Bacteriophage Typing of Staphylococci in the Republic of Korea

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Abstract : A summary is presented of several studies by Korean investigators on the phage typing and antibiotic resistance of staphylococci isolated from varied sources during the years 1960 to 1965. The distribution of phage groups and of strains of the 52/52A/80/81 complex is described, and the relatively high incidence of antibiotic resistant strains is reported.

Introduction

Staphylococci are widely distributed in the human environment. They are members of the normal flora of the skin and mucous membranes, and frequently elicit a variety of infections in man and animals. Coagulase positive staphylococci are considered pathogenic for man, but there is a great deal of difference among them in their ability to produce disease and to cause epidemics. Study of the various aspects of staphylococcal infections is epidemiologically very important and is assisted by distinguishing strains of staphylococci causing infection from other strains isolated from related sources. Biological characteristics can serve as a means for the identification of the species, but are not enough for the differentiation of strains within the species. Immunological techniques can be used to distinguish the strains but they are too complicated to be performed in ordinary laboratories.

Bacteriophage typing has proved to be a useful means for the differentiation of staphylococci from various sources [5, 28, 36, 49, 50], and the value of this technique is increased when applied along with the sensitivity test to antibiotics [6,24,29]. Bacteriophage typing has been extensively used for about two decades in many countries for the identification of staphylococci. However, it was first introduced in Korea by IM [19, 20] who reported the phage types of strains isolated from surgical infections during the period from 1957 to 1959; this was followed by several studies on strains isolated from pathological as well as non-pathological sources All of the reports in Korea, with the exception of one by PAI and YOON [33], came out of the author's laboratory. This paper summarizes the distribution of phage groups and antibiotic resistance of staphylococci of various origins in Korea, based on the reports made during the period from 1960 to the first half of 1965.

Materials and Methods

In all reports referred to this paper, the following general methods of investigation were applied. Staphylococci were isolated from the nose and throat of newborn infants, children and hospital workers, discharges from various pathological sources, and air in the hospital. Nasal and throat swabs were smeared on nutrient agar containing 7.5 per cent sodium chloride, and discharges were inoculated on blood agar or nutrient agar plates. Staphylococci in the air were collected on nutrient agar by the particle fall out method of WILLIAMS et al. [47]. After incubation for one to two days at 37°C, suspected colonies were isolated and were examined for their morphological and biological characteristics; strains identified as coagulase positive staphylococci were maintained on nutrient agar slants for further study. One strain was isolated from each specimen, and on some occasions, especially in the case of HAN's study [15–18], two or more strains were collected from each sample.

The basic set of bacteriophages was kindly supplied in dried form by Dr. M. T. PARKER, Central Public Health Laboratory, London, United Kingdom and propagated in this laboratory. The phages used were 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 42D, 81, and 187. Strains were first typed with the routine test dilutions of the phages and those untypable at R. T. D. were re-typed with phage preparations 1,000 times more concentrated. The techniques of propagation and typing were carried out by the method described by BLAIR and WILLIAMS [9].

Antibiotic sensitivity was tested on nutrient agar plates containing the desired concentrations of the respective antibiotics, inoculated with a 1:100 dilution of broth culture. Resistant strains were identified according to the slightly modified criteria proposed by BYNOE et al. [10].

Results

Healthy carriers : Some reports described the rates of isolation of staphylococci from the nose and throat of healthy persons; the results are summarized in Table 1. The incidence of carriers among children in an urban area [15], hospital workers [22] and newborn infants [27] in Kyungpook University Hospital vary significantly from one report to another, and range from 24.3 to 63 per cent. The rates of isolation from hospital air [23] and various materials associated with newborn infants, such as delivery blankets and maternity and nursery rooms [27] are 40.1 to 47.2 per cent. The rate of carriage of staphylococci in the nose and throat of healthy persons is known to vary, but shows no significant geographical differences, as reported by WILLIAMS [46]. The results in this paper clearly reveal that the carrier rates in the nose and throat of hospi-

Table 1. Isolation of coagulase positive staphylococci from various sources

Reported by	Source		No. of case tested	Positive case No.	Positive case %
Han, 1961 (15)*	Children	Throat	494	120	24.3
Kim, 1964 (22)	Hospital worker	Nose	557	351	63.0
		Throat	557	318	57.1
Kim, 1964 (23)	Hospital	Air	1,109	523	47.2
Lee, 1965 (27)	Newborn infant	Nose & throat	55	30	54.5
	Materials related to newborn infant		277	111	40.1
Total			3,049	1,453	47.7

* Reference No.

tal personnel and hospitalized newborn infants are considerably higher than those of healthy persons outside the hospital, and also indicate that the environment in Kyungpook University Hospital is heavily contaminated. In the reports referred to above, only one culture was made from one specimen; the rates would be increased if cultures had been made repeatedly from the same source.

Phage groups of staphylococci isolated from nose, throat and hospital air : In the reports of HAN [17], KIM [22, 23], PAI and YOON [33] and LEE [25, 27], strains isolated from the nose and throat of healthy and sick (with inflammatory changes in throat) children, newborn infants and hospital workers, and from hospital air were studied; the results are shown in Table 2. Among the staphylococci from these sources, strains

Table 2. Phage groups of staphylococci isolated from nose, throat and hospital air

Reported by	Source		No. of strains	Phage group (%)					
				I	II	III	IV	Mixed	N. T.*
Han, 1961 (17)**	Children	Throat	511 (2)***	2.4 (0.4)	11.0	31.3	0	39.1 (0)	16.2
Kim, 1964 (22)	Hospital worker	Nose & throat	669 (139)	21.2 (8.5)	7.9	21.5	0	31.3 (12.3)	18.1
Kim, 1964 (23)	Hospital	Air	523 (101)	17.2 (9.2)	9.9	20.3	0	32.3 (10.1)	20.3
Pai & Yoon 1964 (33)	Hospital worker	Nose & throat	29 (?)****	0	3.4	10.3	0	27.7	58.6
Lee, 1965 (25)	Sick + Chilbren	Nose & throat	366 (109)	8.2 (4.4)	11.8	21.6	0	33.6 (25.4)	24.8
Lee, 1965 (27)	Newborn & others	Nose, throat & air	141 (60)	7.7 (2.8)	12.0	7.0	0	57.3 (39.7)	16.0
Total			2,239 (411)	12.7 (6.8)	9.9	22.4	0	35.2 (11.7)	19.8

* Non-typable.
** Reference No.
*** Strains belonging to 52/52A/80/81 complex.
**** Not listed.
\+ Inflammatory changes in throat.

of the mixed group occurred most frequently, representing 25.2 per cent of all strains tested. Next most predominant are strains of group III, followed by group I and group II, in that order. Strains belonging to the 52/52A/80/81 complex ("epidemic" strains) were included in either group I or the mixed group according to their phage patterns. If epidemic strains are excluded from the mixed group, strains of that group then show almost the same proportion as those of group III.

As reviewed by BLAIR [4] and BLAIR and CARR [6], many workers in the United States reported the predominance of strains belonging to group III among hospital strains. BYNOE et al. [10] in Canada, VOGELSANG and HAALAND [43] in Norway, and workers in other countries also reported a marked predominance of gronp III among strains isolated from the naso-pharynx of healthy individuals. However, there are a considerable number of reports [2, 3, 11, 42, 45, 48] in which group III is less predominant than the other groups. From these and the reports cited in this paper, it is assumed that the group

distribution of staphylococci varies according to the origin of the strains.

No strain belonging to group Ⅳ was observed, and non-typable strains were present in about 20 per cent. In HAN's report [17], which included strains isolated in 1960 and 1961 from the throat of healthy and sick children, only two epidemic strains are noted. As the study was made on children in an urban area, this result may indicate the distribution of epidemic strains outside the hospital at the time. The proportion of epidemic strains increased to about 20 to 40 per cent of all strains examined in the reports of KIM [22, 23] and LEE [25, 27], revealing the prevalence of epidemic strains in a hospital environment in Korea. The prevalence of these strains in the hospital and their easy transmissibility have been mentioned in reports from the United States and other countries [6, 7, 29, 32, 38, 39].

Phage groups of staphylococci isolated from pathological specimens : Six reports [1, 12, 13, 19, 33, 41] describe the phage typing of strains isolated from various pathological sources, and the results are listed in Table 3. In general, strains of the mixed group predominated, followed by strains

Table 3. Phage groups of staphplococci isolated from pathological sources

Reported by	Infection	No. of strains	Phage group (%)					
			Ⅰ	Ⅱ	Ⅲ	Ⅳ	Mixed	N.T.*
Im, 1960 (19)**	Surgical	150 (45)***	33.3 (30.0)	7.4	13.3	2 7	27.3 (0)	16.0
Song, 1963 (41)	Various	68 (17)	8.8 (7.4)	11.9	27.9	0	39.5 (17.6)	11.9
Ahn & Suh 1963 (1)	Secondary	18 (2)	0	11.1	27.8	0	27.8 (11.1)	33.3
Chi et al. 1963 (12)	Otitis media chronica	42 (13)	14.3 (14.3)	14.3	28.5	0	16.7 (16.7)	26.2
Pai & Yoon 1964 (33)	Secondary	85 (?)	7.0	8.2	22.4	0	34.2	28.2
Chun et al.. 1965 (13)	Surgical	588 (217)	9.5 (2.4)	11.7	13.6	0	46.8 (34.5)	18.4
Total		951 (294)	13.0 (7.4)	10.8	16.3	0.4	40.5 (23.3)	19.0

* Non-typable.
** Reference No.
*** Strains belonging to 52/ 52A/80/81 complex.

of group Ⅲ. Exceptions are the results reported by IM [19] and by CHI et al. [12] who found a predominance of strains of group Ⅰ and group Ⅲ, respectively, with strains of the mixed group occurring next in frequency. In most instances, strains of groups Ⅰ and Ⅱ were encountered much less frequently, and the incidence of the two groups was about the same. Small numbers of strains belonging to group Ⅳ were identified and almost one-fifth of strains tested were non-typable.

Epidemic strains represent around 25 to 37 per cent of all strains from pathological sources tested, with the exception of the results of AHN and SUH [1] and PAI and YOON [33] who found these strains only in small numbers. IM [19] found an incidence of abont 30 per cent of epidemic strains among the staphylococci isolated from 1957

through 1959 from surgical sources in hospitalized patients, and subsequently in 1963 through 1965. These results would indicate the presence from the late 1950's of epidemic strains in high percentages in the hospital environment.

Strains belonging to the mixed group occur in almost the same proportion as those of group Ⅲ, if epidemic strains are excluded from the mixed group. Comparing the staphylococci isolated from the nose, throat and air with those from pathological sources (Tables 2 and 3), the latter sources show a higher proportion of mixed group strains and a slightly lower proportion of group Ⅲ than was found among strains from the nose and throat. The incidence of epidemic strains is higher among staphylococci from pathological sources than among strains isolated from nasal and throat carriers; the other groups occur in almost the same frequency among strains from those sources (Tables 2 and 3).

Phage groups of staphylococci isolated from surgical infections : IM [19] and CHUN et al. [13] correlated the phage groups of staphylococci isolated from various surgical infections with the origin of the strains; the results are summarized in Table 4. In IM's report [19], strains from abscess, carbuncle and myositis are dominated by group Ⅰ, in which 45 strains out of 150 are classified as epidemic strains. Strains from phlegmone and osteomyelitis show a predominance of the mixed group, and no epidemic strain was identified. In the report of CHUN et al. [13], strains from various sources are dominated by the mixed group,

Table 4. Phage groups of staphylococci isolated from various surgical infections

Reported by	Surgical infection	No. of strains	Phage group (%)					
			Ⅰ	Ⅱ	Ⅲ	Ⅳ	Mixed	N. T.*
Im, 1960 (19)	Abscess	16	37.5	0	18.8	0	18.8	25.0
	Phlegmone	14	14.3	0	21.4	0	35.7	28.6
	Carbuncle	31	41.9	12.9	16.1	3.2	19.4	6.5
	Myositis	37	48.6	2.7	5.4	0	24.3	18.9
	Osteomyelitis	10	30.0	10.0	0	0	40.0	20.0
	Secondary infection	4	50.5	0	0	0	50.0	0
	Miscellaneous	38	15.8	13.2	18.4	7.9	31.5	13.2
	Total	150 (45)**	33.3 (30.0)	7.4	13.3	2.7	27.3 (0)	16.0
Chun et al., 1965 (13)	Abscess	461	9.1	13.2	13.7	0	47.3	16.7
	Osteomyelitis	24	8.3	4.2	8.3	0	50.0	29.2
	Secondary infection	23	8.7	4.3	13.1	0	47.8	26.1
	Miscellaneous	80	12.5	7.5	15.0	0	42.5	22.5
	Total	588 (217)	9.5 (2.4)	11.7	13.6	0	46.8 (34.5)	18.4
Grand total		738 (262)	14.3 (8.0)	10.8	18.6	0.6	42.8 (27.5)	17.9

* Non-typable.
** Strains belonging to 52/52A/80/81 complex.

of which about three-quarters are classified as epidemic strains. There is no other significant finding in either report on the relation between group and the origin of the strains, but the slightly greater proportion of epidemic strains reported by CHUN et al. [13] is to be noted. Some workers have studied the distribution of strains according to their origins [10, 21, 29, 35, 40, 51] but the results are varied. However, most of them mentioned the importance of epidemic strains in surgical infections, and this fact was also noted in the reports cited in this paper.

Distribution of phage patterns among the epidemic strains : In this paper, staphylococci with phage typing patterns showing various combination within the 52/52A/80/81 complex are regarded as epidemic strains. As shown in Table 5, strains belonging to

Table 5. The distribution of phage patterns belonging to 52/52A/80/81 complex

Reported by	Source	No. of strains tested	Phage pattern															
			52	52/52A	52/52A/80	52/52A/80/81	52/80	52/80/81	52/81	52A	52A/80	52A/80/81	52A/81	80	80/81	81	Total	
Im, 1960 (19)	Surgical infection	150	0	3	0	0	0	0	0	1	0	0	0	41	0	0	45	36.0
Han, 1961 (22)	Children*	511	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.4
Song, 1963 (41)	Various	68	1	0	0	3	1	1	0	2	1	0	2	0	1	5	17	25.0
Ahn & Suh 1963 (1)	Secondary infection	18	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	11.0
Chi et al. 1963 (12)	Otitis med. chronica	42	1	0	0	1	0	0	0	0	1	0	0	4	4	2	13	31.0
Kim (22) 1964	Hospital worker*	669	0	9	24	11	0	0	0	8	14	0	0	27	35	11	139	20.8
(23)	Hospital air	523	0	5	5	4	0	0	0	0	0	10	0	37	19	20	101	19.3
Lee (25)	Sick child.**	336	0	0	8	7	1	3	0	0	0	0	0	8	56	27	109	29.8
1965 (27)	Newborn***	141	1	0	0	5	0	1	15	0	0	0	0	2	25	10	60	42.5
Chun et al. 1965 (13)	Abscess	461	0	0	1	11	1	3	1	2	2	22	13	4	48	64	172	37.5
	Miscellaneous infection	127	0	0	0	4	1	0	0	1	0	4	3	3	8	22	45	35.4
Total		3,076	5	17	38	46	4	8	16	14	18	36	18	126	198	161	705	22.9

* Throat.
** Nose and throat of children who have inflammatory changes in throat.
*** Nose, throat and other materials in newborn infants and their surroundings.

this group are most predominant among staphylococci isolated from the nose and throat of hospitalized newborn infants, followed by those from surgical and miscellaneous infections, and otitis media chronica. Considerable numbers of hospital workers are also carrying epidemic strains, and they are found in the hospital air. Epidemic strains are very rare among strains isolated from the nose and throat of healthy individuals in urban areas. It is clear from these results that this university hospital environment is heavily contaminated and large numbers of surgical and other infections are due to epidemic strains, while carriers among healthy persons outside the hospital are

very few. Epidemic strains are reported by others to account for about 24 to 50 per cent of hospital strains [6, 29, 31, 32, 36] and vary according to the year of isolation [7, 38, 44].

Phage type 80/81 is the most predominant among the epidemic strains, with 198 strains out of 705, followed by types 81 and 80 with 161 and 126 strains, respectively. These three types comprise about 70 per cent of the epidemic strains and the others are very small in numbers. The in-vitro conversion of one subtype of epidemic strain into another by artificial lysogenization was described by Comtois [14], Rountree and Asheshov [37], Rosenblum and Jackson [34] and Blair and Carr [8] and other workers. It was also observed by Nahmias et al. [31] that two types of epidemic strains were isolated from the same or different sites, and different types within this group were isolated on alternate culturing periods; this finding would suggest type conversion in-vivo. Therefore, although the distribution of types within this group is not especially significant, it is true that types 80, 81 and 80/81 are the most frequent, possibly suggesting that these three types are the most stable forms within the group.

Antibiotic resistant strains in each group: All of the reports referred to this paper described the incidence of antibiotic resistant strains in each phage group, but only four reports are cited in this paper. Table 6 lists the results of Im [20] and Chun et al. [13]. Strains resistant to penicillin and streptomycin, which were used in Korea from the early days of the antibiotic era,

Table 6. Rates of antibiotic resistant staphylococci isolated from surgical infections in each phage group

Reported by	Resistant to	No. of strains	Phage group						
			I	II	III	IV	Mixed	N. T.*	Total
			No. of strains						
			50	11	20	4	40	24	150
Im, 1960 (20)	CP**	133	88.0***	90.0	100.0	100.0	85.4	83.3	88.7
	SM	95	64.0	36.4	60.0	100.0	70.7	58.3	63.3
	AM	36	28.0	27.3	10.0	75.0	24.4	12.5	26.3
	TM	46	40.0	36.4	25.0	75.0	26.8	12.5	30.7
	CM	15	18.0	18.2	11.1	0	4.9	0	10.0
	EM	8	10.0	0	0	0	2.4	0	5.3
			No. of strains						
			56	69	80	0	275	108	588
Chun et al, 1965 (13)	CP	581	96.4	98.6	98.8		98.9	100.0	98.8
	SM	374	67.9	43.5	60.0		67.3	67.6	63.6
	AM	330	44.6	44.9	58.8		60.0	57.4	56.1
	TM	511	71.4	87.0	77.8		85.8	96.4	86.9
	CM	355	67.8	57.7	52.5		61.5	61.1	60.3
	EM	180	32.1	24.6	32.5		28.7	37.0	30.6

* Non-typable.
** CP — penicillin, SM — streptomycin, AM — aureomycin, TM — terramycin, CM — chloramphenicol, EM — erythromycin.
*** Percentages of resistant strains in each group.

show markedly high rates in both reports and occur in almost the same percentages. The incidence of strains resistant to aureomycin, terramycin, chloramphenicol and neomycin obviously increased between 1960 and 1965, as shown by the reports of IM (1960) and of CHUN et al. (1965). These antibiotics were introduced in Korea around the late 1950's and were widely used beginning of the 1960's. The increase in the rate of resistant strains in CHUN's report indicates that the appearance of resistant strains roughly parallels the history of the use of antibiotics in the area.

Strains resistant to the respective antibiotics were almost equally distributed among each phage group. There was no special reletionship between antibiotic resistance and phage group.

Table 7 shows the results of the reports of KIM [22] and LEE [26]. The incidence of resistant strains is generally high in KIM's report. As KIM's study was made on strains from hospital workers and LEE studied strains from sick children who visited the hospital, the difference in the rates of resistant strains between the two reports may be indicative of the distribution of resistant strains in the hospital environment and patients who are not hospitalized. Since antibiotics can freely be purchased from drug stores in Korea without prescription, it is easy to suppose that resistant strains are present in a fairly high proportion of

Table 7. Rates of antibiotic resistant staphylococci isolated from nose and throat of carriers and patients in each phage group

Reported by	Resistant to	No. of strains	Phage group						
			I	II	III	IV	Mixed	N. T.*	Total
			No. of strains						
Kim, 1964 (22)			142	53	144	0	209	121	669
	CP**	649	98.6***	49.0	96.5		98.1	95.6	97.0
	SM	492	81.0	67.9	61.8		82.3	66.1	73.5
	AM	584	91.5	81.1	84.7		88.4	86.0	77.3
	TM	618	97.2	79.2	93.1		90.4	92.6	92.4
	TC	621	97.2	84.9	95.1		90.9	91.7	92.8
	CM	375	64.8	34.0	33.3		66.4	64.5	56.1
	EM	132	26.8	18.9	18.8		20.0	11.6	19.7
			No. of strains						
Lee, 1965 (26)			30	43	79	0	126	91	366
	CP	302	83.3	81.3	82.3		78.9	87.9	82.5
	SM	169	46.7	46.5	46.8		46.3	46.1	46.2
	AM	170	46.7	46.5	46.8		45.5	47.3	46.4
	EM	306	86.7	83.7	84.8		80.4	85.7	83.6
	TC	201	53.5	48.9	50.6		60.1	55.0	54.9
	CM	227	60.0	62.8	60.8		63.4	61.5	62.0
	EM	75	20.0	18.6	20.2		21.1	20.8	20.5

* Non-typable.

** CP — penicillin, SM — streptomycin, AM — aureomycin, TM — terramycin, TC — tetracycline, CM — chloramphenicol, EM — erythromycin.

*** Percentages of resistant strains in each group.

the general population. As for the distribution of resistant strains in each phage group, no special relationship is observed, as was the case also in the reports of I[illegible] [20] and CHUN et al. [13]. This finding is different from the reports of BLAIR and CARR [6], BYNOE et al. [10], ISHIHARA et al. [21] and MESSINGER et al. [30] who found that strains resistant to antibiotics are present in a higher proportion among staphylococci belonging to phage group III than in the other groups.

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韓国におけるブドウ球菌のバクテリオファージ型別，全熹基，韓国慶北大学校医科大学細菌学教室

摘　　要

韓国におけるブドウ球菌ファージ型別は1960年，任によって最初に行なわれ，その後現在まで10数編の報告があるが，1編を除くほかのすべては著者の研究室においてなされた．本綜説は以上の諸報告によって1960—1965年の期間韓国で各種の材料から分離されたブドウ球菌のファージ型と抗生物質抵抗性を概覧したものである。記述は健康保菌者，鼻腔，咽頭および病院の空中分離株のファージ群，同じく病的材料分離株，特に創傷感染分離株，流行株のファージ型，各群菌の抗生物質抵抗性と順次進められているが，主としてファージ群の分布，特にファージ型52/52A/80/81を示す菌株の分布に論旨が向けられている．抗生物質抵抗性を示す菌株の出現頻度は比較的高い。

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