New York City Medium for Enhanced Recovery of *Mycoplasma pneumoniae* from Clinical Specimens

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Modified New York City (MNYC) medium and PPLO medium without methylene blue (PPLO agar) were compared for their ability to support the growth of *Mycoplasma pneumoniae* from clinical specimens. Pharyngeal specimens were collected from 1,070 college students who visited the Syracuse University Student Health Center. Of these patients, 623 were symptomatic for respiratory infection, and the remaining 447 were asymptomatic for respiratory illness. Throat swabs were inoculated into PPLO broths, and these broths were subcultured onto MNYC medium and PPLO agar after 3 and 14 days of incubation. A total of 222 (20.7%) clinical isolates of *M. pneumoniae* were recovered on these solid media, with the majority of the isolates (196) recovered from symptomatic patients. All isolates grew on MNYC medium, whereas five isolates failed to grow on PPLO agar. All isolates of *M. pneumoniae* recovered from symptomatic patients were detected on MNYC medium within 1 to 5 days of incubation, whereas 5 to 7 days of incubation were required before mycoplasmal growth was detected on PPLO agar. Over 86% of these mycoplasma isolates were detected on MNYC medium within 3 days of incubation and before the detection of any mycoplasmal growth on PPLO agar. A similar pattern of recovery times was observed for mycoplasmas isolated from asymptomatic patients. The results of this study have shown that MNYC medium is better than PPLO agar in supporting the rapid growth of *M. pneumoniae* from clinical specimens after 72-h blind subculture in PPLO glucose broth.

*Mycoplasma pneumoniae* is generally recognized as a cause of a benign, self-limited respiratory infection that occurs primarily in children and young adults (2). Although severe mycoplasmal respiratory illness has been uncommon, some reports (15, 16) have suggested that the disease spectrum attributable to *M. pneumoniae* is more serious than previously thought and that severe pulmonary involvement can occur in otherwise healthy children and adults of all age groups. In addition, extrapulmonary complications involving the skin, cardiovascular, gastrointestinal, or central nervous systems (or any combination of these) are frequently associated with *M. pneumoniae* infections (1, 15, 16).

The diagnosis of an *M. pneumoniae* infection is confirmed by either culture or specific serology (13). Conventional culture methods involve the use of specialized media such as E agar (13) and modified formulations of Hayflick’s PPLO broth and agar (19), whereas serological assays test for significant changes in specific mycoplasmal antibody titers in acute and convalescent patient sera (14). Each of these procedures is not without its limitations, since several weeks to a month may elapse before a laboratory diagnosis of mycoplasmal infection can be established. In addition, these methods involve the use of specialized procedures which are not readily available in most clinical laboratories.

In a previous investigation (9), we performed a preliminary study that compared modified New York City (MNYC) medium with conventional mycoplasma media for ability to support the growth of stock strains of *M. pneumoniae*. The results of that study showed that MNYC medium is suitable for supporting the growth of stock strains of *M. pneumoniae* and that growth can be detected a minimum of 5 to 6 days sooner than with conventional mycoplasmal media.

Since the MNYC formulation was able to support the growth of *M. pneumoniae* within a significantly shorter period of time, we reasoned that perhaps this medium could be used in the recovery of *M. pneumoniae* from clinical specimens. The purpose of this investigation was (i) to compare MNYC medium with a conventional mycoplasmal medium, such as PPLO agar with-
out methylene blue (PPLO agar), for ability to support the growth of *M. pneumoniae* from clinical specimens, and (ii) to determine which of these two media was capable of supporting detectable mycoplasmal growth within the shortest period of incubation time.

**MATERIALS AND METHODS**

**Media.** MNYC medium was prepared as previously described (9) and consisted of a Proteose Peptone, cornstarch, phosphate-buffered agar base supplement ed with agamna horse serum, yeast dialysate, dextrose, and an antimicrobial mixture of vancomycin, colistin, amphotericin B, and trimethoprim lactate. PPLO without methylene blue (PPLO) agar was prepared by standard methods (12) and Hayflick’s PPLO glucose broth with phenol red indicator (PPLO glucose broth) was prepared according to the modification described by the Centers for Disease Control (19).

**Clinical specimens.** The patient population consisted of 1,070 college students who visited the Syracuse University Student Health Center during the period of December 1980 to April 1981. There was a comparable number of male and female patients whose ages ranged from 17 to 30 years. Of these patients, 95% were 17 to 22 years old.

Of the 1,070 patients, 623 were symptomatic for respiratory infection with chief complaints of sore throat, nasal congestion, ear discomfort, or cough. The remaining 447 asymptomatic patients had no respiratory complaints for at least 2 weeks before presentation at the clinic. These asymptomatic patients visited the clinic for other medical reasons and served as the control population.

**Protocol.** Pharyngeal swab specimens were collected from all patients by clinic physicians or trained nurses. Each swab was placed immediately in a screw-cap tube containing 2 ml of PPLO glucose broth. The swab was twirled in the broth to elute clinical material, pressed against the side of the tube to express the excess fluid, and then discarded. All tubes were incubated at 35°C for a period of 14 days. The PPLO glucose broths were examined daily for the appearance of mycoplasmal growth and were routinely subcultured after 3 and 14 days of incubation. All subcultures were performed on MNYC and PPLO agar plates by transferring 0.2 ml of the PPLO broth to the surface of each agar medium. The inoculum was distributed evenly over the surface of each medium, and the plates were incubated at 35°C. Each medium was examined daily with a microscope (×100 magnification) to detect the appearance of mycoplasmal growth. When growth was observed, the colonies were presumptively identified as *M. pneumoniae* by their ability to hemadsorb a 1% suspension of guinea pig erythrocytes (19). All provisional isolates were confirmed as *M. pneumoniae* by the serological growth inhibition test (19).

**RESULTS**

A total of 222 (20.7%) clinical isolates of *M. pneumoniae* were recovered from the patient population of 1,070 college students. The majority of these isolates (196) were recovered from the 623 symptomatic patients, whereas the remaining 26 isolates were recovered from the 447 asymptomatic patients.

Table 1 shows the comparative recovery rates of *M. pneumoniae* on MNYC and PPLO media from the symptomatic patients after subculture from the PPLO enrichment broth after 72 h of incubation. All isolates of *M. pneumoniae* were detected on MNYC medium within 1 to 5 days of incubation, whereas 5 to 7 days of incubation were required before mycoplasmal growth was detected on PPLO agar. Importantly, 86.7% of all *M. pneumoniae* isolates were detected on MNYC medium within 3 days of incubation and before the detection of any mycoplasmal growth on PPLO agar. Two isolates of *M. pneumoniae* failed to grow on PPLO agar but were recovered on MNYC medium after subculture from the PPLO broth. There were no isolates of *M. pneumoniae* that grew on PPLO agar but failed to grow on MNYC medium.

The comparative recovery rates of *M. pneumoniae* on MNYC and PPLO media from the control patients after 3-day blind subculture from the PPLO glucose broths are shown in Table 2. All isolates of *M. pneumoniae* were recovered on MNYC medium within 3 to 5 days of incubation, with 77% of the isolates recovered within 4 days of incubation. PPLO medium required 5 to 7 days of incubation before the detection of mycoplasmal growth. All isolates of *M. pneumoniae* that were recovered on PPLO agar grew on MNYC medium. However, three isolates that were recovered on MNYC medium after blind subcultures from the PPLO broth failed to grow on PPLO medium.

All isolates of *M. pneumoniae* were recovered
on solid media after blind subculture from the PPLO glucose broth after 3 days of incubation, even though many of these broth did not show any evidence of mycoplasmal growth as evidenced by color change or turbidity. Prolonged incubation of the PPLO glucose broth for 14 days followed by blind subculture onto MNYC and PPLO media did not result in any additional isolates of *M. pneumoniae*.

Only 13 (1.2%) of the 1,070 clinical specimens were observed to give false-positive reactions in the PPLO glucose broth, as evidenced by a color change of the broth indicator but with failure to recover mycoplasma on solid media after subculture. All of these false-positive reactions occurred within 4 days of incubation of the PPLO glucose broth. Five of these 13 specimens were from asymptomatic patients.

**DISCUSSION**

Within recent years, new formulations of media have been developed that have been reported to support the improved growth of mycoplasma from clinical specimens. SP-4 medium, originally developed by Tully and his co-workers (18) for the cultivation of spiroplasmas, was found to enhance the isolation of *M. pneumoniae* from clinical specimens (17). Although this medium was reported to be superior to diphasic medium with methylene blue for the isolation of *M. pneumoniae*, 2 to 3 weeks of incubation was required for the detection of mycoplasma growth on this medium.

New York City (NYC) medium has also been reported to support the growth of various human mycoplasmas (5, 7). Although Faur and her colleagues initially developed this medium for the isolation of pathogenic *Neisseria* spp. (3, 4, 6), these same investigators (7) subsequently found that this medium would support the growth of *Ureaplasma* sp. and large-colony mycoplasma. We modified the original formulation of NYC medium and found that the MNYC medium would support the rapid growth of stock strains of *M. pneumoniae* (9). In addition, we also found that modified formulations of NYC medium were superior to Martin-Lewis medium for the recovery of *Neisseria gonorrhoeae* from clinical specimens (8, 10, 11).

The results of our current study have shown that MNYC medium is better than PPLO agar in supporting the rapid growth of *M. pneumoniae* from clinical specimens after 72-h blind subculture from PPLO glucose broth. These findings indicate that the combined use of preincubated PPLO glucose broth followed by subculture onto NYC medium results in the detection of colony growth within 4 to 6 days of specimen inoculation. As such, significant improvement can be achieved in reducing the incubation times required to isolate *M. pneumoniae* from clinical specimens.

No advantage was found in prolonged incubation of the PPLO glucose broth beyond 3 days. However, the PPLO glucose broth should be subcultured routinely onto MNYC medium after 3 days of incubation, since all isolates of *M. pneumoniae* were recovered on solid media after this subculture, even though no evidence of mycoplasmal growth was apparent in many of the broths.

There were no isolates of *M. pneumoniae* that grew on PPLO agar but failed to grow on MNYC medium. However, five isolates were recovered on MNYC medium that failed to grow on PPLO agar. Two of these isolates were recovered from asymptomatic patients, whereas the remaining three strains were recovered from the control patient population. This discrepant isolation rate between the two media was not statistically significant.

Although MNYC medium supported a more rapid growth of *M. pneumoniae* than did PPLO agar for isolates from both the symptomatic and the asymptomatic patients, longer incubation times were required to detect mycoplasma growth for isolates from the asymptomatic patient population. Over 96% of the *M. pneumoniae* isolates from the symptomatic patients were recovered on MNYC medium within 4 days of incubation, whereas 77% of the isolates were recovered from the control patients within the same time period. This result is probably attributable to inoculum effect since sympto-

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of isolates recovered on the following day of incubation:</th>
<th>Total isolates (%)</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
<td>4</td>
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<tr>
<td>MNYC</td>
<td>9 (34.6)</td>
<td>11 (42.3)</td>
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<tr>
<td>PPLO agar</td>
<td>0</td>
<td>0</td>
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* Number in parentheses shows the percent recovery rate.
* Three *M. pneumoniae* isolates failed to grow on PPLO agar.

**TABLE 2. Comparative recovery rates of *M. pneumoniae* from asymptomatic patients on MNYC medium and PPLO agar**
matic patients are likely to be carrying fewer mycoplasma than individuals symptomatic for respiratory disease.

In summary, the results of this study have shown that MNYC medium supports the enhanced growth of \textit{M. pneumoniae} from clinical specimens and, when used in conjunction with PPLO glucose broth, significantly reduces the incubation times required to establish the cultural diagnosis of \textit{M. pneumoniae} infection.

\section*{LITERATURE CITED}