Streptococcus pneumoniae and Haemophilus influenzae type b Carriage, Central Asia

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A study of children was conducted in 3 Central Asian Republics. Approximately half of the Streptococcus pneumoniae isolates were serotypes included in available vaccine formulations. Approximately 6% of children carried Haemophilus influenzae type b (Hib). Using pneumococcal and Hib conjugate vaccines may decrease illness in the Central Asian Republics.

The Study

In January 1997, we obtained nasopharyngeal swabs from a convenience sample of both ill and well children, ages 2–59 months, who were visiting outpatient clinics in Taraz City (formerly Djambul), Kazakhstan; Fergana, Uzbekistan; and Osh, Kyrgyz Republic. Before swabs were obtained, written parental consent was obtained in Russian, Kazak, Kyrgyz, or Uzbek under a protocol approved by a local institutional review board and the Centers for Disease Control and Prevention (CDC).

Nasopharyngeal swab collection and pathogen isolation have been described previously (1). Briefly, a flexible calcium alginate swab was inserted through the nares to the nasopharynx, rotated 180°, and withdrawn. While in the field, the swabs were first streaked on chocolate agar (CA) plates containing bacitracin to isolate H. influenzae, and then onto Trypticase soy 5% sheep blood agar plates containing gentamicin to isolate S. pneumoniae. All plates were brought back to the laboratory and incubated appropriately. Pure H. influenzae cultures were isolated and spread onto quad plates. Those colonies that grew on only the XV and blood quadrants were considered to be H. influenzae and were saved on CA slants. Suspected S. pneumoniae colonies were streaked onto conventional 5% sheep blood agar plates with an optochin disk added. After appropriate incubation, α-hemolytic isolates with an optochin inhibition zone >14 mm were considered to be S. pneumoniae and saved.
on CA slants. CA slants of both H. influenzae and S. pneumoniae were transported to CDC in Atlanta. Isolates of H. influenzae were serotyped with Difco H. influenzae serotype-specific rabbit antisera (BD, Sparks, MD, USA), and S. pneumoniae isolates were serotyped with CDC-prepared antiserum. S. pneumoniae cultures were tested for antimicrobial susceptibility to penicillin with broth dilution MIC testing by using the guidelines of the Clinical and Laboratory Standards Institute (formerly NCCLS) and customized MIC panels.

Results were similar in all 3 sites, so data were combined. The method of isolate storage and transport resulted in different survival rates among isolates (Tables 1 and 2). Low rates of S. pneumoniae isolates among children receiving antimicrobial drugs prevent any conclusions about that group. Among S. pneumoniae and H. influenzae isolates, survival was negatively associated with duration of storage. Among S. pneumoniae isolates, survival was positively associated with increasing age. However, the lack of any trends in Hib colonization and S. pneumoniae nonsusceptibility by age and duration of storage suggests that differential survival did not produce bias.

Of 630 children swabbed, 375 (59%) were colonized with S. pneumoniae. Of the 375 isolates, 224 S. pneumoniae isolates were available for susceptibility testing and serotyping. Of the 224 isolates, 54 (24%) were nonsusceptible to penicillin. The 9 most common serotypes in decreasing order were 19F (17% of isolates), 6B (15%), 6A (9%), 14 (6%), 23B (4%), 19A (3%), 23F (3%), 18C (2%), and 4 (2%). These accounted for 61% of all isolates.

In our sample, the 7-valent pneumococcal conjugate vaccine would cover 47% of pneumococcal isolates, the 9-valent would cover 48%, and the 11-valent would cover 51%. Of all the serotypes covered in these vaccines, serotypes 6B, 14, 19F, and 23F account for all nonsusceptible strains. Because all 3 vaccines contain these 4 serotypes, each vaccine would cover 33 (61%) of 54 nonsusceptible isolates of S. pneumoniae. An additional 13% of nonsusceptible strains are vaccine-related (strains 6A [4 of 54, 7%] and 23B [3 of 54, 6%]).

Of the 630 children from whom nasopharyngeal swabs were obtained, 357 (57%) were carrying H. influenzae. Of the 300 isolates available for serotyping, 34 (11%) were Hib. When Hib carriage is determined by multiplying the percentage of children colonized with H. influenzae times the percentage of Hib among all H. influenzae isolates tested, the carriage rate is 6% (Table 2).

**Conclusions**

Our survey showed that most children in these Central Asian Republics were colonized with at least 1 potential respiratory pathogen. Approximately half of the S. pneumoniae isolates and more than half of the penicillin-nonsusceptible S. pneumoniae isolates are included in the available pneumococcal conjugate vaccine formulations. Approximately 6% of the children in this convenience sample were carrying Hib.

The colonization rate of Hib found in our study is similar to rates observed in industrialized populations before Hib conjugate vaccines were widely used. Carriage rates for Hib before widespread vaccination in Finland, the

| Variable | % SP colonization (n/N) | % SP isolate survival (n/N) | % SP PCN nonsusceptible isolates (n/N) | Calculated % colonization with PCN-nonsusceptible SP† |
|----------|------------------------|-----------------------------|----------------------------------------|---------------------------------------------|
| Age (mo) |                        |                             |                                        |                                             |
| 2–5      | 49 (47/95)             | 49 (23/47)                  | 17 (4/23)                              | 8                                           |
| 6–11     | 64 (74/115)            | 54 (40/74)                  | 38 (15/40)                             | 24                                          |
| 12–23    | 66 (94/142)            | 60 (56/94)                  | 27 (15/65)                             | 18                                          |
| 24–35    | 62 (65/105)            | 62 (40/65)                  | 23 (9/40)                              | 14                                          |
| 36–47    | 58 (61/106)            | 64 (39/61)                  | 18 (7/39)                              | 10                                          |
| 48–59    | 51 (34/67)             | 79 (27/34)                  | 19 (5/27)                              | 10                                          |
| Sex      |                        |                             |                                        |                                             |
| Male     | 60 (197/331)           | 60 (119/197)                | 27 (32/119)                            | 16                                          |
| Female   | 60 (176/299)           | 60 (106/178)                | 22 (22/106)                            | 13                                          |
| Reported use of antimicrobial drugs in past 7 days | |                             |                                        |                                             |
| Yes      | 49 (34/70)             | 29 (10/34)                  | 10 (1/10)                              | 5                                           |
| No       | 61 (335/552)           | 64 (213/335)                | 24 (52/213)                            | 15                                          |
| Weeks storage before transport | |                             |                                        |                                             |
| 3        | 65 (72/110)            | 25 (18/72)                  | 11 (2/18)                              | 7                                           |
| 2        | 56 (175/315)           | 62 (108/175)                | 24 (27/108)                            | 13                                          |
| 1        | 62 (128/205)           | 77 (99/128)                 | 26 (26/99)                             | 16                                          |
| Total    | 59 (375/630)           | 60 (225/375)                | 24 (55/225)                            | 14                                          |

*SP, S. pneumoniae; PCN, penicillin.
†Result obtained by multiplying the percentage of children colonized with SP times the percentage of SP isolates that are nonsusceptible to penicillin (percentages in column 1 multiplied by the percentages in column 3).
United Kingdom, and the United States were 2%–6% (10–13). In these countries, introduction of the Hib vaccine virtually eliminated Hib invasive disease (13).

Assessing the prevalence of disease due to specific respiratory pathogens is difficult; blood cultures are insensitive, and other diagnostic tests are not specific. Nasopharyngeal colonization surveys of groups of children identify the predominant organisms circulating in the community and the presence or absence of antimicrobial-drug resistance. The presence of *S. pneumoniae* serotypes found in the pneumococcal conjugate vaccine suggests this vaccine may decrease some illness from acute respiratory infection. The experience in other countries with similar prevaccination Hib nasopharyngeal carriage rates suggests that the Hib conjugate vaccine may also decrease illness. These findings may be helpful in the decision-making process regarding the value of introducing conjugate vaccines for Hib and pneumococcal disease prevention.

Funding for this work was provided by the US Agency for International Development under a participating agency service agreement with CDC.

Dr Factor was an Epidemic Intelligence Service officer in the Respiratory Diseases Branch of CDC when she led the field investigations in the Central Asian Republics. She is currently a medical epidemiologist in the CDC Bioterrorism Preparedness Response Program assigned to the New York City Department of Health and Mental Hygiene to develop emergency response plans for New York City.

**Table 2. Haemophilus influenzae in convenience sample, Central Asian Republics, January 1997***

| Variable                  | % HI colonization (n/N) | % HI isolate survival (n/N) | % Hib among all HI isolates (n/N) | Calculated % colonization with Hib† |
|---------------------------|-------------------------|-----------------------------|----------------------------------|-----------------------------------|
| Age (mo)                  |                         |                             |                                  |                                   |
| 2–5                      | 45 (43/95)              | 77 (33/43)                  | 6 (2/33)                         | 3                                 |
| 6–11                     | 59 (68/115)             | 76 (52/68)                  | 17 (9/52)                        | 10                                |
| 12–23                    | 60 (85/142)             | 89 (78/85)                  | 8 (6/76)                         | 5                                 |
| 24–35                    | 60 (63/105)             | 87 (55/63)                  | 13 (7/55)                        | 8                                 |
| 36–47                    | 58 (62/106)             | 85 (53/62)                  | 13 (7/53)                        | 4                                 |
| 48–59                    | 54 (36/67)              | 86 (31/36)                  | 10 (3/31)                        | 5                                 |
| Sex                      |                         |                             |                                  |                                   |
| Male                     | 57 (187/331)            | 82 (154/187)                | 13 (20/154)                      | 7                                 |
| Female                   | 57 (170/299)            | 86 (146/170)                | 10 (14/146)                      | 8                                 |
| Reported use of antimicrobial drugs in past 7 days |                   |                             |                                  |                                   |
| Yes                      | 57 (40/70)              | 82 (33/40)                  | 9 (3/33)                         | 5                                 |
| No                       | 56 (310/552)            | 84 (260/310)                | 12 (31/280)                      | 7                                 |
| Weeks storage before transport |                   |                             |                                  |                                   |
| 3                        | 59 (65/110)             | 71 (46/65)                  | 20 (9/46)                        | 12                                |
| 2                        | 57 (178/315)            | 83 (147/178)                | 10 (14/147)                      | 6                                 |
| 1                        | 56 (114/205)            | 94 (107/114)                | 10 (11/107)                      | 6                                 |
| Total                    | 57 (357/630)            | 84 (300/357)                | 11 (34/300)                      | 6                                 |

*HI, Haemophilus influenzae; Hib, H. influenzae type b.
†Result obtained by multiplying percentage of children colonized with HI times the proportion of Hib isolates (percentages in column 1 multiplied by percentages in column 3).

United Kingdom, and the United States were 2%–6% (10–13). In these countries, introduction of the Hib vaccine virtually eliminated Hib invasive disease (13).

Assessing the prevalence of disease due to specific respiratory pathogens is difficult; blood cultures are insensitive, and other diagnostic tests are not specific. Nasopharyngeal colonization surveys of groups of children identify the predominant organisms circulating in the community and the presence or absence of antimicrobial-drug resistance. The presence of *S. pneumoniae* serotypes found in the pneumococcal conjugate vaccine suggests this vaccine may decrease some illness from acute respiratory infection. The experience in other countries with similar prevaccination Hib nasopharyngeal carriage rates suggests that the Hib conjugate vaccine may also decrease illness. These findings may be helpful in the decision-making process regarding the value of introducing conjugate vaccines for Hib and pneumococcal disease prevention.

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**References**

1. Centers for Disease Control and Prevention, World Health Organization. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world [monograph on the Internet]. 2003 [cited 2005 Jul 5]. Available from www.who.int/csr/resources/publications/drugresist/en/IAMRmanual.pdf

2. Kyrgyz Republic demographic and health survey, 1997. Calverton (MD): Ministry of Health of the Kyrgyz Republic and Macro International Inc; 1998. p. 109.

3. Uzbekistan demographic and health survey, 1996. Calverton (MD): Ministry of Health of Uzbekistan and Macro International Inc; 1997. p. 115.

4. Pavin M, Nurgozhin T, Hafner G, Yusufy F, Laing R. Prescribing practices of rural primary health care physicians in Uzbekistan. Trop Med Int Health. 2003;8:182–90.

5. Stratchounski LS, Andreeva JV, Ratchina SA, Galkin DV, Petrochenkova NA, Demin AA, et al. The inventory of antibiotics in Russian home medicine cabinets. Clin Infect Dis. 2003;37:498–505.

6. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N, et al. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. N Engl J Med. 2003;349:1341–8.

7. Cutts FT, Zaman SMA, Enwere G, Jaffar S, Levine OS, Okoko JB, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. Lancet. 2005;365:1139–46.

8. Wenger JD, DiFabio JL, Landaverde JM, Levine OS, Gaffar T. Introduction of Hib conjugate vaccines in the non-industrialized world: experience in four ‘newly adopting’ countries. Vaccine. 1999;18:736–42.

9. Peltola H. Worldwide Haemophilus influenzae type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. Clin Microbiol Rev. 2000;13:302–17.
10. Takala AK, Eskola J, Leinonen M, Kayhty H, Nissinen A, Pekkanen E, et al. Reduction of oropharyngeal carriage of Haemophilus influenzae type b (Hib) in children immunized with an Hib conjugate vaccine. J Infect Dis. 1991;164:982–6.

11. Michaels RH, Poziviak CS, Stonebraker FE, Norden CW. Factors affecting pharyngeal Haemophilus influenzae type b colonization rates in children. J Clin Microbiol. 1976;4:413–7.

12. Howard AJ, Dunkin KT, Musser JM, Palmer SR. Epidemiology of Haemophilus influenzae type b invasive disease in Wales. BMJ. 1991;303:441–5.

13. Wenger JD. Epidemiology of Haemophilus influenzae type b disease and impact of Haemophilus influenzae type b conjugate vaccines in the United States and Canada. Pediatr Infect Dis J. 1998;17(9 Suppl):S132–6.

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