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Carrier status for Hemophilus spp.: epidemiology in a general hospital population and a group of geriatric patients

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SUMMARY

Hemophilus species are of growing clinical interest in upper respiratory tract and middle ear infections especially in the elderly. The results of 400 throat swabs done in hospitalized patients, 200 from throat swabs submitted to the laboratory from the general hospital population and 200 from patients over age 65 are reported. The total isolation rate on 204 positive swabs was 123 Hemophilus influenzae versus 121 of H. parainfluenzae, 40 of each in association. Isolation rate in group I was 83 and 75, in group II 40 and 46 for HI and HP respectively. The lower rate in the geriatric patients was significant.

INTRODUCTION

Hemophilus influenzae was first been isolated by Koch in 1883 and Pfeiffer in 1890. It is a delicate gram negative bacilllus, unable to synthesize porphyrin from delta-aminolevulinic acid requiring both factors X (=Hemine) and V (=NADPH) for growth. It can be capsulated, 6 capsular antigens have been recognized so far, in vivo transformation through DNA-transfer between capsulated and unencapsulated strains is possible (Davies 1980). Most pathogenic strains are capsulated and of type b, but lately uncapsulated non-typable strains are on the rise in upper airway infections (Sturm 1990). Nontypable strains show greater adherence to cells of the nasopharyngeal mucosa (Harada 1990). The Hemophilus is involved in a variety of infections of otolaryngologic interest (Halperin 1990), specially in the pediatric and immundepressed host, as well as in geriatric patients. Bacterial meningitis, epiglottitis, pericarditis and otitis are frequent in the infant, upper respiratory tract infections and pneumonia have been largely recognized in the elderly were also a fall in antihemophilus antibodies has been seen (Dorff 1973). Hemophilus spp. are the second most frequent agent in

geriatric pneumonia (Everett 1977). Nontypable organisms are seen with increasing frequency (Musher 1982). In fact most strains isolated from unsymptomatic persons or those with upper airway infections after the pediatric age, are unencapsulated. Penicillin resistance recognizes two pathways, one by the usual ß-lactamase, frequent in capsulated strain, one through Penicillin-Binding-Proteins (PBP), most frequent in unencapsulated strains. ß-lactamese is transmitted through plasmids (Trieu 1990), the PBP's are transmitted mainly through DNA--transfer by transformation (Gleckman 1984, Mendelman 1990). Recently chloramphenicol as well as erythromycin resistant strains have been isolated (Heney 1990). The organism's fastidious growth requirements have since now opposed a routine isolation from nasal and pharyngeal swabs. Its seemingly minor importance did not justify the major workload and costs of a routine isolation. Rapid tests have been described (Palladino 1990, Murphy 1990), like a rapid immunologic test for type b antigen, but its validity is restricted to cases with encapsulated b-strains. Immunologic search for production of ß-lactamase mediated penicillin resistance is not exhaustive since it does not cover PBP mediated resistance. In this paper we describe both a cost effective selective enrichment method for pure colony isolation and discuss the results of its application in an epidemiologic study in a geriatric population (Tortora 1986, Broich 1986).

MATERIALS AND METHODS

I.The enrichment procedure. Tryptic Soy Broth was chosen as the base. A 2 ml aliquot was transferred into a sterile 16x125 mm screw top tube just prior to use. One BVX differentiation disk (Difco, Detroit, Mich.) and two 10 unit bacitracin disks were added. In the first part of the study, 100 throat swabs (Culturette, Marion Scientific, Kansas City, Missouri) submitted to the University Hospital Clinical Microbiology Laboratory, primarily for group A streptococcus screening in patients of all age groups, were tested through selective enrichment. Control broth was inoculated with S.aureus ATCC 25923 and a clinical isolate of H.influenzae for each days testing. The tubes were prepared fresh, inoculated, vortexed for 15 seconds and incubated in 10% CO_2 atmosphere for 24 hours at 35°C. The day after, it was subcultered to a chocolate agar plate (Columbia agar base with 5% chocolatized human blood and 15% yeast supplement C-Difco). A bacitracin 10U disk was added for further selectivity. The plates were than re-incubated at 35°C in a candle jar for 24 hours. The typical colonies were subcultured to Miller-Hinton Plates to which an X disk and a Staph. aureus colony were added for growth requirements and differentiation between the Hemophilus influenzae (HI) and parainfluenzae (HP) types. For the purpose of this study an organism was classified HP if not requiring factor X (Satellite growth) and HI if requiring it. Collaterally ALA test was performed on all positive colonies (Lund 1977). No attempt has been made to classify concurrent non Hemophilus growth.

II.In the second part of the study, an additional 100 throat cultures submitted primarily for group A streptococcus screening were processed utilizing the experimental selective enrichment technique in parallel with comeercially prepared selective chocolate agar containing 300 mg/l of bacitracin. The selective chocolate agar was incubated at 35°C overnight in a candle extinction jar. Typical Hemophilus spp. colonies were subcultured to tryptose blood agar base on which BV and BX differentiation disks were placed to ascertain growth factor requirements. No attempt was made to serotype or biotype the isolates.

III.Epidemiology in a population aged 65-90 years. In the third phase of the study an age selected population was screened for Hemophilus spp. using the selective enrichment technique. 200 randomly selected throat cultures from patients aged 65 to 90 years were subjected to the experimental technique. The swabs were processed using the technique described at point I. The first 30 specimen have been also in parallel directly inoculated directly on chocolate agar and processed as usual without the enrichment step.

RESULTS

In the first phase of the study a total of 42 isolates of HI and 35 isolates of HP were recovered, mainly in pure culture (Table.1). Commonly encountered gram negative rods not inhibited by bacitracin were the most common contaminating flora. Next, the experimental technique was compared to an established selective medium, chocolate agar with bacitracin.

Tab.1: Hemophilus isolates on chocolate agar subcultures of selective enrichment broth(^) from 100 randomly selected throat swabs

Type of Isolated culture	HI	HP	HI+HP
Only HI or HP:	23	21	6
in mixed culture:			
GNR+	7	5	3
Staph+	4	1	0
Yeast+	2	2	0
total isolates	**36**	**29**	**6**
Total positive swabs HI or HP		71	

(^) Tryptic Soy Broth+two 10 Unit Bacitracin Disks and one BVX differentiation Disk, HI=H.influenzae, HP=H.parainfluenzae, GNR=gram negative rods other than Hemophilus spp., Staph=Staphylococcus spp.

Tab.2: Comparison of recovery rates of Hemophilus spp. by the selective enrichment broth(^) and selective agar(*) from 100 randomly selected throat cultures

Procedure	HI	HP	(HI+HP)	Total pos.swabs
Experimental	**34**	**33**	**7**	**74**
Standard	32	31	6	69

(^) Tryptic Soy Broth with one BVX and two bacitracin disks
(*) Chocolate agar with 300 Units/l bacitracin

Table 3: 200 swabs from Geriatric patients: recovered types

	HI	HP	HI+HP	
Specimen 1-30	3	2	4	enriched
Specimen 1-30	4	4	3	not enriched
Specimen 31-200	9	16	24	enriched
Total 1-200	**12**	**18**	**28**	**enriched only**

Total positive specimen: 29%(58/200), one H.parainfluenzae not recovered with the experimental technique, statistically not significant.

Table 4: Plating passages for the first 30 specimen:

days:	2	3	4	5		
enriched	0	28	2	0	M: 3.06	d.s.=0.062
not enriched	10	5	8	7	M: 3.40	d.s.=1.373

$p<0.05$.

The results in table 2 show a slightly higher recovery rate in the experimental technique (no statistical significance, Chi square test). In the third phase of the study, involving an age selected group, the selective enrichment technique yielded a total of 40 isolates of HI and 46 of HP, in 28 cases with presence of both organisms together and a total of 58 positive cultures of 200. In addition to standard X and V factor requirement testing parallel ALA tests were performed on all isolates. There was 100% agreement between the two methods of determining X factor requirement. The results in a group of hospitalized persons age 65-90 are shown in table 3. The technique without the enrichment step failed to reveal one HI in association with HP. 7 cultures had to be replated tree additional times, bringing the isolation time to 5 days. The time necessary for isolation is reassumed in Table 4. It is

Table 5: Isolation rate:

	HI	HP	Tot positive swabs
General population(200)	83	75	145 (72.5%)
Geriatric population(200)	40	46	58 (29.0%)
Total on 400 cases			**204 (50.75%)**

important to notice that also if the total yield of the two methods is similar, the enrichment method remains highly synchronized (93.3%), this was true for the direct plating only in 33%, with a spread of the work time over five days. This difference is statistically significant ($p<0.05$, t test, Tab.4). Overall isolation rate is shown in Table 5.

CONCLUSIONS

The procedure allows rapid, simple preparation of fresh culture medium with a significantly lower cost. It is microbiologically effective, and simplifies the logistical problems of stocking yet another selective medium with its problems of inventory and quality control. The significant difference in isolation time synchronization (Table 4) between new and the standard techniques is a strong point for laboratory work optimization and labor cost reduction. We consider the technique easy and sufficiently reliable to suggest its use in all specimen of upper airway tract cultures, especially if from patients at risk, as those in pediatric or geriatric age, those who stay in communities or in the immunocompromised host, since multiple antibiotic resistance calls for pure culture isolation of the bacteria and standard antibiograms (Broich 1992). Our results show a distinctively high carrier rate in the pharynx. This rate was unexpectetly lower in the geriatric population than in the general one, the major clinical role of the Hemophilus in that age may be due to a larger amount of multiresistant unencapsulated strains and lower host defenses.

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CARRIER STATUS FOR HEMOPHILUS SPP: EPIDEMIOLOGY IN A GENERAL HOSPITAL POPULATION AND A GROUP OF GERIATRIC PATIENTS

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Hemophilus species are of growing clinical interest in upper respiratory tract and middle ear infections especially in the elderly. This study reports the results of 400 throat swabs done in two groups of hospitalized patients without specific oropharyngeal disease.

The first group consisted in 200 persons between 20 and 64 of age, 100 specimen were treated using the usual plating method for isolation, 100 using an enrichment method described elsewhere by one of us (GB). 200 specimen were taken from geriatric patients age 65 up. In the first group the two techniques yielded comparable results with a technical advantage of the enrichment procedure, with proved to be easier and faster to work with. The isolation rate was 83 Hemophilus influenzae colonies versus 75 of H. parainfluenzae.

In the second group, all done with the enrichment technique, the yield was of 40 H. influenzae and 46 H. parainfluenzae colonies. We stress the unexpected high bacterial count in the general population, with a comparatively lower yield in the geriatric population, were the microorganism appears to be of major and growing clinical importance.

We suggest that this may be due to the larger count of unencapsulated multiresistent organisms in the elderly as described in the literature, combined with a significant importance of host interactions, while the microorganism in the general population behaves more like a facultative pathogen with a high subclinical carrier rate.