# STUDIES ON THE DRUG RESISTANCE OF *STAPHYLOCOCCI* AND *ESCHERICHIA COLI* AGAINST ANTIBIOTICS. III

## MULTIPLE-DRUG RESISTANCES

HIROKAZU OTAYA, SABURO OKAMOTO, EMIKO INOUE*,
YOSHIAKI ADACHI, SUGURU MACHIHARA and MIKIO YOSHIMURA

Shionogi & Co., Ltd., Doshomachi, Osaka, Japan

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The frequencies of multiple-drug-resistant strains were investigated in *Staphylococcus aureus* from 1965 through 1968 and in *Escherichia coli* from 1966 through 1968 classifying them with each of the corresponding drugs. The following changes were found during the periods of investigation.

*S. aureus* (7 drugs tested): (1) In PC-G-resistant strains, there was a tendency for 5-drug-resistant and 6-drug-resistant strains to increase. (2) In SM-resistant strains, a tendency was noted for 5-drug-resistant strains to increase markedly. (3) In EM-resistant strains, for 4-drug-resistant strains showed a tendency to increase. (4) Almost no change was observed in the frequencies of multiple-drug-resistant strains comprising CP resistance. (5) In KM-resistant strains, a tendency was observed for 6-drug-resistant and 7-drug-resistant strains to increase. (6) No change was found in the frequencies of multiple-drug-resistant strains comprising TC resistance. (7) In SIM-resistant strains, there was a tendency for 5-drug-resistant strains to increase.

*E. coli* (6 drugs tested): (1) In SM-resistant strains, there was a tendency for 4-drug-resistant strains to decrease, while 5-drug-resistant strains increased. (2) In CP-resistant strains, a tendency was noted for 4-drug-resistant strains to decrease. (3) No change was found in the frequencies of multiple-drug-resistant strains comprising KM resistance. (4) No change was found in the frequencies of multiple-drug-resistant strains comprising CET resistance. (5) In TC-resistant strains, there was a tendency for 5-drug-resistant strains to increase. (6) In SIM-resistant strains, 3-drug-resistant strains showed a tendency to increase.

The possible processes for the development of the main types of multiple-drug-resistant strains were examined in light of their frequencies. The results suggest that the markers of resistance to TC, PC-G and SIM form a common basis of the various drug-resistance patterns in *S. aureus*. It is inferred that various types of multiple-drug-resistant strains may be formed by the successive addition of SM, EM, CP and KM markers in this order to the TC, PC-G and SIM markers. It is similarly inferred that in *E. coli* TC, SM and SIM markers form a common basis of various drug-resistance patterns and that various types of multiple-drug-resistant strains may be formed by the successive addition of CP and KM or CET markers in this order to the TC, SM and SIM markers. The pattern of KM resistance of various multiple-drug-resistant strains of *S. aureus* and *E. coli* was quite unique among the various drug resistances investigated.

* Present address: Gracia Hospital, Osaka, Japan.

In the preceding paper[1] the general tendency of drug-resistances of *Staphylococcus aureus* (coagulase-positive) and *Escherichia coli* was reported on the strains clinically isolated in Japan during the past four years, 1965~1968. The purpose of this paper is to describe multiple-drug resistance in strains of the two species.

With regard to multiple-drug resistance, a number of studies have been already reported[2~5]. However, few of these reports investigated annually and analysed statistically.

In view of previous results showing that the degrees of drug sensitivity are very different depending on the sources of isolation, we here used only the strains from the same source of isolation: *S. aureus* strains were the isolates from pus specimens and *E. coli* from urine. Also sulfamethoxazole was newly added to the drugs tested. The details of the investigations are described hereunder; first on *S. aureus* and then on *E. coli*.

## I. *Staphylococcus aureus*

### 1. Sources of Isolation

Of the *S. aureus* strains used in the present investigation, about 70% were pus specimens as shown in Table 2 of the preceding paper[1]. The investigation on the multiple-drug-resistant strains in the present report has, therefore, been carried out with the strains from pus specimens.

### 2. Drugs Tested

The drugs tested were penicillin G (PC-G), chloramphenicol (CP), erythromycin (EM), streptomycin (SM), tetracycline (TC), kanamycin (KM), cephalothin (CET), cephaloridine (CER), sulfamethoxazole (SIM) and $\alpha$-aminobenzyl-penicillin (AB-PC). AB-PC was tested only on the strains isolated in 1968.

### 3. Method of Sensitivity Test

The method of sentitivity test was the same as described in the preceding paper[1].

### 4. Differentiation between Sensitive and Resistant Strains

We have differentiated the strains into sensitive and resistant according to the following criteria of their M. I. C. values: The strains showing M. I. C. values equal to or higher than those listed below were regarded as resistant and others as sensitive.

| Drug | M. I. C. | Drug | M. I. C. |
|---|---|---|---|
| PC-G | $\geqq$ 3.13 u/ml | KM | $\geqq$ 25 $\mu$g/ml |
| CP | $\geqq$25 $\mu$g/ml | CET* | $\geqq$ 25 〃 |
| EM | $\geqq$ 3.13 〃 | CER* | $\geqq$ 25 〃 |
| SM | $\geqq$25 〃 | AB-PC | $\geqq$ 3.13 〃 |
| TC | $\geqq$25 〃 | SIM | $\geqq$125 〃 |

* The strains tested were inhibited at $<$3.13 $\mu$g/ml.

### 5. Outline of the Annual Investigation for Four Years

The investigation covered the period June 1965 through May 1969. Each year was divided in two parts in the annual investigation and each annual period for investigation started in June and ended in May in the next year.

The frequencies of multiple-drug-resistant strains were studied annually over these four years. As shown in Table 1, single-drug-resistant and 2-drug-resistant strains showed a tendency to decrease, whereas 4-drug-resistant and 5-drug-resistant strains showed a tendency to increase. Almost no change was observed in the frequencies of 6-drug-resistant and 7-drug-resistant strains.

Table 1. Annual frequencies of single-drug-resistant and multiple-drug-resistant strains of *Staphylococcus aureus* isolated from pus specimens (Drug tested : PC-G, SM, EM, CP, KM, TC and SIM)

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to 7 drugs | 35.3% | 24.3% | 26.5% | 36.5% | 30.6% |
| Total resistant | 64.7 | 75.7 | 73.5 | 63.5 | 69.4 |
| Resistant to single drug | 24.5* | 30.3 | 29.4 | 23.7 | 27.2 |
| Resistant to 2 drugs | 11.6 | 11.8 | 10.4 | 6.9 | 10.2 |
| Resistant to 3 drugs | 20.4 | 11.8 | 13.5 | 12.6 | 14.4 |
| Resistant to 4 drugs | 22.0 | 14.2 | 18.8 | 21.7 | 19.0 |
| Resistant to 5 drugs | 14.7 | 20.6 | 17.1 | 24.8 | 19.2 |
| Resistant to 6 drugs | 5.9 | 8.4 | 7.3 | 7.5 | 7.3 |
| Resistant to 7 drugs | 0.9 | 3.0 | 3.6 | 2.9 | 2.7 |

* Among total resistant strains which are regarded as 100% (*ditto* in Tables 4~11, 14~20 and 22).

Table 2. Theoretically possible combinations and actually observed combinations of drug resistances expressed for each drug resistance in *Staphylcoccus aureus* (Total 4 years of 1965~68)

| Type resistant to | Number of theoretically possible combinations | Number of actually observed combinations including resistance to | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | PC-G | SM | EM | CP | KM | TC | SIM |
| Single drug | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 drugs | 6 | 5 | 5 | 5 | 3 | 2 | 4 | 6 |
| 3 drugs | 15 | 11 | 12 | 10 | 10 | 3 | 10 | 10 |
| 4 drugs | 20 | 13 | 13 | 15 | 11 | 9 | 14 | 13 |
| 5 drugs | 15 | 11 | 13 | 13 | 12 | 11 | 12 | 13 |
| 6 drugs | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 7 drugs | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total | 64 | 48 | 51 | 51 | 44 | 33 | 48 | 50 |

**6.** Frequently Observed Combinations of Drug Resistances

We compared the theoretically possible combinations of drug resistance with those actually observed in the studies on combinations of resistance to the above-listed seven drugs (except CET, CER and AB-PC). The combinations of 3-drug resistance and 4-drug resistance were most abundant in their varieties. Many different combinations were actually observed. If we examine the data for each drug, however, considerable differences are found in the frequencies of the drug-resistant strains (Table 2).

In the frequencies of the actually observed drug-resistant strains, single-drug-resistant strains were the most frequent (27.2%). Four-drug-resistant and 5-drug-resistant strains were 19.0% and 19.2%, respectively. Three-drug-resistant strains were 14.4%, 2-drug-resistant strains 10.2%, 6-drug-resistant strains 7.3%, and 7-drug-resistant strains 2.7%. These frequencies were not related to the types of combinations of drug resistances (Table 1).

Table 3 shows the data on the frequencies of various types of drug-resistant strains for the four years. As seen here, annual changes are not obvious except for some types of drug-resistant strains. In the 2-drug-resistant strains, it is noteworthy that strains resistant to PC-G and SIM were as frequent as 42.6%, strains resistant to TC and SIM 19.8%, and strains resistant to SM and PC-G 9.9%. Indeed these three types together formed 73% of the total 2-drug-resistant strains. In the 3-drug-

Table 3. Main types of combinations of drug resistances and their frequencies in *Staphylococcus aureus*

| Type resistant to | Order | Combination | 1965 | | 1966 | | 1967 | | 1968 | | Total | | (B)/(A) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | No. | % | No. | % | No. (B) | % | % |
| 2 drugs | | All | 51 | 100 | 59 | 100 | 61 | 100 | 31 | 100 | 202 | 100 | 10.20 |
| | 1 | SIM, PC-G | 14 | 27.5 | 35 | 59.3 | 26 | 42.6 | 11 | 35.5 | 86 | 42.6 | 4.34 |
| | 2 | SIM, TC | 17 | 33.3 | 10 | 16.9 | 9 | 14.8 | 4 | 12.9 | 40 | 19.8 | 2.02 |
| | 3 | SM, PC-G | 1 | 2.0 | 4 | 6.8 | 9 | 14.8 | 6 | 19.4 | 20 | 9.9 | 1.01 |
| 3 drugs | | All | 90 | 100 | 59 | 100 | 79 | 100 | 57 | 100 | 285 | 100 | 14.39 |
| | 1 | SIM, TC, PC-G | 39 | 43.3 | 36 | 61.0 | 38 | 48.1 | 27 | 47.4 | 140 | 49.1 | 7.07 |
| | 2 | SIM, SM, PC-G | 12 | 13.3 | 6 | 10.2 | 7 | 8.9 | 6 | 10.5 | 31 | 10.9 | 1.57 |
| | 3 | SIM, TC, SM | 19 | 21.1 | 5 | 8.5 | 3 | 3.8 | 3 | 5.3 | 30 | 10.5 | 1.52 |
| 4 drugs | | All | 97 | 100 | 71 | 100 | 110 | 100 | 98 | 100 | 376 | 100 | 18.99 |
| | 1 | SIM, TC, SM, PC-G | 65 | 67.0 | 31 | 43.7 | 39 | 35.5 | 21 | 21.4 | 156 | 41.5 | 7.88 |
| | 2 | SIM, EM, TC, PC-G | 13 | 13.4 | 18 | 25.4 | 44 | 40.0 | 42 | 42.9 | 117 | 31.1 | 5.91 |
| | 3 | SIM, EM, SM, PC-G | 1 | 1.0 | 5 | 7.0 | 7 | 6.4 | 6 | 6.1 | 19 | 5.1 | 0.96 |
| | 3 | SIM, EM, TC, SM | 5 | 5.2 | 2 | 2.8 | 5 | 4.5 | 7 | 7.1 | 19 | 5.1 | 0.96 |
| 5 drugs | | All | 65 | 100 | 103 | 100 | 100 | 100 | 112 | 100 | 380 | 100 | 19.19 |
| | 1 | SIM, EM, TC, SM, PC-G | 32 | 49.2 | 69 | 67.0 | 62 | 62.0 | 80 | 71.4 | 243 | 63.9 | 12.27 |
| | 2 | SIM, EM, TC, CP, PC-G | 12 | 18.5 | 8 | 7.8 | 16 | 16.0 | 17 | 15.2 | 53 | 13.9 | 2.68 |
| | 3 | SIM, EM, TC, CP, SM | 11 | 16.9 | 9 | 8.7 | 2 | 2.0 | 5 | 4.5 | 27 | 7.1 | 1.36 |
| 6 drugs | | All | 26 | 100 | 42 | 100 | 43 | 100 | 34 | 100 | 145 | 100 | 7.32 |
| | 1 | SIM, EM, TC, CP, SM, PC-G | 21 | 80.8 | 21 | 50.0 | 23 | 53.5 | 12 | 35.3 | 77 | 53.1 | 3.89 |
| | 2 | SIM, KM, EM, TC, SM, PC-G | 0 | 0 | 16 | 38.1 | 13 | 30.2 | 19 | 55.9 | 48 | 33.1 | 2.42 |
| | 3 | SIM, KM, EM, TC, CP, SM | 5 | 19.2 | 4 | 9.5 | 2 | 4.7 | 0 | 0 | 11 | 7.6 | 0.56 |
| 7 drugs | | SIM, EM, TC, CP, SM, KM, PC-G | 4 | | 15 | | 21 | | 13 | | 53 | | 2.68 |
| Total strains resistant to 1~7 drugs | | | | | | | | | | | 1,980 (A) | | 100 |

resistant strains, PC-G, SIM plus TC-type was 49.1 % and TC, SM plus SIM-type and PC-G, SM plus SIM-type were 10.5 % and 10.9 %, respectively. These three types together accounted for 70 % of the total 3-drug-resistant strains. In the 4-drug-resistant strains, PC-G, TC, SM plus SIM-type was 41.5 %, and PC-G, TC, EM plus SIM-type 31.1 %. These two types together were 73 % of the total 4-drug-resistant strains. The latter type of 4-drug-resistant strains showed a tendency to increase. In the 5-drug-resistant strains, PC-G, SM, TC, EM plus SIM-type was 63.9 %, and PC-G, EM, TC, CP plus SIM-type 13.9 %. These two types together formed about 78 % of the total 5-drug-resistant strains. The former type of 5-drug-resistant strains showed a tendency to increase as with 4-drug-resistant strains. In the 6-drug-resistant strains, EM, TC, CP, SM, PC-G plus SIM-type was 53.1 % and KM, EM, TC, SM, PC-G plus SIM-type 33.1 %. These two types together were 86 % of the 6-drug-resistant strains. It is interesting to note that KM resistance appeared here for the first time in the main combinations of drug resistance.

Among the total single-drug-, 2-drug-, 3-drug-, 4-drug-, 5-drug-, 6-drug- and

Table 4. Strains of *Staphylococcus aureus* resistant to penicillin-G

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to PC-G | 59.5% | 45.0% | 42.3% | 50.7% | 49.1% |
| Total PC-G-resistant | 40.5 | 55.0 | 57.7 | 49.3 | 50.9 |
| Resistant to PC-G alone | 14.5* | 18.4 | 23.5 | 16.5 | 18.8 |
| Resistant to PC-G + single drug | 6.5 | 11.8 | 9.1 | 6.0 | 8.5 |
| Resistant to PC-G + 2 drugs | 20.7 | 12.9 | 12.6 | 11.4 | 13.9 |
| Resistant to PC-G + 3 drugs | 30.1 | 16.8 | 20.7 | 23.9 | 22.3 |
| Resistant to PC-G + 4 drugs | 19.2 | 25.5 | 20.7 | 28.8 | 23.6 |
| Resistant to PC-G + 5 drugs | 7.6 | 10.4 | 8.9 | 9.7 | 9.2 |
| Resistant to PC-G + 6 drugs | 1.4 | 4.1 | 4.6 | 3.7 | 3.7 |

Table 5. Strains of *Staphylococcus aureus* resistant to streptomycin

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to SM | 68.8% | 65.4% | 67.5% | 68.4% | 67.5% |
| Total SM-resistant | 31.2 | 34.6 | 32.5 | 31.6 | 32.5 |
| Resistant to SM alone | 1.9* | 4.4 | 4.6 | 6.7 | 4.4 |
| Resistant to SM + single drug | 5.6 | 3.5 | 6.5 | 3.6 | 4.8 |
| Resistant to SM + 2 drugs | 16.4 | 7.0 | 9.7 | 7.6 | 10.0 |
| Resistant to SM + 3 drugs | 37.1 | 19.2 | 23.6 | 19.6 | 24.6 |
| Resistant to SM + 4 drugs | 24.9 | 41.0 | 31.7 | 41.8 | 34.9 |
| Resistant to SM + 5 drugs | 12.2 | 18.3 | 16.2 | 15.1 | 15.6 |
| Resistant to SM + 6 drugs | 1.9 | 6.6 | 8.1 | 5.8 | 5.7 |

7-drug-resistant strains, EM, TC, SM, PC-G plus SIM-type was 12.3%, TC, SM, PC-G plus SIM-type 7.9%, TC, PC-G plus SIM-type 7.1%, EM, TC, PC-G plus SIM-type 5.9%, PC-G plus SIM-type 4.3%, and EM, TC, CP, SM, PC-G plus SIM-type 3.9%. In total, these six types comprised 41% of the total resistant strains.

**7.** Analysis of Data for Each Drug

(1) Penicillin-G (Table 4)

The frequency of the total number of PC-G-resistant strains showed a tendency to increase, although slowly, chronologically. Examination of the data in more detail indicates an increase in 5-drug-resistant and 6-drug-resistant strains and a decrease in 3-drug-resistant and 4-drug-resistant strains. Among the total PC-G-resistant strains, 4-drug-resistant strains and 5-drug-resistant strains were as frequent as 22.3% and 23.6%, respectively. Seven-drug-resistant strains were the least frequent (3.7%).

Combinations of PC-G resistance with other drug resistances: A relatively large number of resistance combinations were observed (Table 4). The most frequent combinations were as follows (the percentage shown is the frequency among the total PC-G-resistant strains): Two-drug-resistant, PC-G plus SIM-type 5.9%; 3-drug-resistant, PC-G, TC plus SIM-type 9.6%; 4-drug-resistant, PC-G, TC, SM plus SIM-type 10.8%; 5-drug-resistant, PC-G, TC, SM, EM plus SIM-type 16.7%; 6-drug-resistant, PC-G, TC, SM, CP, EM plus SIM-type 5.3%; and 7-drug-resistant, PC-G, TC, SM, CP, EM, KM plus SIM-type 3.7%.

(2) Streptomycin (Table 5)

Almost no chronological fluctuation was found in the frequency of the total number of SM-resistant strains. Single-drug-resistant strains and 7-drug-resistant

Table 6. Strains of *Staphylococcus aureus* resistant to erythromycin

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to EM | 79.5% | 69.5% | 67.4% | 63.8% | 69.9% |
| Total EM-resistant | 20.5 | 30.5 | 32.6 | 36.2 | 30.1 |
| Resistant to EM alone | 3.6* | 2.0 | 3.1 | 2.3 | 2.7 |
| Resistant to EM + single drug | 4.3 | 1.5 | 3.1 | 2.3 | 2.7 |
| Resistant to EM + 2 drugs | 10.0 | 4.5 | 7.3 | 7.0 | 7.0 |
| Resistant to EM + 3 drugs | 17.1 | 16.8 | 26.2 | 27.5 | 22.9 |
| Resistant to EM + 4 drugs | 43.6 | 47.5 | 36.2 | 42.6 | 42.0 |
| Resistant to EM + 5 drugs | 18.6 | 20.3 | 16.2 | 13.2 | 16.6 |
| Resistant to EM + 6 drugs | 2.9 | 7.4 | 8.1 | 5.0 | 6.2 |

Table 7. Strains of *Staphylococcus aureus* resistant to chloramphenicol

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to CP | 87.0% | 86.6% | 86.7% | 88.5% | 87.2% |
| Total CP-resistant | 13.0 | 13.4 | 13.3 | 11.5 | 12.8 |
| Resistant to CP alone | 11.2* | 2.2 | 1.9 | 7.3 | 5.5 |
| Resistant to CP + single drug | 2.2 | 2.2 | 0.9 | 2.4 | 1.9 |
| Resistant to CP + 2 drugs | 7.9 | 4.5 | 10.4 | 4.9 | 7.1 |
| Resistant to CP + 3 drugs | 12.4 | 13.5 | 8.5 | 18.3 | 12.8 |
| Resistant to CP + 4 drugs | 32.6 | 31.5 | 30.2 | 32.9 | 31.7 |
| Resistant to CP + 5 drugs | 27.2 | 29.2 | 28.3 | 18.3 | 26.5 |
| Resistant to CP + 6 drugs | 4.5 | 16.9 | 19.8 | 15.9 | 14.5 |

strains showed a slight increase and 3-drug-resistant strains and 4-drug-resistant strains decreased, while 5-drug-resistant strains increased remarkably.

Combinations of SM resistance with other drug resistances: It is noteworthy that 4-drug-resistant and 5-drug-resistant strains were as frequent as 24.6% and 34.9%, respectively (about 60% together), whereas single-drug-resistant and 2-drug-resistant strains were infrequent (9.2% together). The most frequent combinations of SM resistance with resistance to other drugs were as follows (the percentage indicates the frequency among the total SM-resistant strains): Two-drug-resistant, SM plus PC-G-type 2.2%; 3-drug-resistant, SM, PC-G plus SIM-type 3.3%; 4-drug-resistant, SM, TC, PC-G plus SIM-type 16.8%; 5-drug-resistant, SM, TC, PC-G, EM plus SIM-type 26.2%; 6-drug-resistant, SM, TC, PC-G, CP, EM plus SIM-type 8.3%; and 7-drug-resistant, SM, TC, PC-G, CP, EM, KM plus SIM-type 5.7%.

(3) Erythromycin (Table 6)

The frequency of the total EM-resistant strains showed a remarkable chronological increase. In particular 4-drug-resistant strains increased considerably.

Combinations of EM resistance with other drug resistances: The most frequent combinations with EM resistance were 4-drug-resistant strains (22.9%) and 5-drug-resistant strains (42.0%). The following were the most frequent types among the observed combinations (the percentage indicates the frequency among the total EM-resistant strains): Two-drug-resistant, EM plus PC-G type 1.2%; 3-drug-resistant, EM, TC plus SIM-type 1.9%; 4-drug-resistant, EM, TC, PC-G plus SIM-type 13.6%; 5-drug-resistant, EM, TC, PC-G, SM plus SIM-type 28.3%; 6-drug-resistant, EM, TC, CP, SM, PC-G plus SIM-type 9.0%, EM, TC, KM, SM, PC-G plus SIM-type 5.6%; and 7-drug-resistant, EM, TC, CP, SM, PC-G, KM plus SIM-type 6.2%.

Table 8. Strains of *Staphylococcus aureus* resistant to kanamycin

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to KM | 97.1% | 92.0% | 91.7% | 92.4% | 93.2% |
| Total KM-resistant | 2.9 | 8.0 | 8.3 | 7.6 | 6.8 |
| Resistant to KM alone | 10.0* | 0 | 0 | 0 | 1.0 |
| Resistant to KM + single drug | 15.0 | 0 | 1.5 | 1.9 | 2.6 |
| Resistant to KM + 2 drugs | 5.0 | 3.8 | 9.1 | 5.6 | 6.2 |
| Resistant to KM + 3 drugs | 5.0 | 13.2 | 10.6 | 13.0 | 11.4 |
| Resistant to KM + 4 drugs | 20.0 | 15.1 | 16.7 | 14.8 | 16.1 |
| Resistant to KM + 5 drugs | 25.0 | 39.6 | 30.3 | 40.7 | 35.2 |
| Resistant to KM + 6 drugs | 20.0 | 28.3 | 31.8 | 24.1 | 27.5 |

Table 9. Strains of *Staphylococcus aureus* resistant to tetracycline

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to TC | 58.2% | 59.1% | 58.5% | 58.7% | 58.6% |
| Total TC-resistant | 41.8 | 40.9 | 41.5 | 41.3 | 41.4 |
| Resistant to TC alone | 1.8* | 0.7 | 1.2 | 2.0 | 1.4 |
| Resistant to TC + single drug | 7.4 | 4.4 | 4.5 | 2.4 | 4.7 |
| Resistant to TC + 2 drugs | 24.9 | 16.2 | 18.2 | 13.3 | 18.1 |
| Resistant to TC + 3 drugs | 33.7 | 22.1 | 30.3 | 28.2 | 28.7 |
| Resistant to TC + 4 drugs | 21.8 | 35.4 | 27.3 | 38.1 | 30.5 |
| Resistant to TC + 5 drugs | 9.1 | 15.5 | 12.1 | 11.6 | 12.0 |
| Resistant to TC + 6 drugs | 1.4 | 5.5 | 6.4 | 4.4 | 4.5 |

(4) Chloramphenicol (Table 7)

There was no chronological fluctuation in the frequency of the total CP-resistant strains. Characteristic were the findings that single-drug-resistant and 2-drug-resistant strains were infrequent, whereas 5-drug-resistant and 6-drug-resistant strains were the most frequent (31.7% and 26.5%, respectively).

Combinations of CP resistance with other drug resistances: It was found that combinations of CP resistance with other drug resistances were rather infrequent. The following combinations were the most frequent types among the observed combinations (the percentage is the frequency among the total CP-resistant strains): Two-drug-resistant, CP plus PC-G-type 0.8%; 3-drug-resistant, CP, TC plus SM-type 2.2%; 4-drug-resistant, CP, TC, PC-G plus SIM-type 3.6%; 5-drug-resistant, CP, TC, PC-G, EM plus SIM-type 15.0%; 6-drug-resistant, CP, TC, PC-G, SM, EM plus SIM-type 21.0%; and 7-drug-resistant, CP, TC, PC-G, SM, KM, EM plus SIM-type 14.5%.

(5) Kanamycin (Table 8)

The frequency of the total KM-resistant strains showed a tendency to chronological increase, although slight. It is noteworthy that single-drug-resistant and 2-drug-resistant strains were very infrequent, whereas multiple-resistant strains (five, six to seven) were more frequent.

Combinations of KM resistance with other drug resistances: It is also characteristic that only a small number of varieties of combinations of KM resistance with other drug resistances was observed. The following combinations were the most frequent types among the observed combinations (the percentage is the frequency among the total KM-resistant strains): Two-drug-resistant, KM plus SM-type 2.1%;

Table 10. Strains of *Staphylococcus aureus* resistant to sulfamethoxazole

| | 1965 ($n$=682) | 1966 ($n$=662) | 1967 ($n$=797) | 1968 ($n$=712) | Total ($n$=2,853) |
|---|---|---|---|---|---|
| Sensitive to SIM | 48.1% | 40.2% | 50.4% | 54.6% | 48.5% |
| Total SIM-resistant | 51.9 | 59.8 | 49.6 | 45.4 | 51.5 |
| Resistant to SIM alone | 11.9* | 16.9 | 9.6 | 5.0 | 11.1 |
| Resistant to SIM + single drug | 11.3 | 12.6 | 9.9 | 5.3 | 9.9 |
| Resistant to SIM + 2 drugs | 24.0 | 14.1 | 14.7 | 15.5 | 17.0 |
| Resistant to SIM + 3 drugs | 26.6 | 16.7 | 25.3 | 27.2 | 23.7 |
| Resistant to SIM + 4 drugs | 17.8 | 25.3 | 24.3 | 33.4 | 25.0 |
| Resistant to SIM + 5 drugs | 7.3 | 10.6 | 10.9 | 9.6 | 9.7 |
| Resistant to SIM + 6 drugs | 1.1 | 3.8 | 5.3 | 4.0 | 3.6 |

3-drug-resistant, KM, SM plus EM-type 4.7 %; 4-drug-resistant, KM, SM, EM plus TC-type 3.1 %; 5-drug-resistant, KM, SM, EM, PC-G plus SIM-tpye 3.6 %; 6-drug-resistant, KM, SM, EM, TC, PC-G plus SIM-type 24.9 %; and 7-drug-resistant, KM, SM, EM, TC, PC-G, CP plus SIM-type 27.5 %.

(6) Tetracycline (Table 9)

Almost no fluctuation was found chronologically in the frequency of TC-resistant strains. It is noteworthy that 3-drug-resistant, 4-drug-resistant and 5-drug-resistant strains were 18.1 %, 28.7 % and 30.5 %, respectively (totally 77 %), whereas single-drug-resistant and 2-drug-resistant strains were quite infrequent.

Combinations of TC resistance with other drug resistances: The actually observed types of combinations of TC resistance with other drug resistances were rather limited. The most frequent were the following types among various combinations (the percentage is the frequency among the total TC-resistant strains): Two-drug-resistant, TC plus SIM-type 3.4 %, 3-drug-resistant, TC, PC-G plus SIM-type 11.9 %, TC, SM plus SIM-type 2.5 %; 4-drug-resistant, TC, SM, PC-G plus SIM-type 13.2 %, TC, EM, PC-G plus SIM-type 9.9 %; 5-drug-resistant, TC, EM, SM, PC-G plus SIM-type 20.6 %, TC, EM, CP, PC-G plus SIM-type 4.5 %; 6-drug-resistant, TC, EM, CP, SM, PC-G plus SIM-type 6.5 %, TC, EM, KM, SM, PC-G plus SIM-type 4.1 %; and 7-drug-resistant, TC, EM, KM, CP, SM, PC-G plus SIM-type 4.5 %.

(7) Sulfamethoxazole (Table 10)

There was a tendency to chronological decrease of SIM-resistant strains, although slight. Among the SIM-resistant strains, single-drug-resistant strains showed a tendency to decrease, while 5-drug-resistant strains increased. The most frequent were 4-drug-resistant and 5-drug-resistant strains (23.7 % and 25.0 %, respectively).

Combinations of SIM resistance with other drug resistances: The following types were the most frequent among various combinations (the percentage is the frequency among the total SIM-resistant strains): Two-drug-resistant, PC-G plus SIM-type 7.3 %, TC plus SIM-type 3.4 %; 3-drug-resistant, TC, PC-G plus SIM-type 11.9 %, PC-G, SM plus SIM-type 2.6 %; 4-drug-resistant, TC, SM, PC-G plus SIM-type 13.2 %, TC, EM, PC-G plus SIM-type 9.9 %; 5-drug-resistant, TC, EM, SM, PC-G plus SIM-type 20.6 %, TC, EM, CP, PC-G plus SIM-type 4.5 %; and 6-drug-resistant, TC, EM, CP, SM, PC-G plus SIM-type 6.5 %, TC, EM, KM, SM, PC-G plus SIM-type 4.1 %.

## II. *Escherichia coli*

**1.** Sources of Isolation

Among the sources of isolation, the *E. coli* strains used in the present investigation, about 70 % of them were urine specimens as shown in Table 3 of the preceding paper[1]. The investigation on the multiple-drug resistant strains in the present report has, therefore, been carried out with the strains from urine specimens.

**2.** Drugs Tested

The drugs tested were streptomycin (SM), chloramphenicol (CP), kanamycin (KM), cephalothin (CET), cephaloridine (CER), tetracycline (TC), sulfamethoxazole (SIM) and aminobenzyl-penicillin (AB-PC). AB-PC was tested only on the strains isolated in 1968.

**3.** Method of Sensitivity Test

The method of sensitivity test was the same as that described in the preceding paper[1].

**4.** Differentiation between Sensitive and Resistant Strains

We have differentiated the strains into sensitive and resistant according to the following criteria of their M. I. C. values: The strains showing M. I. C. values equal to or higher than those listed below were regarded as resistant and others as sensitive.

| Drug | M. I. C. ($\mu$g/ml) | Drug | M. I. C. ($\mu$g/ml) |
|---|---|---|---|
| SM | $\geqq$ 25 | CER* | $\geqq$ 25 |
| CP | $\geqq$ 25 | TC | $\geqq$ 25 |
| KM | $\geqq$ 25 | AB-PC* | $\geqq$ 25 |
| CET | $\geqq$ 25 | SIM | $\geqq$ 125 |

* Regarding these two, the data is described in Tables 20~23, because of shortness of test period.

**5.** Outline of the Annual Investigation for Four Years

The period of each year for annual investigation was the same as in *S. aureus*.

The frequencies of multiple-drug-resistant *E. coli* strains were compared for three years and, as the results are shown in Table 11, only a slight fluctuation was found in the frequency of total resistant strains. However, single-drug-resistant and 4-drug-resistant strains decreased, while 2-drug-resistant and 3-drug-resistant strains increased. There was no change in the frequency of 6-drug-resistant strains.

**6.** Frequently Observed Combinations of Drug Resistances

In examining the combinations of the resistances to the above-listed 6 drugs (except CER and AB-PC), we have compared the theoretically pos-

Table 11. Annual frequencies of single-drug-resistant and multiple-drug-resistant strains of *Escherichia coli* isolated from urine specimens
(Drugs tested : SM, CP, KM, CET, TC and SIM)

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to 6 drugs | 17.4% | 17.7% | 21.6% | 19.2% |
| Total resistant | 82.6 | 82.3 | 78.4 | 80.8 |
| Resistant to single drug | 20.8* | 13.5 | 13.5 | 15.2 |
| Resistant to 2 drugs | 11.3 | 13.2 | 13.9 | 13.0 |
| Resistant to 3 drugs | 9.2 | 12.8 | 15.0 | 12.8 |
| Resistant to 4 drugs | 45.4 | 42.9 | 40.0 | 42.3 |
| Resistant to 5 drugs | 11.6 | 15.5 | 14.7 | 14.3 |
| Resistant to 6 drugs | 1.8 | 2.1 | 3.0 | 2.4 |

Table 12. Theoretically possible combinations and actually observed combinations of drug resistances experessed for each drug resistance in *Escherichia coli*
(Total 3 years of 1966~68)

| Type resistant to | Number of theoretically possible combinations | Number of actually observed combinations including resistance to | | | | | |
|---|---|---|---|---|---|---|---|
| | | SIM | CP | CET | KM | SM | TC |
| Single drug | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 drugs | 5 | 4 | 4 | 4 | 0 | 4 | 4 |
| 3 drugs | 10 | 5 | 6 | 5 | 1 | 6 | 7 |
| 4 drugs | 10 | 6 | 4 | 6 | 3 | 7 | 6 |
| 5 drugs | 5 | 4 | 3 | 3 | 3 | 4 | 3 |
| 6 drugs | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total | 32 | 21 | 19 | 20 | 9 | 23 | 22 |

Table 13. Main types of combinations of drug resistances and their frequencies in *Escherichia coli*

| Type resistant to | Order | Combination | 1966 | | 1967 | | 1968 | | Total | | (B)/(A) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | No. | % | No. (B) | % | % |
| 2 drugs | | All | 32 | 100 | 57 | 100 | 65 | 100 | 154 | 100 | 13.02 |
| | 1 | SM, SIM | 15 | 46.9 | 26 | 45.6 | 35 | 53.8 | 76 | 49.4 | 6.42 |
| | 2 | TC, SIM | 9 | 28.1 | 17 | 29.8 | 14 | 21.5 | 40 | 26.0 | 3.38 |
| | 3 | TC, SM | 4 | 12.5 | 6 | 10.5 | 7 | 10.8 | 17 | 11.0 | 1.44 |
| 3 drugs | | All | 26 | 100 | 55 | 100 | 70 | 100 | 151 | 100 | 12.76 |
| | 1 | TC, SM, SIM | 17 | 65.4 | 35 | 63.6 | 36 | 51.4 | 88 | 58.3 | 7.44 |
| | 2 | SM, CP, SIM | 4 | 15.4 | 12 | 21.8 | 17 | 24.3 | 33 | 21.9 | 2.79 |
| | 3 | SM, CET, SIM | 4 | 15.4 | 2 | 3.6 | 2 | 2.9 | 8 | 5.3 | 0.68 |
| | 3 | TC, CP, SIM | 0 | 0 | 3 | 5.5 | 5 | 7.1 | 8 | 5.3 | 0.68 |
| 4 drugs | | All | 129 | 100 | 185 | 100 | 187 | 100 | 501 | 100 | 42.35 |
| | 1 | TC, SM, CP, SIM | 121 | 93.8 | 165 | 89.2 | 170 | 90.9 | 456 | 91.0 | 38.55 |
| | 2 | TC, SM, CET, SIM | 4 | 3.1 | 11 | 5.9 | 5 | 2.7 | 20 | 4.0 | 1.69 |
| | 3 | SM, CET, CP, SIM | 3 | 2.3 | 5 | 2.7 | 8 | 4.3 | 16 | 3.2 | 1.35 |
| 5 drugs | | All | 33 | 100 | 67 | 100 | 69 | 100 | 169 | 100 | 14.25 |
| | 1 | TC, SM, CET, CP, SIM | 26 | 78.8 | 53 | 79.1 | 51 | 73.9 | 130 | 76.9 | 10.99 |
| | 2 | TC, SM, KM, CP, SIM | 7 | 21.2 | 13 | 19.4 | 17 | 24.6 | 37 | 21.9 | 3.13 |
| | 3 | TC, SM, KM, CET, SIM | 0 | 0 | 1 | 1.5 | 0 | 0 | 1 | 0.6 | 0.08 |
| 6 drugs | | TC, SM, CET, CP, KM, SIM | 5 | | 9 | | 14 | | 28 | | 2.37 |
| Total strains resistant to 1~6 drugs | | | | | | | | | 1,183 | (A) | 100 |

sible combinations of drug resistances with the actually observed combinations. As seen in Table 12, less varieties of combinations were actually observed than the theoretically possible combinations. This point is characteristic for *E. coli* and quite different from the results with *S. aureus*. If we examine the data for each drug, however, considerable differences are found in the frequencies of the drug-resistant strains (Table 12). Especially for KM, actually observed combinations of drug resistances were much less than the theoretically possible combinations. It is also remarkable that 4-drug-resistant strains were most frequently isolated (Table 11).

If we examine in detail the frequencies of actually observed combinations of drug resistances, we can point out that the most frequent types are as follows (Table 13). In 2-drug-resistant strains, SM plus SIM-type was 49.4 %. In 3-drug-resistant strains, SM, TC plus SIM-type was 58.3 %. In 4-drug-resistant strains, SM, TC, CP plus SIM-type was 91.0 %. In 5-drug-resistant strains, SM, TC, CP, CET plus SIM-type was 76.9 %. Thus it is obvious that a particular type of drug-resistant strains appears frequently in each group of multiple-drug-resistant strains. The above four types together indeed occupy about 63 % of the total drug-resistant strains of *E. coli*. It is quite characteristic for *E. coli* that particular types of multiple-drug-resistant strains are predominant. This tendency was not found in *S. aureus*.

**7.** Analysis of Data for Each Drug

(1) Streptomycin (Table 14)

Almost no chronological change was observed in the frequency of total SM-resistant

strains. In the components of the SM-resistant strains, there was a tendency for the incidence of 4-drug-resistant strains to decrease, while 5-drug-resistant strains increased. A greater part of the SM-resistant strains was composed of 3-drug-resistant (14.6 %), 4-drug-resistant (52.8 %) and 5-drug-resistant strains (18.0 %).

Combinations of SM resistance with other drug resistances: The combinations of SM resistance with other drug resistances were frequent (Table 12). The most frequent types of the observed combinations of SM resistance with other drug resistances were as follows (the percentage indicates the frequency among the total SM-resistant strains): Two-drug-resistant, SM plus SIM-type 8.1 %; 3-drug-resistant, SM, TC plus SIM-type 9.4 %; 4-drug-resistant, SM, TC, CP plus SIM-type 48.5 %; 5-drug-resistant, SM, TC, CP, CET plus SIM-type 13.8 %; and 6-drug-resistant, SM, TC, CP, CET, KM plus SIM-type 3.0 %.

(2) Chloramphenicol (Table 15)

There was almost no chronological fluctuation in the frequency of the total CP-resistant strains. Four-drug-resistant strains, however, showed a tendency to decrease. Four-drug-resistant strains were the most frequent (65.1 %) being followed by 5-drug-resistant strains (22.9 %). It is remarkable that these two types together account for as many as 88 % of the total.

Combinations of CP resistance with other drug resistances: The

Table 14. Strains of *Escherichia coli* resistant to streptomycin

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to SM | 36.6% | 33.6% | 37.2% | 35.8% |
| Total SM-resistant | 63.4 | 66.4 | 62.8 | 64.2 |
| Resistant to SM alone | 1.8* | 1.4 | 1.1 | 1.4 |
| Resistant to SM+single drug | 9.6 | 9.8 | 11.2 | 10.3 |
| Resistant to SM+2 drugs | 11.9 | 14.7 | 16.0 | 14.6 |
| Resistant to SM+3 drugs | 59.2 | 52.3 | 49.6 | 52.8 |
| Resistant to SM+4 drugs | 15.1 | 19.3 | 18.4 | 18.0 |
| Resistant to SM+5 drugs | 2.3 | 2.6 | 3.7 | 3.0 |

Table 15. Strains of *Escherichia coli* resistant to chloramphenicol

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to CP | 50.9% | 48.3% | 50.8% | 49.9% |
| Total CP-resistant | 49.1 | 51.7 | 49.2 | 50.1 |
| Resistant to CP alone | 0 | 0.7 | 0 | 0.3 |
| Resistant to CP+single drug | 1.2* | 1.1 | 1.4 | 1.2 |
| Resistant to CP+2 drugs | 3.0 | 6.3 | 9.2 | 6.7 |
| Resistant to CP+3 drugs | 73.4 | 64.2 | 61.2 | 65.1 |
| Resistant to CP+4 drugs | 19.5 | 24.4 | 23.5 | 22.9 |
| Resistant to CP+5 drugs | 3.0 | 3.3 | 4.8 | 3.8 |

Table 16. Strains of *Escherichia coli* resistant to kanamycin

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to KM | 95.9% | 95.6% | 93.8% | 94.9% |
| Total KM-resistant | 4.1 | 4.4 | 6.2 | 5.1 |
| Resistant to KM alone | 7.1* | 0 | 5.4 | 4.1 |
| Resistant to KM+single drug | 0 | 0 | 0 | 0 |
| Resistant to KM+2 drugs | 0 | 0 | 2.7 | 1.4 |
| Resistant to KM+3 drugs | 7.1 | 0 | 5.4 | 4.1 |
| Resistant to KM+4 drugs | 50.0 | 60.9 | 48.6 | 52.7 |
| Resistant to KM+5 drugs | 35.7 | 39.1 | 37.8 | 37.8 |

combination of CP resistance with other drug resistances were frequent (Table 12). The most frequent were the following combinations (the percentage shows the frequency among the total CP-resistant strains): Three-drug-resistant, SM, CP plus SIM-type 4.5 %; 4-drug-resistant, SM, CP, TC plus SIM-type 61.4 %; 5-drug-resistant, SM, CP, TC, CET plus SIM-type17.7 %; and 6-drug-resistant, SM, CP, TC, CET, KM plus SIM-type 3.8 %.

(3) Kanamycin (Table 16)

There was almost no chronological fluctuation in the frequency of KM-resistant strains. It is characteristic that the majority of KM-resistant strains were composed of 5-drug-resistant and 6-drug-resistant strains.

Combinations of KM resistance with other drug resistances: KM was the least frequent drug to appear in resistance combinations with other drugs (Table 12). Five-drug-resistant strains were 52.7% and 6-drug-resistant strains were 37.8 %. Single-drug-resistant, 2-drug-resistant and 3-drug-resistant strains were almost nil. The most frequent were the following combinations (the percentage is the frequency among the total KM-resistant strains): Five-drug-resistant, KM, SM, TC, CP plus SIM-type 50.0 %; and 6-drug-resistant, KM, SM, TC, CP, CET plus SIM-type 37.8 %.

(4) Cephalothin (Table 17)

The frequency of CET-resistant strains was low all through

Table 17. Strains of *Escherichia coli* resistant to cephalothin

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to CET | 84.0% | 79.6% | 82.4% | 81.8% |
| Total CET-resistant | 16.0 | 20.4 | 17.6 | 18.2 |
| Resistant to CET alone | 14.5* | 12.1 | 9.5 | 11.6 |
| Resistant to CET+single drug | 5.5 | 5.6 | 4.8 | 5.2 |
| Resistant to CET+2 drugs | 9.1 | 4.7 | 7.6 | 6.7 |
| Resistant to CET+3 drugs | 14.5 | 18.7 | 15.2 | 16.5 |
| Resistant to CET+4 drugs | 47.3 | 50.5 | 49.2 | 49.4 |
| Resistant to CET+5 drugs | 9.1 | 8.4 | 13.3 | 10.5 |

Table 18. Strains of *Escherichia coli* resistant to tetracycline

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to TC | 39.8% | 35.3% | 40.9% | 38.6% |
| Total TC-resistant | 60.2 | 64.7 | 59.1 | 61.4 |
| Resistant to TC alone | 6.8* | 4.7 | 4.5 | 5.1 |
| Resistant to TC+single drug | 6.8 | 8.0 | 7.1 | 7.3 |
| Resistant to TC+2 drugs | 8.2 | 11.8 | 14.4 | 12.0 |
| Resistant to TC+3 drugs | 59.9 | 53.1 | 50.7 | 53.7 |
| Resistant to TC+4 drugs | 15.9 | 19.8 | 19.3 | 18.7 |
| Resistant to TC+5 drugs | 2.4 | 2.7 | 4.0 | 3.1 |

Table 19. Strains of *Escherichia coli* resistant to sulfamethoxazole

| | 1966 ($n$=344) | 1967 ($n$=524) | 1968 ($n$=597) | Total ($n$=1,465) |
|---|---|---|---|---|
| Sensitive to SIM | 27.9% | 27.9% | 30.3% | 28.9% |
| Total SIM-resistant | 72.1 | 72.1 | 69.7 | 71.1 |
| Resistant to SIM alone | 12.9* | 5.8 | 7.5 | 8.2 |
| Resistant to SIM+single drug | 9.7 | 11.6 | 13.0 | 11.7 |
| Resistant to SIM+2 drugs | 10.1 | 13.8 | 15.1 | 13.4 |
| Resistant to SIM+3 drugs | 52.0 | 48.7 | 44.5 | 43.8 |
| Resistant to SIM+4 drugs | 13.3 | 17.7 | 16.6 | 16.2 |
| Resistant to SIM+5 drugs | 2.0 | 2.4 | 3.4 | 2.7 |

the years, but a tendency to increase was noted. Many of them were resistant to relatively large numbers of drugs: Four-drug-resistant strains, 5-drug-resistant strains and 6-drug-resistant strains were 16.5 %, 49.4 % and 10.5 %, respectively.

Combinations of CET resistance with other drug resistances: A relatively small number of combinations of CET resistance was found with other drug resistances (Table 12). The most frequent among them were the following types of combinations (the percentage is the frequency among the total CET-resistant strains): Four-drug-resistant, CET, TC, SM plus SIM-type 7.5 %; 5-drug-resistant, CET, TC, SM, CP plus SIM-type 48.7 %; and 6-drug-resistant, CET, TC, SM, CP, KM plus SIM-type 10.5 %.

(5) Tetracycline (Table 18)

No chronological fluctuation was found in the frequency of TC-resistant strains. Three-drug-resistant, 5-drug-resistant and 6-drug-resistant strains showed a tendency to decrease, whereas a tendency to decrease was observed in 4-drug-resistant strains.

Combinations of TC resistance with other drug resistances: It is characteristic for TC resistance that many types of combinations with other drug resistances were found (Table 12), but more than 50 % were composed of 4-drug-resistant strains. The most frequent were the following combinations (the percentage is the frequency among the total TC-resistant strains): Two-drug-resistant, TC plus SIM-type 4.4 %; 3-drug-resistant, TC, SM plus SIM-type 9.8 %; 4-drug-resistant, TC, SM, CP plus SIM-type 50.7 %; 5-drug-resistant, TC, SM, CP, CET plus SIM-type 14.5 %; and 6-drug-resistant, TC, SM, CP, CET, KM plus SIM-type 3.1 %.

(6) Sulfamethoxazole (Table 19)

There was almost no chronological fluctuation in the frequency of SIM-resistant strains. Two-drug-resistant, 3-drug-resistant and 5-drug-resistant strains were infrequent but showed a tendency to increase. Consequently, 4-drug-resistant strains showed a tendency to decrease.

Combinations of SIM resistance with other drug resistances: The combinations of SIM resistance with other drug resistances were observed frequently (Table 12). More than 40 % consisted of 4-drug-resistant strains. The most frequent types of combinations were as follows (the percentage is the frequency among the total SIM-resistant strains): Two-drug-resistant, SIM plus SM-type 7.3 %; 3-drug-resistant, SIM, SM plus TC-type 8.4 %; 4-drug-resistant, SIM, SM, TC plus CP-type 43.8 %; 5-drug-resistant, SIM, TC, SM, CP plus CET-type 12.5 %; and 6-drug-resistant, SIM, TC, SM, CP, KM plus CET-type 2.7 %.

## Discussion

The frequencies of multiple-drug-resistant strains of *S. aureus* were investigated with 7 drugs. The drug-resistance markers most commonly observed in the strains resistant to 4 and 5 drugs were PC-G, SM, TC and SIM. The most common drug-resistance markers in the strains resistant to 4, 5 and 6 drugs were EM and CP and those in the strains resistant to 6 and 7 drugs were KM.

The possible processes for the development of the main types of multiple-drug-resis-

Fig. 1. A model of the main processes for the development of multiple-drug-resistant strains of *Staphylococcus aureus*.

Figures over the line in the circle represent the frequency (%) among the strains resistant to each corresponding number of drugs and figures under the line are the frequency (%) among the total resistant strains.
The data used in calculation are the same as shown in Table 3.

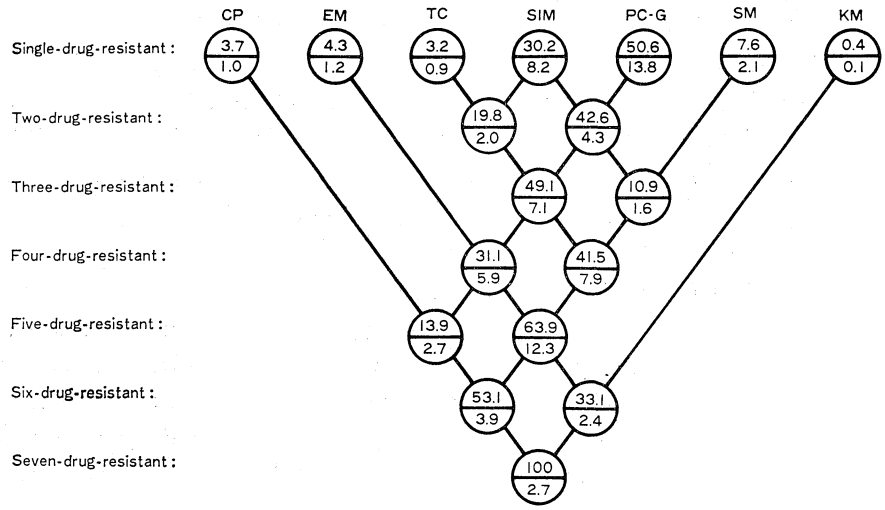



Table 20. Strains of *Staphylococcus aureus* resistant to 1~8 drugs
(Year of isolation : 1968. Total strains tested : 710)

| | All the eight | PC-G | SM | CP | TC | EM | KM | SIM | AB-PC |
|---|---|---|---|---|---|---|---|---|---|
| Total sensitive | 31.8% | 50.6 | 68.3 | 88.2 | 58.6 | 63.7 | 92.4 | 54.3 | 47.5 |
| Total resistant | 68.2 | 49.4 | 31.7 | 11.5 | 41.4 | 36.3 | 7.6 | 45.5 | 52.5 |
| Single-drug-resistant | 17.4* | 3.7 | 5.3 | 4.9 | 2.0 | 1.6 | 0 | 4.0 | 8.6 |
| Two-drug-resistant | 14.1 | 13.7 | 2.7 | 3.7 | 2.0 | 2.3 | 1.9 | 3.4 | 14.7 |
| Three-drug-resistant | 7.2 | 5.7 | 6.2 | 4.9 | 4.4 | 4.7 | 3.7 | 6.8 | 4.8 |
| Four-drug-resistant | 11.4 | 11.4 | 8.9 | 6.1 | 13.6 | 8.9 | 9.3 | 14.6 | 10.7 |
| Five-drug-resistant | 19.6 | 23.6 | 19.1 | 23.2 | 27.9 | 26.0 | 16.7 | 27.6 | 22.3 |
| Six-drug-resistant | 20.9 | 28.8 | 37.3 | 24.4 | 34.4 | 38.8 | 3.7 | 30.3 | 26.8 |
| Seven-drug-resistant | 7.0 | 9.7 | 15.1 | 18.3 | 11.6 | 13.2 | 42.6 | 9.6 | 8.8 |
| Eight-drug-resistant | 2.5 | 3.4 | 5.3 | 14.6 | 4.1 | 4.7 | 22.2 | 3.7 | 3.2 |

(ref.) None of the 710 strains was resistant to CET, CER.

tant strains were examined in light of their frequencies. The drug-resistance markers common to various types of multiple-drug resistance were TC, PC-G and SIM. It is inferred that various types of multiple-drug-resistant strains may be formed by the successive addition of SM, EM, CP and KM markers in this order to the TC, PC-G and SIM markers. This model is shown in Fig. 1.

The frequencies of multiple-drug-resistant strains of *S. aureus* isolated in 1968 are summarized in Table 20 for 10 drugs. Table 21 shows the relationship of 2 drugs.

The frequencies of multiple-drug-resistant strains of *E. coli* were investigated for 6 drugs. The resistance to all the drugs other than KM was most commonly found in 4-drug-resistant and 5-drug-resistant strains, whereas KM resistance was most frequently encountered in 5-drug-resistant and 6-drug-resistant strains.

The possible processes for the development of main types of multiple-drug-resistant strains were examined in light of their frequencies. The drug-resistance markers common to various types of multiple-drug resistances were TC, SIM and SM. It is inferred that various types of multiple-drug-resistant strains may be formed by the successive addition

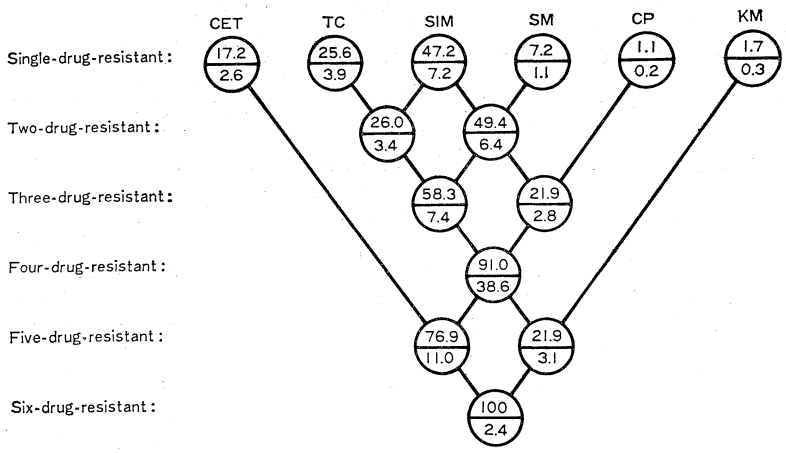
Table 21. Correlation between individual drugs with regard to the incidence of resistant strains of *Staphylococcus aureus*
(Year of isolation : 1968. Total strains tested : 710)

| Resistant to | | PC-G | SM | CP | TC | EM | KM | SIM | CET | CER | AB-PC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PC-G: | 351 * (100)** | —— | 176 * (50.1)** | 59 (16.8) | 248 (70.7) | 213 (60.7) | 41 (11.7) | 268 (76.4) | 0 | 0 | 328 (93.4) |
| SM: | 225 (100) | 176 (78.2) | —— | 40 (17.8) | 177 (78.7) | 168 (74.7) | 53 (23.6) | 184 (81.8) | 0 | 0 | 176 (78.2) |
| CP: | 82 (100) | 59 (72.0) | 40 (48.8) | —— | 68 (82.9) | 65 (79.3) | 20 (24.4) | 64 (78.0) | 0 | 0 | 60 (73.2) |
| TC: | 294 (100) | 248 (84.4) | 177 (60.2) | 68 (23.1) | —— | 223 (75.9) | 45 (15.3) | 269 (91.5) | 0 | 0 | 243 (82.7) |
| EM: | 258 (100) | 213 (82.6) | 168 (65.1) | 65 (25.2) | 223 (86.4) | —— | 51 (19.8) | 223 (86.4) | 0 | 0 | 212 (82.2) |
| KM: | 54 (100) | 41 (75.9) | 53 (98.1) | 20 (37.0) | 45 (83.3) | 51 (94.4) | —— | 38 (70.4) | 0 | 0 | 40 (74.1) |
| SIM: | 323 (100) | 268 (83.0) | 184 (57.0) | 64 (19.8) | 269 (83.3) | 223 (69.0) | 38 (11.8) | —— | 0 | 0 | 266 (82.4) |
| CET: | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | —— | 0 | 0 |
| CER: | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | —— | 0 |
| AB-PC: | 373 (100) | 328 (87.9) | 176 (47.2) | 60 (16.1) | 243 (65.1) | 212 (56.8) | 40 (10.7) | 266 (71.3) | 0 | 0 | —— |

* No. of resistant strains among the 710 strains.
** Their frequency (%) when the figures in left column are expressed as 100 %.

Fig. 2. A model of the main processes for the development of multiple-drug-resistant strains of *Escherichia coli*.

Figures over the line in the circle represent the frequency (%) among the strains resistant to each corresponding number of drugs and figures under the line are the frequency (%) among the total resistant strains.
The data used in calculation are the same as shown in Table 13.




of CP and KM or CET markers in this order to the TC, SIM and SM markers. This model is shown in Fig. 2.

The presence of R factors[6] with all the drug-resistance markers is already known. We assumed from the statistic data written in this paper that the multiple-drug-resistance is mainly controlled by R factors.

The frequencies of multiple-drug-resistant strains of *E. coli* isolated in 1968 are summarized in Table 22 for 8 drugs. Table 23 shows the relationships of 2 drugs.

Table 22. Strains of *Escherichia coli* resistant to 1~8 drugs
(Year of isolation : 1968. Total strains tested : 586)

| | All the eight | TC | CP | SM | SIM | KM | CER | CET | AB-PC |
|---|---|---|---|---|---|---|---|---|---|
| Total sensitive | 20.8% | 40.3% | 50.2% | 36.5% | 29.9% | 93.7% | 94.5% | 82.3% | 84.6% |
| Total resistant | 79.2 | 59.7 | 49.8 | 63.5 | 70.1 | 6.3 | 5.5 | 17.7 | 15.4 |
| Single-drug-resistant | 12.7* | 4.6 | 0 | 1.1 | 6.6 | 5.4 | 0 | 8.7 | 1.1 |
| Two-drug-resistant | 14.4 | 6.9 | 1.4 | 11.0 | 13.4 | 0 | 0 | 5.8 | 4.4 |
| Three-drug-resistant | 13.6 | 13.4 | 7.2 | 14.8 | 14.1 | 0 | 0 | 6.7 | 1.1 |
| Four-drug-resistant | 34.5 | 44.0 | 53.4 | 42.5 | 38.2 | 5.4 | 0 | 5.8 | 7.8 |
| Five-drug-resistant | 12.7 | 16.3 | 19.5 | 15.6 | 14.1 | 16.2 | 9.1 | 31.7 | 25.6 |
| Six-drug-resistant | 6.3 | 7.4 | 9.2 | 7.8 | 7.1 | 32.4 | 18.2 | 16.3 | 31.1 |
| Seven-drug-resistant | 3.9 | 4.9 | 6.2 | 4.8 | 4.4 | 16.2 | 45.5 | 16.3 | 18.9 |
| Eight-drug-resistant | 1.9 | 2.6 | 3.1 | 2.4 | 2.2 | 24.3 | 27.3 | 8.7 | 10.0 |

Table 23. Correlation between individual drugs with regard to the incidence of resistant strains of *Escherichia coli*
(Year of isolation : 1968. Total strains tested : 586)

| Resistant to | SIM | CP | CER | CET | KM | SM | TC | AB-PC |
|---|---|---|---|---|---|---|---|---|
| SIM: 411* (100)** | —— | 283 * (68.9)** | 32 (7.8) | 88 (21.4) | 33 (8.0) | 354 (86.1) | 314 (76.4) | 86 (20.9) |
| CP: 292 (100) | 283 (96.9) | —— | 29 (9.9) | 77 (26.4) | 33 (11.3) | 280 (95.9) | 265 (90.8) | 81 (27.7) |
| CER: 33 (100) | 32 (97.0) | 29 (87.9) | —— | 31 (93.9) | 13 (39.4) | 33 (100) | 28 (84.8) | 29 (87.9) |
| CET: 104 (100) | 88 (84.6) | 77 (74.0) | 31 (29.8) | —— | 16 (15.4) | 85 (81.7) | 79 (76.0) | 44 (42.3) |
| KM: 37 (100) | 33 (89.2) | 33 (89.2) | 13 (35.1) | 16 (43.2) | —— | 34 (91.9) | 34 (91.9) | 26 (70.3) |
| SM: 372 (100) | 354 (95.2) | 280 (75.3) | 33 (8.9) | 85 (22.8) | 34 (9.1) | —— | 306 (82.3) | 81 (21.8) |
| TC: 350 (100) | 314 (89.7) | 265 (75.7) | 28 (8.0) | 79 (22.6) | 34 (9.7) | 306 (87.4) | —— | 77 (22.0) |
| AB-PC: 90 (100) | 86 (95.6) | 81 (90.0) | 29 (32.2) | 44 (48.9) | 26 (28.9) | 81 (90.0) | 77 (85.6) | —— |

* No. of resistant strains among the 586 strains.
** Their frequency (%) when the figures in left column are expressed as 100%.

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