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Drug Resistance and R Plasmids of *Escherichia coli* in Patients and Healthy Individuals in Korea

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=국문초록=

韓國의 患者및 健康人에서 分離한 E.coli의 藥劑耐性 및 R Plasmids

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薛 盛 用

抗菌劑로 治療한 患者와 抗菌劑를 投與받지 않은 醫師 및 學生群으로부터 總 665株의 E. coli를 分離하여 藥劑耐性 및 R Plasmid의 分布를 調査했다.

分離株의 約 25—41%가 chloramphenicol, tetracycline, streptomycin sulfisomidine 및 ampicillin(AP)에 耐性이었고 9.5%가 kanamycin에 耐性이었으며 nalidixic acid에 耐性인 菌株는 극히 드물었다.

耐性株는 醫師 및 學生群에서보다 患者群에서 越等히 높았다.

4 또는 그 以上의 藥劑에 耐性인 多劑耐性株는 醫師 및 學生群보다 患者群 分離株에서 越等히 많은 반면 3劑 또는 그 以下의 藥劑에 耐性인 菌株의 頻度는 3群 分離株 사이에 큰 差異가 없었다.

4劑 또는 그 以上의 藥劑에 耐性인 菌株를 保有하고 있는 사람은 醫師 및 學生群보다 患者群에서 그 頻度가 더 높았다.

거의 大部分의 藥劑 耐性株는 모든 耐性 特히 AP耐性을 接合에 의하여 藥劑感受性인 E.coli에 傳達했다.

多劑耐性을 가지는 菌株는 그 根源에 關係없이 더 높은 頻度로 藥劑耐性을 傳達하는 傾向이 있었다.

INTRODUCTION

The widespread use of antimicrobial drugs is known as a selective force in the increasing incidence of pathogenic as well as non-pathogenic drug-resistant organisms (2, 21). Since the report of Akiba et al. (1), the drug resistance mediated by R plasmids has been considered to be a major cause in the spread of mutliply drug resistance among members of *Enterobacteriaceae* and many other organisms (6, 7, 14, 19, 22, 27). The multiply resistant and R plasmid-carrying organisms are frequently found among clinical isolates from stools (5, 18, 20, 23), and

are concurrently accompanied by the emergence of R' plasmid-carrying intestinal organisms which are not clinically involved (13, 15, 17, 25).

Non-pathogenic R plasmid-carrying intestinal flora, such as *Escherichia coli*, may transfer their resistance to drug-sensitive pathogenic organisms within the bowel of infected patients. In other cases, drug-resistant enteric organisms which are harmless in intestine may infect urinary tracts or cause other parenteral infections. Under the circumstances, it is desirable to study the present status on the distribution of drug-resistant and R plasmid-carrying *E. coli*, a representative resident in intestine, in patients and general populations.

Owing to the frequent use of antimicrobial drugs without prescription, the drug-resistant and R plasmid-carrying enteric organisms are supposed to be prevalent in Korea. The purpose of this report is to investigate the distribution of drug-resistant and R plasmid-carrying *E. coli* among patients, doctors and students, and to evaluate the result in comparison with the findings which were obtained in other parts of the world.

MATERIALS AND METHODS

Sampling

Subjects were divided into three groups. The first group consisted of 40 patients who were hospitalized with urinary tract infections for more than three weeks in the Kyungpook University Hospital. The male patients outnumbered the female with the ratio of 4 : 1; ages ranged from 20 to 50, and a majority were between 25 and 40. The major causative agents of the infections were *E. coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *and Staphylococcus aureus*. All of them were given various kinds of antimicrobial drugs during the hospitalization, and most of them had taken drugs before visiting the hospital. The drugs used frequently were penicillin, chloramphenicol (CM), tetracycline (TC), vibramycin, ampicillin (AP), gentamicin, and carbenicillin; however, it was difficult to illustrate individually the doses, duration of prescription, and kind of drugs used. The other two groups consisted of 40 doctors in the University Hospital and 53 sophomore medical and nursing students who have no history of taking antimicrobial drugs in the previous two months. The defecated stool was collected in a container and subjected for the study.

Isolation of E. coli

Stools were streaked on eosin methylene blue agar plates. After overnight incubation, five colonies showing the typical appearance of *E. coli* were selected at random from the culture of each specimen, purified on MacConkey agar plates, and then confirmed biochemically to be *E. coli*, but serotyping was not attempted. The number of strains collected were 200 each from patients and doctors, and 265 from students.

Drugs

Solutions of CM, TC, streptomycin (SM), sulfisomidine (SA), AP, kanamycin (KM), and nalidixic acid (NA) were freshly prepared as previously described (6).

Determination of drug resistance

Plate dilution method was used throughout the study. Briefly, brain heart infusion (BHI) agar plates containing each drug (12.5μg of CM, TC, AP, KM, and NA per ml, and 25μg of SM per ml) were prepared. SA was incorporated to be 50μg/ml in Mueller-Hinton (MH) agar plates. Overnight broth cultures of test strains diluted 100 times with buffered saline were inoculated with the multiple inoculator (26), and the strains which grew on drug-contained media after 24h of incubation were considered resistant. If necessary, the minimum inhibitory concentration (MIC) was determined (6).

Transfer of drug resistance

E. coli ML 1410 NA^r kindly supplied to us by S. Mitsuhashi, Gunma University School of Medicine,

Maebashi, Japan, was used as the recipient. Single colonies of donor and recipient strains were cultured separately in BHI broth for 18h at 37°C. One drop of each culture was inoculated in 5 ml of BHI broth and incubated for 3.5h with gentle shaking.

One milliliter of donor and 4 ml of recipient cultures were mixed and conjugated for 18h at 37°C, and then spread on BHI or MH agar plates containing 50μg of NA per ml and either one of the selecting drugs (25μg of CM, TC, AP, and KM per ml, 50μg of SM per ml, and 100μg of SA per ml). After incubation for 24h at 37°C, five colonies were picked at random from each culture, purified on MacConkey agar, confirmed to be *E. coli* by biochemical reactions, and tested for the patterns and levels of drug resistance transferred. No growth of donor strains on media containing 50μg of NA per ml, and of the recipient strain on media containing the given doses of selecting drugs was confirmed.

RESULTS

Of 665 strains isolated, 25.3 to 41.4% of them were resistant to CM, TC, SM, SA, and/or AP, and 9.5% were resistant to KM (Table 1).

NA-resistant strains were only occasionally encountered. Strains resistant to the drugs except NA were found in significantly higher frequencies by Chi square (x^2) test among isolates from patients than those from doctors and students ($P<0.005$), but no difference was noted between those from doctors and students. There were some variations in the level of drug resistance, but MICs of drugs to the majority of resistant strains were 400μg of CM, AP, and KM per ml, 200—400μg of TC and SM per ml, and 1600μg of SA per ml. The distribution of multiple drug resistance to CM, TC, SM, SA, AP, and KM is listed in Table 2. The prevalence of strains resistant to four or more drugs was significantly higher among isolates from patients than those from doctors and students ($P<0.05$—0.005), while there was no difference between those from doctors and students. Strains resistant to three or less drugs were distributed almost evenly among the

Table 1. Resistance to antimicrobial drugs of *E. coli* of human origin

Drug	Resistant strains isolated from:			
	Patients (200)[a]	Doctors (200)	Students (265)	Total (665)
CM	135(67.5)[b]	13(6.5)	28(10.6)	176(26.5)
TC	152(76.0)	56(28.0)	67(25.3)	275(41.4)
SM	115(57.5)	27(13.5)	49(18.5)	191(28.7)
SA	141(70.5)	37(18.5)	50(18.9)	228(34.3)
AP	134(67.0)	16(8.0)	18(6.8)	168(25.3)
KM	54(27.0)	5(2.5)	4(1.5)	63(9.5)
NA	5(2.5)	0	3(1.1)	8(1.2)

a : No. of strains tested.
b : Percent in parentheses.

Table 2. Multiple drug resistance of *E. coli* of human origin

Resistant to	No. of strains isolated from:			
	Patients	Doctors	Students	Total
6 drugs	35(17.5)[a]	3(1.5)	4(1.5)	42(6.3)
5	70(35.0)	7(3.5)	10(3.8)	87(13.1)
4	29(14.5)	6(3.0)	14(5.3)	49(7.4)
3	13(6.5)	11(5.5)	13(4.9)	37(5.6)
2	1(0.5)	12(6.0)	14(5.3)	27(4.1)
1	12(6.0)	20(10.0)	19(7.2)	51(7.7)
0	40(20.0)	141(70.5)	191(72.1)	372(55.9)
Total	200	200	265	665

a : Percent of resistant strains in parentheses.

Table 3. Carriage of drug-resistant *E. coli* in stool

Strain resistant to	No. of persons carrying one or more resistant *E. coli*[a] among:			
	Patients (40)[b]	Doctors (40)	Students (53)	Total (133)
6 drugs	12	3	1	16
5	25	4	5	34
4	15	3	6	24
3	6	9	5	20
2	1	8	8	17
1	6	11	8	25
0	4	20	28	52

a : Five strains were selected at random cultures of each person.
b : No. of persons tested in parentheses.

Table 4. Transfer of drug resistance of *E. coli*[a] to drug-sensitive *E. coli*

Resistant to	Patient		Doctor		Student		Total	
	No. of strains	Resistance transferred	No. of strains	Resistance transferred	No. of strains	Resistance transferred	No. of strains	Resistance transferred
CM	130	62(47.7)[b]	13	5(38.5)	25	10(40.0)	168	77(45.8)
TC	147	60(40.8)	56	12(21.4)	64	25(39.1)	267	97(36.3)
SM	110	51(46.4)	27	8(29.6)	46	10(21.7)	183	69(37.7)
SA	137	57(41.6)	37	9(24.3)	47	10(21.3)	221	86(38.9)
AP	130	125(96.2)	16	14(87.5)	15	15(100.0)	161	154(95.7)
KM	53	39(73.6)	5	4(80.0)	1	1(100.0)	59	44(74.6)

a : Strains resistant to NA are excluded, b : Percent of strains in parentheses.

Table 5. Transfer of multiple drug resistance of *E. coli*

Resistant to	Patient		Doctor		Student		Total	
	No. of strains	Resistance transferred	No. of strains	Resistance transferred	No. of strains	Resistance transferred	No. of strains	Resistance transferred
6 drugs	34	32(94.1)	3	3(100.0)	1	1(100.0)	38	36(94.7)
5	67	61(91.0)	7	5(71.4)	10	10(100.0)	84	76(90.5)
4	29	23(79.3)	6	6(100.0)	14	10(71.4)	49	39(79.6)
3	12	11(91.7)	11	4(36.4)	13	6(46.2)	36	21(58.3)
2	1	0	12	0	14	4(28.6)	27	4(14.8)
1	12	4(33.3)	20	7(35.0)	19	9(47.4)	51	20(39.2)
Total	155	131(84.5)	59	25(42.4)	71	40(56.3)	285	196(68.8)

a : Percent of strains in parentheses.

three groups, except doubly resistant ones. Consequently, strains sensitive to the all drugs tested were found less often among patient isolates (20%) than those from the other two groups (more than 70%, respectively). When the carriage of resistant strains was compared (Table 3), the number of persons carrying one or more strains resistant to four or more drugs was significantly much more common among patients than doctors and students($P<0.05$—0.005).

Since NA was used for differentiating donors from the recipient in the resistance transfer experiments, NA-resistant isolates, all of which were multiply resistant to the other drugs, were excluded from the following experiments. Approximately 96% of strains resistant to AP and 75% of strains resistant to KM transferred conjugally their resistance to the recipient, and strains resistint to CM, TC, SM, and SA less frequently; but no significant difference in the transferability was noted among the three groups of strains (Table 4). There was a tendency of higher transferability of resistance among strains of greater multiplicity of resistance (Table 5).

In order to know the transferred resistance patterns, five colonies selected at random from each conjugated culture were tested, and it was found that the transferred resistance patterns were almost uniform among five colonies, in any cases the transferred resistance was the whole or parts of resistance of donors. Table 6 lists the original resistance patterns and transferred ones to the recipient. The most frequently transferred resistance of donor strains resistant to five and six drugs was the whole resistance and AP resistance. However, the tran-

Table 6. Original and transferred resistance patterns of multiply resistant *E. coli*[a]

Resistance pattern	No. of strains	Resistance pattern transferred	No. of strains
CM, TC, SM, SA, AP, KM	38	CM, TC, SM, SA, AP, KM	26
		CM, SM, SA, AP, KM	1
		TC, SM, SA, AP, KM	1
		AP	8
		NT[b]	2
CM, TC, SM, SA, AP	64	CM, TC, SM, SA, AP	19
		CM, TC, SM, AP	1
		TC, SM, SA, AP	4
		CM, TC, AP	2
		SA, AP	5
		AP	29
		NT	4
CM, TC, SM, SA, KM	3	NT	3
CM, TC, SA, AP, KM	17	CM, TC, SA, AP, KM	5
		CM, SA, AP, KM	10
		CM, AP, KM	1
		NT	1
CM, TC, SM, SA	17	CM, TC	6
		NT	11
CM, TC, SA, AP	11	CM, TC, AP	1
		AP	10
CM, TC, AP, KM	1	AP	1
CM, SM, SA, AP	6	CM, SM, SA, AP	2
		AP	4
TC, SM, SA, AP	14	TC, SM, AP	3
		SM, SA, AP	1
		AP	10

a : Strains resistant to four or more drugs are listed. b : Not transferred.

sfer of AP resistance was most frequently observed among strains resistant to four drugs.

DISCUSSION

The increase of drug-resistant organisms has been reported by many workers since the introduction of antimicrobial drugs (2, 18, 19), and our results showed the marked dominance of drug-rseistant *E. coli* in stools of patients treated with antimicrobial drugs. Datta (9) reported that 52% of people in their home in London carried drug-resistant *E. coli*, and the result is similar with ours (Table 3). However, the overall incidence of drug-resistant strains were low among persons who did not receive drugs recently. Doctors who have frequent chances to contact with antimicrobial drugs are suspected to be contaminated heavily with drug-resistant *E. coli* in their intestine; however, the result of our study that there was no difference in the prevalence of drug-resistant strains between doctors and students who have rare chances in contact with drugs suggested

that more contact without taking drugs does not increase the carriage of drug-resistant *E. coli*. This result is in agreement with the report of Datta who found almost no cross-infection of *E. coli* among patients in a hospital (9). The resistant strains to CM, TC, SM, SA, and AP comprised 6.5 to 28% of total strains isolated from doctors and students, and this result roughly parallels the reports on the incidence of drug-resistant *E. coli* among apparently healthy populations in other parts of the world (11, 15, 16, 18) with the exception of some reports on high incidence (3, 8).

As noted first in *Shigella* (1), most drug resistant *E. coli* from patients were multiply resistant to four or more drugs. The dominant multiplicity of drug resistance in *Shigella* and *E. coli* in 1960s was the resistance to four drugs of CM, TC, SM, and SA, and the resistance to AP has been recently added (12, 18, 29). The multiplicity of resistance in our isolates included quite a large number of strains resistant to five and six drugs including AP and KM. Though the low incidence of resistance to KM was noted in *Shigella*, *Salmonella*, and *E. coli* (4, 6, 8, 30), KM-resistant strains can be expected to increase gradually according to the wide use of KM (5, 10).

The multiple drug resistance suggests that the resistance can be mediated by R plasmids, and 36.3 to 95.7% of our drug-resistant isolates transferred the whole or parts of their resistance to drug-sensitive *E. coli*. No difference in the incidence of transferability among strains from patients, doctors, and students suggests that R plasmid-carrying *E. coli* are selected by the drugs used, and the emergence of transferable resistance does not necessarily lead to the rapid development of resistance to drugs in enteric bacteria (24). The transferability of resistance was more frequent among strains of higher multiplicity of resistance than strains of low multiplicity of resistance (9). In contrast to the report of Cooke (8) who found no coliform of marine origin which transferred the resistance to KM, we observed a very high transferability of KM resistance (19).

The dominance of R plasmid-carrying organisms would be a serious problem if they were to persist in the intestine for long period of time. Fortunately, it was found that the cessation of drug administration results in the gradual decrease of resistant organisms in the intestine (13). This observation may explain the finding that the incidence of drug-resistant and R plasmid-carrying *E. coli* in the feces of Koreans, who have numerous chances of taking these drugs but who did not use them just prior to the study, is not much higher than that observed in countries where antimicrobial drugs are controlled through prescription by physicians. Drug-resistant and R plasmid-carrying *E. coli* of patients are excreted with feces and may reach the sewage and be replaced by strains having normal susceptibility patterns. Many reports have documented the distribution of drug-resistant and R plasmid-carrying *E. coli* in sewage (8, 10, 28), and hospital may play a major role in its contamination with these strains.

SUMMARY

A total of 665 strains of *Escherichia coli* isolated in Korea from stools of patients who were treated with antimicrobial drugs, doctors, and students were tested for the drug resistance and distribution of R plasmids. Approximately 25 to 41% of isolates were resistant to chloramphenicol, tetracycline, streptomycin, sulfisomidine and ampicillin (AP), and 9.5% were resistant to kanamycin. Nalidixic acid-resistant strains were only rarely encountered. The prevalence of resistant strains was significantly higher among patients than doctors and students. Strains multiply resistant to four or more drugs were significantly more prevalent among patient isolates than those from doctors and students, while no difference on the incidence of strains resistant to three or less drugs was noted among isolates from the three groups. The persons carrying strains resistant to four or more drugs were more frequently found among patients than doctors and students. Quite large proportions of drug-resistant strains transferred their resistance

to drug-sensitive *E. coli*, with frequent transfer of whole resistance and AP resistance. Strains having higher multiplicity of resistance showed a tendency toward higher incidence of resistance transfer, irrespective of the origins of strains.

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