JOURNAL OF CLINICAL MICROBIOLOGY, May 1978, p. 426-427
0095-1137/78/0007-0426$02.00/0

Vol. 7, No. 5
Printed in U.S.A.

Gentamicin-Blood Agar for Isolation of *Streptococcus pneumoniae* from Respiratory Secretions

R. E. SCHMID, J. A. WASHINGTON II, AND J. P. ANHALT*

Section of Clinical Microbiology, Department of Laboratory Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 2 February 1978

Previous studies have suggested that the yield of *Streptococcus pneumoniae* from respiratory secretions can be increased by using a 5% sheep blood agar plate supplemented with 5 μg of gentamicin (GBA) per ml. We report our experience with 245 lower respiratory specimens in which this method was compared with 5% sheep blood agar (SBA) alone. Of 35 specimens with growth of *S. pneumoniae* on either plate, 21 were detected exclusively on SBA, whereas only 3 were detected on GBA alone ($P < 0.01$). By subculturing representative alpha-hemolytic colonies from the final 169 specimens, the yield of *S. pneumoniae* was increased by 27% compared with the number of identifications that could be made directly from the primary culture. Minimal inhibitory concentrations of gentamicin for the last 25 isolates were ≥8 μg/ml. Our results do not substantiate the previous observations that *S. pneumoniae* from respiratory secretions gives an increased yield in cultures on GBA.

Dilworth et al. (1) and Sondag et al. (2) found that addition of 5 μg of gentamicin per ml to 5% sheep blood agar (GBA) increased the yield of *Streptococcus pneumoniae* from respiratory secretions apparently by suppressing growth of other organisms that may obscure colonies of *S. pneumoniae* on 5% sheep blood agar lacking gentamicin (SBA). Our preliminary experience with this method in an unrelated study of the diagnosis of pneumococcal pneumonia indicated that GBA was less sensitive than SBA for isolating *S. pneumoniae*. We designed this study to compare critically the two media.

MATERIALS AND METHODS

All sputa, bronchial washings, and tracheal aspirates cultured for bacteria in the Clinical Microbiology Laboratory of the Mayo Clinic from 12 October through 17 November 1977 were included in the study. Expectorated sputa from which a Gram-stained smear showed more than 25 squamous epithelial cells per low-powered field (×100) on microscopic examination were considered unacceptable and were not cultured. Specimens that were acceptable for culture were inoculated by the quadrant isolation technique onto SBA containing tryptic soy agar base (Gibco Diagnostics, Madison, Wis.), 5% chocolate blood agar (GIBCO), eosin methylene blue agar (GIBCO), and GBA. GBA plates were prepared by adding 5 μg of gentamicin (Schering Corp., Kenilworth, N.J.) to Trypticase soy agar (BBL, Becton, Dickinson and Company, Cockeysville, Md.). After autoclaving and cooling of the medium to 50°C, 5% (vol/vol) defibrinated sheep blood was added and mixed. The medium was poured to a depth of 4 to 6 mm into plates 90 mm in diameter. After their inoculation, all media were incubated in 5 to 8% CO_2 at 35°C. Cultures were examined after overnight incubation (18 to 24 h) by a technologist. The GBA plates were reincubated and examined again at 48 h by one of us (R.E.S.) and by a technologist who was unaware of the results using the other media. *S. pneumoniae* was identified by colonial morphology and by the bile solubility test (3).

During the final 3 weeks additional studies were done. A representative of each morphological type of alpha-hemolytic colony growing on SBA or GBA plates at 24 h was subcultured to SBA. An optochin disk (Taxo P Disc, BBL) was placed on the agar, and the plates were incubated in 5 to 8% CO_2 at 35°C as described in the package insert for the optochin disks. Subcultures were examined without knowledge of the other culture results after 24 h, and *S. pneumoniae* was identified by colonial morphology, bile solubility, and optochin disk sensitivity. If the optochin disk produced a zone of growth inhibition of <15-mm diameter, the Dreft test (3) was used to confirm identification.

The susceptibility of the final 25 isolates of *S. pneumoniae* to 2, 4, and 8 μg of gentamicin per ml was determined by the agar-dilution method in Mueller-Hinton agar containing 4% sheep blood.

RESULTS

Of 489 lower respiratory specimens received in the laboratory during the study period, 245 were included in the study. Those specimens excluded consisted of 82 expectorated sputa which did not fulfill the microscopic screening criteria for acceptability for culture plus 162 specimens for which some of the many technologists involved forgot to inoculate the GBA or were unaware of the study. The acceptable specimens were comprised of tracheal aspirates (23%), bronchial washings (10%), and expectorated sputa (67%). *S. pneumoniae* was identified in 35 specimens on one or both media (Table 1). There were 9% (3/35) false-negative results with SBA versus 60% (21/35) with GBA ($P < 0.01$ by

TABLE 1. *Comparison of media for detection of S. pneumoniae in 245 lower respiratory secretions*

SBA	GBA	
	+	−
+	11	21
−	3	210

sign test). A total of 11 specimens were positive with both media and 210 specimens were negative with both media.

Subculture of alpha-hemolytic colonies isolated from the final 169 specimens was performed. A mean of 2.1 colony morphological types were subcultured from each of the 105 SBA and 117 GBA plates. Compared to the yield from the original SBA and GBA plates, subculture increased the yield of *S. pneumoniae* by 27% (Table 2). In one case, *S. pneumoniae* was isolated initially from GBA alone, but on subculture *S. pneumoniae* was also identified from SBA. The reverse situation occurred in two cases. *S. pneumoniae* was identified on GBA in two cases in which the SBA plate was overgrown by other organisms. Combining results from the original plates and subculture, *S. pneumoniae* was identified on SBA alone from 11 specimens compared with 3 specimens from which it was identified only on GBA ($P < 0.10$ by sign test). Of the six *S. pneumoniae* isolates identified only from subculture, three had a zone of inhibition to optochin of <15 mm, a negative bile solubility test, and a positive Dreft test.

The gentamicin susceptibility for 25 isolates of *S. pneumoniae* tested was >8 μg/ml, except for one which was 8 μg/ml. Three of these isolates had been identified from GBA alone and six had been isolated from SBA alone.

DISCUSSION

Dilworth et al. reported that in their study of 62 expectorated sputa containing polymorphonuclear leukocytes, but no epithelial cells, *S. pneumoniae* was isolated from 33 on GBA compared with 20 on SBA (1). Sondag et al. found that from 844 unscreened respiratory secretions, *S. pneumoniae* was isolated from 44 on GBA versus 28 on SBA (2). In our study of 245 lower respiratory specimens, *S. pneumoniae* was isolated from 14 on GBA and from 32 on SBA.

The cause for the lower detection rate on GBA in our study is obscure. The reliability of the bile solubility test in identifying colonies of *S. pneumoniae* may be questioned, but further identification techniques generally supported the results of that test. Colonies of *S. pneumoniae* were characteristically large and mucoid on SBA but small on GBA. It is not known whether the smaller colonies were more difficult to detect and lowered the detection sensitivity. None of 25 organisms studied were susceptible to gentamicin at the concentration used in GBA.

TABLE 2. *Comparison of methods of identification of S. pneumoniae from 169 lower respiratory specimens*

Method	SBA	GBA	
		+	−
Isolation plate alone[a]	+	9	10
	−	3	147
Any[b]	+	14	11
	−	3	141

[a] Identification by colonial morphology and bile solubility.

[b] Results of identification on isolation plate and subculture plate by colonial morphology, bile solubility, optochin disk sensitivity and, in four, Dreft test.

Whether inoculation of two SBA plates would give a yield of *S. pneumoniae* from respiratory secretions comparable to inoculating one SBA and one GBA plate remains to be studied. Additionally, non-blind reading of primary culture plates may increase yield by allowing findings from one plate to substantiate suspicions from another.

Our data from subcultures might indicate that the bile solubility test is less sensitive than the other methods used to identify *S. pneumoniae*. That three of the six isolates identified only from subculture were bile soluble, however, seems to show that some of the increased yield may be due to sampling. Some other explanation seems necessary to account for the three isolates identified from subculture that were bile insoluble. This study was not designed to determine the sensitivity and accuracy of the various tests used to identify *S. pneumoniae*. The possibility that use of the Dreft test may have resulted in the false identification of three isolates as *S. pneumoniae* cannot be excluded.

In conclusion, GBA may have limited usefulness in isolating *S. pneumoniae* from respiratory secretions when the SBA plate is overgrown by other organisms, as occurred in two of our cases. However, we do not feel that GBA's limited usefuless justifies routine use, since our data suggest that GBA is generally less sensitive than SBA for *S. pneumoniae*.

ACKNOWLEDGMENT

We thank D. M. Ilstrup for assistance in statistical analysis.

LITERATURE CITED

1. **Dilworth, J. A., P. Stewart, J. M. Gwaltney, Jr., J. O. Hendley, and M. A. Sande.** 1975. Methods to improve detection of pneumococci in respiratory secretion. J. Clin. Microbiol. **2**:453-455.
2. **Sondag, J. E., R. K. Morgens, J. E. Hoppe, and J. J. Marr.** 1977. Detection of pneumococci in respiratory secretions: clinical evaluation of gentamicin blood agar. J. Clin. Microbiol. **5**:397-400.
3. **Washington, J. A., II.** 1974. Media, reagents and tests, p. 461-462. Laboratory procedures in clinical microbiology, 1st ed. Little, Brown & Company, Boston.