Comparison of Modified New York City Medium with Martin-Lewis Medium for Recovery of *Neisseria gonorrhoeae* from Clinical Specimens

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A modified formulation of New York City medium was comparatively evaluated with Martin-Lewis medium for the recovery of *Neisseria gonorrhoeae* from clinical specimens. A total of 240 strains of gonococci were recovered from 1,250 specimens collected from walk-in patients attending a sexually transmitted disease clinic. *N. gonorrhoeae* was cultivated on both of these media from 182 clinical specimens with an additional 58 gonococcal strains isolated on either of the media. Of these discrepant gonococcal isolates, 27 strains were recovered on only modified New York City medium, whereas the remaining 31 strains were recovered on only Martin-Lewis agar. The differences in these isolation rates were not statistically significant. The overall results showed that modified New York City and Martin-Lewis media were comparable in their ability to grow gonococci from clinical material. Since modified New York City medium is capable of supporting the growth of *N. gonorrhoeae, Mycoplasma pneumoniae*, and urogenital mycoplasmas and inhibiting the growth of commensal microorganisms, it is possible that it may have considerable application as a multifunctional plating medium within the clinical laboratory.

Within the last 15 years, the laboratory diagnosis of gonorrhoea has been greatly facilitated by the use of selective media for the improved recovery of *Neisseria gonorrhoeae* from clinical specimens. Thayer and Martin (22) were one of the first successful developers of such a medium in 1964 by using a chocolate agar plate made selective by the addition of ristocetin and polymyxin B. Since that time, the original formulation of Thayer-Martin medium has undergone a number of modifications and improvements (2, 11, 14, 16-19, 23, 24). The development of Martin-Lewis (ML) medium (13) represents a more recent modification in the original Thayer-Martin formulation which has resulted in the enhanced recovery of gonococci from clinical material.

In 1973, Faur and her colleagues (5-7) developed a new selective medium, called New York City (NYC) medium, which could also be used for the primary isolation of *N. gonorrhoeae* from clinical specimens. Although the combination of antimicrobial selective agents on NYC and ML media was similar, the basic formulation of NYC medium differed markedly from ML agar in that it contained a proteose-peptone agar base supplemented with several enrichments. Nevertheless, the NYC medium has been reported (8, 20, 21, 25) to be a suitable alternative isolation medium to improved modifications of the Thayer-Martin formulation for the isolation of pathogenic *Neisseria*.

Previously, the ability of a modified formation of NYC (MNYC) medium to support the growth of stock strains of *Mycoplasma pneumoniae* was evaluated in our laboratory. The results (10) showed that these strains grew well on MNYC medium in that colonial growth could usually be detected within 48 h of incubation.

Since the original formulation of NYC medium was changed to enhance the growth of *M. pneumoniae*, it was uncertain whether the MNYC medium would still be reliable for supporting the growth of gonococci. The purpose of this prospective study is to comparatively evaluate MNYC medium with ML medium for the recovery of *N. gonorrhoeae* from clinical specimens.

**MATERIALS AND METHODS**

**Media.** The ML medium was prepared by the method of Martin and Lewis (13) and obtained commercially (Tidewater Biologicals, Inc., Chesapeake, Va.). MNYC medium and its various components were prepared in-house as originally described by Faur and her co-workers (5-7) with several modifications: (i) lincomycin hydrochloride (Lincocin, Upjohn) at a final
concentration of 4 μg/ml was substituted for vancomycin hydrochloride; (ii) agamma horse serum (Flow Laboratories, Rockville, Md.) was substituted for conventional horse serum; and (iii) an equal volume of distilled water was substituted for 3% hemolyzed horse erythrocytes (10).

The ML and MNYC media were prepared in Jembec plates (12), and each batch or lot number of media was performance-tested before use for its ability to support the growth of N. gonorrhoeae as well as inhibit the growth of E. coli and enterococci. All media were stored at 4°C and warmed to room temperature before use.

Specimen collection and processing. A total of 1,250 clinical specimens was collected from walk-in patients attending the Sexually Transmitted Disease Clinic at the Onondaga County Department of Health. Clinical samples were collected by trained nurses from either the urethra, cervix, pharynx, and/or rectum. Urethral specimens were collected with calcium alginate-tipped applicators (Becton, Dickinson & Co., Rutherford, N.J.) whereas specimens from the other anatomical sites were collected with cotton-tipped swabs.

Three different protocols were used for the inoculation of clinical specimens onto each of the test media. In protocols I and II the same swab used to collect the clinical specimen was used to inoculate each of the test media. In protocol I, 500 clinical samples were processed by inoculation of the ML medium first, followed by the inoculation of the MNYC medium. In protocol II another group of 500 clinical specimens was processed by reversing the order of medium inoculation. For each Jembec plate inoculated, the swab was rolled slowly over the surface of each medium in a large "Z" pattern to provide adequate transfer of microorganisms to the agar surface.

Protocol III was used to process the remaining 250 clinical samples. In this method the swab used to collect the specimen was placed in a tube containing 0.5 ml of a sterile 0.4% gelatin solution in distilled water. The swab was twirled in the solution to elute the clinical material, and then the swab was discarded after rinsing against the side of the tube to remove the excess fluid. Separate cotton-tipped applicators were dipped into the sample solution and then inoculated onto each test medium in the Z pattern as described above.

For each protocol used, a CO2-generating tablet was placed in the well of each Jembec chamber directly after media inoculation. Each plate was sealed in an individual plastic zip-locked environmental pouch and immediately placed in a 35°C incubator. All Jembec plates were incubated for 72 h and examined daily for the appearance of bacterial growth. Isolates were identified as N. gonorrhoeae on the basis of colonial morphology, oxidase reaction, gram stain and pattern of carbohydrate utilization on cystine-tryptophan agar sugars.

Statistical analysis. The comparative results obtained were analyzed by the chi-square test of dissimilar pairs (3).

RESULTS

The comparative recovery rates of N. gonorrhoeae on each of the media from the 500 clinical samples that were processed by protocol I are shown in Table 1. A total of 74 specimens showed gonococcal growth on both of the media. However, discrepant isolations of gonococci were found for 34 clinical samples. Specifically, 21 isolates of gonococci were recovered on the ML medium only, whereas the remaining 13 strains were isolated only on the MNYC medium. Although the ML medium which was inoculated first in this protocol appeared to offer an advantage in the cultivation of gonococci, statistical analysis of these data indicated that these variations in results were not significant ($\chi^2 = 1.441$).

Table 1 shows the results of the second group of 500 clinical specimens which were processed by protocol II. In this experiment, a total of 88 gonococcal strains were recovered on both of the media. Discrepancies in the isolation of gonococci on each of the media were noted for 16 specimens: 10 strains of gonococci were recovered on the MNYC medium only, whereas the remaining 6 isolates grew only on the ML medium. As was observed in the protocol I study, the medium that was inoculated first provided the highest recovery rate. In this case, however, the MNYC medium appeared to have the advantage in recovery rates, but these results were not significant when analyzed statistically ($\chi^2 = 0.562$).

The results of the remaining 250 clinical specimens which were processed by protocol III are also shown in Table 1. This method differed from protocols I and II in that the swab used to collect the clinical specimen was not used to directly inoculate the test media. Instead, the swab was incubated in a diluent to elute the clinical sample, and separate swabs were dipped into the resultant microbial suspension to inoculate each of the test media.

In this protocol, N. gonorrhoeae was isolated on both media from 20 clinical specimens. There was a total of eight discrepant gonococcal isolations: four grew only on ML medium, and an equal number of strains were recovered only from the MNYC medium. Analysis of these data showed that the results were not statistically significant ($\chi^2 = 0.125$).

The cumulative results for the entire 1,250 clinical specimens evaluated in this study are summarized at the bottom of Table 1. N. gonorrhoeae was cultivated on both of these media from the 182 clinical specimens, with an additional 58 gonococcal isolates which were recovered on only one of the media. However, the
distribution of discrepant isolations was comparable for each of the media with no particular formulation affording a statistically significant ($\chi^2 = 0.07$) advantage in recovery rates. Furthermore, both media appeared to be equally selective in inhibiting the growth of saprophytic microorganisms. Although the antibiotic combinations were different in each of the media, the contamination rate was below 3% for each. In addition, the MNYC medium appeared to be comparable to ML medium in supporting the growth of 63 strains of *N. meningitidis* recovered from pharyngeal cultures processed in this study.

**DISCUSSION**

The results of this study have demonstrated that the modified formulation of NYC medium was comparable to ML medium in supporting the growth of *N. gonorrhoeae* from clinical specimens. A total of 209 and 213 strains of gonococci were recovered on the MNYC and ML media, respectively, with 182 of these isolates recovered simultaneously on both media.

A surprising finding in this study was the relatively large number of discrepant isolations of gonococci. A total of 58 gonococcal strains, or 25% of the isolates, was recovered on only one of the media. Even though the distribution of the discrepant isolates was comparable on each of the media and the differences were not statistically significant, the individual variation between the media may be cause for concern.

Originally, we considered that the differences in isolation rates observed between the two media might be partially attributable to the absence of hemoglobin (lysed horse erythrocytes) in the MNYC medium. However, in a subsequent study (P. A. Granato, C. Schneible-Smith and L. B. Weiner, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., Abstr. no. 324, 1980), we comparatively evaluated NYC medium, NYC medium without lysed horse erythrocytes, and ML medium for their ability to support the growth of *N. gonorrhoeae* from clinical specimens. The results of this study showed that the presence of lysed horse erythrocytes in NYC medium was not
necessary for the primary isolation of gonococci from clinical material.

In all likelihood, the discrepancies observed in this study are attributable to the differences in specimen processing in protocols I and II and/or to the differences in the antimicrobial mixtures in each of the media that could selectively affect the growth of gonococci.

In protocols I and II, the same applicator that was used to collect the clinical specimen was also used to inoculate each of the media. As such, the medium that was processed first in either of these protocols received the larger inoculum which resulted in that particular medium having the higher recovery rate.

This hypothesis was supported by the results from protocol III. In this protocol the clinical sample was eluted from the swab used to collect the specimen and separate applicators were dipped into the resultant microbial suspension to transfer a standardized inoculum to each of the test media. Although there were eight discrepant strains isolated, the overall recovery rate for each of the media was identical, with no particular formulation affording an apparent advantage.

Conceivably, the differences in the antibiotic mixtures in each medium, particularly vancomycin in ML agar and lincomycin in MNYC medium, could account for some of the individual variations in gonococcal recovery rates observed. Several studies (1, 4, 15) have shown that the 4 µg/ml concentration of vancomycin in ML medium may be inhibitory to the growth of as many as 10% of gonococcal strains. As such, some investigators (1, 15) have reported that the substitution of lincomycin for vancomycin in the medium resulted in a 7 to 10% increase in gonococcal isolation rates without an appreciable increase in contamination rate. For this reason, we used lincomycin instead of vancomycin as one of the modifications (10) that were made in the original formulation of NYC medium.

On the other hand, Faur and her colleagues (9) have shown that the concentration of lincomycin (4 µg/ml) that we used in the MNYC medium was inhibitory to approximately 6% of the gonococcal strains that they tested. The variability in gonococcal recovery rates that were experienced in this study could be partially attributable to the selective inhibition of particular gonococcal strains to either vancomycin or lincomycin. This explanation could account for the eight discrepancies observed in protocol III. However, as the results indicate, the overall recovery rate for each of the media was not appreciably affected by the use of either of these antibiotics.

Even though the antimicrobial mixtures were different in each medium, both formulations were comparable in their selective abilities to inhibit the growth of commensal microorganisms. The ML medium contained the antimicrobial mixture of vancomycin, colistimethate, anisomycin, and trimethoprim lactate, whereas the MNYC medium contained lincomycin, colistimethate, amphotericin B, and trimethoprim lactate. The contamination rate experienced on each medium was below 3%. In addition, MNYC medium appeared to be equivalent to ML agar in supporting the growth of meningococci from clinical specimens.

In a recent report (10) we had shown that stock strains of M. pneumoniae were capable of growing on a modified formulation of NYC medium and that the colonial growth of most of these mycoplasmas could be detected microscopically within 48 h of incubation. The overall results of this present study have shown that this MNYC medium was comparable to ML medium for the isolation of N. gonorrhoeae from clinical specimens. Since MNYC medium is capable of supporting the growth of pathogenic Neisseria, M. pneumoniae, and urogenital mycoplasmas and inhibiting the growth of commensal microorganisms, it is possible that MNYC medium may have considerable application as a multifunctional plating medium within the clinical laboratory.

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LITERATURE CITED