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Selective medium that distinguishes *Haemophilus influenzae* from *Haemophilus parainfluenzae* in clinical specimens: its value in investigating respiratory sepsis

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SUMMARY A medium is described, which is selective for the haemophilus genus and also distinguishes between the species *Haemophilus influenzae* and *Haemophilus parainfluenzae* isolated in primary culture from clinical material.

Haemophilus influenzae can be distinguished from *Haemophilus parainfluenzae* by its growth requirements. *H influenzae* requires both "X" factor (haemin) and "V" factor (coenzyme). *H parainfluenzae* only requires the "V" factor. Such tests using growth factor requirements can be carried out with impregnated discs[1] available from Oxoid Ltd, Basingstoke. Most nutrient agar, however, contains small amounts of "X" factor, which enables *H influenzae* to grow around the disc containing "V" factor alone, thereby making it seem to be independent of the "X" factor and leading to its misclassification as *H parainfluenzae*.[2] One way to overcome this problem is to use a less rich medium, but then more fastidious strains fail to grow.

Material and methods

Blood agar base No 2 (Oxoid) (40 g), 10 g sucrose, and 25 mg phenol red and 1 litre distilled water were heated for 15 minutes at 121°C. After cooling 100 mg bacitracin (to which haemophilus is resistant) and 3 mg haemin were added as sterile solutions and the resulting medium allowed to set in Petri dishes.

Clinical specimens were plated out in the conventional manner on surface dried medium and a "V" factor disc placed on the surface. After overnight incubation at 37°C haemophilus colonies satellited around the "V" factor disc. *H parainfluenzae* fermented the sucrose and grew as bright yellow colonies; *H influenzae* grew as white colonies (no indicator change).

Whenever a selective method,[3] which uses anaerobic conditions to isolate haemophilus in primary culture from patients with chronic respiratory sepsis complicated by carriage of pseudomonas, is required, the method described here to distinguish *H influenzae* from *H parainfluenzae* should be used at the subculture stage: the sucrose fermentation reaction and indicator change does not occur under anaerobic conditions, and any carbon dioxide present such as when an Oxoid gas pack is used to achieve anaerobic conditions) causes non-specific indicator colour change.

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SPUTUM SPECIMENS

One hundred and fifty isolates of *Haemophilus* (from 107 specimens of sputum expectorated by 74 patients with chronic purulent sputum production, most with confirmed bronchiectasis, were identified by the selective method described above.

Results

The table shows the results of testing a variety of strains of haemophilus isolated in our laboratory, using a selective medium[3] that incorporated no growth factors. The reference strain (NCTC 7857) and two clinical isolates (W and 253) of *H parainfluenzae* fermented sucrose and were easily distinguishable from the reference strain (NCTC 8143) and three clinical isolates (C, A, N) of *H influenzae*—that is, there was good correlation between sucrose fermentation, and growth factor requirements.

Using this technique 107 sputum specimens (produced by 74 patients with chronic bronchial sepsis) yielded 40 isolates of *H influenzae* and 60 isolates of *H parainfluenzae*, both strains being found in eight patients.

Table *Fermentation of sucrose by Haemophilus species grown on growth factor free selective medium*

Strain	Growth around disc			Sucrose fermentation
	"X" factor	"V" factor	"X + V" factor	
NCTC 8143 (*H influenzae*)	−	−	+	−
C	−	−	+	−
A	−	−	+	−
N	−	−	+	−
NCTC 7857 (*H parainfluenzae*)	−	+	+	+
W	−	+	+	+
253	−	+	+	+

− = no growth; + = growth.

Discussion

The clinical importance of distinguishing *H influenzae* from *H parainfluenzae* lies in the widely held interpretation of *H parainfluenzae* as non-pathogenic.[4] According to this, misclassification of *H influenzae* as *H parainfluenzae* would result in treatment to eradicate a candidate pathogen being withheld.

We advise against over-ready acceptance of this interpretation of *H parainfluenzae* as non-pathogenic for four reasons: firstly, *H parainfluenzae* has recently been reported as a pathogen in respiratory infections;[5] secondly, *H parainfluenzae* is inhibitory for human ciliary function in vitro;[6] thirdly, the sputum specimens in our study yielded a surprisingly high proportion of isolates (and a study in progress documenting its presence in the bronchial tree by double-lumen bronchial brushing is yielding a similar incidence of isolates); fourthly, recent work[7] has shown that the progressive bronchial damage associated with chronic bronchial sepsis may be due to a "vicious circle" of tissue damaging inflammatory host response to a persistent microbial flora. This microbial load is largely non-invasive, avirulent, and well contained in the lung but cannot be eliminated by natural defences. It colonises an ecological niche opened by initial lung damage rather than actively invading previously normal respiratory tract in the manner of "classical" acute infection, such as pneumonia. According to this hypothesis,[7] any micro-organism capable of inciting an inflammatory response in the host's respiratory tract will lead to damage from its presence alone. Conventional but inadequate antimicrobial treatment may even provoke abnormal forms of organism,[8] which, while evading host defences, may still evoke a tissue damaging inflammatory response. Our results showing *H parainfluenzae* alone to be associated with chronic bronchial sepsis in the case of 40 patients would support the case for it being a pathogen—at least under some circumstances.

For those subscribing to the "vicious circle" hypothesis, the selective medium described here may be useful in identifying the relative roles of these micro-organisms in those processes which progressively damage the lung. For others, it may be a simple, inexpensive method of studying the carriage of such micro-organisms in health and disease.

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