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Epidemiology of Plasmid-Mediated β-Lactamases in Enterobacteria in Swedish Neonatal Wards and Relation to Antimicrobial Therapy

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TEM-1, OXA-1, SHV-1, and related β-lactamases in fecal isolates from 953 infants in 22 Swedish neonatal intensive care units were studied by DNA hybridization. TEM-1- and OXA-1-positive isolates were always *Escherichia coli* and represented 86 and 8%, respectively, of the ampicillin-resistant isolates of this species. SHV-1 was found in 16% of the *Klebsiella* sp. (mainly *Klebsiella pneumoniae*) isolates. TEM-1 and SHV-1 occurred in 14 and 16 units and in up to 64 and 26% of the neonates, respectively. On average, two to four different biochemical phenotypes per species per ward were positive for each β-lactamase. All but 1 of the 33 *E. coli* phenotypes found to be TEM-1 positive were uniformly positive for the β-lactamase gene, whereas some of the phenotypes found to be positive for OXA-1 (2 of 3) and SHV-1 (6 of 70) were occasionally negative for the respective genes. The occurrence of the three β-lactamases studied tended to be associated with local ampicillin usage (correlation coefficient, 0.31 to 0.39; $P > 0.05$). Of the neonates receiving ampicillin, 30% carried TEM-1-positive *E. coli*, compared with 13% for cephalosporin-treated neonates and 15% for untreated neonates ($P \leq 0.001$). The corresponding rates for SHV-1 in *Klebsiella* spp. were 18, 13, and 9% ($P \leq 0.01$). Ampicillin is thus a significant risk factor for the maintenance of the most prevalent gram-negative plasmid-mediated β-lactamases in hospitalized neonates.

Since the discovery of R plasmids, a wide variety of plasmid-mediated β-lactamases have been described in gram-negative enterobacteria and play an important role in their acquisition of resistance to β-lactam agents. Of these enzymes, TEM-1, SHV-1, and OXA-1 seem to be among the most commonly occurring (2, 4, 5, 11, 15), and the gene specifying TEM-1, encoded by transposon Tn*3*, has also been disseminated to distantly related bacteria, such as *Haemophilus influenzae* and gonococci (12). In recent years, mutations leading to the hyperproduction of TEM-1 and thus resistance to β-lactamase inhibitors (10, 16) or dramatically broadened substrate specificity of TEM-1 and SHV-1 have been described (2, 3). Strains of gram-negative bacteria carrying such plasmid-mediated extended-spectrum β-lactamases are currently being disseminated in hospitals on four continents (13, 14).

Most epidemiological studies of plasmid-mediated β-lactamases concern clinical isolates from single hospitals or local areas. Thus, large-scale studies of bacterial carriage of plasmid-mediated β-lactamases are rare, and few attempts have been made to correlate their occurrence with local antimicrobial consumption, e.g., in hospitals or with antimicrobial therapy. Furthermore, the different modes of emergence of gram-negative plasmid-mediated β-lactam resistance (spread of transposons or plasmids between strains, spread of identical resistant strains between hospitalized patients or other individuals) have not been distinguished.

In a 22-center study of neonatal intensive care units (NICU) located across Sweden, we analyzed the nationwide epidemiology of TEM-1, SHV-1, OXA-1, and related enzymes in enteric bacteria by DNA hybridization. We were particularly interested in any regional differences and correlations with different antimicrobial agents used in the NICUs and in determining whether the local emergence of plasmid-mediated β-lactam resistance was due to the occurrence of each enzyme in different strains or to the local spread of a single resistant strain between babies. It has been reported that of the enteric bacteria isolated from fecal specimens from neonates, *Klebsiella* spp. dominate and are followed by *Escherichia coli* and *Enterobacter* spp. (19, 20). This study therefore focused on these organisms.

MATERIALS AND METHODS

Bacterial isolates. We monitored 22 NICUs for a mean period of 4 (2 to 18) months. Fecal specimens were obtained at discharge from all neonates with a minimum stay of 5 days ($n = 953$). A total of 641 *E. coli*, 749 *Klebsiella* sp., and 87 *Enterobacter* sp. colonies, representing the dominant aerobic gram-negative flora, were isolated and identified as described previously (17, 19, 20).

Biochemical fingerprinting. All isolates were biochemically phenotyped by a recently published method offering high reproducibility and discrimination (7–9). In brief, bacterial suspensions were added to microtiter wells containing dehydrated reagents selected to yield optimal discrimination for each species or group (22 for *E. coli* and 16 for *Klebsiella* and 16 for *Enterobacter* spp.). During incubation at 37°C, the reactions were read at intervals with a microplate reader, and the absorbance values were recorded and processed by a microcomputer. The values obtained for each of the isolates (the biochemical fingerprints) were compared pairwise. A correlation coefficient of ≥0.98 defined identity between fingerprints. For the isolates studied, more than 200 *E. coli* and 400 *Klebsiella* or *Enterobacter* sp. biochemical phenotypes were identified (17).

Antimicrobial resistance testing. All isolates were tested against 16 μg of ampicillin, cephalexin, cefuroxime, and

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TABLE 1. Occurrence of plasmid-mediated β-lactamases in fecal enterobacteria isolated from 953 neonates in 22 Swedish NICUs

Organism	No. of isolates				
	Studied	That were Amp[ra]	With		
			TEM-1	OXA-1	SHV-1
E. coli	641	133	114	10	0
Klebsiella spp.	749	595	0	0	94[b]
Enterobacter spp.	87	44	0	0	0

[a] Resistant to 16 μg of ampicillin per ml.
[b] *K. pneumoniae*, 90 isolates; *K. oxytoca*, 4 isolates.

TABLE 2. Occurrence of TEM-1 and SHV-1 β-lactamases in different enterobacterial biochemical phenotypes in 22 Swedish NICUs

Organism	No. of NICUs	No. of phenotypes	
		Total	Per ward[a]
TEM-1[+] *E. coli*	14	33	2.4 (1–5)
OXA-1[+] *E. coli*	1	3	3.0
SHV-1[+] *Klebsiella* spp.	16	70[b]	4.4 (1–13)

[a] Mean (range).
[b] *K. pneumoniae*, 67 phenotypes; *K. oxytoca*, 3 phenotypes.

cefotaxime per ml by agar dilution in PDM antibiotic sensitivity medium (AB Biodisk, Solna, Sweden) by use of a multipoint inoculator providing 5×10^3 CFU per application site (20). Plates were incubated overnight at 37°C in air and then examined for growth.

Detection of β-lactamase genes. All isolates resistant to ampicillin (133 *E. coli*, 595 *Klebsiella* sp., and 44 *Enterobacter* sp. isolates) were analyzed for plasmid-mediated β-lactamase genes by colony hybridization by use of DNA probes prepared from plasmids pBR322 (TEM-1), pMON301 (OXA-1), and pMON38 (SHV-1) as previously described (6). The probes were ^{32}P labelled by nick translation. Isolates positive for TEM-1, OXA-1, or SHV-1 could produce these β-lactamases or the closely related TEM-2, OXA-4, or SHV-2, respectively (6), or other extended-spectrum β-lactamases of each type. However, antimicrobial resistance testing did not suggest the presence of extended-spectrum enzymes among the colony hybridization-positive isolates. Isolates yielding ambiguous hybridization results were subjected to isoelectric focusing of their β-lactamase(s) as previously described (6).

Antimicrobial usage. Information regarding the antimicrobial therapy of each neonate was recorded when the fecal sample was collected and was used to describe the antimicrobial usage policy, expressed as percentage of neonates treated, of each NICU studied (16). Overall, 38% of the neonates received antimicrobial therapy. Ampicillin (with or without gentamicin) was predominantly used in 12 NICUs (median, 30%, and range, 16 to 51%, of the neonates), and cephalosporins (86% cefuroxime) were predominantly used in 8 NICUs (median, 31%, and range, 11 to 50%, of the neonates), whereas both of these regimens were used in 2 NICUs. Other antimicrobial drugs were used only sporadically (17).

Statistical analysis. Spearman's rank correlation test, the chi-square test, and Fisher's exact test were used for statistical analyses when appropriate.

RESULTS

Species specificity. A majority of the ampicillin-resistant *E. coli* isolates hybridized with the TEM-1 probe (86%), 8% hybridized with the OXA-1 probe, and none hybridized with the SHV-1 probe (Table 1). Among the *Klebsiella* sp. isolates, neither TEM-1 or OXA-1 nor related β-lactamases contributed to resistance, whereas 16% of the isolates reacted with the SHV-1 probe (Table 1). A majority of the SHV-1-positive isolates were *Klebsiella pneumoniae* (96%), the remaining being *K. oxytoca*. None of the *Enterobacter* sp. isolates carried β-lactamase genes related to the three prototypes studied.

General epidemiology. The general epidemiology of the plasmid-mediated β-lactamases (type of enzyme occurring and local rates of carriage of TEM-1- or SHV-1-positive isolates) showed no apparent geographical pattern. TEM-1- and SHV-1-positive isolates were found to be widely spread (in 14 and 16 NICUs, respectively), whereas OXA-1-positive isolates occurred in only 1 NICU (Table 2). TEM-1-positive *E. coli* and SHV-1-positive *Klebsiella* sp. isolates usually occurred together in the same 12 NICUs. TEM-1-positive *E. coli* isolates only and SHV-1-positive *Klebsiella* sp. isolates only occurred in two and four NICUs, respectively, and none of the three β-lactamase genes studied was found in four NICUs.

Local epidemiology. The local prevalence of TEM-1-positive *E. coli* isolates was highly variable (0 to 50% of the neonates and 0 to 100% of the ampicillin-resistant *E. coli* isolates; Table 3). Similarly, SHV-1-positive *Klebsiella* sp. isolates were found in 0 to 24% of the neonates and 0 to 40% of the ampicillin-resistant *Klebsiella* sp. isolates (Table 3). In the NICU with OXA-1-positive *E. coli* isolates, 15% of the neonates carried such isolates and 69% of the ampicillin-resistant isolates contained OXA-1.

Intraspecies epidemiology. Biochemical fingerprinting of the isolates showed that the plasmid-mediated β-lactamases usually occurred in several different phenotypes per species in the same ward (in 1 to 13 phenotypes per ward; Table 2). For most of these phenotypes, the respective β-lactamase gene was found in all isolates from neonates carrying the same phenotype. Of 33 phenotypes with TEM-1-positive *E. coli*, all isolates from 32 phenotypes were uniformly positive for this gene. A widely disseminated ampicillin-resistant *E. coli* phenotype (carried by 39 of 103 babies in one of the

TABLE 3. Local prevalence of TEM-1 and SHV-1 β-lactamases among 953 neonates in 22 Swedish NICUs and their fecal enterobacteria

β-Lactamase	Prevalence (% of neonates or isolates)		
	Mean	Median	Range
TEM-1 in:			
Neonates (n = 953)	10	4	0–50
Neonates with *E. coli* (n = 412)	22	10	9–69
E. coli isolates (n = 641)	18	7	0–67
Amp[ra] *E. coli* isolates (n = 133)	86	80	0–100
SHV-1 in:			
Neonates (n = 953)	8	6	0–24
Neonates with *Klebsiella* spp. (n = 620)	12	12	0–36
Klebsiella sp. isolates (n = 749)	12	10	0–30
Amp[ra] *Klebsiella* sp. isolates (n = 595)	16	13	0–40

[a] Resistant to 16 μg of ampicillin per ml.

TABLE 4. Relationship between local antimicrobial usage and the local prevalence of TEM-1 and SHV-1 β-lactamases among 953 neonates in 22 Swedish NICUs and their fecal enterobacteria

β-Lactamase	Correlation coefficient[a] for:		
	Ampicillin[b]	Cephalosporins[c]	All antimicrobial agents
TEM-1 in:			
Neonates with *E. coli*	0.37	−0.15	0.44[d]
E. coli isolates	0.32	−0.10	0.44[d]
SHV-1 in:			
Neonates with *Klebsiella* spp.	0.31	−0.28	0.33
Klebsiella sp. isolates	0.39	−0.34	0.34

[a] Spearman rank correlation coefficient between the percentage of neonates or isolates with plasmid-mediated β-lactamases in each ward and the percentage of neonates in the same ward receiving the respective antimicrobial therapy.
[b] Ampicillin alone or together with gentamicin.
[c] Cefuroxime in 86%.
[d] $P \leq 0.05$ (significant).

TABLE 5. Relationship between antimicrobial therapy and the occurrence of TEM-1, OXA-1, and SHV-1 in fecal enterobacteria isolated from treated neonates

Antimicrobial therapy of host	No. (%) of isolates of:				
	E. coli			*Klebsiella* spp.	
	Total	Positive for:		Total	Positive for SHV-1
		TEM-1	OXA-1		
Ampicillin[a]	118	30[b,c]	3	186	18[d]
Cephalosporins[e]	63	13	0	83	13
None	453	15	1	464	9

[a] Ampicillin alone or together with gentamicin.
[b] $P \leq 0.001$ compared with no antimicrobial therapy.
[c] $P \leq 0.01$ compared with cephalosporin therapy.
[d] $P \leq 0.01$ compared with no antimicrobial therapy.
[e] Cefuroxime in 86%.

NICUs studied) was TEM-1 negative in one of the carrier neonates. OXA-1 appeared to be less prevalent and stable in *E. coli*. Apart from being found in *E. coli* recovered from only one ward, two of the three phenotypes with OXA-1-positive *E. coli* included isolates negative for this β-lactamase gene. Among the 70 phenotypes with SHV-1-positive *Klebsiella* spp., 6 phenotypes included occasional SHV-1-negative isolates.

Association with antimicrobial usage. The impact of antimicrobial therapy on the occurrence of plasmid-mediated β-lactamases was analyzed from two perspectives. First, the overall antimicrobial usage in each ward (percentage of babies treated with ampicillin and cephalosporins) was correlated with the local rates of carriage of enterobacteria positive for TEM-1 and SHV-1. As expected, these local rates of carriage tended to be associated with ampicillin usage and to be negatively correlated with cephalosporin (cefuroxime) usage (Table 4). Also, in the single NICU with OXA-1-positive *E. coli*, ampicillin-gentamicin was mainly used. Second, the impact of antimicrobial therapy was studied in the individual neonates treated. Neonates who had received ampicillin therapy had a doubled rate of fecal carriage of TEM-1-positive *E. coli* (30%; $P \leq 0.001$) and/or SHV-1 positive *Klebsiella* spp. (18%; $P \leq 0.01$) (Table 5), compared with untreated neonates.

As expected, cephalosporin (cefuroxime) therapy was not associated with the carriage of TEM-1-, OXA-1-, or SHV-1-positive enterobacteria. None of the plasmid-mediated β-lactamase-positive isolates identified showed an extended spectrum of resistance, including for cefuroxime. Nevertheless, cefuroxime therapy did not eliminate TEM-1- or SHV-1-positive enterobacteria from the host or prevent their colonization of the babies (Table 5).

DISCUSSION

As expected, TEM-1-related β-lactamases explained most of the ampicillin resistance in *E. coli* and SHV-1-related β-lactamases explained part of the β-lactam resistance in *Klebsiella* spp. Our Swedish strains showed less promiscuity of TEM-1-, OXA-1-, and SHV-1-type enzymes than was anticipated from the literature (5), with TEM-1- and OXA-1-type enzymes being confined to *E. coli* and SHV-1-type enzymes being confined to *Klebsiella* spp., mainly *K. pneumoniae*. This result could have been due to the host specificities of carrier plasmids, the host specificity of DNA, or other genetic barriers between bacterial species. Previously published reports on plasmid-mediated β-lactamase epidemiology may represent a bias towards clinical isolates with unusual properties, whereas we studied an unselected population of fecal isolates from hospitalized neonates.

In each ward, several different biochemical phenotypes of *E. coli* and *Klebsiella* spp. carried the respective type of plasmid-mediated β-lactamase gene, which was generally present in all isolates belonging to that phenotype. This result suggested that the emergence of plasmid-mediated β-lactam resistance involved both the spread of identical resistant strains between babies and the dissemination of identical β-lactamase genes among different host strains.

The highly variable local prevalence of TEM-1- and SHV-1-positive strains showed no apparent geographical pattern but correlated with the total ampicillin usage in the ward and, in particular, with ampicillin therapy of neonates. Although the isolates positive for plasmid-mediated β-lactamases were uniformly susceptible to cefuroxime, including isolates from neonates given the drug, cephalosporin (cefuroxime) therapy showed no tendency to eliminate TEM-1- or SHV-1-positive strains from the fecal flora of the treated neonates or to prevent their colonization of the babies. These observations indicated that the impact of cefuroxime on these components of the fecal flora of the neonates was negligible. The negative correlation between the local rates of carriage of isolates with these plasmid-mediated β-lactamases and increasing cephalosporin usage in the wards probably reflected the concomitant decline of local ampicillin usage, i.e., cefuroxime replacing ampicillin-gentamicin as the main policy.

This study demonstrated that ampicillin usage increases the prevalence of the major gram-negative plasmid-mediated β-lactamases in the fecal flora of hospitalized neonates. Thus, ampicillin maintains a pool of common plasmid-mediated β-lactamases present in a variety of bacterial phenotypes, among which mutants with wider substrate profiles for the enzymes, including broad-spectrum cephalosporins or enzyme hyperproduction mediating resistance to β-lactam–β-lactamase inhibitor combinations, may be selected. We have also observed that ampicillin therapy but not cefuroxime therapy of neonates carrying *Enterobacter* spp. selects chromosomal mutants with stably derepressed β-lactamase production and thus resistance to newer broad-

spectrum penicillins and extended- and broad-spectrum cephalosporins (1). Taken together, this study and our previous studies of NICUs (1, 17, 18) indicate that the use of ampicillin increases the potential for the emergence of resistance to various other β-lactam antimicrobial agents in enteric bacteria. We suggest that carefully considering the use of broad-spectrum penicillins is advisable from an ecological point of view.

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