Drug Resistance of Enteric Bacteria

IX. Distribution of R Factors in Gram-negative Bacteria from Clinical Sources

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Many isolates belonging to the Enterobacteriaceae were collected in 1965 from the inpatients at geographically scattered hospitals in Japan. Among 2,650 Shigella strains examined, 58.4% were found to be drug-resistant; 95.0% of these resistant strains were multiply resistant. Among 434 resistant strains examined, 81% carried R factors that were transferable by cell-to-cell contact. Of 160 isolates of other enteric bacteria, drug-resistant strains included 84.2% of the Escherichia coli, 93.0% of the Klebsiella, and 90.0% of the Proteus cultures. Among these resistant strains, 70.3% of the E. coli, 66.7% of the Klebsiella, and 52.0% of the Proteus were multiply resistant. Of these resistant strains, 84.0% of the E. coli, 88.0% of the Klebsiella, and 50.0% of the Proteus strains carried R factors. These results indicate that R factors are widespread among gram-negative bacteria of clinical significance.

It was found in Japan that drug resistance was transferred between Shigella and Escherichia coli by mixed cultivation (1, 14). This transfer was not mediated by a filterable agent as are the phenomena of transduction and transformation, but required cell-to-cell contact, i.e., conjugation (8). The transmissible drug resistance could be lost spontaneously or eliminated by treatment with acridine dyes (11-13). Hence, these genetic determinants share many of the properties of bacterial episomes or plasmids and the term "R" (resistance) was proposed for this transmissible property (5).

It is known that the R factor is transferred to many gram-negative bacteria, including most genera of the Enterobacteriaceae (4). In the clinical fields, multiply resistant strains of gram-negative bacteria, including E. coli, Klebsiella, Proteus, and Pseudomonas, have become an increasingly serious problem.

This paper reports the distribution of R factors among the gram-negative bacteria isolated in 1965 from inpatients in Japan.

MATERIALS AND METHODS

The bacterial strains used were all from clinical sources and represent strains thought to be of clinical significance. Shigella strains were isolated by the members of the Research Committee on Shigella Infection (Chief, M. Ezaki). Other gram-negative bacteria, including E. coli, Proteus, Klebsiella, and Pseudomonas aeruginosa, were isolated from inpatients by the members of the Research Committee on the Infections Caused by Gram-negative Bacteria (Chief, S. Ishiyama). Some cultures were isolated by us from inpatients at the hospital, Faculty of Medicine, Gunma University.

Media. Brain Heart Infusion (BHI; Difco) was used as propagating media for R-factor transfer. Heart Infusion Agar (Difco) was used for the assay of drug resistance. AL-agar medium and Salmonella-Shigella agar medium were used for selection of recipients that had acquired the R factor. The AL-agar medium consisted of 1,000 ml of medium A (3), 20 g of lactose, 40 ml of 0.2% bromothymol blue, and 13 g of agar.

Drugs. Chloramphenicol (CM), tetracycline (TC), dihydrostreptomycin (SM), sulfonamide (SA), kanamycin (KM), fradiomycin (FM), aminobenzylpenicillin (ABPC), cephalothin, colistin, paromomycin, dextromycin, gentamicin, furadantin, furazolidone, and nalidixic acid (NA) were used as working standards for the assay of resistance (supplied by H. Umezawa, Department of Antiotics, National Institute of Health, Tokyo, Japan).

Drug resistance. Resistance of the organisms was determined by the method described previously (4) and was expressed as the maximal concentration allowing bacterial growth. Mueller Hinton Medium (Difco) was used for the determination of SA resistance.

Transfer of R factor. E. coli K-12 W3630 and E. coli O-26 were used as the recipients of the R factor from Shigella strains; 0.1 ml of an overnight BHI broth culture of each donor (Shigella) and recipient were mixed and inoculated in 1 ml of fresh BHI broth. After incubation for 18 hr at 37 C, 0.1 ml of the appropriately diluted culture was spread on AL-agar plates containing CM (25 µg/ml), TC (25 µg/ml), SM (12.5

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μg/ml, or SA (200 μg/ml). The mixed culture had a
viable count of about $2 \times 10^6$ organisms per milliliter.
After 2 days of incubation at 37 C, each of the colonies
which developed on the selective plates was picked,
and its drug resistance was determined after two suc-
cessive single-colony isolations.
Salmonella typhi S-57 was used as a recipient for the
R factor from the strains of E. coli, Klebsiella pneu-
moniae, and Proteus mirabilis. Salmonella-Shigella
agar containing CM (25 μg/ml), TC (25 μg/ml), or
SM (12.5 μg/ml) was used for selection. The mixed
cultivation of donor and recipient strains for the
transfer of R factor was the same as described above.
Ability to transfer the R factor was investigated by
mixed cultivation of E. coli W3630 (NA-resistant)
with those recipients which had acquired the R factor
from the tested strains. Heart Infusion Agar con-
taining NA (50 μg/ml) and CM (25 μg/ml), TC (25
μg/ml), or SM (12.5 μg/ml) was used as the selective
medium. AL-plates containing NA (25 μg/ml) and
SA (200 μg/ml) were used as a selective medium when
SA was the selective marker.

RESULTS
Among 2,560 Shigella strains examined, 58.4% were
found to be drug-resistant; 95.0% of the resistant
strains were multiply resistant. The de-
tails of cross-resistance will be described else-
where. The distribution of R factors among the
resistant shigellae was investigated by using 434
resistant strains; 81% of these were found to carry R factors transferable by mixed cultivation
(Table 1). Almost all of the multiply resistant
strains, i.e., resistant to TC.CM.SM.SA, CM.SM.SA,
or TC.SM.SA, were found to carry R factors. This survey of drug resistance in
Shigella isolates disclosed that resistance to the four different drugs, TC, CM, SM, and SA, or to
various combinations thereof, was encountered
most frequently. Resistance to other drugs was
very rare, being only 0.35% for ABPC resistance
and 0.24% for NA resistance (unpublished data).
The isolation frequency of R factors with reference
to resistance patterns is shown in Table 2. R factors that harbor the determinants for

| Table 1. Distribution of R factors among drug-
resistant Shigella strains |
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<td>Resistance pattern</td>
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<td>TC.CM.SM.SA</td>
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<td>CM.SM.SA</td>
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<td>TC.SM.SA</td>
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<td>TC.SA</td>
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<td>Total</td>
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TC.CM.SM.SA resistance were isolated most
frequently. Therefore, the high frequency of iso-
lation in Japan of Shigella strains resistant to four
drugs, i.e., TC, CM, SM, and SA, was accounted
for by the wide distribution of the R(TC.CM.
SM.SA) factor in such bacteria. The resistance of
Shigella strains also can be ascribed to the wide
distribution of R factors.

Among 160 isolates of E. coli, K. pneumoniae,
and P. mirabilis, 84.2, 93.0, and 90.0%, respec-
tively, were found to be drug-resistant, and most
were resistant to the four drugs, TC, CM, SM,
and SA. This survey disclosed that, among 69
tested strains of E. coli, K. pneumoniae, and P.
mirabilis, 84.0, 88.0, and 50.0%, respectively,
carried R factors (Table 3).

| Table 2. Drug resistance patterns of R factors
isolated from Shigella strains |
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<tr>
<td>Resistance pattern of R factors</td>
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<tr>
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<td>TC.CM.SM.SA</td>
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| Table 3. Detection of R factors from drug-resistant
strains of Escherichia coli, Klebsiella pneumoniae,
and Proteus mirabilis |
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<tr>
<td>Organism</td>
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<td>E. coli</td>
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<td>K. pneumoniae</td>
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<td>P. mirabilis</td>
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| Table 4. Drug resistance patterns of R factors
isolated from strains of Escherichia coli, Klebsiella pneumoniae,
and Proteus mirabilis |
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<td>Organism from which R factors were isolated</td>
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<td>E. coli</td>
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<tr>
<td>TC.CM.SM.SA</td>
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<tr>
<td>TC.CM.SM.SA</td>
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<tr>
<td>KM.FM</td>
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<tr>
<td>P. mirabilis</td>
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<td>TC.CM.SM.SA</td>
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Resistance patterns of the R factors isolated from these strains are shown in Table 4. It is a striking feature that R factors which harbor genetic loci that specify the determinants of cross-resistance to TC, CM, SM, SA are isolated most frequently. R(TC, CM, SM, SA, K.M. FM) and R(TC, CM, SM, SA, ABPC) factors also were isolated in this survey. A detailed study of these R factors will be described elsewhere.

DISCUSSION

The resistance to SA in Shigella strains isolated in Japan can be traced back to 1949 (9). The effectiveness of SA against dysentery lasted only for about 5 years after 1945, and SA-resistant Shigella strains then appeared rapidly, reaching a maximum in 1952 when 80 to 90% of Shigella isolates were found to be highly resistant to SA.

The production of antibiotics such as SM, TC, and CM started in Japan around 1951, and these agents were quite effective against dysentery. The antibiotic-resistant Shigella strains have appeared with the increased production of these antibiotics. However, antibiotic-resistant isolates were few in number between 1951 and 1955, with just 7, 11, and 4 isolates resistant to SM or TC reported in Japan in 1953, 1954, and 1955, respectively (6, 7, 9).

The first isolation of a multiply resistant Shigella strain occurred in 1955 (9). This multiple resistance involved TC, SM, CM, and SA. Thereafter, the isolation frequency of multiply resistant strains increased rapidly, reaching 95.0% in 1965. The isolation frequency of Shigella strains resistant to drugs other than TC, SM, CM, and SA was still very low in 1965 (AB-PC and NA resistance were only 0.35 and 0.24%, respectively).

The results of these studies on the distribution of R factors in Shigella isolates in Japan have indicated that the wide distribution and rapid increase of resistant Shigella strains, especially of multiply resistant isolates, can be ascribed primarily to the appearance and distribution of Shigella strains carrying R factors.

The data concerning the drug resistance of other gram-negative bacteria, including E. coli, Klebsiella, and Proteus, are very limited. In 1957, we isolated E. coli strains resistant to TC, CM, SM, and SA during an epidemic caused by S. flexneri 3a resistant to these four drugs (9, 10). Also, in 1958 we isolated an E. coli strain resistant to CM, SM, and SA from a patient infected with S. flexneri 2a, a strain resistant to the same agents. From another patient, S. flexneri 2a, E. coli, and E. freundii strains resistant to TC, CM, SM, and SA were isolated (9). These observations stimulated our interest in the origin and genetics of this multiple resistance.

A previous paper (10) from our laboratory showed that 1.4% of 1,145 healthy human subjects carried multiply resistant E. coli. Another survey in 1960 also disclosed that 1.3% of healthy human subjects carried multiply resistant E. coli (10). In contrast, 58.9% of inpatients treated with chloramphenicol, and 20.5% of inpatients with tuberculosis, carried multiply resistant E. coli (10). Of 93 drug-resistant E. coli strains isolated from tuberculosis patients, 53.7% were multiply resistant, i.e., resistant to TC, CM, SM, SA, TC, SM, SA, or CM, SM, SA.

These surveys also disclose that inpatients often excreted multiply resistant Shigella or E. coli strains after treatment with a single drug, and that CM- and CM-SA-resistant strains have never been isolated.

These results strongly suggested that multiple resistance in E. coli, just as in Shigella, did not appear little by little but rather all at once, mainly owing to R factors. A survey of enteric bacteria isolated from urine (Egawa et al., in preparation) and the present results indicate that R factors are widespread in all gram-negative bacteria thought to be clinically significant.

Enteric bacteria isolated from patients with infections of the genitourinary tract at the Boston City Hospital were multiply resistant, and 34% of the isolates carried R factors (unpublished data). These results and other reports (2, 15) indicate that R factors are now widespread all over the world.

The origin of the R factor is quite obscure. It was suggested that a transfer factor picked up resistance genes from the chromosome of bacteria step by step, increasing the resistance genes from one to two, from two to three, and so on (16).

According to our data, the first isolation of R(TC, CM, SM, SA) factor, which is isolated most frequently, can not be traced back earlier than 1955, despite the extensive use of sulfanilamide since 1945 and of SM, TC, and CM since 1950. Consequently, one of us postulated that R factors have not appeared with increasing numbers and types of resistance genes, but rather all at once with TC, CM, SM, SA resistance (6, 7).

ACKNOWLEDGMENT

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LITERATURE CITED


