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A between-Species Comparison of Antimicrobial Resistance in Enterobacteria in Fecal Flora

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Enterobacteria in fecal flora are often reported to be highly resistant. *Escherichia coli* is the main species; resistance data on other species are rare. To assess the effect of the host's environment, antimicrobial resistance was determined in fecal species of the family *Enterobacteriaceae* from three populations: healthy people (HP) ($n = 125$) with no exposure to antimicrobials for 3 months preceding sampling, university hospital patients (UP) ($n = 159$) from wards where the antibiotic use was 112 defined daily doses (DDD)/bed/month, and geriatric long-term patients (LTP) ($n = 74$) who used 1.8 DDD/bed/month. The mean length of hospital stay was 5 days for the UP and 22 months for the LTP. The isolates were identified to at least genus level, and MICs of 16 antimicrobials were determined. From the university hospital, resistance data on clinical *Enterobacteriaceae* isolates were also collected. Resistance data for on average two different isolates per sample (range, 1 to 5) were analyzed: 471 *E. coli* isolates and 261 other *Enterobacteriaceae* spp. Resistance was mainly found among *E. coli*; even in HP, 18% of *E. coli* isolates were resistant to two or more antimicrobial groups, with MIC patterns indicative of transferable resistance. Other fecal enterobacteria were generally susceptible, with little typically transferable multiresistance. Clinical *Klebsiella* and *Enterobacter* isolates were significantly more resistant than fecal isolates. The resistance patterns at both hospitals mirrored the patterns of antibiotic use, but LTP *E. coli* isolates were significantly more resistant than those from UP. Conditions permitting an efficient spread may have been more important in sustaining high resistance levels in the LTP. *E. coli* was the main carrier of antimicrobial resistance in fecal flora; resistance in other species was rare in the absence of antimicrobial selection.

High frequencies of antimicrobial resistance have been found in enterobacteria, in fecal flora as well as in clinical isolates. *Escherichia coli* isolates from numerous environments have been studied. Data on other enterobacterial species are usually found only for clinical strains, which tend to be relatively resistant. Very patchy data on other enterobacteria in the fecal flora have been published, and thus we know surprisingly little about how their resistance is acquired and maintained.

Part of the problem is the lack of specificity of the data in studies screening for resistance in fecal flora or environmental strains. They usually focus on coliforms, or aerobic gram-negative rods isolated on, e.g., MacConkey agar (8, 10, 15, 16, 27) or concentrate on *E. coli* (1, 14, 22). There could be differences in the spread of resistance genes in different species, which are not detected when studying coliforms. Since endogenous (chromosomal) resistance is common among enterobacteria, it is imperative to know which species are tested. For example, a high level of ampicillin resistance is very significant in *E. coli*, while it would be natural in most other enterobacteria. *E. coli* and *Shigella*, *Salmonella*, and *Klebsiella* spp. are the only ones generally susceptible to narrow-spectrum cephalosporins (4, 17, 18). Similarly, *Proteus* spp. and *Providencia rettgeri* are naturally resistant to tetracycline (3) and *Morganella morganii* is naturally resistant to sulfamethoxazole (36), while these resistance factors are a sign of transferable resistance in other species.

When studying enterobacteria isolated from food, we found that *Enterobacter*, *Klebsiella*, and *Citrobacter* spp. and *Hafnia alvei*, which were the members of the *Enterobacteriaceae* most commonly encountered, were susceptible to most antibiotics tested (26, 29). The lack of multidrug-resistance patterns, indicative of transferable resistance and typically seen in clinical strains, and fecal *E. coli* strains (27), was particularly noted. There is no evidence to show that environmental strains of a species are completely different from human strains; on the contrary, human, animal, and environmental clones have been shown to be indistinguishable in *E. coli* and *Pseudomonas aeruginosa* (25, 31, 33). Thus, the question arose as to what species were responsible for the high levels of resistance in fecal flora. We decided to do a between-species comparison of antimicrobial resistance in enterobacteria in human fecal flora. Three different human populations were included to study how resistance varies depending on the host's environment.

MATERIALS AND METHODS

Collection of isolates. Human fecal samples were collected from three populations: (i) healthy persons (HP) that had not been treated with antibiotics for at least 3 months preceding sampling, in 1993 and 1994, as previously described (29); (ii) long-term patients (LTP) (mean duration of hospitalization, 22 months) at two medicine wards at Turku City Hospital in 1994 (13); and (iii) university hospital patients (UP) (mean duration of hospitalization, 5 days) at the Turku University Hospital in 1997. The UP, who were treated in the Departments of Medical Intensive Care, Cardiology, Gastroenterology, Infectious Diseases, Hematology, and Nephrology at the Turku University Hospital, were taking part in a screening program for carriage of fecal vancomycin-resistant enterococci; at

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the same time, gram-negative isolates were collected. Briefly, the samples were diluted in saline and plated onto MacConkey agar. Plates with discrete colonies were chosen, and at least five colonies per sample, or all different-looking colonies, were selected. Enterococci were ignored.

From the university hospital, data on antimicrobial consumption were obtained from the hospital pharmacy, and data on hospitalization times were taken from the hospital records. Data on overall antimicrobial use at the long-term hospital were calculated from an earlier work by our group (13); data on individual use was extracted directly from patient records.

Identification. Isolates that were found to be gram-negative rods, oxidase negative, and able to ferment glucose, and that thus belonged to the *Enterobacteriaceae*, were selected for further identification. All isolates were first screened for β-glucuronidase activity and indole production; isolates positive in both tests were presumed to be *E. coli* and were not further tested. All others were tested for the following: β-galactosidase (with *o*-nitrophenyl-β-D-galactopyranoside [ONPG] discs), production of gas from glucose, citrate and urea utilization; Voges-Proskauer and methyl red; ornithine, arginine, and lysine decarboxylase; production of dihydrogen sulfide; motility; DNase activity at +25°C; pigmentation; and fermentation of the following sugars: mannitol, maltose, lactose, arabinose, sorbitol, inositol, raffinose, and trehalose. Media and reagents were from BBL (Becton Dickinson Microbiology Systems, Cockeysville, Md.), Rosco (Taastrup, Denmark), Sigma Chemical Co. (St. Louis, Mo.), and Oxoid (Unipath Ltd., Basingstoke, United Kingdom). The tests were essentially performed as described in the manufacturer manuals, and in the *Clinical Microbiology Procedures Handbook* (7).

The results were compared with a database, generated by combining the lists of identification of *Enterobacteriaceae* in *Bergey's Manual of Determinative Bacteriology* (6) and the *Manual of Clinical Microbiology* (3), using the Excel 97 spreadsheet program (Microsoft). Unclear cases, with the genus determined at a probability lower than 65%, were retested and in some instances were tested with the API-E test (bioMérieux, Lyon, France).

Determination of susceptibility. MICs were determined by a standard agar dilution method (23) on Mueller-Hinton II medium (BBL). NCCLS breakpoints were applied. The following antimicrobials were tested (range): ampicillin (0.25–256 mg/liter), trimethoprim (0.12–1024 mg/liter), sulfamethoxazole (0.5–1024 mg/liter), chloramphenicol (2–128 mg/liter), cephalothin (0.25–64 mg/liter), cefuroxime (0.06–64 mg/liter), cefotaxime (0.06–32 mg/liter), gentamicin (0.25–64 mg/liter), tetracycline (0.12–64 mg/liter), and nalidixic acid (0.5–128 mg/liter), all from Sigma; amoxicillin-clavulanic acid (0.5–64 and 0.25–32 mg/liter, respectively; SmithKline Beecham Pharmaceuticals, Rixensart, Belgium), aztreonam (0.25–64 mg/liter, Bristol-Myers Squibb, Italy), imipenem (0.25–64 mg/liter; Merck, Sharp & Dohme, Westpoint, Pa.), ciprofloxacin (0.06–8 mg/liter; Bayer, Leverkusen, Germany).

Resistance data on clinical enterobacterial isolates. Resistance data were collected from the same wards as those from which the patients were sampled at the Turku University Hospital, and from the same time period when the fecal samples were collected. All types of isolates were included, except fecal cultures. The data were obtained with the WHONET program (available from J. Stelling, World Health Organization/EMC, Geneva, Switzerland) from the records of the Microbiological Laboratory. These isolates had been tested with the disk diffusion method, using Oxoid disks with Iso-Sensitest medium (Oxoid). The NCCLS interpretive criteria were used (23).

REA. Restriction enzyme analysis (REA) was performed on all trimethoprim-resistant *E. coli* isolates from the LTP. Bacterial DNA was isolated from overnight cultures in Luria broth by the method of Wilson (37). The DNA was cut with restriction enzymes (*Hin*dIII, *Eco*RI, *Bam*HI, *Sac*I, *Sal*I, and *Pst*I; Promega, Madison, Wis.) overnight with an oil overlay and run into 0.6% agarose gels at 15 to 20 V for 20 h. The patterns were visually compared.

Statistics. The sample groups were compared in a pairwise fashion, using the chi-square analysis-of-contingency table test or Fisher's exact test as applicable. A P value of <0.05 was considered statistically significant.

RESULTS

Samples and isolates. We studied 125 HP, 74 LTP, and 159 UP fecal samples. Of the five isolates picked per sample, isolates that appeared to be of the same strain based on biochemical and MIC profiles were excluded from the analysis. On average two isolates per sample were included in the resistance analysis. *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* were the most common genera (Table 1). A total of 471 *E. coli* isolates and 261 other enterobacterial isolates were studied. Data on clinical isolates from the university hospital were obtained for *E. coli* and *Klebsiella* and *Enterobacter* spp.

Antimicrobial use. In the HP group, no antibiotics had been used for at least 3 months preceding sampling. At the University Hospital, β-lactams were the most frequently used (Table 2). Antimicrobial use at the long-term hospital was comparatively low, but individual total use could be high. Of the LTP, 21% had been treated with trimethoprim, with 15% of patients receiving this treatment for >14 days, sometimes for yearlong periods as urinary tract infection prophylaxis.

Antimicrobial resistance levels. Resistance frequencies varied from 40% resistance to trimethoprim in the LTP group, to

TABLE 1. Identity of bacterial isolates

Species	No. of isolates in sample population		
	HP	UP	LTP
Citrobacter braakii		4	
Citrobacter diversus	1	1	
Citrobacter farmeri		2	
Citrobacter freundii		4	
Citrobacter sedlakii	1	1	
Citrobacter youngae	4	9	4
Citrobacter sp.[a]	2	14	2
Total	11	35	6
Enteric group 58	1	2	0
Enterobacter aerogenes	1	2	
Enterobacter amnigenus	1		
Enterobacter cloacae-Enterobacter dissolvens	18	29	3
Enterobacter sakazakii	2		
Enterobacter taylorae		1	
Enterobacter sp.	4		
Total	26	32	3
Escherichia coli	171	188	112
Escherichia fergusonii-Escherichia hermanii	0	0	4
Hafnia alvei	1	5	0
Klebsiella ornithinolytica	1	2	
Klebsiella oxytoca	8	9	
Klebsiella planticola		3	
Klebsiella pneumoniae	23	33	3
Klebsiella terrigena	1		
Klebsiella sp.	3	1	1
Total	35	47	4
Kluyvera ascorbata		1	
Kluyvera cryocrescens	2		
Kluyvera sp.	1		
Total	3	1	0
Morganella morganii	2	1	0
Proteus mirabilis		1	3
Proteus penneri		1	
Proteus vulgaris		3	
Proteus sp.		4	
Total	0	9	3
Salmonella sp.		1	
Serratia fonticola	2	5	
Serratia liquefaciens	1	1	
Serratia marcescens		1	
Serratia sp.		1	
Total	3	8	0
Yersinia fredriksenii		1	2
Yersinia intermedia		1	
Yersinia sp.	2		
Total	2	2	2

[a] Not determined to species level.

TABLE 2. Characteristics of the wards sampled at the two hospitals

Characteristic	University Hospital	Long-term hospital
Length of stay		
mean	5.1 days	22 mo
max	55.2 days	137 mo
min		1 mo
median	3 days	
No. of beds	122	74
Antibiotic group (DDD[d]/bed/month)		
Aminoglycosides	7.3	0
Penicillins, penicillin with inhibitor	11.3	0.1
Cephalosporins	20.9[a]	0.2
Meropenem, imipenem, aztreonam	42.3	0
Quinolones[b]	19.4	0.1
Trimethoprim-Sulfamethoxazole	2.9	1.4
Others[c]	7.5	0
Total (DDD/bed/month)	112	1.8

[a] 55% broad spectrum.
[b] Mainly ciprofloxacin.
[c] Tetracyclines, rifampin, fusidic acid, and nitrofurantoin.
[d] DDD, defined daily doses.

0 to 4% resistance to broad-spectrum β-lactams, gentamicin, and ciprofloxacin in all groups (Table 3 and 4).

Resistance in *E. coli* compared to other enterobacteria. Among HP, *E. coli* (Table 3) was responsible for most of the resistance found. For all other enterobacterial species together (Table 4), resistance did not exceed 5% for any of the antibiotics tested (antibiotics to which these species have intrinsic resistance were not included). The difference was statistically significant for resistance to streptomycin, tetracycline, sulfamethoxazole, trimethoprim, and trimethoprim and sulfamethoxazole resistance combined (Fig. 1A).

In the UP, *E. coli* was still slightly more resistant than the other enterobacterial species to sulfamethoxazole and trimethoprim, but the other species were more resistant than *E. coli* to chloramphenicol, cefuroxime, and aztreonam (Fig. 1B). Among the clinical isolates it is noteworthy that *E. coli* and the other species were equally resistant to trimethoprim. The only significant differences were for cefuroxime and trimethoprim-sulfamethoxazole (Fig. 1C), the latter evidently as a consequence of sulfamethoxazole resistance being rarer in the other species, since trimethoprim resistance levels were equal.

Comparison of *E. coli* isolates from different sources. *E. coli* isolates from the HP samples were the most susceptible, while LTPs yielded the most resistant isolates (Table 3): trimethoprim resistance in particular was unusually high (40%, compared to 9% in the HP). The UP fecal samples were less resistant than the clinical isolates. The HP and the UP populations differed only in nalidixic acid resistance. In the LTP samples, nalidixic acid resistance was even higher.

In the LTP, a group ($n = 10$) of nalidixic acid-resistant strains with trimethoprim MICs in the range of 16 to 128 mg/liter were prominently featured. These were shown by REA to represent a single clone, which had spread to eight patients in five rooms; the samples yielding these strains were collected over 2 weeks. Thus, 22% of the LTP's trimethoprim resistance was caused by this clone.

Comparison of other enterobacteria from different sources. A similar comparison of other *Enterobacteriaceae* showed a greater difference between UP and HP; a more prevalent cefuroxime, chloramphenicol, and tetracycline resistance and also nalidixic acid resistance among the UP was noticeable (Table 4). On the other hand, sulfamethoxazole resistance remained rare at 5%. In the clinical data, more trimethoprim, and trimethoprim-sulfamethoxazole resistance was seen. Although there were only 22 LTP isolates, two things are noteworthy: the resistance frequency of trimethoprim was as high as 36%, paralleling the high frequency in LTP *E. coli*, and

TABLE 3. Resistance frequencies in *E. coli* from fecal samples and clinical isolates from the university hospital[a]

Antimicrobial (resistance breakpoint [mg/liter])	HP		UP		LTP		C		P[d]					
	No. tested	*R*%	No. tested	*R*%	No. tested	*R*%	No. tested	*R*%	HP-UP	HP-LTP	HP-C	UP-LTP	UP-C	LTP-C
AMP (≥32)	171	12	188	14	112	25[f]	136	32[f]		0.05	<0.0001	0.03	0.0002	
AMC (≥32/16)	165	0	188	3	112	1	19	5						
CEX (≥32)	165	1	188	5	112	8[e]	154	18[f]		0.008	<0.0001		<0.0001	0.01
CXM (≥32)	170	0	188	1	112	3	146	1						
CTX (≥64)	165	0	188	0	112	0	ND	ND						
IPM (≥16)	165	0	188	0	112	0	139	0						
ATM (≥32)	165	0	188	0	112	0	ND	ND						
GEN (≥16)	165	0	188	3	112	0	151	1						
STR (≥32)[b]	156	18	188	14	ND	ND	ND	ND						
CHL (≥32)	171	4	188	7	112	3	ND	ND						
TET (≥16)	171	14	188	13	112	25[e]	ND	ND		0.05		0.2		
SUL (≥512)	171	16	188	13	112	28[e]	ND	ND		0.02		0.002		
TMP (≥16)	171	9	188	12	112	40[f]	127	26[e]		<0.0001	0.0001	<0.0001	0.002	0.03
SXT (≥4/76)[c]	171	8	188	8	112	15	150	24[f]			0.0001		<0.0001	
NAL (≥32)	171	1	188	4[e]	112	13[f]	ND	ND	0.04	<0.0001		0.008		
CIP (≥4)	171	0	188	0	112	0	143	4[e]			0.008		0.006	0.04

[a] Abbreviations for antimicrobial agents: AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CEX, cephalothin; CXM, cefuroxime; CTX, cefotaxime; IPM, imipenem; ATM, aztreonam; GEN, gentamicin; STR, streptomycin; CHL, chloramphenicol; TET, tetracycline; SUL, sulfamethoxazole; TMP, trimethoprim; SXT, co-trimoxazole; NAL, nalidixic acid; CIP, ciprofloxacin. Other abbreviations: *R*%, resistance frequency; C, clinical isolates (university hospital); ND, not determined.
[b] Set by authors, on the basis of histogram distribution.
[c] Tested in combination only for UP and C; for HP and LTP the numbers are for strains resistant to SUL and TMP, tested separately. The UP were tested for both SUL and TMP as well as SXT resistance; the result of combining SUL and TMP resistance was the same as that produced by SXT.
[d] Comparison between subject groups (significant values).
[e] Significantly more resistant.
[f] Very significantly more resistant.

TABLE 4. Resistance frequencies in *Enterobacteriaceae* other than *E. coli* from fecal samples and clinical isolates from the university hospital[a]

Antimicrobial (resistance breakpoint [mg/liter])	HP		UP		C		LTP		*P*					
	No. tested	*R*%	No. tested	*R*%	No. tested	*R*%	No. tested	*R*%	HP-UP	HP-C	HP-LTP	UP-C	UP-LTP	C-LTP
CMX (≥32)[a]	83	2	143	26[b]	62	26[b]	22	0	<0.0001	0.0007			0.002	0.02
CTX (≥64)	64	0	143	0	ND	ND	22	0						
IPM (≥16)	83	1	143	0	61	0	22	0						
ATM (≥32)	84	0	143	3	ND	ND	22	0						
GEN (≥16)	84	0	143	0	53	0	22	0						
STR (≥32)	83	5	143	10	ND	ND	ND	ND						
CHL (≥32)	83	2	143	20[b]	ND	ND	22	5	0.0003					
TET (≥16)	83	2	134	11[b]	ND	ND	22	5	0.02					
SUL (≥512)	83	5	143	5	ND	ND	22	14						
TMP (≥16)	82	0	143	5	33	24[b]	22	36[b]		<0.0001	<0.0001	0.006	<0.0001	
SXT (≥4/76)	82	0	143	3	67	11[b]	22	14[b]		0.005	0.008			
NAL (≥32)	83	0	143	10[b]	ND	ND	22	0	0.008					
CIP (≥4)	58	0	143	1	62	2	22	0						

[a] See footnotes to Table 3.
[b] Significantly more resistant.

cefuroxime resistance was missing, similar to the HP's other enterobacteria.

Multidrug resistance and indications of transferable resistance. Multidrug resistance patterns among the HP, UP, and LTP populations were compared to give a picture of the prevalence of transferable resistance in the different populations. Strains resistant (not intermediately resistant) to at least two of the following antimicrobials—ampicillin (only in *E. coli*), tetracycline, sulfamethoxazole, trimethoprim, streptomycin, and chloramphenicol—were designated multidrug resistant. This selection was based on the fact that these resistance traits are very often found together on transferable elements, e.g., *Tn*21 and related transposons (21).

(i) *E. coli*. The numbers of multidrug resistant *E. coli* strains were the same in the HP and UP populations (18 and 19%, respectively, of all isolates), and sulfamethoxazole resistance occurred in 83 and 71%, respectively, of these. In the LTP strains multidrug resistance had increased to 33% (of which 81% were sulfamethoxazole resistant). The MICs were in all cases within the highest part of the tested range for at least one of the antibiotics.

(ii) Other enterobacteria. In the HP group only 3 isolates (4%) were multidrug resistant. In the UP group, 18 isolates (13%) were multidrug resistant, but only 9 had MIC patterns that were similar to that of the multidrug resistant *E. coli*. The others had low-level chloramphenicol (32 to 64 mg/liter) and tetracycline (16 mg/liter) resistance, together with a borderline nalidixic acid MIC (16 to 32 mg/liter).

DISCUSSION

We found that *E. coli* was the main carrier of the resistance combinations that are typically transferable in the fecal flora of all three human populations in this study. The lack of resistance in other enterobacteria was particularly noticeable in the samples from the HP. The high levels of resistance reported in fecal gram-negative flora in HP (16, 19, 27) are thus at least in Finland specific for the *E. coli* part of the flora. It should be noted that the high levels were maintained even in the absence of direct antimicrobial selection. Similar species differences have previously been reported in detail only by Platt et al. (30), in fecal isolates from hospital patients.

Patterns of multidrug resistance, with combinations of ampicillin, sulfamethoxazole, trimethoprim, tetracycline, streptomycin, and chloramphenicol, were seen in one-fifth of the HP and UP *E. coli* isolates, but they were nearly completely missing from other enterobacteria. Approximately half of all sulfamethoxazole resistance is mediated by the *sulI* gene (32), which has so far only been found as an integrated part of the class 1 integron structure. This is a recombinatorial hot spot for transferable resistance, often found on transposons (5). A 13 to 16% sulfamethoxazole resistance frequency in *E. coli* would indicate a presence of integrons in about 7% of isolates. In contrast, although other enterobacteria from the UP were more resistant than those from the HP, sulfamethoxazole resistance remained at 5%, indicating that integrons were rare. In the clinical *Klebsiella* and *Enterobacter* isolates on the other hand, trimethoprim-sulfamethoxazole resistance was much higher (11%), suggesting a fundamental difference between the way resistance is acquired in clinical and commensal isolates of species other than *E. coli*. Enterobacteria other than *E. coli* are in fact often perceived as very resistant, as a result of the spread of multidrug-resistant epidemic clones through hospitals (24, 34). In reports of resistance levels in clinical isolates, there are generally no large differences in resistance between *E. coli* and other enterobacteria (e.g., see reference 20). Either pathogenic strains of other enterobacteria are better at acquiring transferable resistance than their commensal counterparts or the laboratory data are somehow biased. One source of bias might be that samples yielding bacteria are commonly obtained from patients with treatment failures.

Since the isolates in this study were selected in the same way as in our previous work on resistance in bacteria from food (26, 29), it is possible to compare the data directly. (We found practically no *E. coli* in the food, therefore it remains unknown if resistance in strains from nature would be as common in the absence of antimicrobial selection as it is in the HP.) The resistance levels found in this study in other *Enterobacteriaceae* from the feces of the HP were in fact as low as those found on vegetables and in hamburger meat. There is thus no major input of resistance from these foods in Finland, but occasional transfer of resistance genes might still occur. The low levels of resistance in feces indicate that at least in the absence of antimicrobial selection, there is no enrichment of such resistance in *Enterobacter*, *Klebsiella*, and *Citrobacter* in fecal flora. This is no reason for complacency, however; under the right circumstances, even minority strains can be dangerous.

In the hospital environment, antimicrobial use plays an essential role in the emergence of resistant bacterial strains and, subsequently, in producing a selection pressure causing the

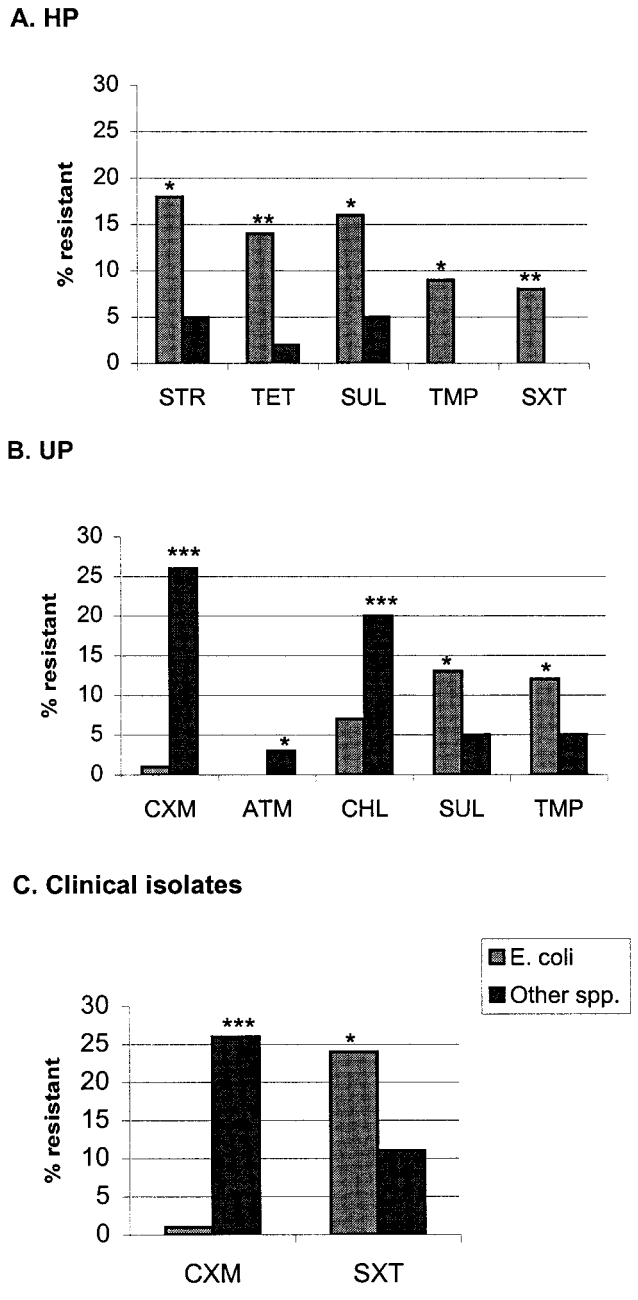

FIG. 1. Statistically significant differences in resistance between *E. coli* and other *Enterobacteriaceae*. Abbreviations are explained in footnote *a* to Table 3. *, $P < 0.05$; **, $P < 0.005$; *** $P < 0.0005$.

spread of resistant clones. The effects of antibiotic use were seen in both of the study hospitals. In the university hospital, species other than *E. coli* had high levels of cefuroxime resistance, probably as a consequence of the extended use of β-lactams. These species generally have inducible class C β-lactamases, which frequently mutate to a derepressed state, conveying resistance to, e.g., cefuroxime (18). In this hospital environment, mutant strains would have a selective advantage. From our data, however, it is not possible to determine which factor is the most important: spread, caused by selection pressure, or de novo emergence of mutant strains. In the long-term hospital, a prolonged use of trimethoprim by 15% of the patients was probably the cause of the unusually high trimethoprim resistance level (40%) in *E. coli*. The trimethoprim use also caused a rise in multidrug resistance by the coselection of other resistance traits, thus causing the difference between the HP and LTP populations.

Besides antimicrobial use, other factors are evidently important for patient colonization in the hospital. A person's fecal flora is not an isolated entity but is part of the immediate environment, and all factors affecting the total bacterial flora in this environment are gradually also affecting the individual flora. This was clearly exemplified in the present study. Although the university hospital used over 60 times more systemic antimicrobials than the long-term hospital, resistance was higher at the latter. Nevertheless, the impact of the magnitude of antimicrobial therapy as compared to other factors cannot be assessed here, since there were major differences between the two inpatient groups: the UP had a shorter hospital stay (mean, 5 days), were in general younger, and had acute diseases, while the LTP (mean hospital stay, 22 months) were geriatric and had chronic diseases. Patient-to-patient transmission via staff hands could have been one major factor contributing to the spread of resistant strains on the geriatric wards. The finding of the multidrug-resistant *E. coli* clone which had spread through five rooms at one ward at the long-term hospital supports such an assumption.

The increase in resistance in enterobacteria in the fecal flora of hospital patients has been described by several authors (2, 11, 12, 35). Yet in those previous studies, the nature of the changes in the flora remained unclear, since isolates were not classified further than the level coliforms or aerobic gram-negative bacilli. The main reason for the increase in resistance could have been a shift in species distribution, towards species naturally more resistant; a decrease of the proportion of *E. coli* has in fact been observed in hospital patient flora (9). This study shows that resistance increased very markedly in the *E. coli* population; consequently any species shift would be only partly responsible.

In conclusion, *E. coli* was the main carrier of resistance in fecal flora. The apparent absence of transferable multidrug resistance in other *Enterobacteriaceae* species in fecal flora is intriguing, and the differences between commensal and resistant pathogenic strains should be investigated on a population level. Resistance increased as a consequence of antimicrobial use, but conditions permitting an efficient spread may have been more important in sustaining high resistance levels.

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