Comparison of Four Charcoal Media for the Isolation of
Bordetella pertussis

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Charcoal-horse blood agar with 40 μg of cephalixin per ml, charcoal-horse blood agar with 3 μg of lincomycin per ml, charcoal agar with 3 μg of lincomycin per ml, and Legionella (buffered charcoal-yeast extract) agar with 3 μg of lincomycin per ml were compared for isolation of Bordetella pertussis. Charcoal-horse blood agar with 40 μg of cephalixin per ml gave the best results, with a B. pertussis recovery rate of 100%. Growth was most rapid and the mean number of colonies was highest on this agar, and growth of pharyngeal flora was completely suppressed.

Using seven stock strains of Bordetella pertussis, Hayes et al. (P. S. Hayes, J. C. Feeley, S. E. Johnson, M. L. Graves, and G. W. Ajello. Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C188, p. 331) tested buffered charcoal-yeast extract (BCYE) agar, a blood-free charcoal medium developed for Legionella sp., supplemented with lincomycin and anisomycin. Legionella agar supported growth of B. pertussis well. In the study described here, we evaluated Legionella agar with lincomycin (L-L) more extensively by using mixtures of B. pertussis and saliva (thus simulating nasopharyngeal specimens). For comparison, we included charcoal-horse blood agar with cephalixin (CB-C), charcoal-horse blood agar with lincomycin (CB-L), and charcoal agar with lincomycin (C-L).

For preparation of CB-C, 51 g of charcoal agar base (Oxoid Ltd., Basingstoke, United Kingdom) was dissolved in 1 liter of distilled water. After the mixture was autoclaved and cooled to 50°C, 100 ml of whole defibrinated horse blood and 40 mg of cephalixin were added. CB-L contained 3 mg of lincomycin (Serva, Heidelberg, Federal Republic of Germany) instead of cephalixin. C-L was CB without horse blood. For preparation of L-L, 2.5 g of Legionella CYE agar base (Oxoid) was dissolved in 90 ml of distilled water. One vial of Legionella BCYE supplemented [N-(2-acetamido)-2-aminoethanesulfonic acid (ACES) buffer-potassium hydroxide, 1.0 g; ferric pyrophosphate, 0.25 g; L-cysteine hydrochloride, 0.04 g; a-ketoglutarate, 0.1 g; Oxoid] was dissolved in 10 ml of sterile distilled water. After the agar was autoclaved and cooled to 50°C, BCYE supplement and 0.3 mg of lincomycin were added. Bordet-Gengou agar (36 g of Bordet-Gengou agar base [Oxoid] containing 10 g of Proteose Peptone per liter) was dissolved in 1 liter of distilled water, and 10 ml of glycerol was added. After the agar was autoclaved and cooled to 50°C, 200 ml of whole defibrinated sheep blood was added. Plates of all media were stored at 4°C under appropriate sterility control conditions.

Fourteen fresh clinical isolates of B. pertussis were cultivated on antibiotic-free Bordet-Gengou agar. A suspension of each strain in sterile saline was prepared and adjusted to a photometric extinction equivalent to that of a nephelometric McFarland 0.5 standard. Saliva specimens of four people were diluted with sterile saline and adjusted to the same extinction. Each B. pertussis suspension was mixed 1:1 with each saliva suspension and further diluted 1:1,000 with sterile saline. A total of 56 experiments were performed.

The inoculum, consisting of 20 μl of the final dilution (ca. 105 Bordetella), was spread evenly on one plate of each of the test media. Plates were incubated for 7 days at 36°C in air protected from desiccation. Growth of Bordetella and flora was recorded daily. The colony size of B. pertussis was assessed semiquantitatively. B. pertussis was identified by colony morphology, oxidase reaction, and slide agglutination with specific antisera (Wellcome Diagnostics, Dartford, United Kingdom), which included positive and saline controls. For statistical evaluation, the chi-square test was used.

The recovery rate of B. pertussis on CB-C was 100%, and no growth of flora occurred on this medium (Table 1). L-L showed a recovery rate of 85.7% (48 of 56 samples) (P < 0.01), but growth of flora was observed in nearly half (27 of 56) of the samples. Furthermore, growth of B. pertussis was significantly more rapid on CB-C than on L-L, and only 57.1% of the strains grew to mature colonies on L-L, in contrast to 100% on CB-C (P < 0.0035). In addition, the mean number of colonies was significantly higher on CB-C (149; range, 11 to 400) than on L-L (30; range, 0 to 93). Identification by agglutination of B. pertussis strains grown on L-L was usually not possible because of autoglutination.

Only 57.1% of the strains were recovered on CB-C (P < 0.0035), and growth of flora was observed in 67.9%. Development of mature colonies was not seen on this agar, and the mean number of colonies was significantly lower (28; range, 0 to 200) than on CB-C.

The lowest recovery rate (21.4%) was observed on C-L. Growth of B. pertussis on this medium was slow, and mature colonies did not develop at all. The mean number of colonies was 1.7 (range, 0 to 18), but growth of flora occurred in 64.3%.

Most strains of B. pertussis are highly resistant to cephalxin (2). A concentration of 40 μg/ml is used in Regan-Lowe transport medium (4), which is now considered the transport medium of choice (3). Since MICs for occasional strains of B. pertussis are below 40 μg/ml (2), these strains may not survive transport in Regan-Lowe medium; however, this factor is unlikely to be a major consideration. All strains used in our study had originally been recovered from Regan-Lowe medium, which may have slightly biased our results.

The MIC of lincomycin against B. pertussis is 3.12 to 50

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TABLE 1. Recovery of *B. pertussis* and growth of flora on four charcoal-containing media

<table>
<thead>
<tr>
<th>Agar medium</th>
<th>Recovery of <em>B. pertussis</em></th>
<th>Growth of flora</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>%</td>
</tr>
<tr>
<td>CB-C</td>
<td>56</td>
<td>100</td>
</tr>
<tr>
<td>CB-L</td>
<td>32</td>
<td>57.1</td>
</tr>
<tr>
<td>C-L</td>
<td>12</td>
<td>21.4</td>
</tr>
<tr>
<td>L-L</td>
<td>48</td>
<td>85.7</td>
</tr>
</tbody>
</table>

* Fourteen *B. pertussis* strains were individually mixed with four separate saliva samples, for a total of 56 mixtures.

In addition to yielding a lower recovery rate than did CB-C, L-L had the disadvantage of producing autoagglutination of *B. pertussis* colonies. Nevertheless, it would be interesting to compare in detail charcoal-horse blood agar and *Legionella* agar, both antibiotic free and both supplemented with cephalixin, to characterize their effectiveness in isolating *B. pertussis*.

A field study conducted in parallel with these laboratory experiments yielded similar results. *Bordetella* isolation rates were 21.9, 14, 10.8, and 7.4% on CB-C, L-L, CB-L, and C-L, respectively. Details have been reported elsewhere (Proceedings of the FEMS Symposium on Pertussis, in press).

**LITERATURE CITED**