and *Ps. aeruginosa* (2.2%) were low and the strains were probably acquired from mothers. Strains of *E. coli* and *Klebsiella* spp. were also probably initially acquired from mothers, as in the series reported by Bettelheim *et al.* (1974) who showed that 22 of 28 babies became colonised by a strain of *E. coli* identical with that isolated from the respective mother. However, in our studies we have shown that the isolation rate of antibiotic-resistant strains of *E. coli* and *Klebsiella* spp. increased in relation to the length of hospital stay and this suggests that other sources or factors were important. The carriage of *Klebsiella* spp. was much higher in special care units but the origin of these organisms was uncertain. Milk feeds were unlikely to be the major source, for if this were the case similar rates would be expected in special care units as in wards for normal babies receiving feeds from the same milk kitchen. Isolations of *Klebsiella* spp. from samples of milk were rare. A high carriage rate was also found in Hospital B where milk was

terminally sterilised. Milk was also terminally sterilised in Hospital D, where the carriage rate of *Klebsiella* spp. was low (10%). This figure corresponds with the findings of Dailey, Sturtevant and Feary (1972) who isolated resistant *Klebsiella* spp. from 13% of neonates.

Klebsiella spp. and other Gram-negative bacilli have been isolated in some units from solutions or equipment that might have constituted a single source, e.g., hexachlorophane soaps. Environmental studies in Hospital A rarely yielded *Klebsiella* spp. and as in most other investigations (Selden *et al.*, 1971; Gardner and Smith, 1967) no common source was found. It is also possible that the intestinal tract of babies is particularly susceptible to colonisation with *Klebsiella* spp. (Pall, Milch and Szecsenyi-Nagy, 1969). The bacteriocine typing patterns suggest the presence of a rather diffuse and changing reservoir of endemic strains in these wards and units. Differences in the predominant type of *Klebsiella* spp. found, on the one hand in wards and on the other hand in special care units, have been shown by serotyping of *Klebsiella* spp. and enteropathogenic *E. coli*; distinct antibiotic-sensitivity patterns, involving bacterial resistance in special care units to kanamycin and in ordinary wards to chloramphenicol have also been shown. A kanamycin-resistant strain of *E. coli* O86 spread around the Special Care Unit of Hospital A unknown to the clinician, since there was no evidence of clinical infection. The main source of resistant Gram-negative bacilli in the nursery was probably babies' faeces, or possibly the upper respiratory tract which was not examined in this study, and the organisms were probably transmitted on the hands of staff members.

A number of factors were found to influence the rates of acquisition of these organisms. Length of stay in hospital and antibiotic treatment were obviously important, particularly in special care units. A significantly higher incidence of both *Klebsiella* spp. and of resistant *E. coli* was found in babies treated with antibiotics than in untreated babies or mothers. A similar selection of *Klebsiella* spp. has been observed in some published studies (e.g., Gardner and Smith, 1967; Selden *et al.*, 1971) but not in others (Datta, 1969). Ampicillin and cloxacillin were the most frequent antibiotics given and ampicillin might be expected to select *Klebsiella* spp. (Price and Sleight, 1970). Colonisation of the faeces with Gram-negative bacilli was more rapid when the mothers' membranes had been ruptured for long periods before delivery, presumably as a result of contamination of the amniotic fluid. Babies delivered by Caesarean section were colonised more slowly by sensitive strains than were normal babies, probably because they were not exposed to their mothers' vaginal-faecal flora; on the other hand, more of them subsequently acquired resistant strains, even in the absence of antibiotic treatment.

Many different antibiotic-sensitivity patterns were found among strains of *E. coli*, but strains resistant to ampicillin (and carbenicillin), tetracycline, streptomycin and often sulphonamide were common, and resistance to these agents was often transferable. This is a common pattern of transferable resistance in *E. coli* (Datta, 1969) and corresponds to the most frequently used antimicrobial agents (Ayliffe, 1973). As in other studies (e.g., Datta, 1971), 54% of 142 strains of *E. coli* resistant to tetracycline showed transferable

resistance, in comparison with only 23% of 56 strains of *E. coli* resistant to drugs other than tetracycline. Most strains of *Klebsiella* spp. were resistant to ampicillin (and carbenicillin) and this resistance was rarely transferable; thus, only 6.25% of *Klebsiella* spp. transferred resistance, compared with 45.4% of resistant strains of *E. coli*. However, tetracycline resistance was transferred by 52% of 25 tetracycline-resistant strains of *Klebsiella* spp. The presence of transferable chloramphenicol resistance and the possible risk of transfer to *Salmonella* spp. has been referred to by Moorhouse and McKay (1968). Transferable chloramphenicol resistance was found in all four hospitals and was most common in *Klebsiella* spp. in Hospital C, although there is no record of chloramphenicol being used therapeutically in that hospital. The isolation of strains of *E. coli* with a similar antibiotic-sensitivity pattern, including resistance to chloramphenicol, suggested intergeneric transfer from *Klebsiella* spp. to *Escherichia* in the unit.

The possibility of transfer of resistance to enteropathogenic strains of *E. coli* is also of major importance and is one of the few instances in which transferable resistance is clinically relevant. Antibiotic treatment of infantile gastroenteritis, although not generally recommended, may be necessary in some severe infections. Highly resistant enteropathogenic strains may then fail to respond to treatment with conventional antibiotics, with possibly unfortunate results (e.g., Anderson, 1968a).

Antibiotic-resistant strains of Gram-negative bacilli are evidently acquired soon after birth, mainly from mothers, and are likely to be increased selectively if babies are treated with antibiotics. The frequency of carriage increases with length of stay in hospitals. Cross-infection is common between babies, particularly in special care units or in units for the care of premature babies.

SUMMARY

A total of 880 samples of faeces from 584 neonates in four hospitals were examined for the presence of antibiotic-resistant Gram-negative bacilli. The proportion of strains of *Escherichia coli* and *Klebsiella* spp. carrying R factors was also determined. Some resistant strains of *E. coli* and *Klebsiella* spp. were rapidly acquired after birth and the number of isolates increased in relation to the length of hospital stay. The proportion of isolates of *Klebsiella* spp. increased to over 60% in babies in special care units but increased to a lesser extent (less than 30%) in other wards. The four hospitals showed differences in the proportion of babies carrying antibiotic-resistant strains and this was partly attributable to the inclusion of samples from special care units in three of the hospitals. Antibiotic-resistant Gram-negative bacilli other than *E. coli* and *Klebsiella* spp. were isolated much less frequently. Transferable resistance was demonstrated in 49% of 208 resistant strains of *E. coli* and in only 6.5% of 240 strains of *Klebsiella* spp.

An increased frequency of resistant strains was found in babies associated with abnormal deliveries and in babies treated with antibiotics. Resistant strains were probably acquired initially from the mothers, but although there

was evidence of cross-infection with *E. coli* and *Klebsiella* spp. particularly in special care units, no common source was identified.

We wish to thank the staff of the four hospitals for providing the samples and Dr I. Ørskov for serological typing of *Klebsiella* strains.

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