

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

### GENERAL TENDENCY OF RESISTANT STRAINS

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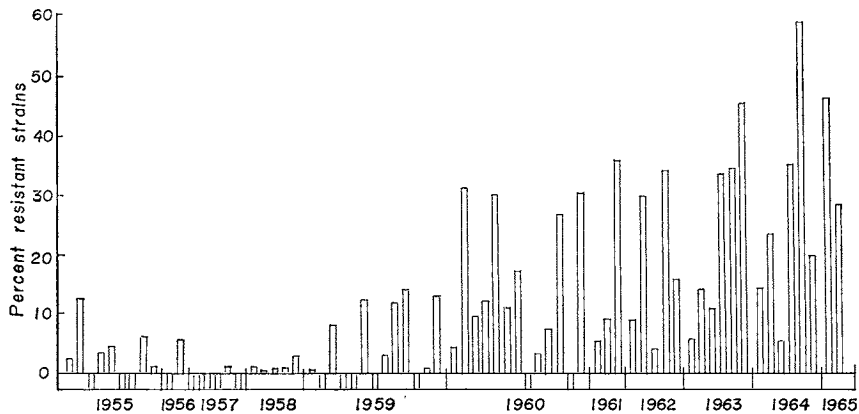
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To evaluate the changes of the susceptibilities of *Staphylococcus aureus* and *Escherichia coli* clinical isolates over a four-year period from 1965 through 1968, we have conducted a series of studies. Results from these studies were used to assess the changes of bacterial sensitivities within groups of bacteria classified according to minimal inhibitory concentrations (MIC) values or having high sensitivity, intermediate sensitivity, or low sensitivity. We were able to compare changes in antibiotic activities more accurately by this technique than by our previous method of comparison of the averages of the MIC values only.

For more than a decade, several investigators have performed studies on sensitivities of various pathogenic bacteria to antibiotics and chemotherapeutic agents used in Japan. Results of several long-term studies, including the first extensive investigation by the National Hospital Research Group on pathogenic staphylococci, headed by KOSAKAI<sup>1)</sup>, are published. The work by the Resistant Staphylococci Research Group, headed by ICHIKAWA<sup>2)</sup>, has continued since 1961. Each of these many studies has had a different purpose of its own; therefore, as one manufacturer of antibiotics, we have not been fully satisfied with the results reported. For example, the data shown in Fig. 1 summarizes the results on the resistance of staphylococci to erythromycin as reported in Japanese scientific journals<sup>3)</sup> between 1955 and 1965. The general trend of a gradual increases in the rate of resistant strains is evident; however, there is a marked fluctuation among individual tests caused by differences in methods of sampling, testing, etc.

To more accurately evaluate the trends of bacterial resistance to antibiotics, we have conducted a series of studies since 1964 by a different procedure that is described below. Bacterial isolates have been collected twice yearly (Nov.~May and June~Oct.) from central clinical laboratories throughout Japan. Nine studies have been completed so far to October 1969. Because data from the first study (Nov.~May 1964) were not obtained with uniform methods or drugs, our present report includes results obtained on staphylococcal and *Escherichia coli* isolates from the eight subsequent studies.

Fig. 1. Erythromycin-resistant staphylococci reported in journals between 1955~1966.



One block in the figure is related to one paper. The height of the blocks on the zero line shows percentage of resistant strains reported in the papers cited. The blocks extending below the zero line, without regard to its length, means no resistant strain.

## I. Design of Investigation

### 1. Objective

Our objective was to investigate the annual trends of bacterial resistance to antibiotics and sulfonamides frequently used in hospitals.

### 2. Methods

- (1) Bacterial isolates and hospitals were selected by a statistical sampling method.
- (2) Bacterial isolates were sampled in the central clinical laboratories or other equivalent laboratories of hospitals.
- (3) Testing method
  - 1) Identification of isolate.
  - 2) Isolated strains were propagated in a modified MULLER-HINTON'S broth medium (37°C, 18 hours).
  - 3) A loopful from each culture was suspended in 5 ml of saline with a 1 mm standard platinum loop.
  - 4) Culture Media: Modified MULLER-HINTON'S medium was mainly used except for the sulfanamide testing *E. coli* isolates where SAUTON medium was employed.

Drug-sensitivity tests were performed with the two-fold agar-dilution method with the same media.

1 mm platinum loop. Agar streak method. (about  $10^4$  cells, 37°C, 18 hours)  
MULLER-HINTON Medium (Modified)

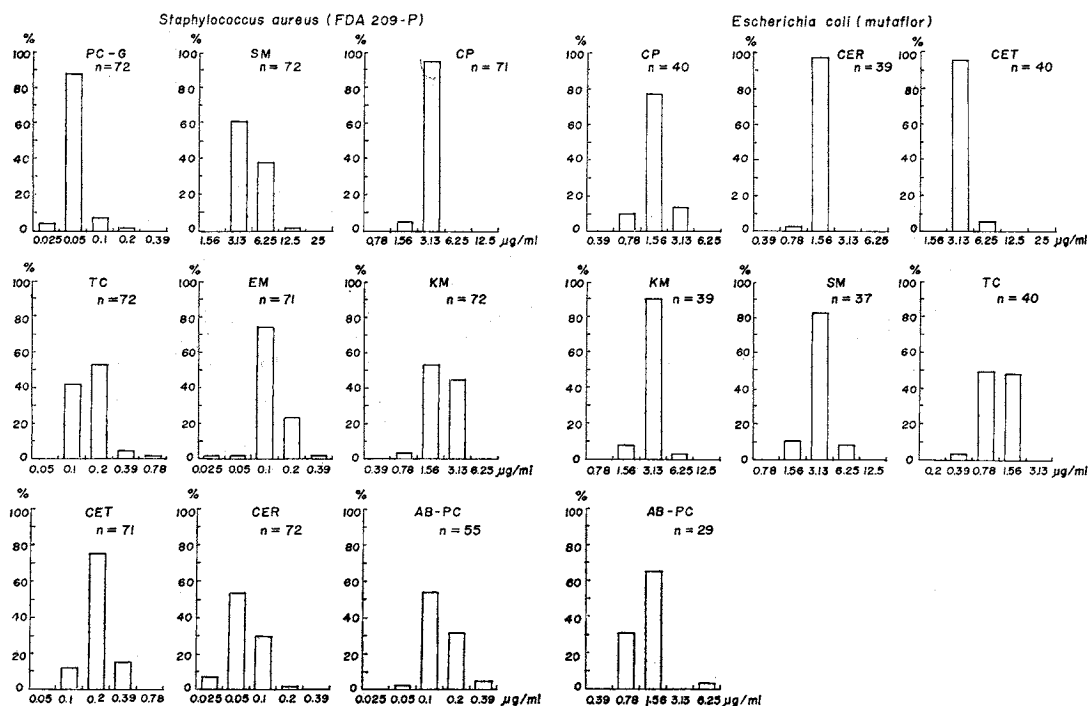
Beef Heart Infusion	200.0 ml	L-Cystine	0.05 g
Casamino Acids	16.5 g	Biotin	5.0 $\mu$ g
Starch	1.5 g	Agar	15.0 g
Glucose	2.0 g	Aq. Dest	1,000.0 g
L-Tryptophan	0.05 g		(pH 7.4 $\pm$ 0.1)

Inoculum About  $3\sim 4 \times 10^6$  cells, 37°C, 18 hours.

SAUTON Medium (Modified) KRUGER-THIMER

Asparagine	4.0 g	Ferric ammonium citrate	0.05 g
KH <sub>2</sub> PO <sub>4</sub>	0.5 g	Glycerol	20.0 ml
Citric Acid	2.0 g	Aq. Dest	1,000.0 ml
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5 g		(pH 7.0)

Fig. 2. Result of tests on control strains.



5) For the control, standard strains *S. aureus* FDA 209-P and *E. coli* Mutaflor (KRÜGER)<sup>4</sup> were used. The results with these strains are shown in Fig. 2.

### 3. Chemotherapeutic agents tested

#### *S. aureus*:

Erythromycin (EM), Penicillin G (PC-G), Streptomycin (SM), Chloramphenicol (CP), Tetracycline (TC), Kanamycin (KM), Cephalothin (CET), Cephaloridine (CER), Sulfisomezole (SIZ), Aminobenzyl-penicillin (AB-PC)

#### *E. coli*:

CP, TC, SM, KM, CET, CER, SIZ, AB-PC

4. Period of study: June 1965~May 1969

#### 5. Items for analysis

- (1) Results of sampling
- (2) Changes: (a) General tendency, (b) Multiple drug resistance
- (3) Grouping by districts (7)
- (4) Grouping by the size of hospitals (expressed in number of beds)
- (5) Grouping by inpatients and outpatients
- (6) Grouping by the age
- (7) Grouping by the sources of strains

## II. Sampling

Hospitals from which bacterial isolates were collected: Among the general

hospitals equipped with central clinical laboratories, 10 % of the larger hospitals were selected in each prefecture.

### III. Results of sampling

1. Districts, sampling hospitals and number of strains (Tables 1, 2 and 3).
2. Correlation analysis of the results of sampling (Table 4).

The correlation analysis of the factors involved have made possible the assumption of the drug sensitivities of the isolates submitted to central clinical laboratories of the hospitals in Japan.

Table 1.

District	Population (000)	Hospitals No.	Sampling hospitals No.	No. of strains		
				<i>S. aureus</i>	<i>E. coli</i>	Total
I Chiba, Tokyo, Kanagawa	18,710	568	60	1,205	753	1,958
II Kyoto, Shiga, Osaka, Hyogo, Nara, Wakayama	16,498	594	62	776	489	1,265
III Hokkaido, Aomori, Iwate, Miyagi, Akita, Yamagata, Fukushima	14,751	340	50	512	257	769
IV Gifu, Shizuoka, Aichi, Mie	11,433	274	45	622	320	942
V Tottori, Shimane, Okayama, Hiroshima, Yamaguchi, Tokushima, Kagawa, Ehime, Kochi	11,188	324	27	322	169	491
VI Fukuoka, Saga, Nagasaki, Kumamoto, Oita, Miyazaki, Kagoshima	12,770	246	37	415	298	713
VI Ibaragi, Tochigi, Gumma, Saitama, Toyama, Niigata, Ishikawa, Fukui, Yamanashi, Nagoya	16,639	293	64	622	410	1,032
Total	101,989	2,639	345	4,474	2,696	7,170

Table 2. Numbers of *Staphylococcus aureus* (Coagulase positive) strains classified by years and sources. (Period: June 1965~May 1969)

Specimen	1965		1966		1967		1968		Total	
	No. of strains	%	No. of strains	%	No. of strains	%	No. of strains	%	No. of strains	%
Pus	707	71.2	671	71.3	827	67.4	733	71.1	2,938	70.1
Pharyngeal mucus	101	10.2	90	9.6	151	12.3	111	10.8	453	10.8
Sputum	80	8.0	63	6.7	92	7.5	113	10.9	348	8.3
Urine	58	5.8	66	7.0	89	7.2	27	2.6	240	5.7
Feces	4	0.4	2	0.2	14	1.1	8	0.8	28	0.7
Blood	11	1.1	12	1.3	8	0.7	7	0.7	38	0.9
Spinal fluid	6	0.6	2	0.2	7	0.6	4	0.4	19	0.5
Punctate	4	0.4	8	0.8	8	0.7	2	0.2	22	0.5
Ascites	2	0.2							2	
Thoracic fluid	7	0.7	7	0.7	5	0.4	4	0.4	23	0.5
Bile	4	0.4	2	0.2	8	0.7	8	0.8	22	0.5
Joint fluid	1	0.1	4	0.4	3	0.2	3	0.3	11	0.3
Washout liquid	2	0.2	1	0.1	1	0.2			4	0.1
Skin piece	3	0.3	1	0.1					4	0.1
Others	4	0.4	13	1.4	13	1.1	11	1.0	41	1.0
Total	994	100.0	942	100.0	1,226	100.0	1,031	100.0	4,193	100.0

Table 3. Numbers of *Escherichia coli* strains classified by years and sources. (Period: June 1965~May 1969)

Specimen	1965		1966		1967		1968		Total	
	No. of strains	%	No. of strains	%	No. of strains	%	No. of strains	%	No. of strains	%
Pus	48	12.1	71	12.8	92	11.8	75	9.2	286	11.3
Pharyngeal mucus	11	2.8	5	0.9	13	1.7	13	1.6	42	1.7
Sputum	5	1.2	6	1.1	12	1.5	22	2.7	45	1.8
Urine	262	66.1	374	67.6	543	70.0	619	76.1	1,798	70.8
Feces	37	9.3	67	12.1	75	9.7	46	5.6	225	8.9
Blood	2	0.5	1	0.2	6	0.8	2	0.2	11	0.4
Spinal fluid			1	0.2	1	0.1	3	0.4	5	0.2
Punctate							1	0.1	1	
Ascites	1	0.3	1	0.2	2	0.3	2	0.2	6	0.2
Thoracic fluid	1	0.3	1	0.2	1	0.1			3	0.1
Bile	22	5.6	22	4.0	20	2.6	26	3.2	90	3.5
Joint fluid										
Washout liquid	1	0.3	1	0.2			1	0.1	3	0.1
Skin piece										
Others	6	1.5	3	0.5	11	1.4	5	0.6	25	1.0
Total	396	100.0	553	100.0	776	100.0	815	100.0	2,540	100.0

#### IV. Results of Annual Investigations of Drug Sensitivities

##### 1. Methods

In comparing the data of annual investigations of bacterial drug sensitivities, we compared the average values of the M. I. C. of the total isolates studied as we have reported previously<sup>6</sup>. This comparison was made on the assumption that the levels of sensitivities of the isolates to each drug give a normal distribution curve, but it has been found that this assumption does not fit all the drugs used. We have, therefore, classified the bacterial isolates into groups of high sensitivity, intermediate sensitivity and low sensitivity according to their M.I.C. values in the present investigation. Annual changes of the bacterial drug sensitivities were compared statistically in terms of the frequencies of these three groups. The bacteria studied were those isolated from various specimens. The results are reported below for each drug.

##### 2. *Staphylococcus aureus*

###### (1) Penicillin-G

*S. aureus* cultures of moderate susceptibility to penicillin were isolated less frequently in 1968 than in 1965. The isolates seem to be more commonly either highly susceptible or highly resistant to the antibiotic. A decrease in isolation of cultures placed in the high sensitivity group occurred in 1966 compared with 1965, whereas an increase was observed for isolates in the low sensitivity group. An increase only of cultures in the low sensitivity group was noted between 1966 and 1967. Between 1967 and 1968, isolates in the high sensitivity group

Table 4. Multiple correlation coefficients for the items shown in Table 1.

Items	Level of significance
A — B C D E	P=0.01
B — A C D E	"
C — A B D E	"
D — A B C E	"
E — A B C D	"

(Remarks)

- A : Population
- B : Total number of hospitals
- C : Number of sampling hospitals
- D : Number of *S. aureus* strains
- E : Number of *E. coli* strains

increased and cultures in the intermediate sensitivity group decreased. Thus a marked increase in numbers of cultures isolated in the low sensitivity group occurred between 1965 and 1968.

M. I. C. u/ml	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	624	483	592	563	58.81	48.54	45.23	52.37
3.13 ~ 12.5	301	289	367	201	28.37	29.05	28.04	18.70
25.0 ~ $\geq$ 100	136	223	350	311	12.82	22.41	26.74	28.93
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56	**	**	**		—	•	**	
3.15 ~ 12.5	—	—	**		—	**	**	
25.0 ~ $\geq$ 100	**	**	**		*	**	—	

Note : \*\* P=0.01   \* P=0.05   • P=0.1   — no significance

## (2) Streptomycin

High-sensitivity group decreased between 1965 and 1966, while a remarkable increase in intermediate-sensitivity group took place in this period.

Almost no change was found in any group between 1966 and 1967, whereas a slight increase in high-sensitivity group and a decrease in intermediate-sensitivity group were observed between 1967 and 1968. As a result, a profound decrease in high-sensitivity group and a marked increase in intermediate-sensitivity group occurred between 1965 and 1968. Highly resistant group with M.I.C. above 100  $\mu$ g/ml somewhat decreased in this period.

M. I. C. $\mu$ g/ml	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~3.13	602	414	578	526	57.39	41.19	43.92	48.93
6.25 ~12.5	104	257	333	219	9.91	25.57	25.30	20.37
25.0 ~100.0	120	147	154	145	11.44	14.63	11.70	13.49
>100	223	187	251	185	21.26	18.61	19.07	17.21
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~3.13	**	**	**		—	**	*	
6.25 ~12.5	**	**	**		—	**	**	
25.0 ~100.0	*	—	—		*	—	—	
>100	—	—	*		—	—	—	

## (3) Chloramphenicol

High-sensitivity group showed some increase between 1965 and 1966, but both intermediate-sensitivity group and low-sensitivity group decreased in this period. There was no change in the frequencies of the three groups between 1966 and 1967. Between 1967 and 1968, high-sensitivity group decreased and intermediate-sensitivity group increased considerably, whereas low-sensitivity group did not show a change. Both high-sensitivity group and low-sensitivity group decreased between 1965 and 1968, while intermediate-sensitivity group exhibited a remarkable increase in this period.

M. I. C. $\mu$ g/ml	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 3.13	700	700	904	592	65.79	70.21	68.69	55.07
6.25 ~ 12.5	201	161	235	356	18.89	16.15	17.86	33.12
25.0 ~ $\geq$ 100	163	136	177	127	15.32	13.64	13.45	11.81

	'65~'66	'65~'67	'65~'68	'66~'67	'66~'68	'67~'68
0.0125~ 3.13	*	—	**	—	**	**
6.25 ~ 12.5	—	—	**	—	**	**
25.0 ~ $\geq$ 100	—	—	*	—	—	—

## (4) Tetracycline

A considerable increase in high-sensitivity group and a decrease in both intermediate-sensitivity group and low-sensitivity group were noted between 1965 and 1966. Almost no change was observed in the frequencies of the three groups between 1967 and 1968. An identical tendency was found also between 1967 and 1968. Thus a marked increase in high-sensitivity group and a decrease in both intermediate-sensitivity group and low-sensitivity group were observed between 1965 and 1968.

M.I.C. $\mu$ g/ml	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	529	604	792	651	49.95	60.10	60.64	60.56
3.13 ~ 12.5	64	18	11	10	6.04	1.79	0.84	0.93
25.0 ~ $\geq$ 100	466	383	503	414	44.00	38.11	38.51	38.51
	'65~'66	'65~'67	'65~'68	'66~'67	'66~'68	'67~'68		
0.0125~ 1.56	**	**	**	—	—	—		
3.13 ~ 12.5	**	**	**	*	.	—		
25.0 ~ $\geq$ 100	**	**	**	—	—	—		

## (5) Erythromycin

A decrease in high-sensitivity group and an increase in low-sensitivity group occurred between 1965 and 1966. Intermediate-sensitivity group decreased and low-sensitivity group increased between 1966 and 1967. An increase in low-sensitivity group was found between 1967 and 1968 but no change was observed in the other groups in this period. Consequently, a marked decrease in high-sensitivity group and a decrease in intermediate-sensitivity group occurred between 1965 and 1968 resulting in a remarkable increase in low-sensitivity group.

M.I.C. $\mu$ g/ml	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	819	726	907	714	76.90	72.24	69.24	66.42
3.13 ~ 12.5	56	52	34	23	5.26	5.17	2.60	2.14
25.0 ~ $\geq$ 100	190	227	369	338	17.84	22.59	28.17	31.44
	'65~'66	'65~'67	'65~'68	'66~'67	'66~'68	'67~'68		
0.0125~ 1.56	*	**	**	—	**	—		
3.13 ~ 12.5	—	**	**	**	**	—		
25.0 ~ $\geq$ 100	**	**	**	**	**	.		

## (6) Kanamycin

High-sensitivity group decreased considerably between 1965 and 1966, and intermediate-sensitivity group and low-sensitivity group increased in this period. There was almost no change in any group between 1966 and 1967 and between 1967 and 1968. Consequently, a considerable decrease in high-sensitivity group and a remarkable increase in intermediate-sensitivity group were observed between 1965 and 1968. An increase in intermediate-sensitivity group was also found in this period.

M.I.C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	869	676	670	572	82.84	68.15	51.15	53.26
3.13 ~ 12.5	144	244	546	428	13.73	24.60	41.68	39.85
25.0 $\sim\geq$ 100	36	72	94	74	3.43	7.26	7.18	6.89
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56	**	**	**		**	**	—	
3.13 ~ 12.5	**	**	**		**	**	—	
25.0 $\sim\geq$ 100	**	**	**		—	—	—	

## (7) Cephalothin

Only high-sensitivity group was found to this drug and no change was observed from 1965 through 1968.

M.I.C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 0.78	543	992	1294	1073	99.82	99.60	99.31	99.81
1.56 ~ 12.5	1	4	9	2	0.18	0.42	0.69	0.19
25.0 $\sim\geq$ 100	0	0	0	0	0.00	0.00	0.00	0.00
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 0.78	—	—	—		—	—	.	
1.56 ~ 12.5	—	—	—		—	—	.	
25.0 $\sim\geq$ 100								

## (8) Cephaloridine

Only high-sensitivity group was found also to this drug and no change was observed from 1965 through 1968.

M.I.C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 0.78	545	994	1297	1075	100.00	99.80	99.69	100.00
1.56 ~ 12.5	0	2	4	0	0.00	0.20	0.31	0.00
25.0 $\sim\geq$ 100	0	0	0	0	0.00	0.00	0.00	0.00
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 0.78	—	—	—		—	—	.	
1.56 ~ 12.5	—	—	—		—	—	.	
25.0 $\sim\geq$ 100								

## (9) Aminobenzyl-penicillin

No change was found in the frequencies of the three groups between 1967 and 1968.

M.I.C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	0	0	200	533	0.00	0.00	48.19	49.67
3.13 ~ 12.5	0	0	110	275	0.00	0.00	26.51	25.63
25.0 $\sim\geq$ 100	0	0	105	265	0.00	0.00	25.30	24.70
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56							—	
3.13 ~ 12.5							—	
25.0 $\sim\geq$ 100							—	

3. *Escherichia coli*

## (1) Chloramphenicol

Between 1965 and 1966, high-sensitivity group decreased markedly and intermediate-sensitivity group increased considerably, whereas no change was found in the frequency of low-sensitivity group. A slight decrease was observed in intermediate-sensitivity group between 1966 and 1967. Thus a remarkable



decrease in high-sensitivity group and a moderate increase in intermediate-sensitivity group took place between 1965 and 1968, while no change was found in low-sensitivity group in this period.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 3.13	81	53	92	103	19.24	8.62	11.37	12.20
6.25 ~ 12.5	149	295	336	354	35.39	47.97	41.53	41.94
25.0 $\sim \geq 100$	191	267	381	387	45.37	43.41	47.10	45.85
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 3.13	**	**	**		.	*	—	
6.25 ~ 12.5	**	*	*		*	*	—	
25.0 $\sim \geq 100$	—	—	—		—	—	—	

## (2) Cephaloridine

A decrease in high-sensitivity group and a considerable increase in intermediate-sensitivity group occurred between 1966 and 1967. A similar tendency was observed also between 1967 and 1968; high-sensitivity group decreased and intermediate-sensitivity group increased. Accordingly, a marked decrease in high-sensitivity group was accompanied by a profound increase in intermediate-sensitivity group between 1966 and 1968. There was some increase in low-sensitivity group in this period.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	12	360	403	370	80.00	59.41	50.12	43.84
3.13 ~ 12.5	3	228	375	433	20.00	37.62	46.64	51.30
25.0 $\sim \geq 100$	0	18	28	41	0.00	2.97	3.23	4.86
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56	.	*	**		**	**	*	
3.13 ~ 12.5	—	*	*		**	**	.	
25.0 $\sim \geq 100$	—	—	—		—	.	.	

## (3) Cephalothin

There were some increase in high-sensitivity group, a great increase in intermediate-sensitivity group and a marked decrease in low-sensitivity group between 1965 and 1966. Intermediate-sensitivity group decreased and low-sensitivity group increased between 1966 and 1967. A slight increase in high-sensitivity group and a slight decrease in low-sensitivity group were found between 1967 and 1968. Consequently, a tendency of increase in high-sensitivity group and decrease in low-sensitivity group was observed between 1965 and 1968.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	1	10	11	23	0.32	1.66	1.36	2.73
3.13 ~ 12.5	249	515	636	684	80.06	85.69	68.81	81.04
25.0 $\sim \geq 100$	61	76	160	137	19.61	12.65	19.83	16.23
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56	.	—	**		—	—	.	
3.13 ~ 12.5	*	—	—		**	*	—	
25.0 $\sim \geq 100$	**	—	—		**	.	.	

## (4) Kanamycin

Intermediate-sensitivity group decreased between 1965 and 1966, and high-sensitivity group and low-sensitivity group increased in this period.

High-sensitivity group further increased between 1966 and 1967. High-sensitivity group decreased between 1967 and 1968 and low-sensitivity group showed a tendency of increase in this period. Thus a decrease in intermediate-sensitivity group and an increase in both high-sensitivity group and low-sensitivity group were observed between 1966 and 1968.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	0	23	54	32	0.00	3.75	6.69	3.79
3.13 ~ 12.5	115	565	720	762	99.14	91.69	89.22	90.28
25.0 $\sim \geq 100$	1	28	33	50	0.86	4.56	4.09	5.92
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56	*	**	*		*	—	**	
3.13 ~ 12.5	**	**	**		—	—	—	
25.0 $\sim \geq 100$	*	.	**		—	—	.	

#### (5) Streptomycin

A slight decrease in high-sensitivity group and some increase in intermediate-sensitivity group were found between 1965 and 1966. Both high-sensitivity group and intermediate-sensitivity group decreased and low-sensitivity group increased between 1966 and 1967. A decrease in intermediate-sensitivity group and an increase in high-sensitivity group took place between 1967 and 1968. The overall tendency between 1965 and 1968 was that almost no change occurred in this period.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~3.13	95	137	139	231	27.94	22.91	17.18	27.37
6.25 ~12.5	57	132	165	115	16.76	22.07	20.40	13.63
25.0 ~100	107	161	286	280	31.47	26.92	35.35	33.18
>100	81	168	219	218	23.82	28.09	27.07	25.83
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~3.13	.	**	—		**	.	**	
6.25 ~12.5	.	—	—		—	**	**	
25.0 ~100	—	—	—		**	*	—	
>100	—	—	—		—	—	—	

#### (6) Tetracycline

High-sensitivity group increased considerably and both intermediate-sensitivity group and low-sensitivity group decreased between 1965 and 1966. An increase in low-sensitivity group and a decrease in intermediate-sensitivity group occurred between 1966 and 1967. Low-sensitivity group decreased and intermediate-sensitivity group increased between 1967 and 1968. The overall change between 1965 and 1968 was a marked decrease in high-sensitivity group, but there was no change in low-sensitivity group.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 1.56	19	129	173	172	4.66	20.98	21.54	20.38
3.13 ~ 12.5	168	158	135	200	41.18	25.69	16.81	23.70
25.0 $\sim \geq 100$	221	328	495	472	54.17	53.33	61.64	55.92
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 1.56	**	**	**		—	—	—	
3.12 ~ 12.5	**	**	**		**	—	**	
25.0 $\sim \geq 100$	—	*	—		**	—	*	

## (7) Aminobenzyl penicillin

A considerable decrease in intermediate-sensitivity group accompanied by an increase in high-sensitivity group and low-sensitivity group was observed between 1967 and 1968.

M. I. C. $\mu\text{g/ml}$	Number of strains				Percentage			
	'65	'66	'67	'68	'65	'66	'67	'68
0.0125~ 3.13	0	0	80	599	0.00	0.00	54.79	72.00
6.25 ~ 12.5	0	0	50	111	0.00	0.00	34.25	13.34
25.0 $\sim \geq 100$	0	0	16	122	0.00	0.00	10.96	14.66
	'65~'66	'65~'67	'65~'68		'66~'67	'66~'68	'67~'68	
0.0125~ 3.13							**	
6.25 ~ 12.5							**	
25.0 $\sim \geq 100$							—	

## Discussion and Conclusion

As we have described above, we have been able to learn the changes of bacterial sensitivities to each drug more accurately through comparing the annual changes of the high-sensitivity group, intermediate-sensitivity group and low-sensitivity group, which were classified according to the M. I. C. values of the bacterial isolates, than by our previous method of comparison of the annual averages of the M. I. C. values.

(1) In *S. aureus*, both high-sensitivity group and intermediate-sensitivity group decreased and low-sensitivity group increased against penicillin-G, erythromycin and kanamycin. A decrease in high-sensitivity group and a considerable increase in intermediate-sensitivity group accompanied by either a decrease or no change in low-sensitivity group were found in *S. aureus* against chloramphenicol and streptomycin. High-sensitivity group increased and both intermediate-sensitivity group and low-sensitivity group decreased against tetracycline.

(2) In *E. coli*, both high-sensitivity group and low-sensitivity group increased against aminobenzyl-penicillin and kanamycin. Only high-sensitivity group increased against cephalothin. High-sensitivity group decreased and both intermediate-sensitivity group and low-sensitivity group increased against cephaloridine. A marked decrease in high-sensitivity group and an increase in intermediate-sensitivity group without a

Table 5.

	Group	Drug								
		PC-G	AB-PC*	SM	CP	TC	KM	EM	CET	CER**
<i>S. aureus</i>	High-sensitivity group	↓	→	↓	↓	↑	↓	↓	→	→
	Intermediate-sensitivity group	↓	→	↑	↑	↓	↑	↓	→	→
	Low-sensitivity group	→	→	→	↘	↓	↑	↑	→	→
<i>E. coli</i>	High-sensitivity group		↑	→	↓	↑	↑		↑	↓
	Intermediate-sensitivity group		↓	→	↑	↓	↓		→	↑
	Low-sensitivity group		↑	→	→	→	↑		→	↑

PC-G: Penicillin. AB-PC: Aminobenzyl-penicillin. SM: Streptomycin. CP: Chloramphenicol.  
 TC: Tetracycline. KM: Kanamycin. EM: Erythromycin. CET: Cephalothin. CER: Cephaloridine.  
 ↑ Increase ↓ Decrease → No change ↘ Slight decrease  
 \* 1967, 1968 \*\* 1966, 1967, 1968

change in low-sensitivity group were found against chloramphenicol. A tendency opposite to that against chloramphenicol was found against tetracycline. No change was observed in any of the three groups against streptomycin.

These data can be summarized as in Table 5.

We believe that it has become possible to perform more accurate comparative studies of the annual changes of the bacterial drug sensitivities with the method described in the present paper than with our previous method.

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