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Epidemiology of Fecal Strains of the Family *Enterobacteriaceae* in 22 Neonatal Wards and Influence of Antibiotic Policy

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The gram-negative fecal floras from 953 infants were studied upon discharge of the infants from 22 neonatal wards. More than 600 distinct phenotypes of *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. were distinguished by high-resolution biotyping. The colonization patterns observed showed considerable local and temporal variation. The major (M) strains (phenotypes), which colonized more than 10% and up to 78% of the infants in a ward (median, 23%), were *Klebsiella oxytoca* (15 strains), *E. coli* (4 strains), *Klebsiella pneumoniae* (1 strain), and *Enterobacter cloacae* (1 strain). Resistance to beta-lactam antibiotics was more pronounced among M strains than among strains of enteric bacteria colonizing few or single infants only. Local antibiotic policy influenced the colonization patterns. Despite the fact that M strains of *Klebsiella* spp. were usually resistant to ampicillin as well as to cephalexin and cefuroxime, their local dissemination was associated with the use of ampicillin with or without gentamicin but not with the use of cefuroxime. It thus appeared that in the neonatal setting, ampicillin posed a greater risk of local spread of certain drug-resistant bacterial clones than a newer cephalosporin, such as cefuroxime.

Gram-negative bacterial infections represent a major cause of morbidity and mortality in neonatal wards. A lack of efficient typing methods has prevented thorough epidemiological studies of enterobacteria in the neonatal setting during endemic periods. We have observed that the nosocomial spread of certain virulent P-fimbriated clones of *Escherichia coli* can result in protracted outbreaks of pyelonephritis or septicemia among infants cared for in a neonatal ward (17, 18). More typical, however, are the many reported outbreaks of severe neonatal infection due to various other members of the family *Enterobacteriaceae*, such as *Klebsiella* spp. (3, 8, 10, 15) and *Enterobacter* spp. (3, 11), and other gram-negative bacteria, such as *Flavobacterium* spp. (4).

Apart from hand carriage of bacteria by the staff (5, 13), the institutional factors and bacterial properties that contribute to endemic and epidemic spread of gram-negative bacteria among newborns remain poorly understood. In some outbreaks, antibiotic usage has been implicated as a predisposing factor, e.g., *Klebsiella* infections have been associated with ampicillin usage (14) and *Enterobacter* infections have been associated with ampicillin or modern cephalosporin usage (3, 11).

In a recent survey of the aerobic gram-negative fecal floras of babies discharged from 22 neonatal wards in Sweden, we found that a majority of the infants were colonized with *Klebsiella* spp., *E. coli*, or *Enterobacter* spp. (K. Tullus, B. Fryklund, B. Berglund, G. Källenius, and L. G. Burman, J. Hosp. Infect., in press). However, the local patterns of fecal colonization differed greatly between the wards. There was marked dominance of *Klebsiella* spp. in 17 wards, slight dominance of *E. coli* in 2 wards, dominance of *Enterobacter* spp. in 1 ward, and a balanced distribution of floras in 2 wards. *Klebsiella* spp. were particularly prevalent among infants aged 1 to 2 weeks, whereas *E. coli* gradually replaced *Klebsiella* spp. in a competitive manner as the infants became older.

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The aim of the present work was to study the fecal epidemiology of individual *E. coli*, *Klebsiella*, and *Enterobacter* strains occurring in the 22 neonatal wards by a novel fingerprinting method and to describe each local colonization pattern. We also analyzed the influence of local antibiotic policy on the epidemiology of enterobacterial clones in these wards.

MATERIALS AND METHODS

On the day of discharge, rectal swabs were taken from all infants with a minimum stay of 5 days (n = 953) in 22 neonatal wards as previously described (Tullus et al., in press). About 10% of all children born in the catchment area of each hospital were admitted to the wards. Colonization in each ward was monitored for 2 to 18 months (mean, 4 months).

From each fecal specimen, five gram-negative isolates were collected for further study. Colonies with different appearances were sought and identified by standard methods. If they were available, at least two *E. coli* and two *Klebsiella* or *Enterobacter* colonies from each specimen were biochemically phenotyped (9). Briefly, the spectrum of biochemical activities of each isolate was determined with 24 substrates for *E. coli* and 48 substrates for *Klebsiella* and *Enterobacter* spp. These substrates were selected for optimal discrimination of individual phenotypes. The assays were performed in microdilution plates with an automatic optical reader (Titertek Multiskan, model MCC/340; Flow Laboratories, Inc.) and with microcomputer storage and handling of readings. Isolates showing a correlation coefficient of >0.975 when the sets of readings were compared in pairs were regarded as identical and assigned to the same phenotype.

Nosocomially spread strains (major [M] strains or clones) were defined as biotypes which colonized ≥10% of the infants discharged from a particular ward during the study period. In wards contributing fewer than 30 fecal samples, M strains were defined as biotypes found in at least three infants. In addition, we defined a cluster as a phenotype

TABLE 1. Carriage of S or nosocomially spread M strains by infants in each ward upon discharge

Organism	Carriage (% of infants)	
	Range	Median
Total M strains	0–78	23
Total S strains	14–100	51
Total *E. coli*	16–72	40
E. coli M strains	0–36	0
Total *Klebsiella* spp.	27–100	77
Klebsiella spp. M strains	0–57	17

carried by at least two infants. Because both definitions of nosocomial transmission yielded similar results, only the stricter definition (that for M strains) is used below for simplicity and brevity.

The susceptibilities of 1,369 isolates to ampicillin, cefotaxime, cefuroxime, cephalexin, and gentamicin were determined by the agar dilution method with Paper Disk Message (PDM) antibiotic susceptibility medium (AB Biodisk; Solna, Sweden); 16 mg/liter was used as the break point to define bacterial drug resistance. Higher levels of ampicillin (up to 1,024 mg/liter) were tested against selected isolates in order to determine the MICs for 50 and 90% of isolates. On the basis of these criteria, the high-level-ampicillin resistance of all isolates of M strains was compared with that of all isolates of sporadic (S) strains obtained from the wards where M strains were found.

Information on any antibiotic treatment of each infant was recorded as the fecal sample was collected. These data were used to describe the antibiotic policy of each neonatal unit.

The chi-square test and Spearman rank correlation test were used for the statistical analyses with an ABC 800 personal computer and the statistical program MULREG 800.

RESULTS

Colonization patterns. *Klebsiella* species were more common than *E. coli* in the fecal floras of neonates and occasionally colonized 100% of the studied infants in a ward (Table 1). A total of 641 *E. coli* colonies and 881 *Klebsiella* or *Enterobacter* colonies obtained from the 953 infants were further studied, yielding more than 200 *E. coli* biotypes and 400 *Klebsiella* or *Enterobacter* biotypes.

The median proportion of infants carrying M strains in the 22 wards was 23% (range, 0 to 78%) (Table 1). In total, 4 distinct M strains of *E. coli*, 15 M strains of *Klebsiella oxytoca*, 1 M strain of *Klebsiella pneumoniae*, and 1 M strain of *Enterobacter cloacae* were isolated. Colonization of more than 25% of the infants in a ward by a particular biotype occurred with one M strain of *E. coli* and six M strains of *Klebsiella* spp. The median values and ranges for local carrier rates of *E. coli* M strains and *Klebsiella* spp. M strains also showed that *Klebsiella* spp. were more prone to spreading than *E. coli* (Table 1). Similarly, individual M strains of *Klebsiella* spp. tended to colonize higher proportions of infants in a ward than M strains of *E. coli*, with a maximum of 57% of the infants in a ward carrying the same *K. oxytoca* clone (Table 1).

The gram-negative bacterial colonization patterns varied both between wards and over time with respect to species domination and distribution of S and M strains. For example, in one ward, first one *Klebsiella* strain and then one *E. coli* strain dominated (Table 2). In 8 of the 22 wards no M strain of the family *Enterobacteriaceae* was isolated during the study period (Table 3). There was an association between the spread of M strains of *E. coli* and that of M strains of *Klebsiella* spp. in the same ward (correlation coefficient $[r] = 0.39$, $P < 0.05$).

Antibiotic policy. Among the 22 wards, the median proportion of infants with stays of more than 5 days who had received antibiotics was 38% (range, 14 to 67%). There were marked differences in antibiotic policy between wards. Three major antibiotic regimens were recorded, namely, ampicillin only (2 wards), ampicillin plus gentamicin (10 wards), and cephalosporins (8 wards). In two wards, ampicillin and newer cephalosporins were used equally often. Ampicillin was received by up to 43% and cephalosporins were received by up to 50% of the infants in a ward. Of the children treated with cephalosporins, 71% had received cefuroxime.

TABLE 2. Fecal carriage of enteric bacteria in 104 neonates discharged from ward 16

Organism	Carriage (no. of infants)																
	1984												1985				
	Ja ($n = 3$)[a]	F ($n = 7$)	Mr ($n = 11$)	Ap ($n = 5$)	My ($n = 9$)	Jn ($n = 0$)	Jl ($n = 1$)	Au ($n = 6$)	S ($n = 7$)	O ($n = 13$)	N ($n = 11$)	D ($n = 6$)	Ja ($n = 7$)	F ($n = 4$)	Mr ($n = 4$)	Ap ($n = 6$)	My ($n = 4$)
E. coli	2	1	6	0	3	0	1	5	4	11	10	6	7	4	3	6	4
Klebsiella spp.	2	4	4	5	7	0	0	0	2	6	1	0	2	0	3	1	2
K 1[b] (M)	2	2	4	4	5					2							
K 2		2															
Ec 1 (M)								4	3	4	8	4	4	1	1	5	4
Ec 2	1	1	1														
Ec 3		2															
Ec 4							1	1	1								
Ec 5							1								1	1	
Ec 6											2	1	4	2			
Ec 7										1		1			1	2	
Ec 8												1	1				

[a] Ja, January; F, February; Mr, March; Ap, April; My, May; Jn, June; Jl, July; Au, August; S, September; O, October; N, November; D, December; *n*, number of infants studied.

[b] K, *Klebsiella* spp.; Ec, *E. coli*. Numbered strains represent distinct biotypes (clones) occurring in the ward.

TABLE 3. Fecal carriage of enteric bacteria in neonates discharged from ward 8

Organism	Carriage (no. of infants) in 1984					
	Ap (n = 1)[a]	M (n = 13)	Jn (n = 3)	Jl (n = 7)	Au (n = 3)	S (n = 6)
E. coli	0	6	1	1	2	3
Klebsiella spp.	1	10	2	5	2	4
K 1[b]		2				
K 2		2				
K 3				2		
K 4				1		1
Ec 1		3				

[a] Ap, April; M, May; Jn, June; Jl, July; Au, August; S, September; n, number of infants studied.
[b] K, *Klebsiella* spp.; Ec, *E. coli*. Numbered strains represent distinct biotypes (clones) occurring in the ward.

Antibiotic policy and colonization. To study the influence of the local antibiotic policy on the local colonization pattern, the correlation coefficients between the percentage of infants treated and the fecal carrier rates of bacteria were calculated. Antibiotics in general had no influence on the overall colonization pattern in the wards (Table 4). Neither was any of the three major antibiotic regimens associated with colonization with *E. coli* or *Klebsiella* spp. (Table 4).

In contrast, ampicillin alone or in combination with gentamicin as the local regimen was associated with a significantly increased dissemination of M strains (Table 4). A median of 28% (range, 0 to 78%) of neonates in wards with ampicillin policies were colonized with M strains, compared with 14% (range, 0 to 37%) of infants in wards with cephalosporin policies. The selective effect of ampicillin was more marked for the *Klebsiella* spp. M strains (Fig. 1) than for *E. coli* M strains, and no additional ecological impact of gentamicin was observed (Table 4).

Cephalosporin (mainly cefuroxime) usage tended to be negatively correlated with both spreading and S strains of *E. coli* and *Klebsiella* spp. (Table 4 and Fig. 1). However, cephalosporin usage was associated with gram-positive bacteria as the only aerobic floras (r = 0.42, $P < 0.05$) and tended to increase the carrier rate of gram-negative bacteria other than *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. (r = 0.15, $P > 0.05$). Only four of the gram-negative isolates were *Pseudomonas* spp. and *Proteus* spp.

Antimicrobial resistance. None of the 1,369 fecal isolates of *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. tested was found to be resistant to gentamicin. For the beta-lactam antibiotics studied, resistant *E. coli* was found mainly for ampicillin and occasionally for cephalexin, whereas only cefotaxime was generally active against *Klebsiella* strains (Table 5).

Isolates of M strains were resistant to 16 mg of beta-lactam antibiotics per liter more frequently than were non-M strains, particularly with regard to cefuroxime in *Klebsiella* spp. and ampicillin in *E. coli* (Table 5). Resistance to higher levels of ampicillin was used as a semiquantitative indicator of the β-lactamase activity of resistant strains. Also in this respect, isolates of *E. coli* M and *Klebsiella* M strains showed greater beta-lactam resistance than non-M isolates (Table 6).

TABLE 4. Influence of local antibiotic policy on the fecal floras of neonates

Colonization	Correlation coefficients (r)[a] for:			
	Ampicillin alone	Ampicillin plus gentamicin	Cephalosporin[b]	All antibiotics
E. coli	0.10	0.18	−0.06	0.05
Klebsiella spp.	0.11	0.23	−0.21	−0.20
Total M strains	0.52[c]	0.55[c]	−0.21	0.00
E. coli M strains	0.26	0.29	−0.12	−0.18
Klebsiella spp. M strains	0.66[d]	0.51[c]	−0.33	−0.13

[a] Spearman rank correlation coefficient between percent colonized infants in each ward and percent infants in the same ward receiving its major antibiotic regimen.
[b] Mainly cefuroxime; see the text.
[c] $P < 0.01$.
[d] $P < 0.001$.

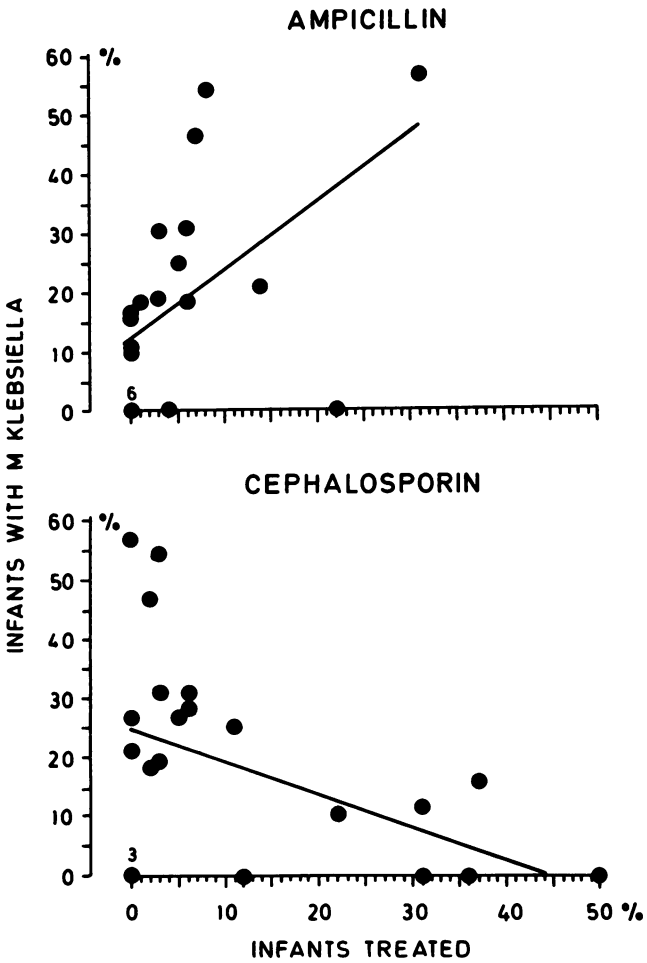

FIG. 1. Local rate of neonates carrying *Klebsiella* spp. M strains versus percentage of infants treated with ampicillin or cephalosporin (mainly cefuroxime) in each ward. The regression lines are $y = 24.7 - 0.55x$ for cephalosporin and $y = 11.7 + 1.2x$ for ampicillin. Each symbol represents one ward, except where a number is indicated.

DISCUSSION

In this study of 953 infants from 22 Swedish neonatal wards, strains of *Klebsiella* spp. were twice as common as *E. coli* strains as early colonizers of the newborn gut. This is in contrast to data from babies cared for in ordinary nurseries, where *E. coli* strains dominate the fecal flora (for a review, see reference 16). Fingerprinting of 1,522 isolates of *Entero-*

TABLE 5. Resistance to beta-lactam antibiotics of gram-negative fecal strains from neonates

Organism	No. of isolates	Resistance (% of isolates)			
		Ampicillin	Cefotaxime	Cefuroxime	Cephalexin
Klebsiella and *Enterobacter* spp.					
M strain	189	89[a]	0	46[b]	49[c]
Non-M strain	662	76[a]	2	12[b]	25[c]
E. coli					
M strain	57	88[d]	0	0	0
Non-M strain	461	18[d]	0	0	7

[a,b,c,d] $P < 0.001$ for values with the same letter.

bacteriaceae from the neonates showed that on the average, half of the infants in each ward were colonized with S strains (found in single infants only) and 23% (range, 0 to 78%) were colonized with nosocomially spread (i.e., M) strains. Thus, only a small minority among the more than 600 biotypes of *Enterobacteriaceae* found showed a potential for spreading. M strains of *K. oxytoca* were more prevalent than M strains of *E. coli*, *K. pneumoniae*, and *E. cloacae*.

Although the colonization patterns showed considerable local and temporal variation, the factors determining the patterns remain poorly understood. We are presently studying bacterial factors that could contribute to the dissemination of M strains of the family *Enterobacteriaceae*. For example, a higher resistance to desiccation may have enhanced the survival of certain M strains of *Klebsiella* spp. on the hands of the ward staff (5), facilitating their nosocomial spread.

The occurrence of M strains of both *Klebsiella* spp. and *E. coli* in the same ward suggested that institutional factors (such as crowding), which are currently under study, also contributed to the nosocomial spread of certain strains. Contrary to the results of previous studies (1, 6, 7), results of the present study showed that antibiotic usage in general had no clear impact on epidemiology of *Enterobacteriaceae*. However, the often-quoted clinical impression that ampicillin usage predisposes to nosocomial *Klebsiella* infections in infants (3) and adults (12) was further clarified here, since ampicillin selected certain epidemic *K. oxytoca* strains and rare strains of *E. coli*, *K. pneumoniae*, and *E. cloacae* that were highly resistant to ampicillin. Gentamicin showed no further ecological impact when added to ampicillin in the local antibiotic regimen.

Although 46% of *Klebsiella* spp. M isolates were resistant to 16 mg of cefuroxime per liter, cefuroxime, in contrast to ampicillin, did not select such strains. The recent reports on outbreaks of neonatal *Enterobacter* infections associated with the use of newer cephalosporins (3, 11) were apparently not parallelled in the present survey, since none of 8 institutions using mainly cefuroxime but 1 of 12 wards using ampicillin showed an increased prevalence of various strains of *Enterobacter* spp., including one M strain of *E. cloacae*.

In contrast to experience with cefotaxime (2), cefuroxime usage did not create a significant *Pseudomonas* problem, since only four infants harbored such bacteria. This could indicate a true difference in ecological potential between these agents or reflect the risk of generalizing from the experience of only one unit, where institutional factors or a particular bacterial strain may have contributed to a local problem. Apparently, multicenter studies of endemic situations are needed as a complement to reports on outbreaks to define the general ecological impact of an antibiotic.

We hypothesize that clusters of nosocomial gram-negative bacterial infections during endemic situations and occasional outbreaks are caused by rare virulent clones that are also equipped with the ability to disseminate in the hospital setting. Because such strains probably tend to acquire multiple-drug resistance, the use of antibiotics with unfavorable ecological impacts may enhance their spread. Hence, the prevention of infections might be aided by the identification and elimination of such strains from neonatal units and other parts of hospitals and by proper selection of antibiotic policy. Our data indicate that both these approaches may prove feasible. Switching from ampicillin to cephalosporin regimens could trade a risk of *Klebsiella* outbreaks and *E. coli* infections for a risk of lesser *Enterobacter*, *Pseudomonas*, and *Citrobacter* infection problems.

TABLE 6. High-level-ampicillin resistance among M and S strains of the family *Enterobacteriaceae*

Organism	No. of isolates	Ampicillin MIC (mg/liter)[a]		% of isolates with MIC >1,024 mg/liter
		50%	90%	
Klebsiella spp. M strains	185	256	>1,024	48[b]
Klebsiella spp. S strains	194	32	256	7[b]
E. coli M strains	55	1,024	>1,024	38[c]
E. coli S strains	49	<16	16	12[c]

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b,c] $P < 0.001$ for values with the same letter.

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