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

MULTIPLE-DRUG RESISTANCES

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In previous papers from this laboratory[1,2] we reported the distribution of MIC values of *Staphylococcus aureus* and *Escherichia coli* isolated from clinical specimens during 1965~1971 and a method was proposed for the determination of the grouping value to differentiate the isolates into sensitive and resistant strains.

In this paper the moving pooling method to determine the grouping values was applied and the distribution and annual change in types of combinations of multiple-drug resistance were examined. A model for the process of the development of multiple-drug resistance in *S. aureus* and *E. coli* is also proposed.

Materials and Methods

Strains

Strains of *S. aureus* isolated from pus and *E. coli* isolated from urine were studied.

Sensitivity test

Sensitivity of isolates was studied by the streak culture method described previously.[2]

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

The grouping value to differentiate the isolates into sensitive and resistant strains was determined as described previously.[3] The grouping values of each antibiotics for *S. aureus* and *E. coli* are listed in Table 1.

Results

1. Frequency of Isolation of Multiple Drug Resistant Strains

The antibiotics studied for four years (1968~1971) were as follows: For *S. aureus*; penicillin G (PC-G), aminobenzylpenicillin (AB-PC), tetracycline (TC), erythromycin (EM), streptomycin (SM), chloramphenicol (CP), kanamycin (KM), cephalothin (CET), cephaloridine (CER) and sulfamethoxazole (SMX). For *E. coli*; SM, TC, CP, AB-PC, KM, CER, CET and SMX.

Various combinations of drug resistance were observed with 1,637 strains of *S. aureus* isolated from pus (Table 2). The greatest number of combinations were observed in

Table 1. Grouping values of drug to differentiate the isolates into sensitive and resistant groups.

S. aureus				*E. coli*			
Drug	≧MIC	Peak MIC		Drug	≧MIC	Peak MIC	
CET	1.56	0.39	2* 3**	CET	50.00	12.50	2* 3**
CER	0.78	0.20	2 3	CER	12.50	3.13	2 3
CEX	12.50	3.13	2 2	CEX	50.00	12.50	2 2
CEG	12.50	3.13	2 2	CEG	25.00	6.25	2 2
CEZ	3.13	0.39	3 2	CEZ	12.50	1.56	3 2
KM	12.50	1.56	3 3	KM	25.00	6.25	2 3
GM	1.56	0.20	3 3	GM	6.25	1.56	2 3
SM	25.00	3.13	3 3	SM	12.50	6.25	1 3
AB-PC	0.39	0.10	2 3	AB-PC	25.00	3.13	3 3
PC-G	0.20	0.05	2 3	CB-PC	50.00	12.50	2 2
MCI-PC	0.78	0.20	2 2	TC	12.50	3.13	2 3
CP	12.50	6.25	1 3	CP	25.00	6.25	2 3
SMX	125.00	31.30	2 3	SMX	3.90	0.50	3 3
TC	3.13	0.39	3 3				
EM	0.78	0.20	2 3				

* Fold index; from peak MIC to resistant MIC.
** Sequential years for a moving pooling method.

Table 2. Theoretically possible combinations and actually observed combinations of drug resistance in *Staphylococcus aureus* (1968~1971)

Type resistance to:—	Number of theoretically possible combinations	Number of actually observed combinations	Frequencies of resistant strains
Single drug	15	6	5.3%
2 drugs	45	7	24.9
3 drugs	120	17	15.3
4 drugs	210	24	9.3
5 drugs	252	21	13.7
6 drugs	210	18	13.9
7 drugs	120	11	7.0
8 drugs	45	2	2.3
9 drugs	10	2	0.1
10 drugs	1	0	0.0
Total	1,023	108	91.7

Total number of strains, 1,637 (100 %); number of resistant strains, 1,501 (91.7 %)

Table 3. Actually observed combinations of drug resistance in *Staphylcoccus aureus* expressed for each antibiotic (1968~1971)

Type resistant to	Number of actually observed combinations which include resistance to:—									
	PC-G	AB-PC	SMX	TC	EM	SM	CP	KM	CET	CER
2 drugs	2	2	5	2	2	0	1	0	0	0
3 drugs	10	10	8	6	4	7	2	2	1	1
4 drugs	16	11	14	11	11	7	7	7	1	0
5 drugs	17	16	17	15	12	12	6	7	0	3
6 drugs	16	15	13	14	15	13	8	10	2	2
7 drugs	11	10	9	9	11	8	6	8	2	3
8 drugs	2	2	2	2	2	2	1	2	0	1
9 drugs	2	2	2	2	2	2	2	2	1	1
Total	76	68	70	61	59	51	33	38	7	11

Table 4. Theoretically possible combinations and actually observed combinations of drug resistance expressed in *Escherichia coli* (1968~1971)

Type resistant to:—	Number of theoretically possible combinations	Number of actually observed combinations	Frequency of resistant strains
Single drug	8	7	10.2%
2 drugs	28	13	9.6
3 drugs	56	16	10.8
4 drugs	70	17	30.1
5 drugs	56	14	7.8
6 drugs	28	12	6.5
7 drugs	8	5	5.7
8 drugs	1	1	1.4
Total	255	85	81.9

Total number of strains, 1,733 (100 %); number of resistant strains, 1,421 (82 %)

4-drug resistant strains (24 combinations) followed by 21 combinations of resistance to 5 drugs and 18 combinations of resistance to 6 drugs; 25% of the isolates were resistant to 2 drugs and 15% 3 drugs. Resistance to 5- and 6-drug was present in 14% of the isolates.

The frequency of each antibiotic appearing in various combinations of drug resistance is summarized in Table 3. PC-G appeared in 76 combinations while SMX, AB-PC, and EM appeared in 70, 68 and 59 combinations respectively.

In 1,733 strains of *E. coli*, the maximum number of combinations of drug resistance was observed in strains resistant to 4 drugs (17 combinations) followed by 16 combinations of resistance to 3 drugs, 14 combinations of resistance to 5 drugs and 13 combinations of resistance to 2 drugs. The percentages of strains showing 4-, 3- and 2- drug resistance were 30%, 11% and 10%, respectively (Table 4).

The frequency of each antibiotic appearing in various combinations of drug resistance is

Table 5. Actually observed combinations of drug resistances in *Escherichia coli* expressed for each antibiotic (1968~1971)

Type resistance to:—	Number of actually observed combinations which include resistance to:—							
	SM	SMX	TC	CP	AB-PC	CER	CET	KM
2 drugs	5	5	5	3	3	0	3	2
3 drugs	9	8	9	6	6	4	3	3
4 drugs	13	10	12	10	8	5	5	5
5 drugs	12	11	11	7	10	10	6	3
6 drugs	11	10	10	11	9	9	7	5
7 drugs	5	5	4	5	4	4	4	4
Total	55	49	51	42	40	32	28	22

Table 6. Annual frequencies of single-drug-resistant and multiple-drug-resistant strains of *Staphylococcus aureus* isolated from pus specimens

Year	Tot	R	%	1R.	2R.	3R.	4R.	5R.	6R.	7R.	8R.	9R.
1965	373	341	91.	31.9	11.3	11.5	13.1	13.9	7.0	2.4	0.3	0.0
1966	662	603	91.	28.5	17.1	8.3	10.3	16.2	7.4	3.3	0.0	0.0
1967	796	723	91.	33.0	11.4	8.8	14.1	13.7	6.0	3.8	0.0	0.0
1968	744	668	90.	34.5	8.7	7.4	14.1	16.5	6.6	1.9	0.0	0.0
1969	521	471	90.	25.7	14.8	9.2	16.3	13.8	7.5	3.1	0.0	0.0
1970	258	244	95.	20.9	29.1	14.3	13.2	9.3	5.8	1.6	0.4	0.0
1971	116	110	95.	21.6	23.3	8.6	11.2	12.9	12.1	4.3	0.9	0.0

(Drugs tested: PC-G, SMX, EM, TC, SM, CP, KM, CER, CET)

Table 7. Annual frequencies of single-drug-resistant and multiple-drug-resistant strains of *Escherichia coli* isolated from urine specimens

Year	Tot	R	%	1R.	2R.	3R.	4R.	5R.	6R.	7R.
1966	344	277	81.	14.5	9.9	7.6	40.1	6.4	1.5	0.6
1967	524	423	81.	10.5	9.9	11.5	38.5	7.1	2.9	0.4
1968	618	483	78.	10.2	10.8	11.3	34.0	6.0	4.5	1.3
1969	432	341	79.	9.5	10.2	10.0	35.6	9.0	3.7	0.9
1970	384	327	85.	8.9	10.4	11.2	33.6	10.2	8.6	2.3
1971	310	263	85.	11.0	6.5	14.8	34.2	8.4	8.1	1.9

(Drugs tested: SMX, SM, TC, CP, CER, CET, KM)

shown in Table 5. SM, TC, SMX and AB-PC appeared in 55, 51, 49 and 49 combinations respectively.

2. Annual Change in Frequency of Isolation of Multiple Drug Resistant Strains.

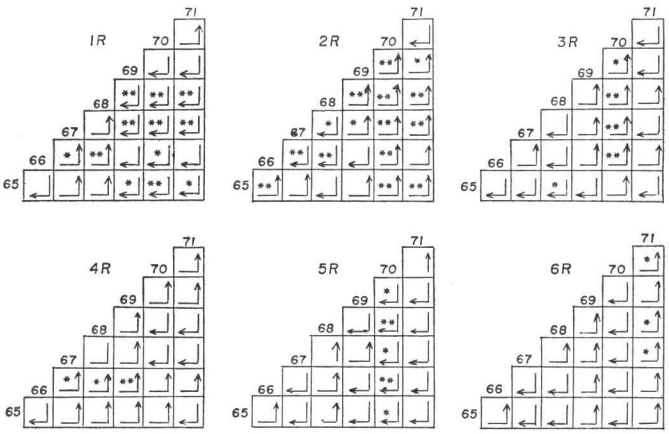
In *S. aureus*, isolates obtained from pus between 1965 and 1971 were tested for sensitivity to PC-G, EM, TC, SM, CP, KM, CET, CER and SMX. As shown in Table 6, single-drug resistant strains decreased while 2-drug resistant strains increased. The test of significance of the values listed in Table 6 is shown in Fig. 1.

In *E. coli*, isolates obtained from urine between 1966 and 1971 were tested for sensitivity to SM, TC, CP, CER, CET, KM and SMX. As shown in Table 7, the number of 5-, 6- and 7- drug resistant strains showed an increase over this period the tendency being most marked with 6-drug resistant strains. The test of significance of the values listed in Table 7 is shown in Fig. 2.

3. Annual Change in Drug Combinations in Multiple Drug Resistant Strains

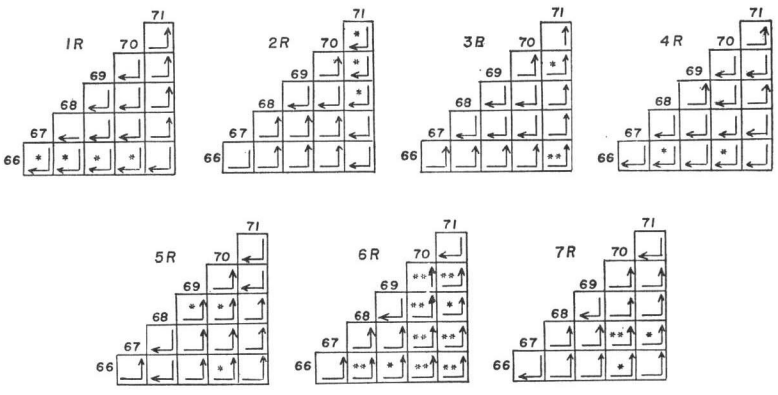
The annual change in isolations of multiple-drug resistant strains showing various combinations of drug resistance was studied. The results are shown in Tables 8 and 9. In *S. aureus*, over the whole period under investigation the most frequent combination of resistance to 2 drugs was PC-G/SMX followed by PC-G/EM. In strains resistant to 3-drug combinations, the most frequent combination was PC-G/TC/SMX while in 4-drug resistant strains the most frequent combination was PC-G/TC/SM/SMX or PC-G/TC/EM/SMX. Of the 5-drug combinations the most important was PC-G/SM/TC/EM/SMX, followed by PC-

Fig. 1



Test of significance : ** P=0.01, * P=0.05, → increased.

Fig. 2



Test of significance : ** P = 0.01, * P = 0.05, → increased.

Table 8. Main types of combinations of drug resistance and their frequencies in *Staphylococcus aureus*

Type resistant to	Order	Combination	1965		1966		1967		1968		1969		1970		1971		Total	
			N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1R.	Total		119	100	189	100	263	100	257	100	134	100	54	100	25	100	1041	100
	1	PC-G	111	93	167	88	243	92	244	95	110	82	33	61	18	72	926	89
	2	SMX	7	6	19	10	15	6	4	2	22	16	21	39	7	28	95	9
2R.	Total		42	100	113	100	91	100	65	100	77	100	75	100	27	100	490	100
	1	PC-G, SMX	20	48	81	72	48	53	22	34	54	70	60	80	23	85	308	63
	2	PC-G, EM	6	14	5	4	9	10	9	14	10	13	6	8	3	11	48	10
	2	PC-G, SM	1	2	12	11	16	18	16	25	2	3	1	1	0	0	48	10
3R.	Total		43	100	55	100	70	100	55	100	48	100	37	100	10	100	318	100
	1	PC-G, SMX, TC	31	72	41	75	40	57	31	56	19	40	7	19	3	30	172	54
	2	PC-G, SMX, SM	3	7	5	9	5	7	4	7	6	13	5	14	1	10	29	9
	3	PC-G, SMX, EM	2	5	2	4	4	6	5	9	5	10	8	22	1	10	27	8
	4	PC-G, EM, TC	0	0	1	2	6	9	3	6	6	13	0	0	0	0	16	5
4R.	Total		49	100	68	100	112	100	105	100	85	100	34	100	13	100	466	100
	1	PC-G, SMX, EM, TC	12	24	20	29	44	39	45	43	48	57	20	59	5	39	194	42
	2	PC-G, SMX, TC, SM	27	55	31	45	38	34	24	23	13	15	0	0	0	0	133	29
	3	PC-G, SMX, EM, SM	1	2	3	4	6	5	8	8	2	2	4	12	1	8	25	5
	4	PC-G, SMX, TC, CP	1	2	6	9	1	1	6	6	5	6	0	0	1	8	20	4
	4	SMX, FM, TC, SM	2	4	1	2	4	4	4	4	8	9	1	3	0	0	20	4
	5	PC-G, EM, SM, KM	3	6	2	3	4	4	4	4	2	2	3	9	1	8	19	4
5R.	Total		52	100	107	100	109	100	123	100	72	100	24	100	15	100	502	100
	1	PC-G, SMX, EM, TC, SM	38	73	80	75	58	53	82	67	42	58	14	58	6	40	320	64
	2	PC-G, SMX, EM, TC, CP	7	14	10	9	18	17	23	19	13	18	5	21	5	33	81	16
	3	PC-G, SMX, EM, SM, KM	0	0	4	4	6	6	0	0	5	7	3	13	0	0	18	4
	4	PC-G, EM, TC, SM, KM	2	4	1	1	4	4	2	2	3	4	1	4	0	0	13	3

6R.	Total		26	100	49	100	48	100	49	100	39	100	15	100	14	100	240	100
	1	PC-G, SMX, EM, TC, SM, CP	20	77	31	63	22	46	14	29	7	18	2	13	4	29	100	42
	2	PC-G, SMX, EM, TC, SM, KM	3	12	18	37	16	33	22	45	25	64	8	53	4	29	96	40
	3	PC-G, SMX, EM, SM, CP, KM	0	0	0	0	3	6	0	0	2	5	4	27	2	14	11	5
	3	PC-G, EM, TC, SM, CP, KM	0	0	0	0	1	2	5	10	5	13	0	0	0	0	11	5
	4	PC-G, SMX, EM, TC, SM, CER	3	12	0	0	1	2	3	6	0	0	0	0	2	14	9	4
7R.	Total		9	100	22	100	30	100	14	100	16	100	4	100	5	100	100	100
	1	PC-G, SMX, EM, TC, SM, CP, KM	9	100	21	96	26	87	14	100	15	94	4	100	4	80	93	93
	2	PC-G, SMX, EM, TC, SM, KM, CER	0	0	0	0	1	3	0	0	1	6	0	0	1	20	3	3
8.R	Total		1	100	0	0	0	0	0	0	0	0	1	100	1	100	3	100
	1	PC-G, SMX, EM, TC, SM, CP, KM, CER	0	0	0	0	0	0	0	0	0	0	0	0	1	100	1	33
	1	PC-G, SMX, EM, TC, SM, CP, KM, CET	0	0	0	0	0	0	0	0	0	0	1	100	0	0	1	33
	1	PC-G, SMX, EM, TC, SM, CP, CER, CET	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	33
		Total strains (resistant)	341		603		723		668		471		244		110		3,160	
		Total strains	373		662		796		744		521		258		116		3,470	

G/CP/TC/EM/SMX, these two combinations accounting for about 80 % of all strains showing 5-drug resistance. Most important of the 6-drug combinations was PC-G/SM/CP/TC/EM/SMX or PC-G/TC/EM/SM/KM/SMX, these two combinations accounting for about 70~80 % of all strains showing 6-drug resistance. Strains showing 7- and 8-drug resistance, were encountered only rarely.

In *E. coli*, of the strains showing resistance to 2 drugs, the most important combination was SM/SMX, this being present in about 50% of all strains with 2-drug resistance. The next most frequent combination was SMX/TC which was present in about 20 ~ 30 % of strains. Of the 3-drug combinations the most important was TC/SM/SMX followed by SM/CP/SMX, these two accounting for 60~80 % of all strains showing resistance to 3 drugs. Since 1968, however, a gradual but steady increase has been noted with SM/TC/CP. The combination of SM/TC/CP/SMX was prominent among the strains resistant to 4 drugs. Among the resistance combinations of 5 drugs, SM/TC/CP/KM/SMX and SM/TC/CP/CER/SMX were the most frequent, both accounting for 30 % of all strains showing 5-drug resistance. Of the less frequent 6-drug combinations, the most important was SM/TC/CP/CER/CET/SMX. The occurrence of 7-drug combinations, also small in the number of strains involved, was found to be increasing year by year.

4. Setting a Model for a Process of the Development of Multiple-drug Resistant Strains

S. aureus

Strains were tested for sensitivity to the following 15 antibiotics: PC-G, AB-PC, MCI-PC, CB-PC, CEX, CET, CER, CEG, CEX, TC, CP, SM, KM, EM and SMX. Of 374 strains of *S. aureus* isolated from pus during 1970~1971, 20 were drug-sensitive and the remaining 354 were drug-resistant. A classification of the latter according to the number of drugs involved (type of multiple-drug resistance) is shown in Table 10. The most frequently observed type was the 3-drug resistance followed by 5-, 4- and 6-drug resistance. As shown in Table 10, the antibiotic most frequently involved was PC-G, followed by AB-PC, SMX, EM, TC and SM. Com-

Table 9. Main types of combinations of drug resistance and their frequencies in *Escherichia coli*

Type resistant to	Order	Combination	1966		1967		1968		1969		1970		1971		Total	
			N	%	N	%	N	%	N	%	N	%	N	%	N	%
1R.	Total		50	100	55	100	63	100	41	100	34	100	34	100	277	100
	1	SMX	32	64	22	40	34	54	18	44	11	32	13	38	130	47
	2	TC	13	26	19	35	17	27	13	32	10	29	10	29	32	30
	3	SM	4	8	9	16	9	14	9	22	9	27	10	29	50	18
2R.	Total		34	100	52	100	67	100	44	100	40	100	20	100	257	100
	1	SMX, SM	17	50	25	48	34	51	20	46	18	45	10	50	124	48
	2	SMX, TC	9	27	16	31	16	24	7	16	6	15	4	20	58	23
	3	SM, TC	5	15	7	13	11	16	11	25	10	25	2	10	46	18
	4	TC, CP	0	0	2	4	4	6	4	9	0	0	3	15	13	5
3R.	Total		26	100	60	100	70	100	43	100	43	100	46	100	288	100
	1	SMX, SM, TC	21	81	44	73	43	61	29	67	23	54	24	52	184	64
	2	SMX, SM, CP	4	15	13	22	17	24	5	12	3	7	9	20	51	18
	3	SM, TC, CP	0	0	0	0	4	6	4	9	10	23	8	17	26	9
4R.	Total		138	100	202	100	210	100	154	100	129	100	106	100	939	100
	1	SMX, SM, TC, CP	135	98	197	98	202	96	142	92	115	89	92	87	883	94
	2	SMX, SM, CP, CER	0	0	0	0	5	2	1	1	4	3	5	5	15	2
5R.	Total		22	100	37	100	37	100	39	100	39	100	26	100	200	100
	1	SMX, SM, TC, CP, CER	2	9	12	32	14	38	21	54	18	46	9	35	76	38
	2	SMX, SM, TC, CP, KM	10	46	12	32	11	30	12	31	9	23	9	35	63	32
	3	SMX, SM, TC, CP, CET	6	27	6	16	6	16	1	3	5	13	6	23	30	15
6R.	Total		5	100	15	100	28	100	16	100	33	100	25	100	122	100
	1	SMX, SM, TC, CP, CER, CET	5	100	7	47	15	54	8	50	16	49	17	68	68	56
	2	SMX, SM, TC, CP, CER, KM	0	0	6	40	13	46	7	44	16	49	3	12	45	37
	3	SMX, SM, TC, CP, CET, KM	0	0	2	13	0	0	1	6	1	3	2	8	6	5
	4	SMX, SM, CP, CER, CET, KM	0	0	0	0	0	0	0	0	0	0	3	12	3	2
7R.	Total	SMX, SM, TC, CP, CER, CET, KM	2	100	2	100	8	100	4	100	9	100	6	100	31	100
Total strains (resistant)			277		423		483		341		327		263		2,114	
Total strains			344		524		618		432		384		310		2,612	

Table 10. Number of multiple-drug-resistant strains of *S. aureus*

Number of total resistant strains, 354	
Single drug 26. 7%	6 drugs 36. 10%
2 drugs 61. 17%	7 drugs 30. 8%
3 drugs 100. 28%	8 drugs 11. 3%
4 drugs 40. 11%	9 drugs 2. 0.5%
5 drugs 47. 13%	10 drugs 1. 0.2%

Table 11. Number of multiple-drug-resistant strains of *E. coli*

Number of total resistant strains, 578	
Single drug 69. 12%	7 drugs 37. 6%
2 drugs 56. 10%	8 drugs 37. 6%
3 drugs 77. 13%	9 drugs 37. 4%
4 drugs 192. 33%	10 drugs 15. 3%
5 drugs 26. 4%	11 drugs 12. 2%
6 drugs 28. 5%	12 drugs 5. 1%

Fig. 3. A model of the main processes for the development of multiple-resistant strains of *S. aureus* (328 strains).
Figures over the line in the square represent the frequency (%) among the strains resistant to corresponding drugs and figures under the line are the frequency (%) among the total resistant strains.

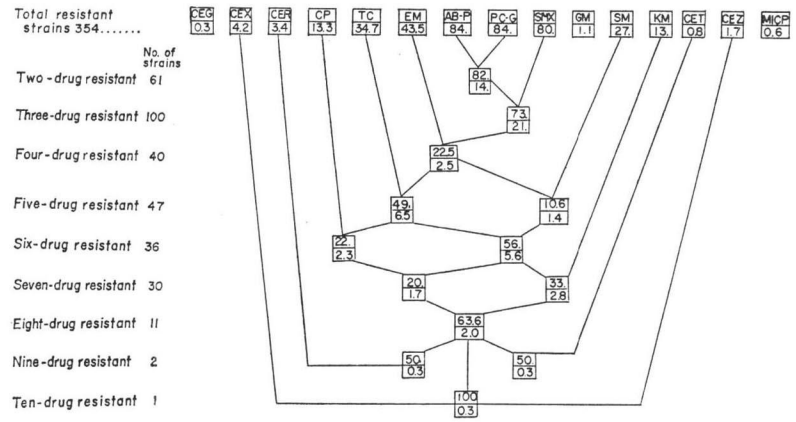


Fig. 4. A model of the main processes for the development of multiple-resistant strains of *E. coli* (509 strains).
Figures over the line in the square represent the frequency (%) among the strains resistant to corresponding drugs and figures under the line represent the frequency (%) among the total resistant strains.

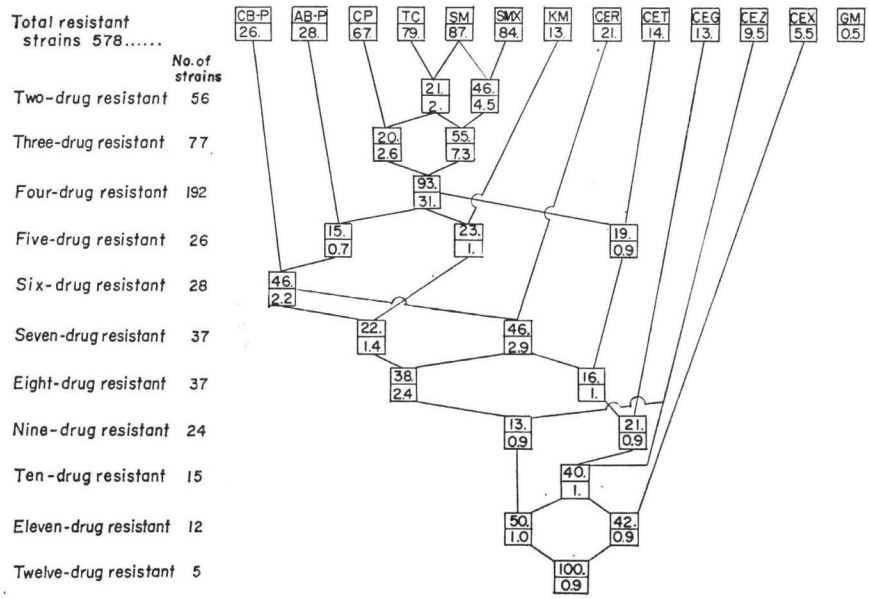


Table 12. The frequencies of single-drug-resistant and multiple-drug-resistant strains of *S. aureus* isolated from pus specimens. (years of isolation: 1970～1971)

T.: number of strains, S.: sensitive strains, R.: resistant strains

	15 drugs	PC-G	AB-PC	SMX	EM	TC	SM	CP	KM	CEX	CER	CEZ	GM	CET	MIPC	CEG
T.	374	374	374	374	374	374	374	374	374	374	374	374	374	374	374	374
S.	20	76	77	92	220	251	279	327	328	359	362	368	370	371	372	373
R.	354	298	297	282	154	123	95	47	46	15	12	6	4	3	2	1
1R.	26	1	0	25	0	0	0	0	0	0	0	0	0	0	0	0
2R.	61	53	53	11	3	1	0	1	0	0	0	0	0	0	0	0
3R.	100	88	87	85	19	11	8	1	0	0	1	0	0	0	0	0
4R.	40	31	34	40	18	10	11	6	2	5	0	1	0	1	1	0
5R.	47	45	44	42	36	33	13	6	7	2	2	3	1	0	1	0
6R.	36	36	35	35	36	33	25	9	4	1	1	1	0	0	0	0
7R.	30	30	30	30	28	23	24	14	19	3	6	0	3	0	0	0
8R.	11	11	11	11	11	9	11	7	11	3	1	0	0	1	0	1
9R.	2	2	2	2	2	2	2	2	2	0	1	0	0	1	0	0
10R.	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0
11R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Log n	5.697	5.694	5.642	5.037	4.812	4.554	3.850	3.829	2.708	2.458	1.792	1.386	1.099	0.693	0.000

Table 13. Percentage of resistant strains.

	15 drugs	PC-G	AB-PC	SMX	EM	TC	SM	CP	KM	CEX	CER	CEZ	GM	CET	MIPC	CEG
R.	354	84.2	83.9	79.7	43.5	34.7	26.8	13.3	13.0	4.2	3.4	1.7	1.1	0.8	0.6	0.3
1R.	26	3.8	0.0	96.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2R.	61	86.9	86.9	18.0	4.9	1.6	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3R.	100	88.0	87.0	85.0	19.0	11.0	8.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
4R.	40	77.5	85.0	100.0	45.0	25.0	27.5	15.0	5.0	12.5	0.0	2.5	0.0	2.5	2.5	0.0
5R.	47	95.7	93.6	89.4	76.6	70.2	27.7	12.8	14.9	4.3	4.3	6.4	2.1	0.0	2.1	0.0
6R.	36	100.0	97.2	97.2	100.0	91.7	69.4	25.0	11.1	2.8	2.8	2.8	0.0	0.0	0.0	0.0
7R.	30	100.0	100.0	100.0	93.3	76.7	80.0	46.7	63.3	10.0	20.0	0.0	10.0	0.0	0.0	0.0
8R.	11	100.0	100.0	100.0	100.0	81.8	100.0	63.6	100.0	27.3	9.1	0.0	0.0	9.1	0.0	9.1
9R.	2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0
10R.	1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0
11R.	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12R.	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13R.	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14R.	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15R.	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

parative figures converted into $\log n$ of the numbers of drug-resistance strains against each antibiotic are given in the lower column of Table 11. Table 13 shows the frequency of resistance to individual antibiotics in strains showing single and multiple drug resistance.

E. coli

Strains were tested for sensitivity to the following 13 antibiotics: SM, TC, CP, AB-PC, CB-PC, CER, CET, KM, CEG, CEZ, GM and SMX. Of 675 strains isolated from urine during 1970 ~ 1971, 97 (14 %) were drug-sensitive and the remaining 578 (86 %) were drug resistant. A classification of the latter according to the number of drugs involved is shown in Table 12. The most frequently

Table 14. The frequencies of single-drug-resistant and multiple-drug resistant strains of *E. coli* isolated from urine specimens. (years of isolation: 1970~1971)
T.: number of strains, S.: sensitive strains, R.: resistant strains

	13 drugs	SM	SMX	TC	CP	AB-PC	CB-PC	CER	CET	KM	CEG	CEZ	CEX	GM
T.	675	675	675	675	675	675	675	675	675	675	675	675	675	675
S.	97	173	192	218	286	511	523	552	592	600	602	620	643	672
R.	578	502	483	457	389	164	152	123	83	75	73	55	32	3
1R.	69	19	24	19	1	2	0	0	3	0	1	0	0	0
2R.	56	39	38	26	6	2	1	0	0	0	0	0	0	0
3R.	77	73	58	62	27	1	2	0	2	6	0	0	0	0
4R.	192	191	188	190	183	1	1	0	2	8	2	0	2	0
5R.	26	24	23	22	24	11	9	2	6	6	2	0	1	0
6R.	28	27	28	21	25	23	22	9	4	4	2	0	2	1
7R.	37	37	35	34	37	33	33	24	3	12	5	3	2	1
8R.	37	36	35	32	31	36	36	34	15	16	13	9	3	0
9R.	24	24	23	20	24	23	19	22	16	8	19	12	5	1
10R.	15	15	14	14	14	15	12	15	15	3	13	14	6	0
11R.	12	12	12	12	12	12	12	12	12	7	11	12	6	0
12R.	5	5	5	5	5	5	5	5	5	5	5	5	5	0
13R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Log n	6.2	6.2	6.1	6.0	5.1	5.0	4.8	4.4	4.3	4.3	4.0	3.5	1.1

Table 15. Percentage of resistant strains.

	13 drugs	SM	SMX	TC	CP	AB-PC	CB-PC	CER	CET	KM	CEG	CEZ	CEX	GM
R.	578	86.9	83.6	79.1	67.3	28.4	26.3	21.3	14.4	13.0	12.6	9.5	5.5	0.5
1R.	69	27.5	34.8	27.5	1.4	2.9	0.0	0.0	4.3	0.0	1.4	0.0	0.0	0.0
2R.	56	69.6	67.9	46.4	10.7	3.6	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3R.	77	94.8	75.3	80.5	35.1	1.3	2.6	0.0	2.6	7.8	0.0	0.0	0.0	0.0
4R.	192	99.5	97.9	99.0	95.3	0·5	0.5	0.0	1.0	4.2	1.0	0.0	1.0	0.0
5R.	26	92.3	88.5	84.6	92.3	42.3	34.6	7.7	23.1	23.1	7.7	0.0	3.8	0.0
6R.	28	96.4	100.0	75.0	89.3	82.1	78.6	32.1	14.3	14.3	7.1	0.0	7.1	3.6
7R.	37	100.0	94.6	91.9	100.0	89.2	89.2	64.9	8.1	32.4	13.5	8.1	5.4	2.7
8R.	37	97.3	94.6	86.5	83.8	97.3	97.3	91.9	40.5	43.2	35.1	24.3	8.1	0.0
9R.	24	100.0	95.8	83.3	100.0	95.8	79.2	91.7	66.7	33.3	79.2	50.0	20.8	4.2
10R.	15	100.0	93.3	93.3	93.3	100.0	80.0	100.0	100.0	20.0	86.7	93.3	40.0	0.0
11R.	12	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	58.3	91.7	100.0	50.0	0.0
12R.	5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0
13R.	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

observed type was 4-drug resistance, accounting for 192 strains (33%). This was followed by 3- and 2-drug resistance in this order.

As shown in Table 14, the antibiotic most frequently involved was SM, followed by SMX, TC and CP. In the bottom column of Table 14, is given the numbers of the drug-resistant strains encountered as comparative figures converted into $\log n$. Table 15 shows the frequency of resistance to individual antibiotics in strains showing single and multiple-drug resistance.

From the above results, models for a process of the development of multiple drug resistance in *S. aureus* and *E. coli* are given in Figs. 3 and 4 respectively.

Discussion and Conclusion

A number of papers have reported on multiple-drug resistance.[3,4] However, the data given therein differ with respect to the antibiotic concentrations used to differentiate the isolated strains into drug-sensitive and drug-resistant groups. In this paper, the grouping values determined as described previously were adopted to differentiate the isolates into sensitive and resistant strains.

In *S. aureus*, the most frequently observed combination among 2-drug resistance was PC-G/SMX. Of the 3-drug combinations, most frequent pattern was PC-G/SMX/TC, although there was a tendency for the number of such strains to decrease over the period of study. Of the 4-drug combinations, the most frequently observed was PC-G/SMX/EM/TC with a tendency to increase in frequency over the period of study. Of the 5-drug combinations, the most frequently observed was PC-G/SMX/EM/TC/SM with a tendency to decrease. Of the 6-drug combinations the most frequently observed was PC-G/SMX/EM/TC/SM/CP also with a tendency to decrease. The 7- and 8-drug combinations were seldom encountered.

The reports of KOSAKAI *et al*[6,7] published in 1960 were the first that dealt with studies on the subject of multiple-drug resistance. The antibiotics studied in their paper were PC-G, SM, TC, CP and EM. Comparison of their paper and the data presented in this paper shows that the occurrence of drug resistant strains has increased, especially strains resistant to more than 3 drugs. A significant increase in the occurrence of strains resistant to EM, CP and TC is also noted.

In *E. coli*, the most frequently encountered of the 2-drug combinations was SM/SMX.

SMX/SM/TC, the most frequently encountered type among the 3-drug combinations, showed a decrease in frequency year by year. The number of strains resistant to the 4-drug combinations was found to be decreasing. The dominant type of combination, SM/SMX/TC/CP was decreasing year by year. Of 5-drug combinations, SMX/SM/TC/CP/CER in addition to SMX/SM/TC/CP/KM which was dominant previously were most frequently encountered. This may be due to the fact as pointed out by MITSUHASHI *et al.* that R-factor resistance to cephalosporin and its derivatives is now widespread. Of the 6-drug combinations, SMX/SM/TC/CP/CER/CET was dominant, accounting for about 50% of all strains with 5-drug resistance.

From the data obtained with *S. aureus* and *E. coli* isolated during 1970~1971, a model for the process of the development of multiple-drug resistance is proposed for *S. aureus* and for *E. coli*. From these models the main combinations of multiple-drug resistance were found to be PC-G/AB-PC/SMX, TC/EM/PCG/SMX and TC/EM/PCG/SMX/SM for *S. aureus* and SM/SMX, TC/SM/SMX and CP/TC/SM/SMX for *E. coli*. This confirms the report of MITSUHASHI *et al.*[5,8] in which they propose that the above mentioned types of combinations were the basis of the development of all types of multiple-drug resistance.

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