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Relation between the Drug-resistance and the Groups and T-types of β -hemolytic Streptococcal Strains Isolated from the Pharyngeal Regions of School Children

Shizue MAEKAWA, Terumasa YAMAUCHI, Keiko TAKAHASHI,
Tomo YAMAGUCHI and Yuko TAKADA
Department of Microbiology, Sapporo Medical College
(Chief : Prof. T. T. A. Hayashi)

The drug-susceptibility of 833 strains of β -hemolytic streptococci (group-A, -B, -C, or -G) isolated from the pharyngeal regions of elementary school children in the City of Sapporo by monthly examinations over a 2 years and 5 months period between 1977 and 1979 with regard to the following 6 antibiotics, penicillin G(PCG), propyl-penicillin (PPC), chloramphenicol (CP), tetracycline (TC), erythromycin (EM) and josamycin (JM), were investigated by the MIC-test, using blood-agar plates.

All of the isolated strains tested were sensitive to PCG and PPC, while some strains were resistant to one or more of the remaining 4 antibiotics. All of the T4-, T12- and T13-group A streptococcal strains isolated were resistant to at least one of the 4 antibiotics. On the other hand, all of the T28- and B3264-group A streptococcal strains isolated were sensitive to all of the 4 antibiotics. Both sensitive and resistant strains were found among the isolated T1-group A streptococcal strains.

Many of the T4 and T12 strains resistant to EM were highly resistant strains with an MIC of EM of $100\mu\text{g/ml}$. From examinations of the MIC of EM to the T12 strains isolated from the same individual carrier at different times, the highly resistant nature of the T12 strains to EM seems to be stable. In the case of T4 strains, however, lability of the highly resistant nature to EM was suggested from the fact that both highly resistant and sensitive strains could be isolated at the same time from the same individual carrier.

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Key words: β -Hemolytic streptococci, Drug resistance, Antibiotic susceptibility

1 Introduction

Investigations of the drug-resistance of β -hemolytic streptococcal strains isolated at various areas in Japan have been carried out for many years by the Research Group for Scarlet Fever, and the results have been reported annually¹⁻³⁾. These results showed that many streptococcal strains are without concern to the type and group of those strains isolated resistant to single or multiple drugs, among tetracycline (TC), erythromycin (EM), josamycin (JM), lyncomycin (LCM) and chloramphenicol (CP). Strains resistant to penicillins, however, have never been isolated.

The present authors carried out monthly examinations of pharyngeal streptococcal carriers from the lower grades of elementary school for 2 years and 5 months, between 1977 and 1979 in the City of Sapporo⁴⁾. The total number of subjects examined was 2921, with 833 strains of β -hemolytic streptococci being isolated from these subjects. Among these, 593 strains were tested for sensitivities to the following antibiotics; CP, EM, JM, TC and the penicillins, PCG and PPC. The results were expressed by the MIC of the antibiotics to each streptococcal strain. The relations between the appearance of antibiotic-resistant strains, the pattern of resistance to various antibiotics, and the groups and T-types of the isolated strains were

investigated. The present paper deals with the results of these experiments.

2 Materials and Methods

2.1 Isolation of β -hemolytic streptococci from the pharyngeal swabs and grouping and typing of isolated streptococcal strains

The methods have been described precisely in our previous paper⁴⁾.

2.2 Titration of MIC of various antibiotics

CP, EM, JM, TC, PCG and PPC were used for the MIC-tests, and the standard agar plate method, established by the Japanese Association of Chemotherapy, was employed⁵⁾. The concentrations of antibiotics ranged in 13 steps from 100 μ g/ml (or 100u/ml) to 0.025 μ g/ml (0.025u/ml) with 2-fold serial dilution. The strains of streptococcus were cultivated at 37°C for 20 hrs in 2 ml of Todd-Hewitt broth (THB) and were inoculated spot-like on the surface of the agar plate, in which a definite concentration of antibiotics was contained, by an automatic inoculator (SAKUMA, Type MIT-P). Twenty-seven strains could be inoculated at one time on one agar plate. The growth of the strains of streptococcus was checked after a 48 hr incubation period at 37°C, after which the MIC of each antibiotic was tested for a definite strain of streptococcus.

3 Results

3.1 The MICs of CP, EM, JM, TC, PCG and PPC against the streptococcal strains isolated from the pharyngeal regions of school children

The MICs of the above-mentioned 6 antibiotics against the 593 streptococcal strains isolated from the

Table 1 *Frequencies of appearance of strains having the MIC of the 6 kinds of antibiotics among the β -hemolytic streptococci isolated from the school children during the period, August 1977-December 1979*

MIC μ g/ml	No. of Strains (%)					
	PCG	PPC	CP	EM	JM	TC
0.025	548(92)	262(44)		6(1)		
0.05	36(6)	263(44)		79(13)		
0.1	9(2)	64(11)		308(52)		
0.2		4(1)		44(7)	45(8)	
0.39			3(0.5)	2(0.3)	416(70)	38(6)
0.78			21(3.5)	6(1)	24(4)	53(9)
1.56			89(15)	2(0.3)	3(0.6)	22(4)
3.13			309(52)	11(2)	1(0.1)	32(5)
6.25			91(15)	42(7)	4(0.7)	28(5)
12.5			4(1)	10(2)	13(2)	85(14)
25.0			60(10)	4(1)	2(0.4)	216(36)
50.0			16(3)	3(0.5)	1(0.1)	106(18)
100.0				1(0.1)		13(2)
>100.0				75(13)	84(14)	
No. of resistant strains(%)	0	0	80(13.4)	156(26.3)	132(22.2)	480(80.9)
Grand total	593	593	593	593	593	593

◻ : Zone of resistant strains.

As shown in Table 2, 497 out of 593 strains were resistant to some of the CP, EM, JM and TC, singly or multiply. The ratio was 83.8%. The patterns of drug resistance were as follows: Three-hundred and thirty-two of the single TC-resistant strains (66.8%) were found with the highest rate of occurrence. The second highest rate of appearance was of strains of triple drug-resistance, with 92 strains (18.5%) being counted as the total of the following 3 kinds of triple drug-resistants: TC-CP-EM, TC-CP-JM, or TC-EM-JM. The third highest rate of occurrence was of double drug-resistant strains, and totaled 39 (7.8%) for the following 4 kinds of double drug-resistants: TC-CP, TC-EM, TC-JM or EM-JM. The lowest rate of appearance was of strains of quadruple drug-resistance, TC-CP-EM-JM, with 34 strains (6.9%) being counted.

The relation between the groups of isolated strains and the frequencies of appearance of the strains resistant to at least one kind of antibiotic among TC, CP, EM and JM was as follows; 411 out of 474 isolated group A strains, 35 out of 57 isolated group B strains, 46 out of 51 isolated group C strains and 6 out of 11 isolated group G strains were resistant strains and, therefore, the ratios of appearance of resistant strains for each group strain were calculated as 86.7, 61.4, 90.1 or 54.5%.

The relation between the T-types of isolated group A streptococcal strains and the patterns of appearance of the resistant strains was follows; all of the isolated T4, T12 and T13 strains were resistant strains, whereas all of the isolated T28 and B3264 strains were sensitive to all of the 6 antibiotics used for the present experiment. Seventy-four percent of the isolated T1 strains were resistant, while the remaining 26% were sensitive strains. The patterns of drug-resistance of the isolated T1, T4, T12 and T13 differed from each other, i. e. 60% of T1-resistant strains were triple drug-resistants (TC-CP-EM), 79.4% of T4-resistant strains were single TC-resistants, and 85% of T12-resistant strains were quadruple drug-resistants (TC-CP-EM-JM). All of isolated T-untypable strains (ut) which were not reactive to any of the 22 T-type typing sera of group A streptococci, showed drug resistance, while 55 strains (91.6%) were single TC-resistants.

4 Discussion

Five-hundred and ninety-three strains of β -hemolytic streptococci, isolated from the pharyngeal regions of children of the lower grades of an elementary school in the City of Sapporo between 1977 and 1979, were tested for the MICs of the following 6 antibiotics; PCG, PPC, TC, CP, EM and JM. Four hundred and ninety-seven strains (83.8%) were found to be resistant to at least one of the 4 antibiotics with the exception of the two kinds of penicillins. Eighty percent of all the isolated β -hemolytic streptococcal strains were group A streptococci, in which 86.7% of drug-resistant strains were contained. These results were almost the same as those for the rate of appearance of drug-resistant group A streptococcal strains in 1021 strains collected from various parts of Japan between 1974-1975, as reported by Nakae and his collaborators⁶⁾. The rate was calculated as 81.6% in their report. It has been shown in the reports of various investigators, not only in Japan but also in other countries, that none of the β -hemolytic streptococcal strains resistant to penicillins have ever been isolated⁷⁾. The same results were obtained also in our present investigation. As shown in our present report, however, there was a tendency towards an increase in the rate of the appearance of strains resistant to CP, EM, JM or TC.

As for the relation between the occurrence of drug-resistant strains and the T-types of group A streptococcal strains, it has been reported that the rates of occurrence of drug-resistance of T4- and T12-strains, which are known as the 2 main strains isolated in Japan, were higher than those of the other T-type strains⁸⁻¹⁰⁾. The same results were obtained in our present investigations and all of the strains of these two T-types were resistant strains. The rates of appearance of drug-resistance in the T28- and B3264-strains, as has also been shown in the report of other investigators^{6,9)}, were very low. In the case of our present

investigations, all of the strains of both of these types of streptococci were sensitive to the antibiotics tested. The frequency of isolations of the group A streptococci of these two T-types was lower than that of the isolations of T1-, T4- or T12 strains throughout Japan. The lower occurrence of drug-resistance in the strains of T28- and B3264-group A streptococci is probably due to the lower frequency of isolations of the strains of both of these two types. In the case of T1-strains, the rate of appearance of drug-resistant strains varied among the reports of various investigators^{1,6,9)}. In our present investigation, 74% were resistant and 26% were sensitive.

Although it has been reported that most of the EM-resistant T12 strains are highly resistant strains, ones which resist concentration of up to 100 μ g per ml, the occurrence of EM-resistant T4 strains has never clearly been reported in our country. In the case of our present investigations, EM-highly resistant strains, which were able to grow under conditions containing 100 μ g of EM, were isolated. It was of much interest in these investigations that examples of the existence of both EM-sensitive T4 colonies of streptococci, which could be inhibited by a concentration of 0.2 μ g, and EM-highly resistant T4 colonies of streptococci, which could grow under conditions of more than 100 μ g, could be found on the same agar plate. These facts suggest that the characteristics of the EM-resistance of T4 strains are not as stable as those of the EM-resistance of T12 strains, and also that the missing colonies of the T4 strain, resistant to EM, may occur unless multiple T4 colony tests from a single specimen are performed.

5 Conclusions

Five-hundred and ninety-three β -hemolytic streptococcal strains, isolated from the pharyngeal regions of children of the lower grades of an elementary school in the City of Sapporo between 1977 and 1979, were tested for their sensitivities to the following 6 antibiotics; PCG, PPC, CP, EM, JM and TC. The results obtained were as follows:

- 1) All of the isolated β -hemolytic streptococcal strains were sensitive to penicillins (PCG, PPC).
- 2) Four hundred and ninety-seven strains (83.8%) were resistant to at least one of the following 4 antibiotics; CP, EM, JM and TC.
- 3) TC-single resistant strains had the highest rate of occurrence, which was 66.8% (332 out of 497 resistant strains). The rate of appearance of triple drug-resistant strains (TC-CP-EM, TC-CP-JM and TC-EM-JM) was the next highest, 18.5% (92 out of 497 resistant strains). This was followed by of double drug-resistant strains (TC-CP, TC-EM, TC-JM and EM-JM), which was estimated as 7.8% (39 out of 497 resistant strains), and lastly by that of quadruple drug-resistant strains (TC-CP-EM-JM), which was estimated as 6.9% (34 out 497 resistant strains).
- 4) Four-hundred and seventy-four strains (79.9%) among the 593 isolated strains were group A streptococcal strains, and 411 strains (86.7%) were strains resistant to at least one of the antibiotics used for the present experiments. Fifty-nine group B strains contained 35 drug-resistant strains (61.4%), 51 group C strains contained 46 drug-resistant strains (90.1%) and 11 group G strains contained 6 drug-resistant strains (54.5%).
- 5) The 474 group A streptococcal strains isolated contained T1-, T4-, T12-, T28-, B3264- and ut-strains. All of the T4-, T12- and T13-strains isolated were resistant to at least one of the above-mentioned 4 antibiotics, with the exception of the penicillins. On the other hand, all of the T28- and B3264-strains were sensitive to all of the 6 antibiotics, including the penicillins. The T1 strains isolated were both sensitive and resistant ones.
- 6) Most of the isolated T4- and T12-strains resistant to EM or JM were highly resistant strains, able to grow under conditions involving concentrations more than 100 μ g per ml.
- 7) It was observed that the characteristic of high EM-resistance of T4 strains was labile and could

easily be released.

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Address for reprint requests :

Shizue Maekawa

Department of Microbiology, Sapporo Medical College
Minami-1, Nishi-17, Sapporo, 060, Japan

学童咽頭分離株である β 溶血レンサ球菌の群別・T型別と薬剤耐性との関係について

前川 静枝 山内 暉真 高橋 圭子
山口 とも 高田 裕子

(札幌医科大学微生物学講座)

要 約

1977年から1979年の2年5カ月間の札幌市内学童の毎月1回実施した咽頭培養検査から分離された β 溶血レンサ球菌(A-, B-, C-, G群を含む)2921株の薬剤感受性試験をした。用いた抗生物質はペニシリンG(PCG), プロピールペニシリン(PPC), クロラムフェニコール(CP), テトラサイクリン(TC), エリスロマイシン(EM), ジョサマイシン(JM)の6剤で, MICは寒天平板希釈法で測定した。

その結果, すべての分離株はPC-感受性であるが, 他の4剤に対して耐性を示した。A群溶血菌のT型別との関係は, T4, T12, T13のすべては耐性を示したが, T28, B3264はすべての感受性であった。T1の場合は, 両者の菌が分離された。

T4, T12のEM-耐性菌の大部分は, $>100 \mu\text{g/ml}$ の高度耐性菌であった。T12のEM-高度耐性の性質は安定であったが, T4の場合は同一保菌者の同一時期の検索から感受性株と高度耐性株が同時に分離されたことから不安定であることが示唆された。