Jpn. J. Infect. Dis., 59, 2006

Laboratory and Epidemiology Communications

Epidemiology and Molecular Analysis of Group A Streptococci from Patients Involved in Food-Borne Disease Outbreaks in Japan between 1996 and 2003

Daisuke Tanaka, Tomoko Shima, Junko Isobe, Masanori Watahiki*, Masakado Matsumoto[1], Miyoko Endoh[2], Rumi Okuno[2], Kikuyo Ogata[3] and Yoshiyuki Nagai

Department of Bacteriology, Toyama Institute of Health, Toyama 939-0363; [1]Department of Microbiology, Aichi Prefectural Institute of Public Health, Nagoya 462-8576; [2]Department of Microbiology, Tokyo Metropolitan Public Health Research Institute, Tokyo 169-0073; and [3]Department of Bacteriology, The Oita Prefectural Institute of Health and Environment, Oita 870-1117, Japan

Communicated by Haruo Watanabe

(Accepted April 12, 2006)

Group A streptococcus (GAS) is a pathogen responsible for human infection through various modes. Acute pharyngitis caused by GAS is common among children. Recently, various molecular techniques have been applied to the epidemiological investigation of GAS isolates. For example, *emm* typing, which involves the sequence analysis of the 5′ end of the *emm* gene that encodes the M protein, has been widely used to characterize GAS isolates because of its high discrimination power and versatility. The Centers for Disease Control and Prevention (CDC) maintains a database (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html) containing >150 *emm* types for GAS. Multilocus sequence typing (MLST) is also a highly discriminatory and unambiguous method of characterizing bacterial isolates. MLST is based on the sequence variation of the internal fragments of housekeeping genes. The different sequences at each locus are assigned different allele numbers, and each unique profile is assigned a sequence type (ST). Enright et al. (1) developed a method used on *S. pyogenes*, and a public database (http://spyogenes.mlst.net/) is maintained.

In Japan, there have been frequent reports of large food-borne outbreaks of streptococcal pharyngitis in recent years. Since the late 1990s, seven food-borne outbreaks caused by GAS have been reported (Table 1), as opposed to only two cases up to and including the early 1990s (2,3). According to a review on food-borne streptococcal pharyngitis (4), the main cause of this infection is the poor handling and preservation of cold salads that generally contain eggs; further, epidemics tend to occur in warm months. We found this to be true of the recent food-borne streptococcal epidemics in Japan as well–egg was the most common ingredient, and all epidemics took place in warm months, i.e., between May and September. Therefore, to prevent food-borne streptococcal pharyngitis as well as other food-borne diseases, food handlers should pay great attention to personal hygiene when handling food.

We performed T serotyping, *emm* typing, and MLST for 16 GAS isolates from seven food-borne outbreaks in Japan during 1996 - 2003 (Table 1). Five T/*emm* types were found: T1/*emm1*, T22/*emm22*, T28/*emm28*, TB3264/*emm68*, and

Table 1. Food-borne outbreaks of group A streptococcal infection in Japan, 1996-2003

Date	Place (Prefecture)	Setting	No. of patients	Vehicle	No. of isolates	T type	*emm* type	ST	allele							Reference
									gki	*gtr*	*murI*	*mutS*	*recP*	*xpt*	*yqiL*	
1996. 5	Aichi	Sports meeting	244	Catering lunch, including boiled eggs, boiled fish paste, fried chicken, and wakame-gohan	3	T1	1	28	4	3	4	4	4	2	4	8
1997. 5	Fukuoka	Police guard on international conference	943	Catering lunch, including rolled omelet	1	TB3264	104	405[1)]	25	37	8	7	2	56	12	9
1997. 7	Kochi	Beer festival	77	Unknown	3	T22	22	45	9	8	1	1	1	3	3	10
1998. 8	Ibaraki	Softball tournament	342	Catering lunch, including thick omelet	3	T22	22	45	9	8	1	1	1	3	3	11
1998. 9	Kumamoto	Convention of a labor union	254	Sandwich, including egg salad	4	T28	28	52	11	6	14	5	9	17	19	12
2003.9	Tokyo	School trip	66	Unknown	1	T28	28	52	11	6	14	5	9	17	19	13
2003.9	Chiba	Funeral	67	Catering lunch	1	TB3264	68	247	11	9	1	7	2	8	3	14

[1)]: New designation by multilocus sequence typing (MLST).
ST, sequence type.

*Corresponding author: Mailing address: Department of Bacteriology, Toyama Institute of Health, Nakataikoyama 17-1, Imizu, Toyama 939-0363, Japan. Tel: +81-766-56-8142, Fax: +81-766-56-7326, E-mail: masanori.watahiki@pref.toyama.lg.jp

TB3264/*emm104*. Types T22/*emm22* and T28/*emm28* matched the types from two independent food-borne outbreaks. In our previous study (5), T1/*emm1* and T28/*emm28* were dominant among throat and skin isolates, respectively; but T22/*emm22*, TB3264/*emm68*, and TB3264/*emm104* were not common among throat and skin isolates. Thus, these results suggest that the presumed source of food contamination was not restricted to the food handler who had pharyngitis or hand wounds. The raw materials might have been contaminated by the pathogen before cooking. Close correlations between *emm* type and MLST were found among the isolates obtained over a long period and/or from different places in Japan. By using *emm* typing and MLST, a similar concordance has been noted in previous studies (1,6,7). Finally, our data generated one new ST (ST405) and provided useful comparative data for future studies on the epidemiological investigation of food-borne streptococcal pharyngitis.

We thank Fukuoka City Institute of Hygiene and Environment, Kumamoto City Environmental Research Institute, Ibaraki Prefectural Institute of Public Health, and Public Health Institute of Kochi Prefecture for providing some isolates.

REFERENCES

1. Enright, M. C., Spratt, B. G., Kalia, A., Cross, J. H. and Bessen, D. E. (2001): Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between *emm* type and clone. Infect. Immun., 69, 2416-2427.
2. Kashiwagi, Y., Hori, M., Bushimata, K., Itoh, T., Yabuuchi, K., Suzuki, S., Ohashi, M., Yamanouchi, A., Tsuchitani, H., Maruyama, O., Tsukahara, Y., Hirota, S., Amano, T., Shimizu, K., Nemoto, H., Tanaka, N. and Tsujita, R. (1986): A foodborne outbreak of streptococcal sore throat occurred in Tokyo, 1983. J. Jpn. Assoc. Infect. Dis., 60, 673-685 (in Japanese).
3. Okuyama, Y. (1982): Epidemiological study of a food-borne epidemic of pharyngitis due to group A, T type 12 streptococcus. J. Jpn. Assoc. Infect. Dis., 56, 1173-1185 (in Japanese).
4. Katzenell, U., Shemer, J. and Bar-Dayan, Y. (2001): Streptococcal contamination of food: an unusual cause of epidemic pharyngitis. Epidemiol. Infect., 127, 179-184.
5. Tanaka, D., Gyobu, Y., Kodama, H., Isobe, J., Hosorogi, S., Hiramoto, Y., Karasawa, T. and Nakamura, S. (2002): *emm* Typing of group A streptococcus clinical isolates: identification of dominant types for throat and skin isolates. Microbiol. Immunol., 46, 419-423.
6. Reinert, R. R., Lutticken, R., Sutcliffe, J. A., Tait-Kamradt, A., Cil, M. Y., Schorn, H. M., Bryskier, A. and Al-Lahham, A. (2004): Clonal relatedness of erythromycin-resistant *Streptococcus pyogenes* isolates in Germany. Antimicrob. Agents Chemother., 48, 1369-1373.
7. McGregor, K. F. and Spratt, B. G. (2005): Identity and prevalence of multilocus sequence typing-defined clones of group A streptococci within a hospital setting. J. Clin. Microbiol., 43, 1963-1967.
8. Matsumoto, M., Miwa, Y., Matsui, H., Saito, M., Ohta, M. and Miyazaki, Y. (1999): An outbreak of pharyngitis caused by food-borne group A streptococcus. Jpn. J. Infect. Dis., 52, 127-128.
9. Ikeda, Y., Tsubakimoto, M., Zaitsu, S., Ishikita, R., Murata, T., Ohta, K., Nakahara, S., Tanigawa, Y., Tsuji, N., Yamamoto, T. and Zaitsu, Y. (1997): An outbreak of group A streptococcal infections caused by contaminated luncheons-Fukuoka Prefecture. Infect. Agents Surveillance Rep., 18, 264 (in Japanese).
10. Yasuoka, T., Kinuta, M. and Nishioka, S. (1997): An outbreak of streptococcal disease caused by group A streptococcus T-22 in Kochi Prefecture. Infect. Agents Surveillance Rep., 18, 307-308 (in Japanese).
11. Yamaguchi, K., Mashiko, K., Nemoto, H., Hujisaki, N., Hiraoka, H. and Murata, A. (1998): An outbreak of group A streptococcal infection caused by contaminated luncheons provided by a caterer in Ibaragi Prefecture. Infect. Agents Surveillance Rep., 19, 279 (in Japanese).
12. Honda, R., Asoshina, S., Matsuoka, Y., Nakamura, T. and Shimoda, K. (1999): An outbreak of group A streptococcal infection caused by contaminated sandwiches in Kumamoto Prefecture. Infect. Agents Surveillance Rep., 20, 115-116 (in Japanese).
13. Endoh, M., Okuno, R., Hatakeyama, K., Mukaigawa, J., Yanagawa, Y., Morozumi, S. and Sasai, T. (2004): An outbreak of infection due to *Streptococcus pyogenes* on a school trip. Infect. Agents Surveillance Rep., 25, 42-43 (in Japanese).
14. Kishida, K., Yokoyama, E., Uchimura, M., Igarashi, N., Sone, M., Makabe, H., Kihara, E., Kudoh, M., Takiguchi, J., Nakashima, H., Takei, N., Kusa, T., Takahashi, K. and Sakuma, H. (2004): An outbreak of group A streptococcal infection caused by contaminated luncheons provided by a caterer in Chiba Prefecture. Infect. Agents Surveillance Rep., 25, 100-101 (in Japanese).