A Study of Haemolytic Streptococci Isolated from Outpatients in Dermatological Clinics

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(Received: November 5, 1990)
(Accepted: December 10, 1990)

Key words: streptococcal skin infection, group A streptococcus, T-type, M-type, ADNB

It appears that skin infections involving streptococci have recently decreased in Japan due to improved sanitary and nutritional conditions, not to mention the wide availability of antibiotics. However, there have as yet been published no reports on the exact status of such infections.

This study was undertaken to provide a better understanding of the current status of skin infections in Japan, with a main focus on the frequency of streptococcal skin infections and the bacteriological characteristics of isolates from patients.

Materials and Methods

Patients: Patients with clinically-suspected streptococcal infections were examined bacteriologically or serologically in the outpatient clinics of the Department of Dermatology, Ohashi Hospital, Toho University School of Medicine and the Department of Dermatology, Kanto Central Hospital, Tokyo, during the one-year period from December, 1988 to December, 1989.

Bacteriological examinations: Direct culture of pus, exudate or crusta on skin or throat swabs from patients suspected of having streptococcal infections were examined for three main aetiological agents. Suspected colonies grown on primary blood agar plates were examined by catalase test, gram staining and microscopical method, followed by the tests specified for each pathogen as described below.

1) Pyogenic streptococcus: For colonies confirmed by beta-hemolysis on a blood agar plate, serological grouping and typing, as well as OF (Opacity Factor) tests were undertaken as previously described1).

Grouping and typing sera supplied for this study included the following:

Grouping sera: group A, B, C, E, F, and G

T-typing sera: polyvalent serum T, U, W, X, and Y. monovalent serum type 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 18, 19, 22, 23, 25, 27, 28, 44, 49, B3264, and Imp19, and Provisional type (Prov.) Thai151).

M-typing sera: type 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 18, 19, 22, 23, 27, 28, 29, 36, 39, 41, 44, 48, 49, 53, 54, 55, 56, 57, 60, 63, Prov. Thai15, and Prov. Matsuyama21662).

R-typing sera: 3R and 28R

2) Staphylococcus aureus: Suspected colony was cultured on No. 110 medium and PS Latex “Eiken”
was used for differentiation of *Staphylococcus aureus*.

3) *Pseudomonas aeruginosa*: Suspected colony was confirmed using NAC medium.

Antibody titration of group A streptococcus in patients: Three kinds of antibodies of group A streptococcus were measured using commercial kits, including Blue ASO, manufactured by FUJIREBIO INC., for anti-streptolysin O (ASO) titration, SERODIA ASK (FUJIREBIO INC.) for anti-streptokinase (ASK) titration and HEMOPROBE-TEST (CARTER-WALLANCE INC.) for anti-deoxyribonuclease B (ADNB) titration.

Immunization study for group A streptococci isolated from patients with skin infections: For the study of M antigens of skin strains, two group A streptococci obtained from patients, namely strains OD8 and OD17, were immunized to rabbits following the ordinary method using heat-killed whole cell vaccines. Strain OD8 was isolated from the pus of a patient with impetigo and typed as T13-49, MNT (Mnon-typeable) and OF-negative [OF(−)]; OD17 was taken from the pus of a patient with erysipelas and typed as T11, MNT, and OF(+).

After absorption of group A antibody, hyperimmune sera were tested with HCl extracts from the following reference strains: M-type 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 26, 27, 28, 30, 33, 34, 36, 37, 39, 40, 41, 42, 43, 44, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, Prov. Thai15, Prov. Matsuyama2166 and R-type strains 3R and 28R.

Corresponding types of absorption antigens, which had shown precipitation lines on micro gel agar method, were supplied for absorption of cross reaction between hyperimmune serum and the reference strains.

Absorption test: To obtain antigens for absorption tests, packed cells of overnight cultures of reference strain (1 g wet weight) were mixed with 1 ml of serum. After incubation at 37°C for 1 hour, the mixture was incubated in a cold room (about 4°C) overnight; the existence of precipitin in the supernatant of the mixture was then tested. For absorption of reaction by group A polysaccharides, strain A 25 Matthew was supplied as the absorption antigen.

### Results

Patient diagnoses: A total of 44 patients were examined in this study; the diagnoses made are shown in Table 1. There were 15 cases (34.1%) of erysipelas, 9 cases (20.5%) of impetigo and 7 cases (15.9%) of infections occurring other than on the skin but with symptoms appearing on the skin, such as scarlet fever.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total</th>
<th>Streptococcus group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>C</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>Impetigo</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Phlegmon</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Furuncle</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ucer</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinea pedis (including secondary infections)</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Psoriasis guttata</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary infection</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Other infections</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>with symptoms on skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>44</td>
<td>22</td>
<td>2</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
or urticaria.

The age distribution of patients with erysipelas or impetigo is shown in Table 2. Relatively older patients were seen to have a tendency to suffer from erysipelas.

Bacteriological findings: As a rule, cultures were attempted with samples taken from injuries: pus, exudate or crusta. But in case of erysipelas, besides pus or erosion, small injuries located near erosions as well as throat swabs were examined for the presence of infectious agents.

Table 1 also shows the bacteria detected in relation to diagnoses made. Among samples from impetigo patients, group A streptococci were identified in 7 of 9 cases. In cases with erysipelas group A streptococci were detected in 4 cases and staphylococci were in 5. They were isolated mainly from pus or erosions. From these results, it can be clearly seen that streptococci still occur in skin infections with classical symptoms.

Type distribution of group A streptococci from skin infections: Table 3 shows serological group, T-type and M-type distributions of haemolytic streptococci isolated from 26 cases of skin infection. Eighteen (69.2%) of the strains were serologically classified as group A. The prevalent T-types were T13, T1 and T11, and 4 strains (15.4%) were unknown T complexes. Except for 5 strains which were typed as M1, M9, and M28, 21 (80.8%) of them were non-typable using M-typing sera normally employed in routine typing. The strains marked with asterisks (*) were later identified by immunization study.

T-type and M-type combinations of group A streptococci: In this study it was confirmed that some group A streptococci produced discrepancies in typing results between T-antigen and M-antigen in antigen number, as shown in the following.
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1) Strain OD82: This strain was isolated from a patient with impetigo, and the same strain was identified in the same patient's pus, throat, and nose. The strain was agglutinated by T11 typing serum, and HCl extract of this strain reacted to M9 typing serum by precipitation reaction but did not react to M11. Confirmation was made by cross-absorption reaction using the antigen-antibody system of M11 and M9 as shown in Fig. 1. On the left side on the photo, HCl extract from OD82 made a precipitation line against M9 typing serum which was completely connected with a line made between M9 extract and M9 typing serum, as well as a faint line against M2 typing serum, which made a spur to a line between M2 extract and M2 typing serum. M9 typing serum was absorbed with the related antigens, such as strains M9, M11, and OD82, and it was confirmed that the antigenicities of strains OD82 and M9 were identical but had no relation to type M11.

2) Strain OD8: Hyperimmune serum with OD8 was absorbed with strain A 25 to remove group A
polysaccharide antibodies; precipitation reaction to extracts from M43 and M56 remained in addition to OD8, as shown in Fig. 2. Both strains M43 and M56 could effectively absorbed the cross reaction, and OD8 antiserum (No. 364) exhibited a precipitation line only to strain OD8, as far as our reference strains for M-typing were concerned. Strain OD8 was typed as T13-49, MOD8, OF (−).

3) Strain OD17: Hyperimmune serum (No. 365) against OD17 whose type was T11, MNT, OF (+), showed a precipitation line to the extract from M62 which clearly connected with the extract from OD17, after the absorption of polysaccharide antibody. It was suspected that the M-type of strain OD17 was M62, as indicated by absorption test of hyperimmune serum with strain M62 (Fig. 3). Strain OD17 was identified as T11, M62, and OF (+). The same precipitation reaction could be observed in strains OD1 and OD2, whose T-types were also T11.

Antibody responses of streptococcal antigens in the sera of patients: Fig. 4 shows the changes of antibody titer of ASK, ASO and ADNB in three patients, two with erysipelas and one with impetigo, from whom paired sera could be taken. At the first visit, group G streptococcus was isolated from an erosion in case (A). The titer of ASK and ASO had greatly increased by the next week, but there were no changes in ADNB titer. In case (B), a patient with erysipelas, group A streptococcus, T1, M1, OF (+), was isolated from the nose at the first visit, at which time three antibody titers were already seen to be increased. Case (C) was a patient with impetigo from group A streptococcus T11, M9, OF (+) was detected from the throat, nose, and pus, with remarkable antibody responses shown by the three titers after two weeks.

From these results, it was seen that both titers ASK and ASO could reflect infections with group A and G streptococci, while ADNB could not reflect group G streptococcal infection.

Discussion

This report revolves around three focal points: 1) confirmation of the continued existence of streptococcal skin infections; 2) clarification of prevalences of etiological agents, especially groups and
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...types of streptococci; 3) discussion of the present status of skin infections in Japan, in comparison with streptococcosis in the upper respiratory tract, and to compare the status of skin infections in a few other Asian countries.

It is widely-commented that streptococcal infections are on the decrease, especially in developed countries, ever since the beginning of "the antibiotic era". In Japan, we have observed this same decreasing tendency as complications of streptococcal infections such as rheumatic fever, rheumatic heart diseases and acute glomerulonephritis are now quite rare and the symptom of scarlet fever and streptococcal sore throat are less severe than before, even though there are still many patients with these diseases as well as carriers of streptococci in the upper respiratory tract. Skin infections with streptococci also seem to have been following similar trends. It is impossible to obtain exact data on the morbidity of streptococcosis on the skin and the upper respiratory tract, so we have undertaken to get statistical resources from mortality trends. According to Vital Statistics, records of the Japanese Government cases of acute rheumatic fever (ICD-9, 390-392) and 1492 cases of chronic rheumatic heart disease (393-398) were registered in 1987, compared to 1025 cases of rheumatic fever (ICD-8, 400-402) and 5332 cases of rheumatic heart disease (410-416) in 1961. As for streptococcal skin infections, it is too difficult to reliably speculate the actual status only from statistical figures, and, unfortunately, a search of the literature did not turn up any reports about the prevalences of these infections. Supposing that all of the 57 deaths attributed to acute glomerulonephritis (ICD-9, 580) in 1987 resulted from streptococcal infections, we can speculate the following: serious infections directly involved with this complication seem to be decreasing, as indicted by the change of patient age; there were two peaks in 1961, one under 20 years of age and the other 70 to 80 years of age, while in 1987, only one peak is found, namely that of the elderly. Therefore, new young patients were thought to be increasingly rare.

Nishiwaki estimated the volume of new patients with erysipelas visiting the average outpatient clinic in Japan as being 0.05-0.08%. In this report, 15 patients with clinically-diagnosed erysipelas were further examined by bacteriological culture. From six of them haemolytic streptococci were isolated, and some were confirmed by antibody responses. Staphylococci and pseudomonas were also found in the other 7 patients, but the etiological relationships were not clear, because these pathogens are frequently observed in our environment. It is said that detection of agents in erysipelas is sometimes difficult. In this report, in addition to erosion or pus, presumable portal of entry such as the throat, external auditory canal or canthus in cases of facial erysipelas, and injuries or surface of the erosion with tinea on foot in cases of erysipelas on the leg were examined by culturing. The results are not yet complete, but it seems that streptococci tend to be isolated mostly from pus or erosions.

In cases of impetigo, group A streptococci were detected in 7 of 9 patients. In a patient from whom group A streptococcus typed as T11, M9, OF(+) was isolated, isolations were possible from many sources: pus on the arm, throat and nose. This case seems to be one of typical transmission, as described by Ferrieri et al. Moreover, in this patient, elevation of antibodies against ASO, ASK, and ADNB could be observed 17 days after the first visit.

As a supplement to culturing, serodiagnostic methods employing ASK, ASO, and ADNB were carried out in some of the patients. Titration of ADNB was preferred because of repeatedly low or non-response of ASK or ASO in cases of skin infections. In this report, responses of ASK and ASO could be observed in infections by both group A and G streptococci, as shown in Fig. 4. In the two cases involving group A streptococci, positive cultures from the nose or throat were observed but in one case from group G, streptococci could not be isolated from these locations. It is difficult to discuss non-responses of titers in patients with skin infection in this report, because the antibody response is usually observed in carriers with streptococci on the throat.
Table 4 Type distribution of group A streptococci isolated from upper respiratory infections in Matsuyama city (Dec. 1988—Dec. 1989)

<table>
<thead>
<tr>
<th>T-type</th>
<th>No. of strains</th>
<th>M-type</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>NT</td>
<td>48</td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>NT</td>
<td>1</td>
</tr>
<tr>
<td>12-28</td>
<td>17</td>
<td>Matsuyama 2,166</td>
<td>16</td>
</tr>
<tr>
<td>28</td>
<td>20</td>
<td>Matsuyama 2,166</td>
<td>5</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>NT</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>NT</td>
<td>3</td>
</tr>
<tr>
<td>B3264</td>
<td>2</td>
<td>NT</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>25-Imp19</td>
<td>1</td>
<td>NT</td>
<td>1</td>
</tr>
<tr>
<td>11-12-44-28</td>
<td>1</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

Total 243 243

Streptococcal infections of the skin are important because of their relation to poststreptococcal nephritis (acute glomerulonephritis) such as streptococcal infections other than the skin, especially in tropical, subtropical and temperate zones throughout the world. Besides climate, other important factors affect the development of acute glomerulonephritis, including the presence or absence of an infectious agent whose M-type is specific to type 2, 49, 55, 57, 59, 60, and 61, as well as the level of hygiene.

As shown in Table 3, the types of group A streptococci isolated from the skin were varied. Compared with our knowledge of streptococcal sore throat, prevalent T-types of isolates were seen to involve various different types being rather similar to types isolated in Thailand and Malaysia. This fact is emphasized by the points of T-complex patterns and M-nontypability. From these results, it was suspected that group A streptococci isolated on the skin might have different characteristics, from those causing upper respiratory infections in Japan, too.

The fact the so-called “impetigo strains” usually exhibit T-complex patterns is well known. T3-13-B3264-12, T8-25-Imp19 and T5-11-12-27-44 have been called “Parker’s impetigo strains” and many types of strains, designated as higher number M-antigen, were related to these agglutination types. Other skin types, T14-49, T15-17-19-23-47, and T4 were thought to have numbers M49, M54, M60, respectively, as in the outbreak at Red Lake. T-typing sera available for routine typing is quite limited. The reason for this is that few studies of T-antigens are performed on higher-numbered strains, because of the lack of a relationship of T-antigen to virulent material. So, sometimes instead of specific, high-number T-protein, common T-antigens which are representative of the types available with T-typing sera, are used for T-type identification. T-protein is shared by many M-types of strains. Consequently it was observed that higher-numbered M-strains have a tendency to be identified as T-complex patterns.

In this study unique combinations of T- and M-antigens could be confirmed. Combinations of T11 and
M9, T11 and M62, and T13-49 and Prov. OD8 seemed to be unique and different from those in previous reports. As for the strain designated as Prov. type OD8, it has a possibility of being a strain already known as a number higher than 63. Because the 34 kinds of M- and R-typing sera supplied for this study only went up to number M63, as did the 52 kinds of reference extracts, sera and strains numbered higher than 63 could not be identified here. Moreover, it has possibility of being a new antigen. Like the strains we found in studies in Thailand\(^{11}\) and Malaysia\(^{14}\), unknown antigenicity differencing from ordinary M-types covered by routine typing sera might exist among wild strains in Japan.

As for the quality control of typing sera using in this study, the type distribution of strains isolated from upper respiratory infections in the same period is shown in Table 4. There are large discrepancies between the results of Table 3 and Table 4. These results led us to suspect that different kinds of group A streptococci were responsible for infections and manifestations in skin infections and upper respiratory infections. Based on the results of previous investigations, the virulence of group A streptococcus decreases when it spreads outside from the mouth, so that strains isolated in the environment were considered to not be M-typable. The question is whether a relationship exists between such strains and those carried on the skin.

Epidemiological studies of streptococcal skin infections are not yet clear. Ferrieri et al.\(^{8}\) put forth a model of how streptococci can exist on the skin and throat. In their model, group A streptococci appers at first on the skin, then after some interval they are detected on the throat. However, this model seems insufficient to explain all of the differences we found in this study. The nephritogenicity of group A streptococci is also well known, moreover the types of group A streptococci found seem to differ according to the type of preceding infection\(^{9}\): type M1, 3, 4, 12, 25, from pharyngitis-associated acute glomerulonephritis (AGN), and type M2, 49, 55, 57, 59, 60, 61 from pyoderma-associated AGN. This might be one proof of differences among indigenous bacteria; moreover there might be some difference in factors concerning infection or manifestation of primary infection and secondary diseases between these types of infection. In the strains isolated from upper respiratory infections or carriers, so-called “pharyngitis associated types” could be seen quite frequently in Japan. However, no information concerning strains on the skin, both with and without infection, is as yet available in Japan. This report deals with groups and types of streptococci isolated from skin infections, which are showing an increased prevalence in older patients even in Japan.

From the philippine Health Statistics published in 1983\(^{17}\), death caused by nephritis, nephrotic syndroms and nephrosis (ICD-9, 580—590) was registered at a rate of 9.3 per 100,000 population. It is certain that this figure is composed not only of AGN cases, but even so, it is clear that many infections and deaths caused by streptococcal sequelae occur frequently in our neighboring countries\(^{18}\)\(^{19}\). So, it seems to be very important to investigate the status of skin infections involving streptococci not only from bacteriological or epidemiological standpoints, but keeping in mind as well effective preventive methods, especially in developing countries.

**Conclusion**

A total of 44 patients suspected of streptococcal infection were studied in our outpatient clinics in Tokyo during the one-year period from December, 1988 to December, 1989.

Our conclusions were as follows:

1) There were 9 cases of impetigo and 15 of erysipelas showing typical clinical manifestations.
2) Some of the skin infections were caused by group A streptococci whose M-types were different from those found in upper respiratory infections in Japan.
3) The results of type identification were quite similar to those in Thailand or Malaysia.
4) There were found group A streptococci which had unique T- and M-type combinations.
Further epidemiological studies on skin infections involving streptococci should be carried out in order to better know the present status of infections in Japan, as well as to help in planning preventive measures against streptococcal diseases and their complications in countries where they are as yet prevalent.

Abstract

A total of 44 patients suspected of streptococcal infections were studied in outpatient clinics in Tokyo during the one year from December 1988 to December 1989. Employing bacteriological culturing and serodiagnosis, the following results were obtained:

1) There were 9 cases of impetigo and 15 cases of erysipelas with typical clinical manifestations and age distributions.
2) It seemed that some of the skin infections were caused by group A streptococci whose M-types were different from those of upper respiratory infections typically occurring in Japan.
3) The type distribution of group A streptococci found were quite similar to those isolated in Thailand or Malaysia.
4) There were found group A streptococci exhibiting unique combinations of T- and M-types, such as T11 and M9, T11 and M62 or T13-49 and MOD8 (Provisional type).
5) As for serodiagnostic method, ADNB (anti-deoxyribonucleas B) titer reflected infection by group A streptococcus only, while ASK (anti-streptokinase) and ASO (anti-streptolysin O) reflected not only group A streptococcal infections but group G infections as well.

References

Group A streptococcal skin infection


皮膚科外来患者より分離された溶血レンサ球菌の研究

レンサ球菌感染症研究会（会長：中島邦夫）
東邦大学医学部公衆衛生学教室
村井 貞子 稲積 温子
東邦大学医学部皮膚科学研究所
西脇 宗一 野田 佳子
関東中央病院皮膚科
日野 治子

（平成2年11月5日受付）
（平成2年12月10日受理）

要 旨
1988年12月より1989年12月の間に、皮膚科外来を訪れた患者のうち、レンサ球菌感染症を疑われるものを含む44例について、細菌学的、血清学的検査を行った。
結果は次の如くであった。
1）膿痂疹9例と丹毒15例が含まれており、共に典型的な臨床症状と年齢分布を示すものであった。
2）A群レンサ球菌を原因菌とするものでは、そのM型は、通常日本でみられる上気道感染由来の菌型とは異っていた。

3）また、T、M型共にタイ国、マレーシア等でみられる菌型と類似していた。
4）A群レンサ球菌の中には従来の報告には見られないT、M抗原の組合せが存在した。つまりT11とM9、T11とM62、T13-49とOD8（今回分離された亜定型）等である。
5）血清診断の方法として用いたADNB（anti-deoxyribonuclease B）価の測定では、A群レンサ球菌の感染のみにより有意に上昇したが、ASK（anti-streptokinase）価、ASO（anti-streptolysin O）価ではA群のみでなくG群レンサ球菌によっても有意な上昇を示した。

平成3年8月20日