ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1985, p. 107–113
0066-4804/85/010107-07$02.00/0

Vol. 27, No. 1

Antibiotic Resistance and Small R Plasmids Among *Escherichia coli* Isolates from Outpatient Urinary Tract Infections in Northern Norway

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Received 14 May 1984/Accepted 18 October 1984

***Escherichia coli* strains from outpatient urinary tract infections in northern Norway over a period of 1 year were examined for resistance to nine commonly used antibiotics. Strains collected during 4.5 months were examined for R plasmid content by using conjugation and in vitro transformation. Of the *E. coli* strains, 42% were resistant to one or more antibiotics. Resistance was highest to sulfonamide (20.8% of all strains), nitrofurantoin (14.5%), and tetracycline (10.1%), whereas less than 6% of the strains were resistant to ampicillin, carbenicillin, cephalothin, nalidixic acid, or trimethoprim-sulfamethoxazole. No strain was resistant to gentamicin. Tetracycline resistance was more common in men than in women. Resistance to cephalothin, nalidixic acid, and sulfonamide was higher in strains from older people. Resistance to sulfonamide was more frequent in the urban community. These was no seasonal variation in antibiotic resistance, although the incidence of urinary tract infection varied with seasons. Plasmid-determined resistance to ampicillin, streptomycin, sulfonamide, and tetracycline was found. About 18% of the resistant strains from the urban municipality carried R plasmids, most of which were small plasmids mediating resistance to sulfonamide and streptomycin. The overall frequency of resistance in strains collected from rural areas was similar to the urban frequency, but in the rural strains, R plasmids were found in only 5% of the resistant strains.**

It has been shown that antibiotic therapy can select for antibiotic-resistant strains in the fecal flora (7, 15, 19), and that R-plasmid-mediated antibiotic resistance can spread in a population subjected to heavy antibiotic therapy (25) and in hospitalized patients (33). In the enterobacteria isolated from hospitalized patients, multiple drug resistance due to transferable, extrachromosomal circular DNA has been shown to be a common character (18, 33, 35).

The incidence of resistant strains in general practice and the efficiency with which resistant strains are spread in such a population are less well known. Some reports (18, 31, 33, 34) have dealt with the incidence of R-plasmid-mediated drug resistance in enterobacteria isolated from patients in general practice, but some of these reports include hospital patients as well (18, 31, 34). The presence of R plasmids was generally inferred in these investigations from transmittance of resistance by conjugation. The frequencies of R plasmids varied in these investigations, and none dealt systematically with nonconjugative R plasmids.

The present investigation is a systematic study of antibiotic-resistant strains causing urinary tract infections (UTI) from a large sample of the general population in northern Norway collected over 1 year. The occurrence of resistant strains in different age and sex groups, in different geographical areas, and at different times of the year was determined. In a subset of the resistant strains, occurrence of both conjugative and nonconjugative R plasmids was determined by using an efficient procedure for extraction of plasmids from clinical isolates and a method of transforming $CaCl_2$-treated cells with DNA directly from an agarose gel without prior elution.

MATERIALS AND METHODS

Strains. *Escherichia coli* K-12(F^+), which was obtained from G. Bertani and into which plasmids pBR322 (37) obtained from the Plasmid Reference Center and R388 (8) obtained from C. Linder were transformed, was used to obtain plasmid size standards. *E. coli* K-12 K43 (F^- *cys his met* Str *trp*) was obtained from G. Bertani. A Nal^r derivative of K43 was isolated and used as a recipient in conjugation. As a transformation recipient, *E. coli* K-12 SK1592, $aT1^r$-derivative of SK1590 (23) (*endA gal hsdR4 hsdM*$^+$ *sbcB15 thi*), was obtained from S. Kushner.

On our request, all practicing physicians (except one in Harstad) in the six participating municipalities shown in Fig. 1 collected and sent urine samples from all patients with symptoms of UTI (frequent, painful urination with or without fever) from 1 June 1979 through 30 May 1980. Samples for R factor determination were collected from 21 May to 30 June and 1 September to 30 November 1979. These months were chosen to avoid the vacation period so that mostly permanent residents were included in the study. The age, sex, domicile, date of collection of urine sample, bacterial species, and resistance patterns of the bacteria were recorded for statistical analysis. The sex of the patient was registered in 1,225 cases (881 patients with *E. coli*), and the age was registered in 1,212 cases (872 patients with *E. coli*).

The urine samples were inoculated by the participating physicians on transport agar (Uricult/Urotube) (27) and incubated overnight at 37°C before being sent to the Microbiological Laboratory, University Hospital, Tromsø. Samples with more than 100,000 bacteria per ml, indicating significant bacteriuria (21), were defined as representing a UTI and were included in the study. *E. coli* was identified by

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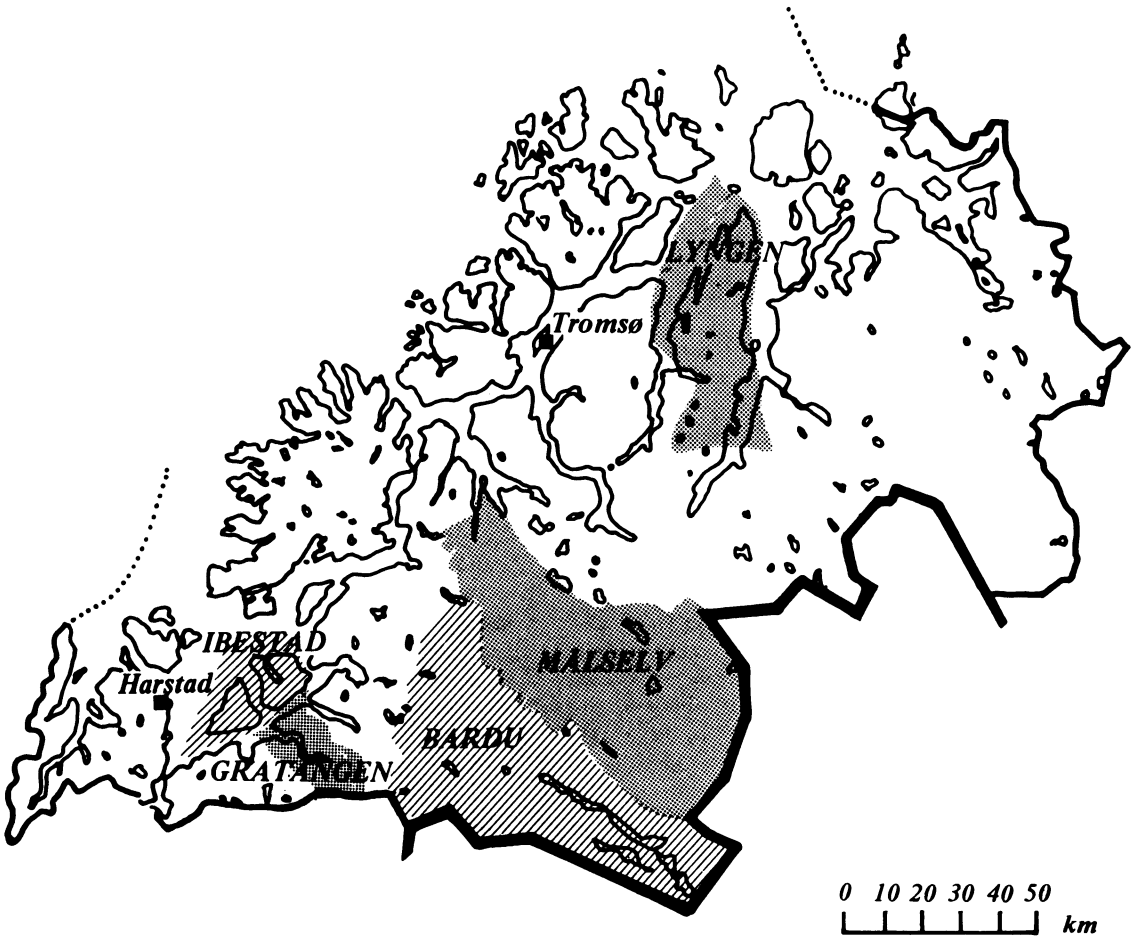

FIG. 1. Map of Troms County, northern Norway. Municipalities participating in the study were Lyngen, Ibestad, and Gratangen (coastal), Målselv and Bardu (inland), and Harstad (city, coastal).

the three-tube test system (24). Bacteria were stored by lyophilization.

Media and reagents. Blood agar, bromothymol blue lactose agar, chocolate agar, and brain heart infusion broth used for propagation and analysis of clinical strains were prepared essentially as described by the manufacturer (Oxoid Ltd.) as modified by the Statens Institutt for Folkehelse, Oslo, Norway. Glucose broth contained glucose (10 g), veal infusion broth (Difco Laboratories) (25 g), $Na_2HPO_4 \cdot 2H_2O$ (2 g), and distilled water to 1 liter, and TE buffer contained 50 mM Tris-hydrochloride (pH 7.4) and 1 mM Na_2EDTA. Agarose was from Bio-Rad Laboratories (catalog no. 162-0100). Paper disks used for determination of antibiotic resistance (9) were obtained from AB Biodisk, and the tests were carried out according to the recommendations of the manufacturer, using PDM agar (AB Biodisk). Resistance determined by the paper disk method in this study corresponds to the following MICs (in micrograms per milliliter): ampicillin, 8; carbenicillin, 16; cephalothin, 16; nalidixic acid, 32; nitrofurantoin, 32; sulfonamide, 64; tetracycline, 3; trimethoprim, 16. When needed for selection of resistant strains, antibiotics were added to the media as follows (in micrograms per milliliter): ampicillin, 50; nalidixic acid, 50; nitrofurantoin, 30; streptomycin, 20; sulfamethoxazole, 250; tetracycline, 25; trimethoprim, 30. Resistance in this communication refers to the percent resistant strains among those strains within a specified group (age of patient, geographical area, etc.) which were tested for the antibiotic in question (all strains were not tested with all antibiotics).

Isolation of plasmids. Cells were grown overnight in 30 ml of brain heart infusion broth, harvested, and lysed essentially as described by Guerry et al. (16), except that final lysis was performed by adding 1 volume of 2% (wt/vol) Triton X-100–50 mM Tris-hydrochloride (pH 8.0)–10 mM Na_2H_2EDTA. The sample was incubated on ice for 30 min and then centrifuged for 15 min at 12,800 × *g*. The viscous chromosomal pellet was removed with a Pasteur pipette, and the supernatant was extracted at room temperature with 1 volume of a 50:50 mixture of distilled water-saturated phenol and chloroform (20) for 5 min by gentle shaking. The phases were separated by a 3 min centrifugation at 12,800 × *g*. Extraction was repeated until the aqueous phase was clear. The number of extractions required for the different isolates varied but did not exceed four.

DNA was precipitated with cold 96% ethanol and the precipitate was collected by centrifugation (5 min, 12,800 × *g*), dried in a vacuum, and dissolved in 40 μl of TE buffer. Several other rapid extraction procedures (5, 16, 17, 28) failed to give reproducible results with the clinical strains. The method described above worked well with all gram-negative strains and species examined so far and gave reproducible recoveries of plasmids in the range from 1 to 93 kilobase pairs (kbp).

TABLE 1. Geographical distribution of patients with UTI

Geographical area	Municipality	Population on 31 December 1979[a]	No. of participating physicians	No. of patients	No. of patients with *E. coli*
City	Harstad	21,605	10	701	511
Rural					
Coastal	Lyngen	3,881	3	250	163
	Ibestad	2,705	2	107	87
	Gratangen	1,712	2	100	70
Inland	Målselv	7,668	4	153	116
	Bardu	4,043	3	122	97

[a] See reference 1.

Horizontal agarose slab gel electrophoresis was performed by the method of Carlson and Nicolaisen (4). Sizes of plasmids were estimated from plots of the logarithm of migration distance versus the logarithm of plasmid size (28) with F, R388, and pBR322 plasmids as references.

Transfer of resistance determinants. (i) Transformation. DNA for transformation was either the ethanol precipitated cellular extract (see above) or material that was cut out from agarose gels during 340-nm UV illumination by using a scalpel in which blades were changed between each band. Each gel piece was placed in a 15-ml tube, further cut into smaller pieces, and used as such in transformation. Cutting the agarose containing the DNA band into three or four pieces seemed to result in somewhat higher transformation efficiency than using either intact or lacerated gel pieces.

Transformation was carried out essentially as described by Cohen et al. (6), using *E. coli* SK1592 grown to 5×10^8 cells per ml in brain heart infusion broth as a recipient. For transformation, 0.2 ml of $CaCl_2$-treated cells was mixed with either 20 μl of cellular extract or the agarose pieces containing a plasmid band obtained upon electrophoresis of 20 μl of cellular extract. After alternative cold and warm incubations in $CaCl_2$, broth was added, and cells were allowed to grow for 1 h at 37°C before being plated out on selective media (6). With plasmid DNA from 15 ml of an overnight brain heart infusion culture per 3×10^8 $CaCl_2$-treated cells, pBR322 (4.4 kbp) cellular extract or agarose-contained covalently closed circular (ccc) DNA transformed 5×10^{-4} of the cells, whereas R388 (33 kbp) cellular extract transformed 10^{-5} of the cells and R388 agarose-contained ccc DNA transformed 10^{-6} of the cells. Our detection limit corresponded to a transformation frequency of about 10^{-7} estimated in this manner.

Cotransformation of resistance determinants was determined by testing all transformants against all antibiotics to which the donor strain was resistant. Extracts were then obtained from all transformants as described above and subjected to gel electrophoresis. Transformation was scored as positive only when resistant transformants were shown to contain a plasmid(s) of a size similar to that found in the original isolate.

(ii) Conjugation. Conjugation was performed as described by Miller (29), using 2 h for conjugation of clinical strains (donors) to *E. coli* K43 Nal^r (recipient) in brain heart infusion broth at 37°C and selecting transconjugants on minimal agar plates (29) supplemented with amino acids and antibiotics as needed. Transconjugants were tested for multiple resistance and plasmid content as described above for transformants.

Statistical methods. Tests of significance in two-way tables were carried out by means of chi-square tests (2). Occasionally, columns or rows of tables were joined together during testing to prevent numbers in single cells from being too small. Three-way tables were analyzed by means of log-linear models (10).

RESULTS

Description of strain collection. The geographical (Fig. 1) distribution of the population, participating physicians, and patients with UTI are shown in Table 1. The participating areas were chosen to allow a comparison between coastal (Lyngen, Ibestad, and Gratangen) and inland (Målselv and Bardu) municipalities with about the same total population and between these five rural municipalities and a city with about the same total population as that of the rural areas combined. The numbers of residents per physician were similar in all areas. Except for *E. coli*, too few strains were collected to allow more detailed analysis.

Table 2 shows the occurrence of resistance to different antibiotics for the *E. coli* strains; 42% of the strains were resistant to one or more antibiotics. The fraction of antibiotic-resistant strains exceeded 10% only for nitrofurantoin, sulfonamide, and tetracycline.

Antibiotic resistance of *E. coli* strains as a function of sex and age. The percentages of resistance to the various antibiotics in the different age and sex groups are shown in Table 3. The basis for Table 3 was the total number of samples where the respective antibiotics were tested and where the sex and age of the patients were known.

Significantly more tetracycline-resistant strains were isolated from men than from women ($P = 0.03$). Resistance to several antibiotics increased with age. This increase was significant for cephalothin ($P = 0.01$), nalidixic acid ($P = 0.002$), and sulfonamide ($P = 0.002$).

Geographical and seasonal variations of resistance in *E. coli*. Since the age distribution of the population varied among the geographical districts (1) and resistance to some antibiotics varied with age (Table 3), geographical variation in resistance was analyzed separately for different age groups and corrected for these differences by using three-way tables (10) where the factors were age, geographical area, and degree of resistance. When rural municipalities at the coast were compared with rural municipalities inland, no systematic or significant differences in antibiotic resistance were found. Comparison between urban (Harstad) and rural municipalities on the coast or inland revealed one significant difference. In strains from patients younger and older than 50 years, respectively, the sulfonamide resistances were: rural coast, 14 and 23%; rural inland, 12 and 19%; Harstad,

TABLE 2. Resistance of *E. coli* to various antibiotics

Antibiotic	No. of strains tested	Resistant strains (% of total ± SE)
Ampicillin	1,044	5.7 ± 0.7
Carbenicillin	583	5.1 ± 0.9
Cephalotin	1,043	3.6 ± 0.6
Gentamicin	591	0.0
Nalidixic acid	1,043	2.4 ± 0.5
Nitrofurantoin	1,042	14.5 ± 1.1
Sulfonamide	1,044	20.8 ± 1.3
Tetracycline	584	10.1 ± 1.2
Trimethoprim-sulfamethoxazole	1,044	1.3 ± 0.4

TABLE 3. Antibiotic resistance of *E. coli* by age and sex of patients[a]

Antibiotic	Age of patient (yr)	Total no. of strains tested		% of strains resistant	
		Men	Women	Men	Women
Ampicillin	0–20	6	193	16.7	3.6
	21–50	10	258	0.0	6.6
	>50	33	372	9.1	4.8
Carbenicillin	0–20	1	67	0.0	3.0
	21–50	7	98	0.0	8.2
	>50	17	234	17.6	4.8
Cephalothin	0–20	6	193	0.0	1.0
	21–50	10	257	10.0	2.3
	>50	33	372	9.1	5.1
Nalidixic acid	0–20	6	193	0.0	0.5
	21–50	10	258	0.0	1.2
	>50	33	371	3.0	4.6
Nitrofurantoin	0–20	6	192	16.7	10.4
	21–50	10	257	10.0	12.1
	>50	33	372	12.1	14.2
Sulfonamide	0–20	6	193	0.0	13.0
	21–50	10	258	20.0	21.7
	>50	33	372	27.3	24.2
Tetracycline	0–20	1	57	0.0	5.3
	21–50	7	96	28.6	10.4
	>50	17	239	29.4	11.7
Trimethoprim-sulfamethoxazole	0–20	6	193	0.0	0.5
	21–50	10	258	0.0	1.6
	>50	33	372	3.0	1.6

[a] Sex was not registered for 161 (carbenicillin), 167 (tetracycline), and 172 (all other antibiotics) patients.

22 and 29%. The higher resistance in Harstad was significant ($P = 0.05$). Resistance against antibiotics other than sulfonamide did not vary significantly between urban and rural regions.

The seasonal variations in UTI incidence and antibiotic resistance were compared (Table 4). Only resistance to nitrofurantoin showed a significant seasonal variation; this variation, however, was the opposite of the variation in UTI incidence. Resistance to other antibiotics did not vary significantly throughout the year. As an example, the resistance to sulfonamide is included in Table 4. Thus, increasing incidence of UTI was not accompanied by increasing incidence of antibiotic resistance.

Plasmid-mediated resistance. During 4.5 months of this investigation we collected 403 *E. coli* strains (215 from the urban area, 188 from rural areas), where the occurrence of R plasmids was investigated. The incidence of antibiotic resistance was similar to that in the complete strain collection (Table 2); 103 resistant strains were urban, and 77 were rural. All resistant strains were tested also for resistance to streptomycin since it is well known that streptomycin resistance often occurs in conjunction with resistance to other antibiotics, especially sulfonamide (3). The antibiotic phenotypes of the resistant strains are shown in Table 5.

Plasmid frequency. Ten of the Fu^r and Su^r strains, all strains resistant to other antibiotics, and an additional twenty nonresistant strains were analyzed for plasmid content and transformable and conjugative antibiotic resistance.

Of the 20 nonresistant strains, 17 contained plasmids (85%), whereas 62 of the 69 tested resistant strains (90%) contained plasmids. The number of plasmid bands from each bacterial strain varied from one to six, with a mean of two.

R-plasmid frequency. Figure 2 shows representative results of gel electrophoresis of plasmid extracts from 10 strains resistant to sulfonamide in combination with other drugs. DNA from all plasmid bands designated by number in Fig. 2B was used for transformation, as were the unfractionated plasmid preparations. Isolates in lanes b, c, d, e, and k (Fig. 2) and DNA from those plasmid bands transformed the recipient to resistance to streptomycin and sulfonamide. The cellular extracts and all transforming bands from one isolate yielded transformants containing the same-size plasmid, suggesting that the different transforming gel bands from one isolate contained different forms of one plasmid. This was not unexpected since the small reference plasmid pBR322 regularly yielded three bands (Fig. 2B, bands 1, open circular and ccc forms; Fig. 2A, lanes a and l), but complicated efforts to estimate the true number of different plasmids in the clinical strains. The analysis of resistance phenotype and plasmid content of the transformants from strains shown in Fig. 2 revealed the presence of one small R plasmid in each strain, 6 or 9 kbp, mediating resistance to streptomycin and sulfonamide. The Su Sm plasmid in lane b or k was transferred also by conjugation from these strains.

The 12 strains resistant only to streptomycin and sulfonamide (Table 5) all carried transformable plasmids mediating Su Sm resistance, as did the Ap Sm Su, Ap Sm Su Tc, Fu Sm Su, and Sm Su Tp strains. The R plasmid in the Ap Sm Su Tc strain could also be transferred by conjugation from this strain. The remaining strains were analyzed in the same manner, and ampicillin- and tetracycline-resistant strains were found to carry transferable resistance plasmids.

The results for all R-plasmid-containing strains are summarized in Table 5. Altogether, 23 resistance plasmids were identified. Seven strains transferred resistance in conjugation experiments, but no plasmid could be visualized in the transconjugants. These presumed R plasmids are not included in the study. Some strains may have carried both chromosomal and plasmid-bound resistance determinants or a large non-self-transmissible plasmid(s) not detected by our methods in addition to the R plasmid we documented. None of the monoresistant sulfonamide strains tested carried plasmid-mediated resistance, and no resistance to nalidixic acid or nitrofurantoin was plasmid mediated.

Sizes of R plasmids. Most plasmids were small (<10 kbp), mediating resistance to ampicillin (~7 kbp), tetracycline and ampicillin (~6 kbp), and sulfonamide and streptomycin (6 or 9 kbp). The different-size Su Sm plasmids occurred together in one strain. One plasmid coding for resistance to tetracycline was about 40 kbp.

TABLE 4. Seasonal variation of UTI and antibiotic resistance[a]

Month	Incidence of *E. coli* (no. of patients) per 1,000 inhabitants	Resistance to nitrofurantoin (% of isolates)	Resistance to sulfonamide (% of isolates)
Jan.–Apr.	8.6	11.2	19.3
May–Aug.	6.2	18.1	21.2
Sept.–Dec.	10.2	15.1	21.8

[a] Significance was analyzed by chi-square tests.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] Not significant.

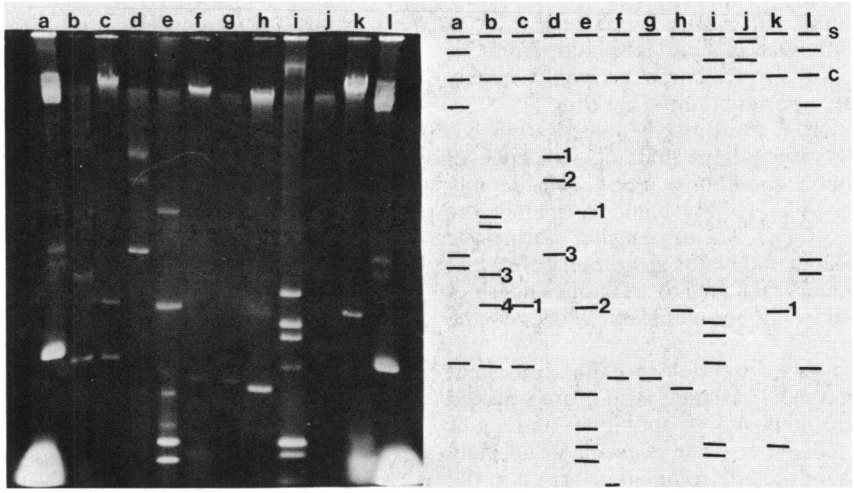

FIG. 2. (A) Electrophoresis of plasmids from strains resistant to sulfonamide in combination with other antibiotics. Lanes: a and l, *E. coli* K-12(F^+, R388, pBR322); b and c, *E. coli* Ap^r Sm^r Su^r; d, *E. coli* Sm^r Su^r Tp^r; e, *E. coli* Fu^r Sm^r Su^r; f, g, i, and k, *E. coli* Sm^r Su^r Tc^r; j and h, *E. coli* Su^r Tc^r. Electrophoresis was from top to bottom. Panel B shows a schematic representation of the bands in the gel in panel A. s, slot; c, cDNA. Numbers within panel B denote plasmid bands found to transform antibiotic resistance. The six bands in lanes a and l correspond to (from top to bottom): F factor, cDNA, R388, linear pBR322, open circular pBR322, and ccc pBR322.

Epidemiology of R plasmids. Table 6 shows the geographical distribution of the R factors. The urban municipality accounted for 53% of all collected strains, 57% of resistant strains, and 83% of R plasmids. This corresponds to 18% R-plasmid-mediated resistance in Harstad and 5% in the rural municipalities. The discrepancy between the frequencies of R plasmids in the urban and rural municipalities was statistically significant ($P < 0.01$).

DISCUSSION

The present study included all UTI isolates from general practitioners in five rural municipalities and most UTI isolates from one urban municipality. The overall frequency of antibiotic resistance was about 40%, somewhat higher than that reported in other studies from general practice (11, 30, 36). For *E. coli* isolates, resistance exceeded 10% only for nitrofurantoin, sulfonamide, and tetracycline (Table 2), which are the three most common generally used drugs in northern Norway (32). Resistance to cephalothin, nalidixic acid, and sulfonamide, which are used extensively to treat UTI (13, 38) and other serious infections (14), was significantly higher in older patients (Table 3). Only resistance to tetracycline showed a sexual bias. The sex ratios for the different diseases usually treated with tetracyclines (bronchitis, skin infections [including acne], and infections of the urogenital system [38]) in northern Norway are not known. Hence, the observed differences in resistance between the sexes cannot be explained at present. Since few strains were collected from men, the bias may be artificial. We observed very little resistance to tetracycline among young people (Table 3).

Resistance to ampicillin, carbenicillin, and nitrofurantoin showed no significant age or sex bias. Nitrofurantoin, like sulfonamide, is a first-line drug to treat UTI (32, 38; the same practice is followed today), but in contrast to sulfonamide (7) and tetracycline (7, 15, 19), it is quickly and completely

TABLE 5. Antibiotic resistance and resistance plasmids

Resistance phenotype of clinical strain[a]	No. of strains	No. of strains with resistance plasmids[b]	Resistance phenotype of transformants or transconjugants
Ap	2	2 T	Ap
Tc	7	1 C	Tc
Ap Fu Su Tc	1	1 T	Ap Tc
Ap Sm Su	2	1 T, 1 T + C	Sm Su
Ap Sm Su Tc	2	2 T	Sm Su
Fu Sm Su	1	1 T	Sm Su
Sm Su	12	12 T	Sm Su
Sm Su Tc	4	1 T + C	Sm Su
Sm Su Tp	1	1 T	Sm Su

[a] Ap, ampicillin; Fu, nitrofurantoin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; Tp, trimethoprim. Plasmid-mediated resistance to nitrofurantoin (18, 26) or sulfonamide (26, 30) alone is rarely, if ever, encountered. Since the probability (P) of observing plasmid-mediated resistance is given by the formula $P = 1 - (1-x)^y$ (2), where x is the fraction of plasmid-mediated resistance and y is the number of examined strains, analysis of 10 strains provides a 90% probability of detecting 20% R-factor-mediated resistance. No R factors were found in strains with the following resistance phenotypes: Fu (59 strains, 10 tested), Su (71 strains, 10 tested), Ap Fu Nx (1 strain), Ap Fu Sm (4 strains), Ap Su Tp (1 strain), Fu Nx Su Tc (2 strains), Fu Sm Su Tc (1 strain), Fu Su Tp (2 strains), Fu Tc (1 strain), Nx Su Tc (1 strain), Sm Tc (3 strains), or Su Tc (2 strains), totaling 148 strains, of which 18 were multiresistant.

[b] T, Transformable; C, conjugative or mobilized by a cryptic conjugative plasmid.

TABLE 6. Geographical distribution of R plasmids

Plasmid resistance phenotype	No. of strains[a]	
	Urban	Rural
Ap	2	0
Ap Tc	1	0
Sm Su	15	1 (Ibestad) 3 (Målselv)
Tc	1	0

[a] Not included are seven conjugative R factors which could not be visualized in transconjugants. Of these, six were urban, and one was rural (Lyngen).

absorbed in the small intestine (12) and may not exert a selective pressure in the bowel. In a previous study from this region (22), resistance to nitrofurantoin was only weakly correlated with previous consumption of the drug.

Analysis of material from the present investigation has showed (manuscript in preparation) that the incidence of UTI depends on climatic conditions, being considerably higher in coastal areas (wet, windy climate) than in the interior (drier, not so windy) and also higher during the severe conditions of autumn and winter in northern Norway. It is striking that the rate of resistance to antibiotics does not follow this pattern but is rather constant whatever the climate (Table 4).

The resistance patterns we observed were thus consistent with the assumption that most resistant strains are selected as a consequence of prior exposure to antibiotics and not of random spread among individuals. The resistant strains may have been picked up from the environment or arisen in the normal flora of the patients.

We concentrated our search on R plasmids that could be visualized by gel electrophoresis. By including also potentially non-self-transmissible plasmids able to transform a recipient to antibiotic resistance, we aimed at obtaining a more complete picture of R plasmid occurrence. Some conjugative resistance could not be verified as plasmid mediated by our criteria (perhaps because the plasmid involved was too large to be visualized by electrophoresis, or less likely because of conjugal transfer of chromosomal resistance or plasmid integration in the recipient); however, including or excluding those as R plasmids would not affect the main conclusions (Table 6). Large non-self-transmissible or strongly repressed plasmids might not have been detected by our screening since they would have transformed poorly and would not have conjugated. This leads to a possible underestimate of R plasmid frequency.

The frequency of R plasmids detected in urban samples was significantly higher than in rural samples. Two factors may contribute to this difference: (i) the population density in urban as compared with the rural areas and (ii) the location of the only hospital in these areas in Harstad. The higher frequency of small R plasmids mediating resistance to sulfonamide and streptomycin in Harstad (Table 6) may account for the distinct geographical distribution of sulfonamide resistance, which was higher in Harstad than elsewhere.

Although the R plasmid frequencies generally were low and varied between the areas, overall frequencies of antibiotic resistance were high and remarkably constant. It would appear that transferable R factors are not a major reason for the high level of antibiotic resistance in general practices in this region.

ACKNOWLEDGMENTS

We thank the general practitioners who participated, Aud Øvervatn and the staff at the Microbiology Laboratory, University Hospital, for technical assistance, and Lars Korsnes and Knut Altmann for helpful discussions.

This investigation was partly supported by a grant from the Norwegian Research Council for Science and the Humanities (to K.C.).

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