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

ACQUIRED MULTIPLE DRUG RESISTANCE AND ITS INFLUENCE ON THE MINIMUM INHIBITORY CONCENTRATION IN THESE STRAINS

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With many clinical isolates of *Staphylococcus aureus* and *Escherichia coli*, we have found with our statistical analysis that there is a correlation between the increase in the minimum inhibitory concentration (MIC) and the number of antibiotics to which strains are resistant. However, detailed examination of the results shows that not only does the MIC increase with acquired multiple drug resistance but the type can be differentiated into three types, both with *S. aureus* and *E. coli*.

Since 1965 we have studied the resistance of *Staphylococcus aureus* and *Escherichia coli* to antibiotics in Japan according to the statistical method and three reports have already been made[1~3]. The present paper is concerned with acquired multiple drug resistance and its influence on the minimum inhibitory concentrations.

It is not known how the minimum inhibitory concentration (MIC) changes when a certain bacterial strain acquires the resistance to antibiotics and progresses from single to double, triple and multiple drug resistance.

Using the method described below we have studied this problem and, from the results of our study, we have confirmed that when bacteria acquire resistance to a number of antibiotics, the MIC values are correspondingly greater.

The details of the present investigation are as follows:

(I) Method of study

By grouping a large number of organisms into sensitive strains, single-, two-, three-, and multiple drug resistant strains the changes in the MIC of each antibiotic for the groups of strains were analysed statistically. The method of sensitivity testing was the same as in the previous reports[2].

(II) Number of isolates

S. aureus, 1,724 strains (isolated from pus)

E. coli, 1,183 strains (isolated from urine)

(III) Period of isolation

August 1968 to December 1969.

(IV) Drugs tested

S. aureus: Penicillin G (PC-G), aminobenzylpenicillin (AB-PC), streptomycin (SM), chloramphenicol (CP), tetracycline (TC), erythromycin

(EM), kanamycin (KM), cephalothin (CET) and cephaloridine (CER).

E. coli: CP, SM, KM, TC, AB-PC, CET and CER.

(V) Differentiation between sensitive and resistant strains.

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

S. aureus:

Drug	MIC	Drug	MIC
PC-G	≧ 3.13 u/ml	KM	≧25 μg/ml
CP	≧25 μg/ml	CET*	≧25 μg/ml
EM	≧ 3.13 μg/ml	CER*	≧25 μg/ml
SM	≧25 μg/ml	AB-PC	≧ 3.13 μg/ml
TC	≧25 μg/ml		

* The strains tested were inhibited at 3.13 μg/ml or less.

E. coli:

Drug	MIC	Drug	MIC
SM	≧25 μg/ml	CER	≧25 μg/ml
CP	≧25 μg/ml	TC	≧25 μg/ml
KM	≧25 μg/ml	AB-PC	≧25 μg/ml
CET	≧25 μg/ml		

(VI) Results of the study.

The results of the statistical analysis show that there is a correlation between the increase in the MIC and the number of antibiotics to which the strains are resistant.

However, further detailed examination of the results shows that not only does the MIC increase with multiple drug resistance but the sensitivity patterns can also be differentiated into three types, both with *S. aureus* and *E. coli*.

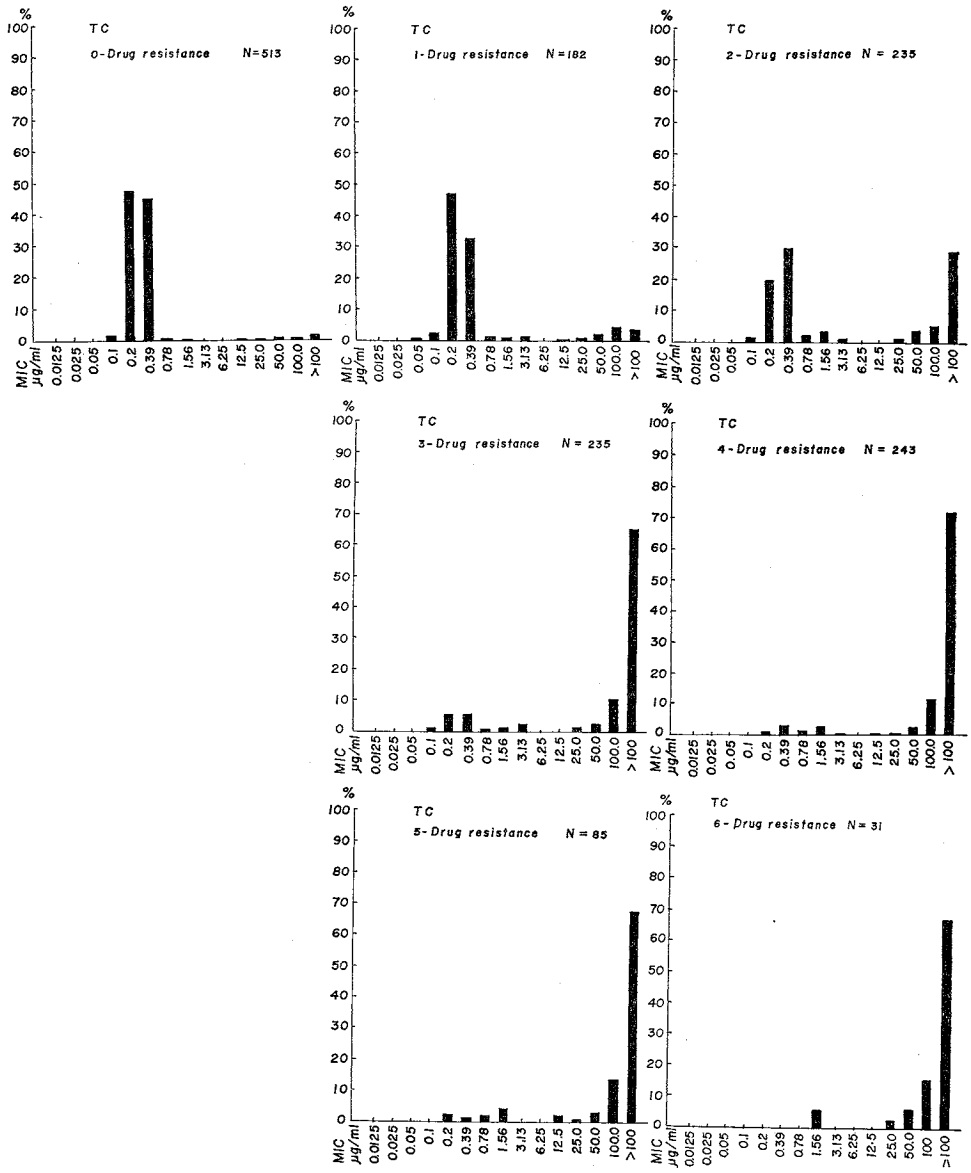
Firstly, in *S. aureus*, these is the TC type in which both the incidence and the level of resistance increases with increase in the number of antibiotics to which the strains are resistant, and with multiple drug resistant strains almost all show a high level of resistance (Fig. 1). Antibiotics belonging to this type include TC, EM and SM (Fig. 2). Secondly, there is the PC-G type in which polyresistant strains retain a normal distribution curve with respect to PC-G resistance but the curve is displaced in the direction of increased resistance. Antibiotics belonging to this type include PC-G and AB-PC (Fig. 3). The third type, namely the CP type, lies between type 1 and type 2. In this type, multiple drug resistant strains show a bimodal distribution of sensitivity to CP, one group of strains being resistant while those in the other group are sensitive. Even among strains resistant to six antibiotics a proportion shows normal sensitivity to CP. Antibiotics belonging to this type include CP and KM (Fig. 4). It will be of interest to observe any changes in these resistance patterns in the future.

With regard to CET and CER, strains of *S. aureus* show essentially the same distribution of sensitivity regardless of the number of antibiotics to which they are

resistant (Fig. 5).

In *E. coli*, resistance to CP is of the type in which multiple drug resistant strains are almost all highly resistant to this antibiotic (Fig. 6). Antibiotics belonging to this type are CP, TC and AB-PC (Fig. 7). SM resistance belongs to the type where as multiple drug resistance increases the distribution of the MIC moves to the high side, but not as completely as in the case of the CP type. Antibiotics belonging to this type are SM, CET and CER (Fig. 8). Thirdly there is the KM type, where even with the increase in multiplicity of drug resistance a highly resistant group and a highly sensitive group always remain. KM belongs to this group (Fig. 9). Thus,

Fig. 1. Distribution of MIC of TC in *S. aureus* grouped by the number of antibiotics to which strains are resistant.



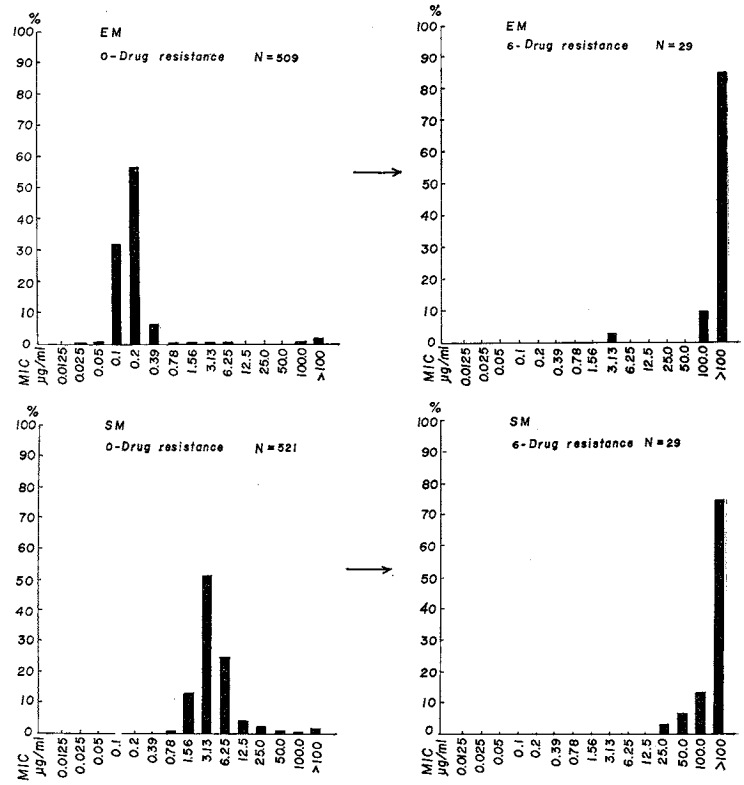


Fig. 2. Distribution of MIC of EM or SM in *S. aureus* grouped by the number of antibiotics to which strains are resistant. (as TC type)

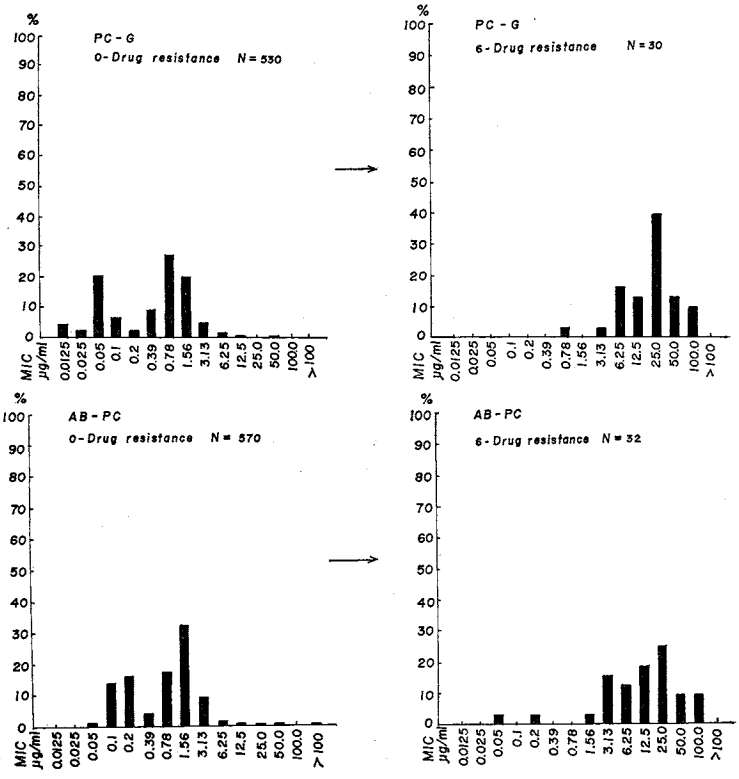


Fig. 3. Distribution of MIC of PC-G or AB-PC in *S. aureus* grouped by the number of antibiotics to which strains are resistant. (as PC-G type)

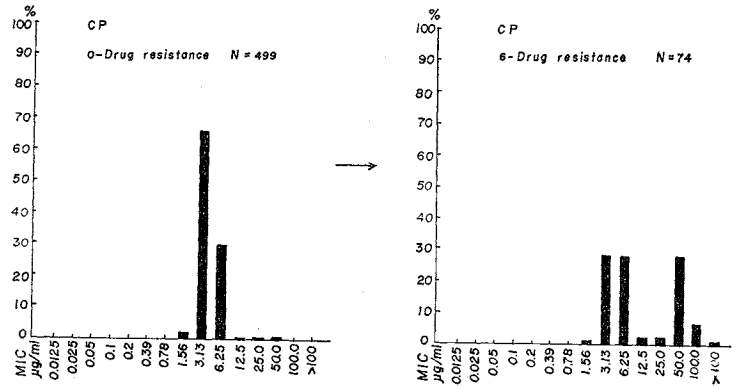


Fig. 4. Distribution of MIC of CP or KM in *S. aureus* grouped by the number of antibiotics to which strains are resistant. (as CP type)

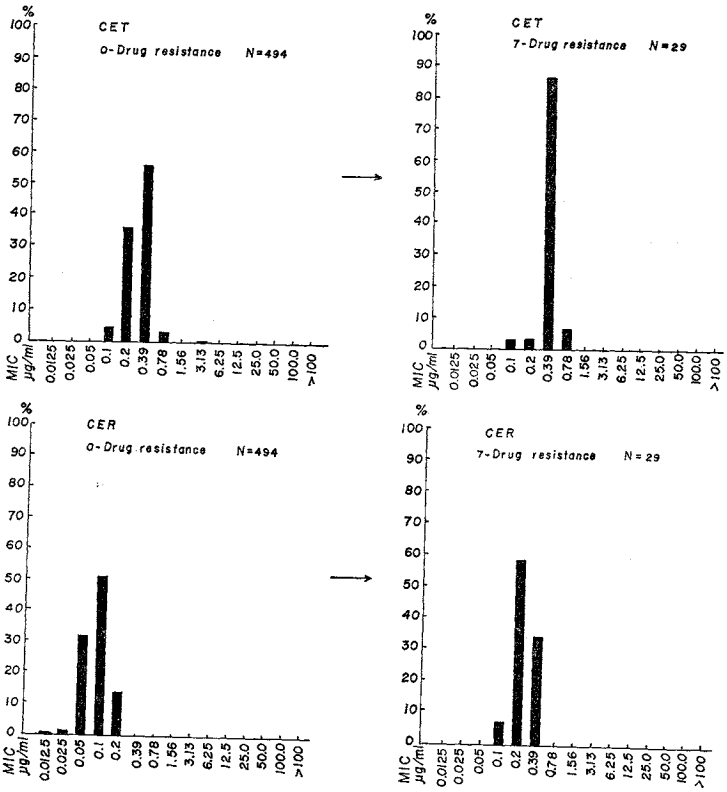
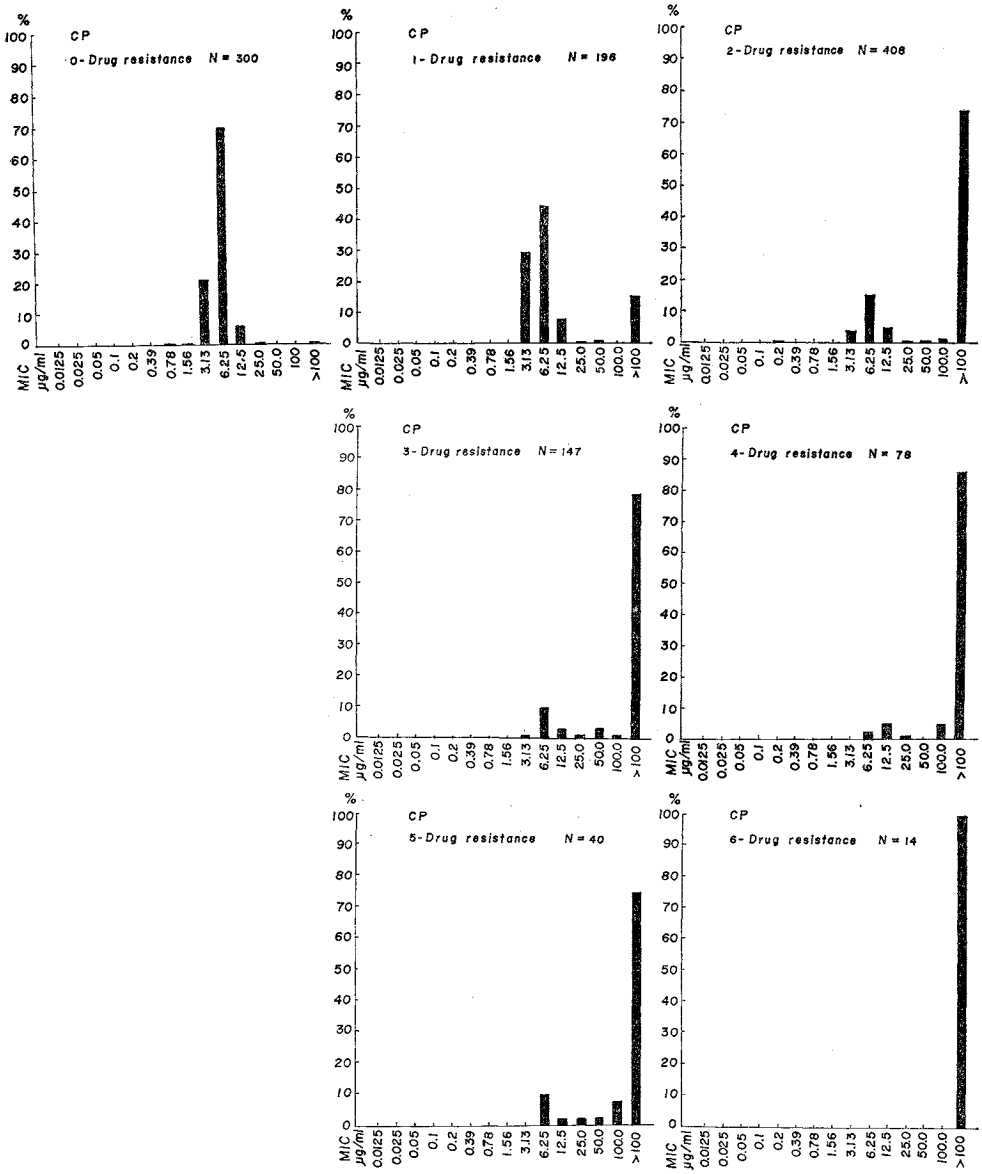


Fig. 5. Distribution of MIC of CET or CER in *S. aureus* grouped by the number of antibiotics to which strains are resistant. (as CET type)

Fig. 6. Distribution of MIC of CP in *E. coli* grouped by the number of antibiotics to which strains are resistant.



both *S. aureus* and *E. coli* show identical types, though the drugs differ.

The details of TC resistance in *S. aureus* and CP resistance in *E coli* are as follows:

(1) *Staphylococcus aureus*

Tetracycline (Fig. 1)

TC has been taken as a typical case and is explained in detail.

Graph 1 is a histogram showing the distribution of the MIC of TC for 513 strains of *S. aureus* sensitive to all 8 antibiotics used in this study other than TC. In this case 94.9 % of the strains were sensitive to 0.39 μg/ml TC or less. Of the remainder, 3.7 % showed an MIC of 25 μg/ml or over and only 2.5 % showed an MIC of 100 μg/ml

Fig. 7. Distribution of MIC of TC or AB-PC in *E. coli* grouped by the number of antibiotics to which strains are resistant. (as CP type)

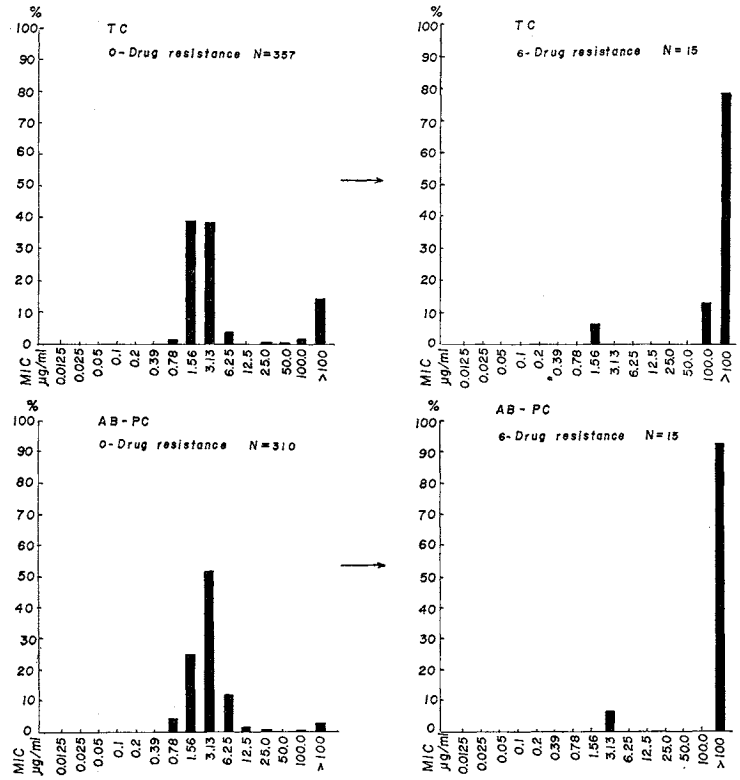
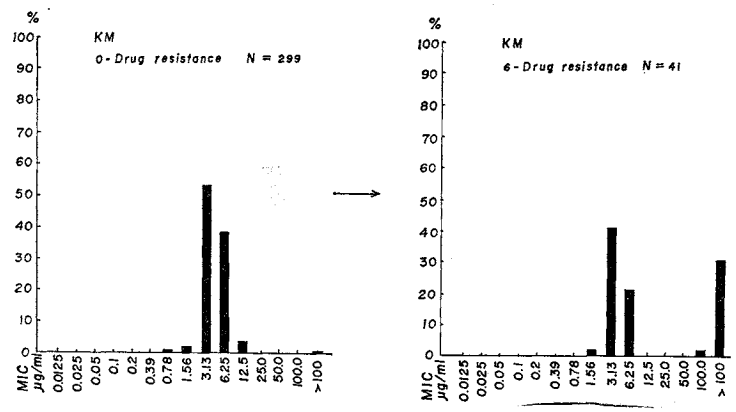


Fig. 8. Distribution of MIC of KM in *E. coli* grouped by the number of antibiotics to which strains are resistant. (as KM type)

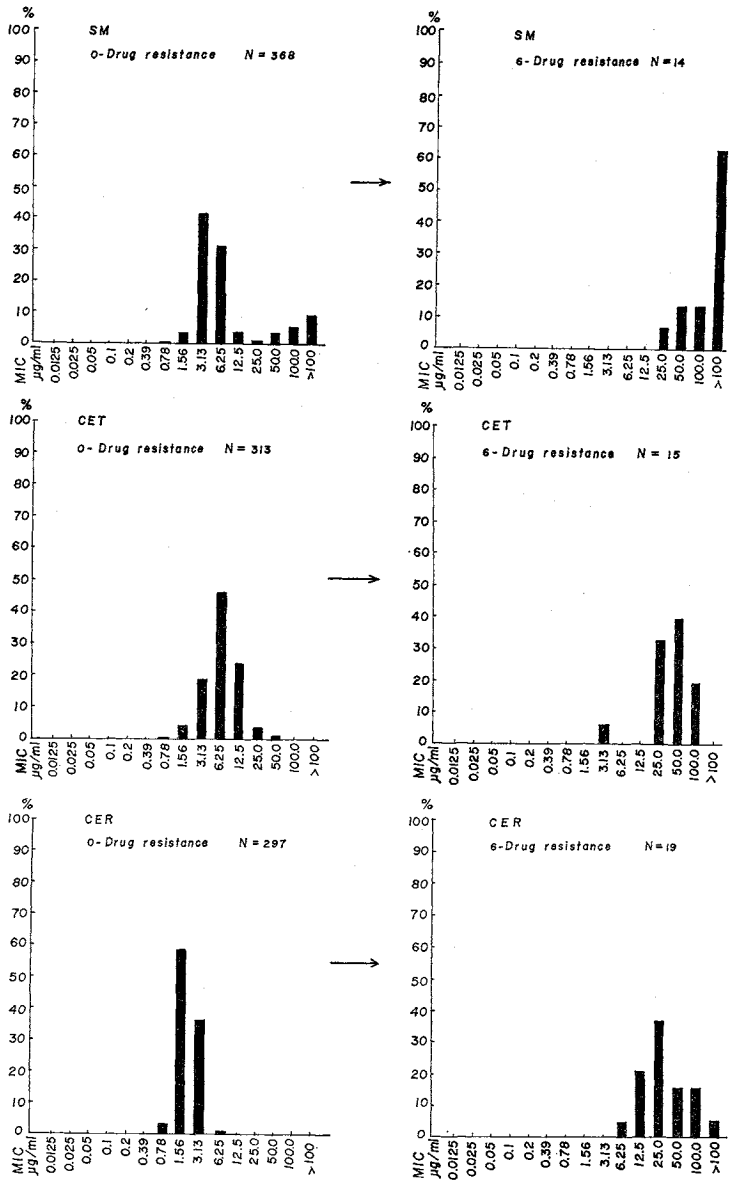


or more.

Graph 2 is a histogram showing the distribution of the MIC of TC for 182 strains resistant to 1 of the 8 antibiotics used. A total of 12.1 % showed an MIC of 25 μg/ml or over while 8.8 % were only inhibited by 100 μg/ml or more.

Graph 3 is a histogram of the MIC of TC for 235 strains showing resistance to 2 of the 8 antibiotics used. Strains showing an MIC of 25 μg/ml or over comprised 40.4 % of the total and 34.9 % showed an MIC of 100 μg/ml or more.

Fig. 9. Distribution of MIC of SM, CET or CER in *E. coli* grouped by the number of antibiotics to which strains are resistant. (as SM type)



Graph 4 shows the distribution of the MIC of TC for 235 strains resistant to 3 of the 8 antibiotics. As many as 81.3 % showed an MIC of 25 μg/ml or more while 76.6 % were only inhibited by 100 μg/ml or greater.

Graph 5 shows the distribution of the MIC of TC for 243 strains resistant to 4 of the antibiotics. Strains showing an MIC of 25 μg/ml or over comprised 88.1 % of the total while the proportion showing an MIC of 100 μg/ml or greater had increased to 84.4 %.

Graph 6 similarly shows the MIC of TC for 85 strains resistant to 5 of the antibiotics in which 87.1 % showed an MIC of 25 μg/ml or over and 82.4 % has an MIC

of 100 μg/ml or more.

Lastly, Graph 7 shows that of the 31 strains resistant to 6 of the antibiotics used, 83.9% showed an MIC for TC of 100 μg/ml or over and only 2 strains (6.5%) were sensitive with an MIC of 1.56 μg/ml.

It should be noted that no strains resistant to CER or CET were found.

The results of similar studies on the other antibiotics are shown in Figs. 2 to 5.

(2) *Escherichia coli*

Chloramphenicol (Fig. 6)

As a typical case, CP is explained in detail.

Graph 1 is a histogram showing the distribution of the MIC of CP for 300 strains of *E. coli* sensitive to the 6 antibiotics used in this study other than CP. Two strains showed an MIC of 25 μg/ml with CP and 1 strain an MIC of over 100 μg/ml. The majority of the strains were sensitive in the range 3.13 μg/ml to 6.25 μg/ml.

Graph 2 shows the distribution of the MIC of CP for 196 strains resistant to 1 of the 6 antibiotics. A total of 17.3% showed an MIC of 25 μg/ml or over and 15.8% an MIC of 100 μg/ml or more.

Graph 3 shows the distribution of the MIC of CP for 408 strains resistant to 2 of the 6 antibiotics. In these strains 75.5% showed an MIC of 100 μg/ml or more.

Similarly Graph 4 shows the distribution of the MIC of CP for 147 strains resistant to 3 of the other antibiotics. Of these strains 80.3% showed an MIC of 100 μg/ml or more.

Graph 5 shows the distribution of the MIC of CP for 78 strains resistant to 4 of the other antibiotics. In these strains the proportion showing an MIC of 100 μg/ml or more had risen to 91.0%.

Graph 6 shows the distribution of the MIC of CP for 40 strains resistant to 5 of the 6 antibiotics. Strains showing an MIC of 100 μg/ml or over accounted for 82.5% of the total.

Graph 7 shows the MIC of CP for 14 strains resistant to all 6 of the antibiotics used. All the strains showed resistance to 100 μg/ml.

The results of similar studies on the other antibiotics are shown in Figs. 7 to 9.

Discussion and Conclusion

We have found with our method of statistical analysis that the MIC values of antibiotics against bacteria increase with the number of antibiotics to which the strains are resistant and that the resistance can be differentiated into three types. It would be desirable if the above findings could be confirmed with another method using one bacterial culture, if possible.

Acknowledgment

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References

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