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Emergence of Cross-Resistance to β-Lactam Antibiotics in Fecal *Escherichia coli* and *Klebsiella* Strains from Neonates Treated with Ampicillin or Cefuroxime

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Both ampicillin and cefuroxime therapy of neonates selected drug- and species-dependent beta-lactam resistance patterns in fecal strains of *Escherichia coli* and *Klebsiella* spp. This was in contrast to our previous findings that ampicillin, but not cefuroxime, contributed to the emergence of beta-lactam resistance also by the promotion of nosocomial spread of resistant strains.

The emergence of bacterial resistance to antibiotics in hospital patients may be due to either selection of drug resistance in the resident microflora or colonization with new resistant strains. We have previously found that high rates of fecal carriage of ampicillin- and cefuroxime-resistant strains of *Escherichia coli* and *Klebsiella* spp. in neonates were associated with the use of ampicillin but that cephalosporin use (86% cefuroxime) was negatively correlated with such carriage (6). This result seemed to be due to the promotion by ampicillin, but not by cefuroxime, of nosocomial spread of certain multiple-beta-lactam-resistant strains (5).

The aim of the present study was to minimize the impact of cross-infection and to compare ampicillin and cephalosporin (cefuroxime) therapies with respect to the emergence of resistance in fecal *E. coli* and *Klebsiella* strains found only in individual treated neonates.

On the day of discharge, rectal swabs were taken from all infants (n = 953) in 22 Swedish neonatal intensive care units (7). Information on any antibiotic received by each infant was recorded at the time of sampling. Five gram-negative colonies on Endo agar per fecal specimen were isolated, and all enterobacterial colonies with different appearances and a minimum of two colonies per specimen were phenotyped biochemically. This was done by determining the individual rates of a set of biochemical activities of each isolate (24 reactions for *E. coli*, 48 reactions for *Klebsiella* isolates) by using an automated photometer and computer analysis of the readings. This fingerprinting method, originally established for *E. coli* (2), has proven to be highly discriminatory also for *Klebsiella* strains (unpublished data). A correlation coefficient of ≥0.98 between sets of readings for compared pairs of isolates was regarded as identity. More than 200 strains (biotypes) of *E. coli* and 400 strains of *Klebsiella* spp. were found among 641 *E. coli* and 881 *Klebsiella* isolates tested.

A biochemical phenotype found in only one infant in a ward was designated an S (sporadic) strain. By focusing on S strains, the dominating influence on the emergence of drug resistance by local spread of certain resistant strains was minimized.

The susceptibilities of the isolates to ampicillin, cefotaxime, cefuroxime, cephalexin, and gentamicin were determined by agar dilution, using PDM antibiotic sensitivity medium (AB Biodisk, Solna, Sweden). The breakpoint used for resistance was 16 μg/ml (4).

A set of *Klebsiella* isolates, comprising all 315 isolates belonging to phenotypes that had colonized a minimum of 10% and occasionally up to 78% of the infants in a ward and all available S strains of *Klebsiella* spp., matched for ward and week of isolation (n = 176), were tested also against higher levels of ampicillin (1,024 μg/ml and twofold dilutions thereof).

Fisher's exact test was used for the statistical analyses.

Infants treated with ampicillin showed higher rates of carriage of ampicillin-resistant S strains of *E. coli* than did untreated infants, whereas cefuroxime therapy was associated with ampicillin and/or cephalexin resistance (Table 1). *E. coli* strains resistant to cefuroxime and cefotaxime were rare.

Among S strains of *Klebsiella* spp., ampicillin resistance was selected only by ampicillin therapy, whereas cefuroxime and/or cephalexin resistance was associated both with ampicillin and cephalosporin therapies (Table 2). Ampicillin therapy also increased the rate of carriage of high-level ampicillin-resistant (MIC, ≥1,024 μg/ml) *Klebsiella* S strains (from 2 to 14%; $P < 0.01$). In contrast, cephalosporin therapy was not significantly associated with high-level ampicillin resistance in *Klebsiella* spp. (data not shown). Strains of *Klebsiella* spp. resistant to cefotaxime were rare.

None of the isolates studied was resistant to gentamicin. Gentamicin therapy always occurred in combination with ampicillin and had no further ecological impact with respect to beta-lactam resistance than did treatment with ampicillin alone (data not shown).

As mentioned above, ampicillin but not cefuroxime promoted the spread of beta-lactam-resistant enterobacterial strains in neonatal intensive care units (5, 6). In the present study, we dissected the ecological situation further and considered the impact of each drug given on resistance patterns among nonspreading phenotypes, i.e., those found in only one infant.

At the level of the individual patient both ampicillin and cefuroxime showed significant, although qualitatively and quantitatively different, ecological impacts. Thus, different patterns of cross-resistance to beta-lactam agents, related both to the drug given and the organism exposed, emerged in vivo. An understanding of these complex ecological findings

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TABLE 1. Influence of ampicillin and cephalosporin therapies on beta-lactam resistance among fecal *E. coli* S strains

Antibiotic received by host	No. of isolates	% of isolates resistant to ≥16 μg/ml	
		Ampicillin	Cephalexin
Ampicillin	37	27[a]	3
Cefuroxime	13	31[b]	15
Total cephalosporins	17	41[c]	12
None	135	8	2

[a] $P < 0.01$ compared with value for no antibiotic.
[b] $P < 0.05$ compared with value for no antibiotic.
[c] $P < 0.001$ compared with value for no antibiotic.

requires the elucidation of the underlying genetic and biochemical mechanisms of resistance in these strains.

Modern cephalosporins, such as cefotaxime, are thought to select multiple-beta-lactam-resistant strains more easily than broad-spectrum penicillins (3, 8, 9). This was not the case in our studies (6). However, cefuroxime and cefotaxime might differ in ecological impact on the fecal flora in neonates, e.g., due to lower biliary excretion of cefuroxime than of cefotaxime, including the microbiologically active desacetyl-cefotaxime metabolite (1), or to the lower stability of cefuroxime to naturally occurring fecal β-lactamases (H. G. de Vries-Hospers, D. van der Waaij, and G. W. Welling, Abstr. Fourth Eur. Confer. Clin. Microbiol., abstr. no. 1134, April 1989).

We conclude that treatment of neonates with either ampicillin or cefuroxime increased the rates of beta-lactam resistance among fecal strains of *E. coli* and *Klebsiella* spp. found only in the individual treated child. The effect of ampicillin was more pronounced among *Klebsiella* strains, and that of cefuroxime was more pronounced among *E. coli* strains.

TABLE 2. Influence of ampicillin and cephalosporin therapies on beta-lactam resistance among fecal *Klebsiella* sp. S strains

Antibiotic received by host	No. of isolates	% of isolates resistant to ≥16 μg/ml			
		Ampicillin	Cefotaxime	Cefuroxime	Cephalexin
Ampicillin	66	89[a]	2	17[b]	20[b]
Cefuroxime	33	79	0	15[a]	21[b]
Total cephalosporins	40	80	3	15[a]	20[a]
None	129	76	0	3	6

[a] $P < 0.05$ compared with value for no antibiotic.
[b] $P < 0.01$ compared with value for no antibiotic.antibiotic.

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