Selective Media for Recovery of *Haemophilus influenzae* from Specimens Contaminated with Upper Respiratory Tract Microbial Flora

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Isolation of *Haemophilus influenzae* from specimens contaminated with upper respiratory tract microbial flora was attempted with three different media: enriched chocolate agar, chocolate agar plus vancomycin, and chocolate agar plus vancomycin, bacitracin, and clindamycin. Recovery rates of *H. influenzae* from 852 pediatric pharyngeal swab specimens were 6.0, 28.5, and 59.9%, respectively.

*Haemophilus influenzae*, a fastidious gram-negative bacillus, is frequently found in the upper respiratory tract (URT) of healthy humans. In addition to various systemic life-threatening infections, *H. influenzae* is a common cause of serious diseases of the upper and lower respiratory tract. Examples of *Haemophilus* respiratory tract infections include acute otitis media, acute maxillary sinusitis, epiglottitis, acute exacerbations of bronchitis in patients with underlying chronic obstructive pulmonary disease, and pneumonia in children and adults. Microbiological diagnosis of these *Haemophilus* infections is often hampered by an inability to recover the organism on primary-plated media due to the frequency and ease with which it is overgrown by the commensal microbial flora of the URT (3). This problem is underscored by the recent observations of Levin and colleagues, who described 24 adult patients with pneumonia from whom *H. influenzae* was recovered from either blood or pleural fluid cultures (4). In only 6 of 17 cases was *H. influenzae* recovered from sputum specimens cultured by using conventional techniques including enriched chocolate agar (CHOC) media, despite the fact that gram-stained smears of sputum specimens often revealed a predominance of pleomorphic gram-negative bacilli. Observations such as these have led to the development of selective media which inhibit URT microbial flora while permitting growth of *H. influenzae*. Approaches utilized most frequently incorporate bacitracin into various enriched basal media (1, 3, 5).

The intent of the present study was to determine recovery rates of *H. influenzae* from contaminated URT specimens by using two selective media: CHOC agar containing 5.0 µg of vancomycin per ml (CHOC-V) and CHOC agar containing 5.0 µg of vancomycin per ml, 300 µg of bacitracin per ml, and 1.0 µg of clindamycin per ml (CHOC-VBC). Recovery rates obtained with these two media were compared with those obtained on CHOC without antibiotics. CHOC contained brain heart infusion agar base (Scott Laboratories, Fiskeville, R.I.), 1% hemoglobin (Scott), and 1% Bio-X supplement (Scott). This study was prompted by observations of breakthrough growth of URT flora on CHOC that contained only bacitracin.

Dacron swab specimens of the posterior pharynx were obtained from 852 children between the ages of 1 month and 13 years in 32 central Massachusetts day care centers. Swabs were immediately placed in Aime transport medium containing charcoal and transported at ambient temperature to the laboratory within 2 h of collection. The content of individual swabs was eluted into 0.5 ml of sterile Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), and 0.1 ml of this suspension was transferred to the surface of each of the three media described previously. Plates were streaked for isolation in four quadrants, incubated for 48 h at 35°C in an atmosphere of 5 to 7% CO₂, and examined daily for the presence of colonies morphologically compatible with *Haemophilus* species. Species identification, biotyping, and determination of capsular serotype were performed by using conventional procedures (2) on five different colonies of each unique colony morphological type.

A total of 609 unique strains of *H. influenzae* were recovered in this study. Fifty-two strains (8.5%) were isolated on CHOC (Table 1). A single strain was recovered from 50 subjects; two different strains were isolated from one individual. CHOC-V yielded a total of 254
strains (41.7%). One strain was recovered from 232 subjects, and two different strains were recovered from 11 children. A total of 589 strains (96.7%) were isolated on CHOC-VBC. In 431 instances, a single strain was recovered. Two and three different strains were recovered from 73 and 4 subjects, respectively. Among the total of 852 children examined in this study, the percentages of subjects found to be URT carriers of at least one strain of *H. influenzae* were 6.0, 28.5, and 59.9% with CHOC, CHOC-V, and CHOC-VBC, respectively.

Among the total of 609 unique strains of *H. influenzae* recovered in this study, 125 (20.5%) were found to possess type b capsular antigen. Seven of these type b strains were isolated on CHOC, 52 on CHOC-V, and 116 on CHOC-VBC. Among the remaining 484 non-type b isolates, 45 were recovered on CHOC, 202 on CHOC-V, and 473 on CHOC-VBC.

Relative recovery rates of different biotypes of *H. influenzae* seemed to be influenced by the medium used for primary isolation, at least with regard to CHOC and CHOC-V (Table 2). For example, whereas 6 of 30 strains (20.0%) of undefined *H. influenzae* biotypes were recovered on CHOC, 0 of 13 strains (0.0%) of biotype 5 were isolated on this medium. Similarly, whereas 70 of 129 biotype 2 strains (54.3%) of *H. influenzae* were recovered on CHOC-V, only 3 of 13 biotype 5 strains (23.1%) were isolated on this medium. The relative recovery rates of different biotypes of *H. influenzae* observed with CHOC-VBC did not appear to vary.

Increased recovery of *H. influenzae* on CHOC-V and CHOC-VBC was thought to be the result of suppression of overgrowth with URT microbial flora rather than enhancement of growth of *H. influenzae* on these media. This conclusion was supported by the following observations. The basal medium in CHOC-V and CHOC-VBC was identical to that used in CHOC. Growth of URT flora was minimal on CHOC-V and completely absent on CHOC-VBC. All strains recovered on either CHOC-V or CHOC-VBC (which were not recovered on CHOC) grew well on CHOC when subcultured to this medium. Also, since plates containing the three media were inoculated in precisely the same manner, with equivalent amounts of a uniform suspension prepared from each pharyngeal swab, comparisons of the quantity and location of *Haemophilus* colonies on the different media were possible. Colonies of *H. influenzae* growing on CHOC-V or CHOC-VBC were frequently located only in the first quadrant. This was also the location of the heaviest growth of URT flora on CHOC. Finally, the colony morphology of *H. influenzae* was not altered on either of the selective media.

The results of this study clearly illustrate the problem of lack of recovery of *Haemophilus* species from specimens contaminated with microbial flora of the URT. Conventional microbiological techniques, which utilize only CHOC, can frequently be expected to yield false-negative results. In the present study, recovery rates of *H. influenzae* from pharyngeal swabs were significantly increased when CHOC-V or CHOC-VBC was used for primary isolation. Maximum recovery rates were obtained with the latter medium. Furthermore, selective recovery of certain biotypes, observed with CHOC and with CHOC-V, was not seen with CHOC-VBC. It is believed that this medium might have some utility in diagnostic microbiology laboratories as an adjunct to suitable nonselective media when seeking *H. influenzae* in respiratory tract specimens likely to be contaminated with URT microbial flora.

**LITERATURE CITED**

