SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* TO ANTIBIOTICS. V

DIFFERENTIATION OF DRUG SENSITIVE- AND RESISTANT-STRAINS BY MINIMAL INHIBITORY CONCENTRATION OF THE DRUG AND ANNUAL CHANGE OF SENSITIVITY OF THE BACTERIA TO VARIOUS DRUGS

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Minimal inhibitory concentration (MIC) values of *Staphylococcus aureus* and *Escherichia coli* isolated from clinical specimens from June, 1965 through May, 1972 were studied and distribution of the values was examined. A method for establishing the appropriate MIC values for grouping the isolates into sensitive and resistant strains is described.

The grouping values thus determined were applied to study an annual change of sensitivity to various antibiotics of *S. aureus* and *E. coli* isolated from clinical specimens.

Materials and methods

Strains

Coagulase-positive strains of *S. aureus* and strains of *E. coli* isolated from various clinical specimens during 1965~1971 were used. The period of study was divided as follows:

1965 : June 1965~May 1966
1966 : June 1966~May 1967
1967 : June 1967~May 1968
1968 : June 1968~May 1969
1969 : June 1969~May 1970
1970 : June 1970~May 1971
1971 : June 1971~May 1972

Clinical specimens

Tables 1 and 2 show the numbers of *S. aureus* and *E. coli* isolated during 1965~1971 from various clinical specimens. Every year both bacteria were isolated from various clinical specimens at a similar rate. About 70 % of *S. aureus* were isolated from pus, 10 % from pharyngeal mucus and 7 % from sputum. In *E. coli* about 70 %, 10 % and 8 % were isolated from urine, pus and feces, respectively. In 1970, the strains of *S. aureus* examined were isolated from pus only. Sensitivity testing of *S. aureus* isolated in 1971 is partly under investigation.

Method of sensitivity test (MIC test)

MIC values for each antibiotic were determined by streak culture on agar using two-fold serial dilutions of antibiotic in modified MÜLLER-HINTON medium[1,2]. For MIC of sulfonamide with *E. coli*, SAUTON medium[2] was used from 1965 through 1968 and then changed to SAUTON-agar using streak culture.

Antibiotics tested

(a) Antibiotics examined for 7 years: Te-

Table 1. *Staphylococcus aureus* isolated from various clinical specimens from June 1965 through May 1971

Year	1965	1966	1967	1968	1969	1970	1971	Total
Number of strains	994	942	1226	1077	751	262	185	5437
Pus	71.0%	71.0%	67.4%	71.2%	71.2%	100%	65.4%	71.0%
Pharyngeal mucus	10.0	9.6	12.3	10.9	11.0		20.5	10.6
Sputum	8.0	6.7	7.5	10.6	5.5		13.0	7.7
Urine	6.0	7.0	7.0	2.6	6.4			5.3
Blood	1.1	1.3	0.7	0.6	0.9			0.8
Chest fluid	0.7	0.7		0.4	0.3			0.5
Ascites	0.6	0.8	0.7	0.2	0.7			0.5
Feces	0.4	0.2	1.0	0.7	0.4			0.6
Spinal fluid	0.6	0.2	0.6	0.4	0.3			0.4
Bile	0.4	0.2	0.1	0.7	0.1			0.4
Skin piece	0.3	0.1	0.4					0.1
Joint fluid	0.1	0.4		0.4	0.4			0.3
Others	0.4	1.4	2.1	1.4	2.9			1.2

Table 2. *Escherichia coli* isolated from various clinical specimens from June 1965 through May 1971

Year	1965	1966	1967	1968	1969	1970	1971	Total
Number of strains	396	553	776	852	599	483	426	4085
Urine	66.0%	67.6%	70.0%	75.0%	73.8%	80.0%	76.8%	72.8%
Pus	12.0	12.8	12.0	9.2	9.8	8.1	11.0	10.6
Feces	9.3	12.0	9.7	6.5	9.2	5.0	1.4	7.8
Bile	5.6	4.0	2.6	3.2	2.7	2.5	1.9	3.1
Sputum	1.3	1.1	1.5	2.7	1.8	2.7	1.9	1.9
Pharyngeal mucus	2.8	0.9	1.7	1.5	1.0	0.6	2.8	1.5
Blood	0.5	0.2	0.8	0.2	0.3	0.2	1.2	0.5
Ascites	0.3	0.2	0.3	0.4	0.5	0.2	0.7	0.3
Chest fluid	0.3	0.2	0.1			0.2		0.1
Others	1.5	0.5	1.4	0.6	0.8	0.2	1.9	0.9

tracycline (TC), chloramphenicol (CP), streptomycin (SM), kanamycin (KM), penicillin G (PC-G), cephalothin (CET), cephaloridine (CER), erythromycin (EM) and sulfamethoxazol (SMX).

(b) Antibiotics examined for 3 or 2 years: Aminobenzylpenicillin (AB-PC), gentamicin (GM), cephaloglycin (CEG), cephalexin (CEX), cefazolin (CEZ), methylchlorchlorophenylisoxazolyl-penicillin (MCI-PC) and carbobenzylpenicillin (CB-PC).

Determination of MIC value to differentiate the isolates into sensitive and resistant strains

For the evaluation of the MIC values of drugs the following conditions should be considered.

1) Variation of size of hospital and of area where the strains tested were isolated[2].

2) Variation of characters of strains due to difference of clinical specimens from which the strains were isolated[2,3].

3) Variation of quality of the medium due to difference of the date produced.

4) Variation of technique of the investigator[4].

To minimize experimental errors due to variation of quality of the medium and variation of technique of the investigator, the MIC of standard strains for both *S. aureus* and *E. coli* were examined together with the isolated strains. *S. aureus* FDA 209P and *E. coli* mutaflora were tested as control strains. These control strains were examined once every 100 experiments.

Fig. 1. Distribution of MIC of *S. aureus* FDA 209P.

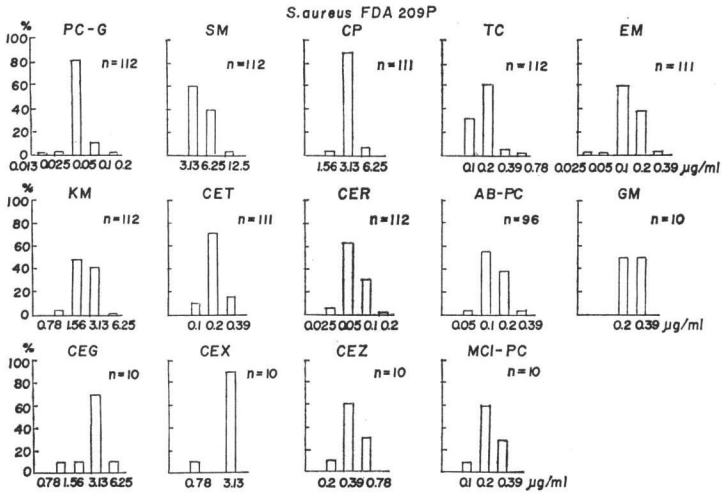
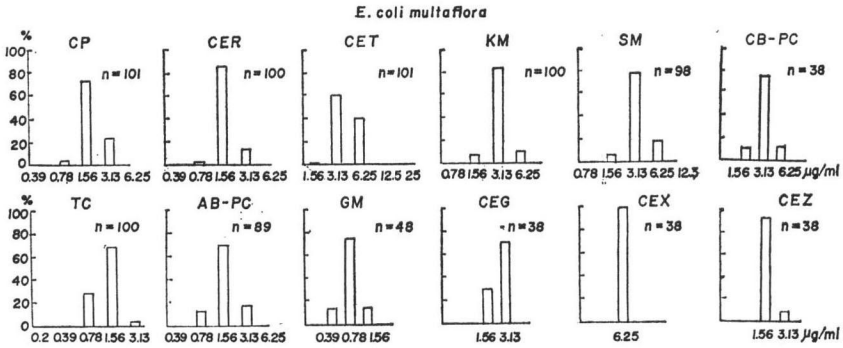


Fig. 2. Distribution of MIC of *E. coli* mutaflora.

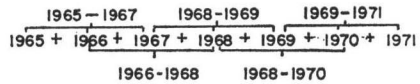


Figs. 1 and 2 show the variation in MIC values for various drugs in the control strains of *S. aureus* and *E. coli*. Due to differences of experimental conditions employed, considerable variations of MIC values were observed in most antibiotics studied.

In a previous paper[1], we have simply classified all the isolates into three groups according to the degree of MIC values of each antibiotic. They were groups of high sensitivity, intermediate sensitivity and low sensitivity. In this grouping, however, sensitivity to specific antibiotics was not considered. In the present study in order to determine the MIC value for grouping the isolates into sensitive- and resistant- groups the following method for the determination was adopted.

MIC values of isolates were pooled for either 2 or 3 years and were ploted in a single figure. The MIC value of the lowest point of the curve between peaks was then used as the grouping value for grouping the isolates into sensitive and resistant.

Fig. 3. A model of moving pooling method for 3 years.



In Fig. 3, a typical model of pooling the MIC values of isolates for 3 years is presented. In this paper we have designated this method as the moving pooling method.

Results

Differentiation of Sensitive and Resistant Strains by the Grouping Value

a) *S. aureus*

Fig. 4-A shows the annual distribution of MIC values for SM. If we determine the lowest point of the curve as the MIC value of SM for grouping of isolates into sensitive and resistant groups, the grouping value for 1965 should be either 12.5 μg/ml or 25 μg/ml. For 1970 and 1971, the grouping values should be 25 μg/ml and 50 μg/ml, respectively.

Fig. 4. Distribution of sensitivity of *S. aureus* to SM. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

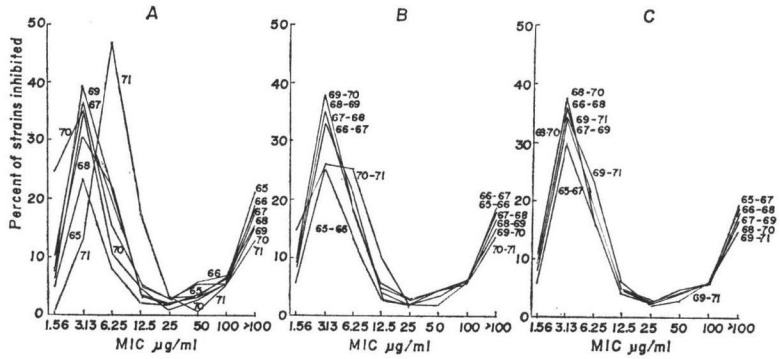
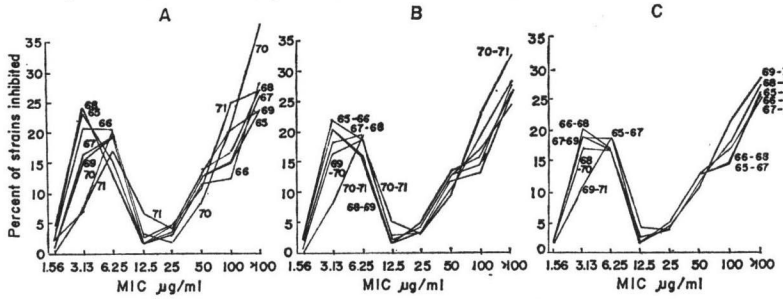


Fig. 5. Distribution of sensitivity of *E. coli* to SM. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.



On the other hand, as shown in the Fig. 4-B, if we pool the MIC values in isolates for 2 years the grouping value for all samples should be 25 μg/ml. Fig 4-C, shows the results of pooling the MIC values in isolates for 3 years. These figures clearly demonstrate that the grouping value for all samples should be 25 μg/ml.

b) *E. coli*

A similar experiment was carried out with *E. coli*, the results being presented in Fig. 5. The annual distribution of MIC values of SM shows that the grouping values for 1970 and 1971 were 25 μg/ml. Pooling the MIC values in isolates for 2 years also show that the grouping values for 1970 and 1971 were 25 μg/ml. However, the grouping values obtained by pooling the MIC values in isolates for 3 years were 12.5 μg/ml.

The above findings indicate that for determination of the MIC value of SM for grouping of the isolates into sensitive and resistant groups, pooling the MIC values for 2 years in *S. aureus* and for 3 years in *E. coli* were necessary.

Determination of the grouping value by pooling the MIC values for more than 3 years was also carried out both in *S. aureus* and *E. coli*, but no shift of the grouping values was observed.

Distribution of MIC values of various antibiotics were studied and the MIC values of each antibiotic for the grouping of isolates into sensitive and resistant groups were determined. The grouping values thus determined are summarized in Table 3. In addition,

Table 3. Grouping values of drugs to differentiate the isolates into sensitive and resistant groups

Staphylococcus aureus			*Escherichia coli*		
Drug	Grouping value	Peak MIC*	Drug	Grouping value	Peak MIC*
CET	1.56	0.39	CET	50.00	12.50
CER	0.78	0.20	CER	25.00	3.13
CEX	12.50	3.13	CEX	50.00	12.50
CEG	12.50	3.13	CEG	25.00	6.25
CEZ	3.13	0.39	CEZ	12.50	1.56
KM	12.50	1.56	KM	25.00	6.25
GM	1.56	0.20	GM	6.25	3.13
SM	25.00	3.13	SM	12.50	12.50
AB-PC	0.39	0.10	AP-PC	25.00	3.13
PC-G	0.20	0.05	CB-PC	50.00	12.50
MCI-PC	0.78	0.20	TC	12.50	3.13
CP	12.50	6.25	CP	25.00	6.25
SMX	125.00	31.30	SMX	3.90	0.50
TC	3.13	0.39			
EM	0.78	0.20			

* Peak MIC of sensitive strains

Fig. 6. Distribution of sensitivity of *S. aureus* to CER. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

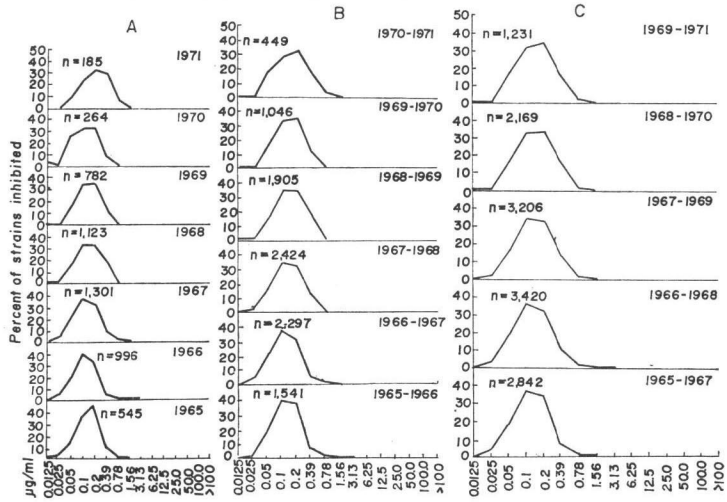


Fig. 7. Distribution of sensitivity of *S. aureus* to AB-PC. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

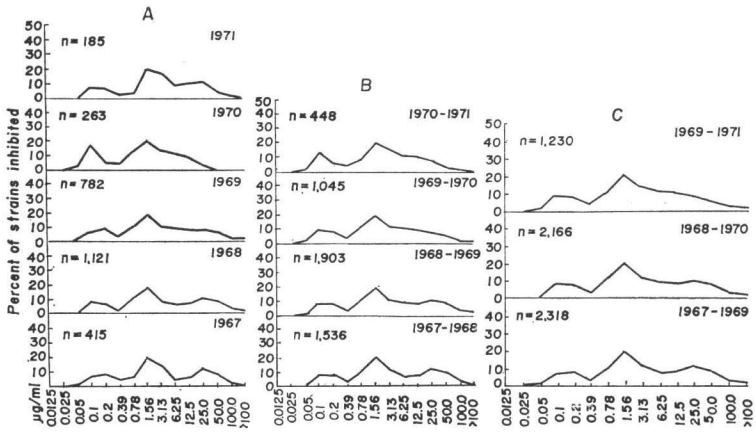


Fig. 8. Distribution of sensitivity of *S. aureus* to TC. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

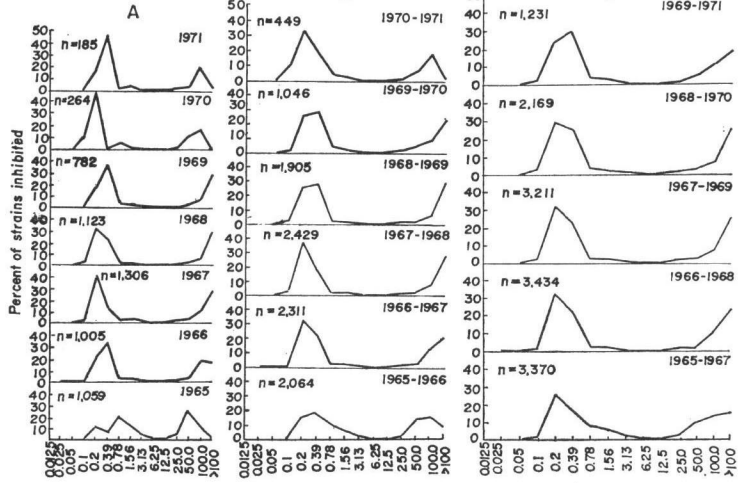


Fig. 9. Distribution of sensitivity of *S. aureus* to EM. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

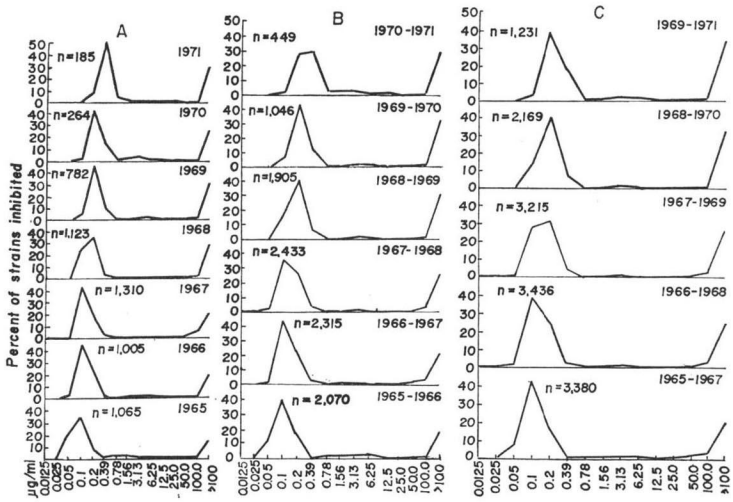
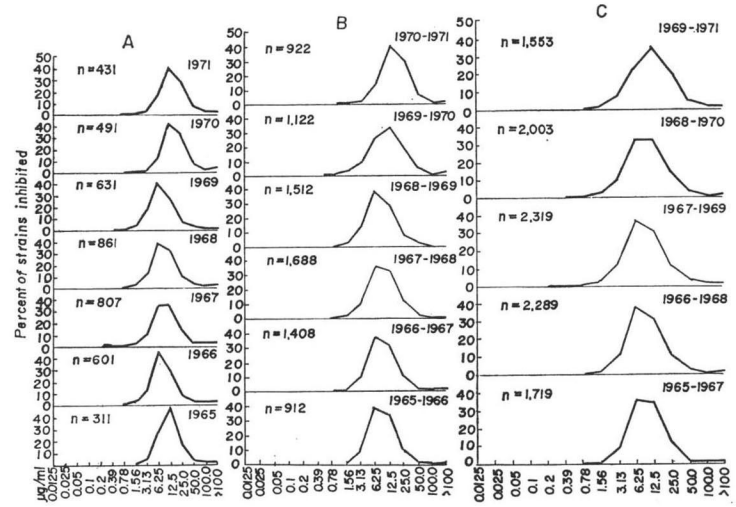


Fig. 10. Distribution of sensitivity of *E. coli* to CET. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.



some of the results of MIC values of various antibiotics determined by the same method are presented in Figs. 6~13. For both *S. aureus* and *E. coli*, the grouping values determined were two or four times higher than the peak MIC values, the latter being the MIC value of the highest number of isoaltes.

Annual Change in Sensitivity of the Bacteria to Various Antibiotics

1) *S. aureus*

Table 4 shows the annual change in sensitivity of *S. aureus* to various antibiotics. The grouping values were determined by the moving pooling method for 2 years. Test of significance of the values shown in Table 4 is presented in Table 5. With PC-G, SM, CP and TC, an increase in sensitivity was observed, while with EM, GM and KM, sensitivity decreased gradually. No significant change was observed in CET, CER and SMX.

2) *E. coli*

A similar determination was made in *E. coli* and the results are presented in Table 6. Increase in sensitivity was not seen with any

Fig. 11. Distribution of sensitivity of *E. coli* to KM. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

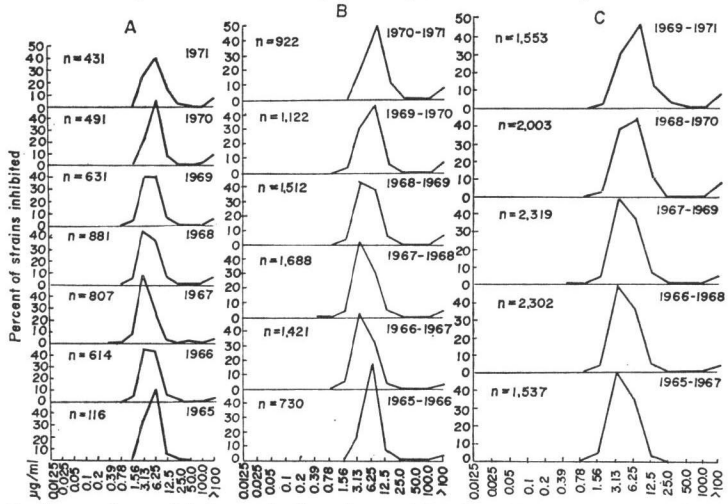


Fig. 12. Distribution of sensitivity of *E. coli* to AB-PC. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

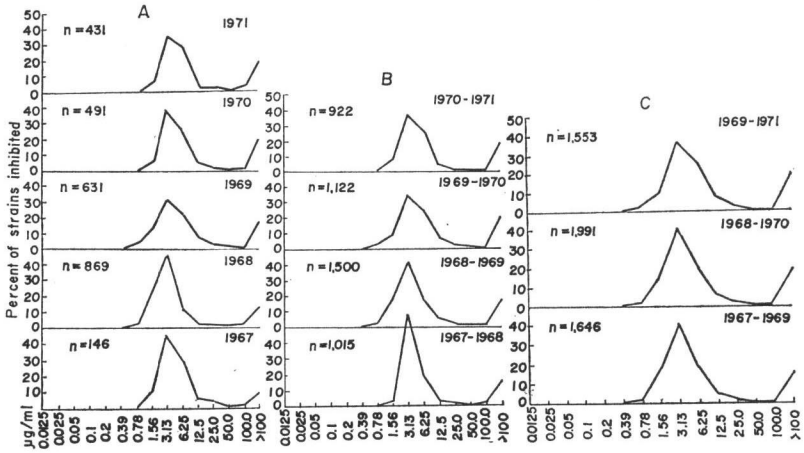


Fig. 13. Distribution of sensitivity of *E. coli* to CP. (A) Annual distribution; (B) Moving pooling method for 2 years; (C) Moving pooling method for 3 years.

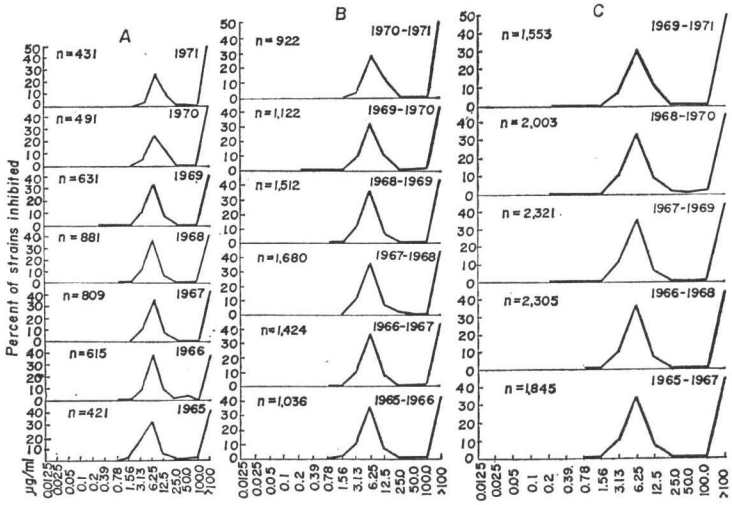


Table 4. Annual change of sensitive strains of *S. aureus* to various drugs*

	'65+'66 (A)		'66+'67 (B)		'67+'68 (C)		'68+'69 (D)		'69+'70 (E)		'70+'71 (F)	
	N	S	N	S	N	S	N	S	N	S	N	S
PC-G	2056	13.5	2304	14.3	2432	14.8	1905	15.7	1046	18.1	449	19.4
SM	2054	67.0	2321	68.2	2439	69.0	1905	70.2	1046	73.3	449	76.6
CP	2061	84.9	2313	86.3	2439	86.8	1904	87.8	1045	89.2	449	87.1
TC	2064	54.9	2311	60.4	2429	60.5	1905	60.1	1046	62.0	449	68.6
EM	2070	70.4	2315	69.1	2433	66.9	1905	63.8	1046	61.2	449	59.7
KM	2041	94.2	2302	91.2	2432	90.7	1903	89.7	1045	99.4	449	88.2
SMX	2060	44.1	2297	48.1	2417	54.5	1901	50.9	1039	38.5	437	25.9
CET	1540	99.7	2299	99.4	2426	99.5	1905	99.7	1046	99.4	449	99.3
CER	1541	99.1	2297	98.7	2424	98.7	1905	99.2	1046	99.1	449	97.1
GM	0	0.0	1601	99.0	0	0.0	0	0.0	0	0.0	449	96.7
AB-PC	0	0.0	0	0.0	0	0.0	1903	16.8	1045	18.7	448	19.6
MCI-PC	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	449	98.9
CEG	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	449	99.9
CEX	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	449	95.1
CEZ	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	449	98.7

Note : N : Number of strains examined S : Percent of sensitive strains.
* The grouping values of each drug were determined by the moving pooling method for 2 years.

Table 5. Test of significance of the experiment in Table 4*

	A-C	A-D	A-E	A-F	B-D	B-E	B-F	C-E	C-F	D-F
PC-G	—	*	**	**	•	**	**	*	*	*
SM	•	*	**	**	•	**	**	**	**	**
CP	*	**	**	—	•	*	—	*	—	—
TC	**	**	**	**	—	—	**	—	**	**
EM	**	**	**	**	**	**	**	**	**	•
KM	**	**	**	**	•	**	*	*	•	—
SMX	**	**	**	**	*	**	**	**	**	**
CET	—	—	—	—	—	—	—	—	—	—
CER	—	—	—		•	—	*	—	*	**
GM							**			
AB-PC										•
MCI-PC										
CEG										
CEX										
CEZ										

Note: ** P=0.01 * P=0.05 • P=0.1 — Not significant
* By the exact probability method by R.A. FISHER

of the antibiotics used. Decrease in sensitivity was observed in CP, TC, KM and CET. A slight decrease in sensitivity was observed in CER and SEX. In AB-PC, though the period of the experiment was not long, decrease of sensitivity was seen. In GM, though the experiment was interrupted once, no change of sensitivity was observed. Test of significance of the values shown in Table 6 is presented in Table 7.

Table 6. Annual change of sensitive strains of *E. coli* to various drugs*

	'65+'66 (A)		'66+'67 (B)		'67+'68 (C)		'68+'69 (D)		'69+'70 (E)		'70+'71 (F)	
	N	S	N	S	N	S	N	S	N	S	N	S
SMX	1008	35.8	1363	34.6	1635	34.5	1473	37.0	1107	35.3	901	31.0
CP	1036	55.8	1424	54.5	1690	53.6	1512	54.1	1122	50.5	922	44.9
CER	0	0.0	1410	93.6	1685	91.8	1512	90.1	1122	85.8	922	82.6
CET	912	95.6	1408	94.9	1688	94.4	1512	94.7	1122	92.4	922	88.3
GM	0	0.0	1259	99.6	0	0.0	0	0.0	0	0.0	901	99.7
KM	0	0.0	1421	95.7	1688	95.0	1512	93.6	1122	90.7	922	88.6
SM	938	43.3	1407	39.2	1690	38.1	1512	38.0	1122	32.1	922	27.3
TC	1023	45.2	1418	40.8	1684	40.1	1512	40.8	1122	36.4	922	34.6
AB-PC	0	0.0	0	0.0	0	0.0	1500	82.0	1122	77.0	922	76.8
CB-PC	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	922	78.0
CEG	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	922	90.2
CEX	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	922	95.8
CEZ	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	922	92.0

Note: N: Number of strains examined S: Percent of sensitive strains
* The grouping values of each drug were determined by the Table 3.

Table 7. Test of significance of the experiment in Table 6

	A-C	A-D	A-E	A-F	B-D	B-E	B-F	C-E	C-F	D-F
SMX	—	—	—	*	—	—	*	—	*	**
CP	—	—	**	**	—	*	**	•	**	**
CER					**	**	**	**	**	**
CET	—	—	**	**	—	**	**	*	**	**
GM							—			
KM					**	**	**	**	**	**
SM	**	**	**	**	—	**	**	**	**	**
TC	**	*	**	**	—	*	**	*	**	**
AB-PC										**
CB-PC										
CEG										
CEX										
CEZ										

Note: ** P=0.01 * P=0.05 • P=0.1 — Not significant

Table 8. Annual change of sensitivity of *S. aureus* and *E. coli*

	S. aureus	*E. coli*
Group 1. Increase of sensitivity	PC-G. AB-PC. SM. CP. TC.	None
Group 2. No change of sensitivity	CET. CER*	GM. SMX*
Group 3. Decrease of sensitivity	EM. GM. KM. SMX	CP. CET. CER. KM. SM. TC. AB-PC.

* Almost no change

Discussion

The MIC values of *S. aureus* and *E. coli* isolated from clinical specimens were examined and the MIC values for the grouping of isolates into sensitive and resistant groups were determined by the moving pooling method for 2 or 3 years. In *S. aureus*, distribution of MIC values of EM and TC gave a two-peak-curve. CP, TC, SMX gave a similar curve in *E. coli*. However, with other antibiotics, the distribution of MIC values gave a normal distribution curve. Thus it is difficult to determine the grouping value of these antibiotics. In a previous paper[1], we determined a certain grouping value for all antibiotics and differentiated the clinical isolates into highly sensitive, intermediate sensitivity and low sensitivity. ERICSSON[6] reported the grouping of bacteria by certain values into 4 groups for each drug. However the values were applied to all types of bacteria isolated and the determination of clinical dosage of antibiotics was discussed in relation to the blood level of the drug. The determination of the grouping values presented in this paper is more ideal than the other determinations reported previously.

It was found that the moving pooling method for 2 or 3 years is ideal to get the grouping value, since pooling for more than 4 years makes it difficult to observe annual changes in sensitivity of the bacteria to the drug.

The grouping value determined in this study might not be applicable as the only consideration in the clinical usage of the antibiotics. However, together with a consideration of other characteristics, method of application and pharmacokinetic properties of the drug, the grouping value might contribute important information for the clinical application of the antibiotics.

In *S. aureus*, an increase in sensitivity to PC-G, SM, CP and TC was seen. This may be due to the recent application of new derivatives of penicillin and cephalosporin to patients. Observation of decrease in sensitivity in *E. coli* not only to various antibiotics which have been used frequently for a long time but also to new antibiotics may be mainly controlled by R factors.

The MIC values of various antibiotics for *S. aureus* and *E. coli* isolated from clinical specimens were determined to enable the isolates to be grouped into sensitive and resistant strains. To minimize the effects of various variable conditions, the grouping values were determined by the moving pooling method for 2 or 3 years. The values were determined on the basis of MIC values of the drugs in *S. aureus* and *E. coli* under investigations. Annual change in sensitivity of the bacteria to various drugs found in this study is summarized (Table 8).

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