JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2001, p. 1021–1024 Vol. 39, No. 3
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.3.1021–1024.2001

Evaluation of a Medium (STGG) for Transport and Optimal Recovery of *Streptococcus pneumoniae* from Nasopharyngeal Secretions Collected during Field Studies

KATHERINE L. O'BRIEN,[1,2]* MELINDA A. BRONSDON,[1] RON DAGAN,[3,4] PABLO YAGUPSKY,[3,4] JACOB JANCO,[3,4] JOHN ELLIOTT,[1] CYNTHIA G. WHITNEY,[1] YONG-HONG YANG,[5] LISA-GAYE E. ROBINSON,[6] BENJAMIN SCHWARTZ,[1] AND GEORGE M. CARLONE[1]

Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases,[1] and Epidemic Intelligence Service, Epidemiology Program Office,[2] Centers for Disease Control and Prevention, and Department of Pediatrics, Egleston Children's Hospital, Emory University,[6] Atlanta, Georgia; Soroka University Medical Center[3] and Faculty of Health Sciences,[4] Ben Gurion University, Beer Sheva, Israel; and Beijing Children's Hospital, Beijing, People's Republic of China[5]

Received 10 October 2000/Returned for modification 20 November 2000/Accepted 22 December 2000

Field studies of *Streptococcus pneumoniae* (pneumococci) nasopharyngeal (NP) colonization are hampered by the need to directly plate specimens in order to ensure isolate viability. A medium containing skim milk, tryptone, glucose, and glycerin (STGG) has been used to transport and store NP material, but its ability to preserve pneumococci has not been evaluated. Our objective was to qualitatively and semiquantitatively evaluate the ability of STGG to preserve pneumococci in NP secretions. Entwined duplicate calcium alginate NP swab samples were obtained from children. One swab was plated directly onto a gentamicin blood agar plate; the other was placed in STGG. Growth from the directly plated specimen was compared with growth from an STGG aliquot immediately cultured or stored at −70°C for 9 weeks, −20°C for 9 weeks, or 4°C for 5 days. Of 186 specimens, 96 (52%) were positive for pneumococci from the direct plating; 94 (98%) of these were positive from the fresh STGG specimen. Pneumococci were recovered from all 38 positive specimens frozen at −70°C, all 18 positive specimens frozen at −20°C, and 18 of 20 positive specimens stored at 4°C. Recovery of pneumococci after storage of NP material in STGG medium at −70°C is at least as good as that from direct plating. Storage at −20°C is also acceptable. Storage at 4°C for 5 days is not ideal.

Streptococcus pneumoniae (pneumococci) is the most important cause of bacterial otitis media, pneumonia, bacteremia, and meningitis among children worldwide (12, 15, 17). Pneumococci are also important because the rate of nonsusceptibility to various classes of antimicrobial agents, such as penicillins and cephalosporins, is rising throughout the United States and worldwide (4, 18, 19). Prevention efforts have been hampered by the lack of a vaccine which is immunogenic for important serotypes in children younger than 2 years of age. A seven-valence pneumococcal conjugate vaccine (Prevnar; Wyeth Lederle Vaccines) which is immunogenic and efficacious in this age group recently has been licensed in the United States for use among children through 9 years of age and is recommended routinely for those under 2 years of age (1, 3, 16). The effect of this and other conjugate pneumococcal vaccines on nasopharyngeal (NP) colonization is a subject of intense investigation.

It is well known that pneumococci are spread from person to person via the respiratory route. NP colonization studies have shown that people acquire pneumococci at a young age, carry these organisms for various periods of time, may carry more than one serotype at a time, and transmit these organisms to others with whom they are in close contact (2, 5, 9–11, 14). Many studies of the dynamics and ecology of pneumococcal NP carriage, particularly in the setting of new conjugate pneumococcal vaccines, will be performed in settings where microbiologic facilities are not readily available.

An optimal medium has not been validated for the transport, preservation, and recovery of pneumococci from NP material. One medium, STGG (skim milk-tryptone-glucose-glycerin), has been used in some epidemiologic field studies of pneumococcal carriage (8, 13). However, culturing of NP material stored in STGG has not been compared with direct plating (DP) of NP material on selective blood agar, considered the standard method for isolating pneumococci.

In this study, we aimed to determine (i) what proportions of NP specimens yield pneumococci by direct inoculation onto culture plates compared with storage in STGG, (ii) what proportion of pneumococci collected on an NP swab is recovered by direct plate inoculation, (iii) whether qualitative and semiquantitative recoveries of pneumococci from NP secretions suspended in STGG are at least equivalent to those from direct plate inoculation, and (iv) the least stringent and optimum conditions for storage of NP secretions containing pneumococci in STGG.

(This research has been presented in abstract form [M. A. Bronsdon, K. L. O'Brien, P. Yagupsky, J. Janco, R. Dagan,

* Corresponding author. Present address: Center for American Indian and Alaskan Native Health, Johns Hopkins School of Hygiene and Public Health, 621 N. Washington St., Baltimore, MD 21205. Phone: (410) 614-3806. Fax: (410) 955-2010. E-mail: klobrien@jhsph.edu.

TABLE 1. Qualitative recoveries of pneumococci (Pnc) from the STGG-Stored samples, DP samples, and STGG-Fresh samples

Storage condition	Pnc[a]	No. of specimens with the indicated result found by:				
		DP	STGG-Fresh		STGG-Stored	
			Pnc +	Pnc −	Pnc +	Pnc −
−70°C, 6 wk	+	38	37	1	38	0
	−	16	0	16[b]	ND	ND
−20°C, 8 wk	+	18	17	1	18	0
	−	25	2	23[b]	2	ND
4°C, 5 days	+	20	18	2	18	2
	−	15	0	15	0	15

[a] + and −, positive and negative for pneumococci, respectively.
[b] Specimens found negative from both DP and STGG-Fresh samples were presumed negative from STGG-Stored samples and therefore were not tested.

J. Elliott, C. G. Whitney, Y.-H. Yang, L. G. Robinson, R. Facklam, G. Carlone, and B. Schwartz, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., Abstr. D-5, p. 128, 1998].)

MATERIALS AND METHODS

Subjects. A convenience sample of 186 children younger than 5 years of age and who were attending clinics or emergency rooms for any complaint was obtained in China (n = 36), Israel (n = 132), and the United States (n = 18).

STGG transport medium. We followed the previously published recipe for the preparation of STGG medium (7), modified from Gherna (6). We mixed and dissolved 2.0 g of skim milk powder (Difco, Detroit, Mich.), 3.0 g of Oxoid tryptone soy broth, 0.5 g of glucose, and 10 ml of glycerol in 100 ml of distilled water. The solution was dispensed in 1.0-ml amounts into screw-cap 1.5-ml vials (Sarstedt Microtube, Newton, N.C.). These were autoclaved at 15 lb/in^2 and 121°C for 10 min. After the vials were cooled, the caps were screwed on tightly and the vials were stored at −20°C or refrigerated at 4°C until used. All vials were used within 6 months of preparation. The medium was tested for sterility by plating the entire volume of one vial from each lot onto Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) and incubating the plate at 37°C for 48 h. If the growth of any organism was observed, the lot was discarded. STGG vials were vortexed for approximately 20 s immediately before being inoculated with an NP swab specimen to draw into suspension the precipitate which develops during storage.

Sample collection and processing. We collected NP specimens on duplicate pediatric calcium alginate swabs (Fisherbrand, catalog number 14–959-78; Fisher Scientific, Pittsburg, Pa.) which were twined together in a sterile manner and passed into the nasopharynx of the subject. The combined swabs were rotated 180°, removed from the nose, and untwined in a sterile fashion.

One swab was plated immediately onto selective Trypticase soy blood agar with 5% sheep blood and 2.5 mg of gentamicin/liter (G-BA; BBL) by rolling the swab over one-quarter of the plate, streaking the sample onto four quadrants using a sterile loop, and incubating the plate at 37°C in 5% CO_2 for 18 to 24 h. This specimen was termed the DP sample. After being used to inoculate the G-BA plate, that same swab was placed in 1.0 ml of STGG; the handle was cut off using sterile scissors, leaving the swab immersed, and the cap was secured. The vial was vortexed for 30 s to elute the NP material from the swab. A 100-μl sample was placed on one-quarter of a G-BA plate, streaked, and incubated as described above. This specimen was termed the DP residual (DP-Residual) sample.

Following untwining, the second swab was immersed in 1.0 ml of STGG and processed as described above. This sample was termed the fresh STGG transport (STGG-Fresh) sample. The remaining 900-μl specimen was stored with the swab in place at a single storage condition (4°C for 5 days, −20°C for 9 weeks, or −70°C for 9 weeks). After storage, the vial was brought to room temperature and vortexed for approximately 30 s. A 100-μl aliquot was inoculated, streaked, and incubated as described above. This sample was termed the stored STGG transport (STGG-Stored) sample.

Pneumococcal identification. Alpha-hemolytic colonies phenotypically suspicious for pneumococci were confirmed as such using optochin disk susceptibility and bile solubility assays. Growth was scored on a semiquantitative scale: scant growth, <25 colonies in quadrant 1; 1+ growth, ≥25 colonies in quadrant 1 and <25 in quadrant 2; 2+ growth, ≥25 colonies in quadrant 2 and <25 in quadrant 3; 3+ growth, ≥25 colonies in quadrant 3 and <25 in quadrant 4; and 4+ growth, ≥25 colonies in quadrant 4.

TABLE 2. Semiquantitative recovery of pneumococci from STGG-Stored samples and DP samples

Storage condition	No. of specimens	No. (%) with the following result:		
		DP > Stored	DP = Stored	DP < Stored
−70°C, 9 wk	38	14 (37)	11 (29)	13 (34)
−20°C, 9 wk	20	4 (20)	12 (60)	4 (20)
4°C, 5 days	20	16 (80)	4 (20)	0 (0)

Ethics and informed consent. This study was approved by the institutional review boards of the Centers for Disease Control and Prevention, Emory University, Soroka University Medical Center, and Beijing Children's Hospital. Written informed consent was obtained from parents, as required by the local institutional review board.

RESULTS

Recovery of pneumococci by DP. Fifty-four NP specimens were tested for the qualitative and semiquantitative recoveries of pneumococci from both the DP and the DP-Residual samples. Of these, 38 (70%) were positive for pneumococci from the DP samples. The qualitative isolation of pneumococci was fully concordant between the DP and the DP-Residual samples. Among the 38 specimens positive for pneumococci, 18 (47%) produced growth from the DP-Residual samples equal or greater than that from the DP samples. Of the 17 specimens which had growth of ≤2+ from the DP samples, 13 (76%) had equivalent or greater growth from the DP-Residual samples.

Recovery of pneumococci from STGG versus DP. Pneumococci were recovered from 96 (52%) of 186 DP samples and from 94 (51%) of STGG-Fresh samples. Six specimens were discordant for the recovery of pneumococci between the DP and the STGG-Fresh samples; in two, pneumococci grew only from the STGG-Fresh samples, and in four, they grew only from the DP samples. Growth was categorized as 1+ in four discordant cases and as 2+ in the remaining two discordant cases.

Seventy-eight pneumococcal specimens were used for the semiquantitative growth comparison between DP and STGG-Fresh samples. Thirty-six (44%) of the STGG-Fresh samples had growth greater than or equal to the growth from the DP samples. Among 26 specimens with growth categorized as ≤2+ from the DP samples, 15 (58%) had equivalent or greater growth from the STGG-Fresh samples.

Recovery of pneumococci from STGG-Stored samples. Of the 132 specimens used to evaluate storage conditions, 76 (58%) were positive for pneumococci from the DP samples; of these, 72 (95%) were also positive from the STGG-Fresh samples. Compared with the results obtained with DP and STGG-Fresh samples, we found no substantial differences in the qualitative recoveries of pneumococci under any of the storage conditions tested (Table 1). Semiquantitative recovery of pneumococci stored at −70°C for 9 weeks and at −20°C for 9 weeks was at least as good as that from the DP samples (Table 2). There was some reduction of pneumococcal growth in the samples stored at 4°C for 5 days.

DISCUSSION

Until now, studies have not been conducted to evaluate the qualitative and quantitative abilities of STGG to sustain pneumococci in NP specimens. To our knowledge, no other transport media have been evaluated in this way either. Previously, STGG has been used for populations with extremely high and dense colonization rates (8, 13). Those studies focused on the gross recovery of pneumococci rather than on low-level colonization or small proportions of multiple serotypes, which are now issues of considerable interest. In many settings, the laboratory facilities necessary for the processing of such specimens are not located on site. The performance of such studies, even when laboratory facilities are readily available, could be enhanced if NP specimens could be tested in batch form. We found that STGG performed well compared to DP, the standard technique for NP carriage studies.

We have shown that substantial NP material remains on a swab after it is used to inoculate a culture plate. We recovered pneumococci from the residual swab material of specimens found positive for pneumococci by DP. The enhanced recovery of pneumococci from the DP-Residual samples compared with the DP samples was especially noted for specimens which had $\leq 2+$ growth in the DP samples.

We also compared the recoveries of pneumococci from the DP samples and from a duplicate swab suspended in STGG. Use of the transport medium resulted in enhanced recovery of pneumococci compared with recovery by DP, especially at low colony counts. We identified a few samples that were negative for pneumococci from the DP samples but positive for pneumococci from the STGG samples and vice versa. The discrepancies between the STGG and the DP samples all occurred at low colony counts and may have represented sampling inconsistency or incomplete mixing of samples. There were no qualitative discrepancies between recoveries of pneumococci from the DP and STGG samples at higher colony counts.

On a qualitative basis, all storage conditions studied were acceptable for the recovery of pneumococci compared with DP. When evaluated on a semiquantitative basis, the specimens maintained at 4°C did not preserve pneumococci as well as those maintained at −20°C or −70°C but were nevertheless acceptable when compared with DP. Although the limited follow-up time did not distinguish between storage at −20°C and that at −70°C, the experience of others has shown that maintaining specimens at −70°C results in long-term survival of pneumococci (E. Stubbs, M. McKinnon, T. M. Shelby James, H. Smith Vaughan, and A. J. Leach, 2nd Int. Symp. Pneumococci Pneumococcal Dis., abstr. P104, 2000). Transporting a specimen in STGG on wet ice (simulated by 4°C) followed by storage at −20°C in the short term (up to 9 weeks) and by maintenance of the specimen at −70°C for the long term is likely acceptable. Optimal conditions consist of freezing the specimen as quickly as possible at −70°C.

The benefits of the STGG storage medium include (i) enhanced recovery of pneumococci compared with DP, especially at low organism concentrations; (ii) ability to conduct multiple assays on a single NP specimen; (iii) long-term storage of the original NP specimen at −70°C without loss of CFU; (iv) manipulation of the inoculum size to adjust the density of colonies on a plate; (v) transport of NP specimens from the site of collection to a distant laboratory; and (vi) assay of NP specimens in batch form.

In conclusion, we have shown equivalent recoveries of pneumococci after suspension in STGG compared with DP of the swab, even at low organism numbers (<50 CFU). Pneumococci from NP secretions are preserved in the short term and in the long term by suspension in STGG when transported and stored at low temperatures. STGG is inexpensive and remains stable for at least 6 months after sterilization. We recommend STGG for transport and storage of field-collected NP specimens in epidemiologic studies.

REFERENCES

1. **American Academy of Pediatrics Committee on Infectious Diseases.** 2000. Policy statement: recommendations for the prevention of pneumococcal infections, including the use of pneumococcal conjugate vaccine (Prevnar), pneumococcal polysaccharide vaccine, and antibiotic prophylaxis. Pediatrics **106:**362–366.
2. **Austrian, R.** 1986. Some aspects of the pneumococcal carrier state. J. Antimicrob. Chemother. **18:**35–45.
3. **Black, S., H. Shinefield, B. Fireman, E. Lewis, P. Ray, J. R. Hansen, L. Elvin, K. M. Ensor, J. Hackell, G. Siber, F. Malinoski, D. Madore, I. Chang, R. Kohberger, W. Watson, R. Austrian, K. Edwards, and Northern California Kaiser Permanente Vaccine Study Center Group.** 2000. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr. Infect. Dis. J. **19:**187–195.
4. **Bulter, J. C., J. Hoffman, M. S. Cetron, J. A. Elliott, R. R. Facklam, and R. F. Breiman.** 1996. The continued emergence of drug-resistant Streptococcus pneumoniae in the United States: an update from the Centers for Disease Control and Prevention's Pneumococcal Sentinel Surveillance System. J. Infect. Dis. **174:**986–993.
5. **Dowling, J. N., P. R. Sheehe, and H. A. Feldman.** 1971. Pharyngeal pneumococcal acquisitions in "normal" families: a longitudinal study. J. Infect. Dis. **124:**9–17.
6. **Gherna, R. L.** 1981. Preservation, p. 208–217. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
7. **Gibson, L. F., and J. T. Khoury.** 1986. Storage and survival of bacteria by ultra-freeze. Lett. Appl. Microbiol. **3:**127–129.
8. **Gratten, M., K. Manning, J. Dixon, F. Morey, P. Torzillo, J. Hanna, J. Erlich, V. Asche, and I. Riley.** 1994. Upper airway carriage by *Haemophilus influenzae* and *Streptococcus pneumoniae* in Australian Aboriginal children hospitalised with acute lower respiratory infection. Southeast Asian J. Trop. Med. Public Health **25:**123–131.
9. **Gratten, M., J. Montgomery, G. Gerega, H. Gratten, H. Siwi, A. Poli, and G. Koki.** 1989. Multiple colonization of the upper respiratory tract of Papua New Guinea children with Haemophilus influenzae and Streptococcus pneumoniae. Southeast Asian J. Trop. Med. Public Health **20:**501–509.
10. **Gratten, M., H. Gratten, A. Poli, E. Carrad, M. Raymer, and G. Koki.** 1986. Colonisation of Haemophilus influenzae and Streptococcus pneumoniae in the upper respiratory tract of neonates in Papua, New Guinea: primary acquisition, duration of carriage, and relationship to carriage in mothers. Biol. Neonate **50:**114–120.
11. **Gray, B. M., G. M. Converse, and H. C. Dillon.** 1980. Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. J. Infect. Dis. **142:**923–933.
12. **Klein, J. O.** 1994. Otitis media. Clin. Infect. Dis. **19:**823–833.
13. **Leach, A. J., T. M. Shelby-James, M. Mayo, M. Gratten, A. C. Laming, B. J. Currie, and J. D. Mathews.** 1997. A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of Streptococcus pneumoniae. Clin. Infect. Dis. **24:**356–362.
14. **Lloyd-Evans, N., T. J. D. O'Dempsey, I. Baldeh, O. Secka, E. Demba, J. E. Todd, T. F. McArdle, W. S. Banya, and B. M. Greenwood.** 1996. Nasopharyngeal carriage of pneumococci in Gambian children and in their families. Pediatr. Infect. Dis. J. **15:**866–871.
15. **Mulholland, K.** 1999. Magnitude of the problem of childhood pneumonia. Lancet **354:**590–592.
16. **Rennels, B. M., K. M. Edwards, H. Keyserling, K. S. Reisinger, D. A. Hogerman, D. V. Madore, I. Chang, P. R. Paradiso, F. J. Malinoski, and A. Kimura.** 1998. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. Pediatrics **101:**604–611.
17. **Schuchat, A., K. Robinson, L. H. Harrison, M. Farley, A. L. Reingold, L. Lefkowitz, and B. A. Perkins.** 1997. Bacterial meningitis in the United States

in 1995. N. Engl. J. Med. **337:**970–976.
18. **Song, J. H., N. Y. Lee, S. Ichiyama, R. Yoshida, Y. Hirakata, W. Fu, A. Chongthaleong, N. Aswapokee, C. H. Chiu, M. K. Lalitha, K. Thomas, J. Perera, T. T. Yee, F. Jamal, U. C. Warsa, B. X. Vinh, M. R. Jacobs, P. C. Appelbaum, and C. H. Pai.** 1999. Spread of drug-resistant Streptococcus pneumoniae in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) Study. Clin. Infect. Dis. **28:**1206–1211.
19. **Whitney, C. G., M. M. Farley, J. Hadler, L. H. Harrison, C. Lexau, A. Reingold, L. Lefkowitz, P. R. Cieslak, M. Cetron, E. R. Zell, J. H. Jorgensen, A. Schuchat, and R. R. Facklam.** 2000. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. N. Engl. J. Med. **343:**1917–1924.