associated infection 29 (43%) and 3 (20%), for presence of urinary calculi 3 (4.4%) and 2 (13%), and for appropriate antimicrobial therapy prior to urine culture 5 (7.3%) and 3 (20%), respectively. These differences were not significant when compared using the standard error of percentage.

Discussion

This study revealed that at least 18% of patients with urinary tract infections associated with bacteremia had quantitative urine cultures <10^5 CFU/mL of urine. The results of this study are similar to those obtained in a report from a smaller community teaching hospital. This would suggest that quantitative urine cultures must be able to detect levels <10^5 CFU/mL and that these may be clinically significant. The majority of the 18% had concentrations between 10^4 and 10^5 CFU/mL. This may be simply a reflection of the sensitivity of the MS-2 method used to screen some of the specimens. Because of the limitations of the MS-2, many patients with bacteremia and urinary tract infections with undetected small concentrations of bacteria in the urine may have been disqualified from the study. The finding of only one patient with a negative urine culture is evidence of the difficulty in making a diagnosis of urinary tract infection when the urine culture is reported negative.

The results of this study demonstrated that a significant number of serious urinary tract infections are associated with urine quantitative counts below 10^5. Counts as low as 1 X 10^4 must be detected by any method used and considered potentially significant. Quantitative counts below 10^4 occurred in 6% in this study and in 8.8% in another study. Further studies will be required to determine the true incidence of significant urine cultures with counts less than 10^4 CFU/mL.

References


Detection of L-Forms of Neisseria gonorrhoeae in Pure and Mixed Culture Suspensions by an Enzyme Immunoassay

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An enzyme immunoassay (EIA) (Gonozyme™, Abbott Laboratories, Chicago, IL) was evaluated for its ability to detect L-forms of Neisseria gonorrhoeae in pure and mixed culture suspensions. A total of 15 L-form strains that were induced from fresh clinical isolates of N. gonorrhoeae on an L-form medium were tested by EIA at antigen levels equivalent to those found in 2 × 10^6, 3 × 10^4, and 2 × 10^3 parental cells per milliliter. The results showed the sensitivity of the EIA for L-forms and parental cells to be the same, exhibiting positive results in all pure culture suspensions of parental cells at 2 × 10^6 and 3 × 10^4 cells per milliliter and their corresponding L-form preparations.

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At 2 × 10^3 cells per milliliter, three parental and two of their respective L-form preparations yielded positive EIA results. Incorporation of a mixture of heterologous organisms that can be found in the normal human genital flora, each at a concentration of 1 × 10^6 cells per milliliter into the L-form preparations, did not affect the sensitivity of the EIA for detection of L-forms.

The results of the present study indicate that the EIA is an equally sensitive method for detecting gonococcal L-forms and parental cells. A further study, however, is needed to ascertain its usefulness under actual clinical conditions. (Key words: Gonococcal L-form detection; Pure and mixed culture suspensions; Enzyme immunoassay; Sensitivity) Am J Clin Pathol 1986; 85: 618–622
IN 1972, GNARPE AND CO-WORKERS\(^4\) reported that of the 197 gonorrhea patients examined, 4.5% yielded \emph{Neisseria gonorrhoeae} on osmotically stabilized media only. In addition, Mavrov\(^5\) recently noted gonococcal L-forms from 3.1% of the patients with postgonococcal urethritis. L-forms also have been isolated from "sterile" synovial fluid in gonococcal arthritis\(^6\) and from a case of untreated gonococcal urethritis.\(^5\) In the latter,\(^5\) a cell-wall-defective form of \emph{N. gonorrhoeae} was isolated that was incapable of reverting to a parental gonococcus.

These studies\(^4-6,9\) seem to suggest that gonococcal L-form infections do exist \emph{in vivo}. Elucidation of the role of L-forms in asymptomatic and symptomatic gonococcal infection, however, is hampered by the fact that none of the conventional culture methods for the primary isolation of \emph{N. gonorrhoeae} are adequate for detecting L-forms of \emph{N. gonorrhoeae}.

It is, therefore, possible that some of the culture-negative, enzyme immunoassay (EIA)-positive (so-called false positive EIA) findings observed in recent studies\(^1,10-12\) with Gonozyme™ (Abbott Laboratories, Chicago, IL), an EIA system for detection of gonococcal antigen, might actually have represented cases of asymptomatic gonococcal L-form infections.

The present study was initiated to determine whether the EIA (Gonozyme) is able to detect L-forms of \emph{N. gonorrhoeae}, and whether there are differences in test sensitivity for the detection of L-form and parental gonococcus.

**Materials and Methods**

**Strains**

A total of 38 strains of \emph{N. gonorrhoeae}, isolated from the various clinical specimens at the Long Island College Hospital, were used in the study. All strains were identified as \emph{N. gonorrhoeae} by Gram stain, oxidase reaction, fluorescent antibody (Difco, Detroit, MI) test, and fermentation of glucose, but not maltose, sucrose, or lactose, as well as by growth characteristics on Trypticase soy sheep blood (5%) agar plates (BBL). In addition, a strain each of \emph{Acinetobacter calcoaceticus} var. \emph{anitratus}, \emph{Corynebacterium hoffmannii}, \emph{Escherichia coli}, \emph{Klebsiella oxytoca}, \emph{Proteus mirabilis}, \emph{Staphylococcus epidermidis}, \emph{Streptococcus faecalis}, \emph{Candida albicans}, \emph{Moraxella osloensis}, \emph{Haemophilus influenzae}, and \emph{Neisseria} species was used as heterologous control organisms.

**Media**

Trypticase soy broth (BBL) supplemented with 15% (v/v) glycerol frozen\(^2\) and Trypticase soy agar slant (BBL) were used for the maintenance of the 38 gonococcal strains and the heterologous organisms. For gonococcal L-form induction, a medium consisting of brain–heart infusion with PABA (Difco), 1% noble agar (Difco), 7% polyvinylpyrrolidone (Eastman Kodak Co., Rochester, NY), 10% horse serum (heat-inactivated and certified to allow growth of \emph{Mycoplasma}) (M.A. Bioproducts, Walkersville, MD), and 100 units of penicillin G (Eli Lilly and Co., Indianapolis, IN) per mL (PVP-L-medium) was used.\(^8\) The latter two ingredients were added after sterilization of the basal medium at 121 °C for 15 minutes followed by cooling of the medium to 50 °C. PVP-L-medium devoid of 1% agar and penicillin G (PVP-broth) was used for suspension, as well as dilution of the organisms for EIA and PVP-L-medium without penicillin G was used for growth of parental gonococci. Subculturing of stock cultures of \emph{N. gonorrhoeae} was done to chocolate agar plates (BBL).

**L-Form Induction Procedure**

Stock cultures of \emph{N. gonorrhoeae} were thawed at room temperature, subcultured onto chocolate plates (BBL), and incubated for 24 hours to 48 hours at 37 °C under 10% CO\(_2\). The growth of \emph{N. gonorrhoeae} was then suspended in PVP-broth, and the turbidity was adjusted to that of 0.5 McFarland standard. Further dilutions of 1:10 and 1:10,000 of the standard were made in PVP-broth, and 0.1-mL aliquots of each diluted organism suspension were spread onto PVP-L-medium in triplicate for L-form induction and PVP-L-medium without penicillin G for growth of parental gonococci, respectively.

**Confirmation of L-Form Induction**

As soon as growth became visible on the PVP-L-medium, the colonies were examined microscopically at \(\times\)100 magnification, stained in situ by the Dienes method,\(^3\) and Gram-stained smears were prepared for assessment of microscopic morphology. Maintenance of L-form colonies was carried out by the agar block transfer.\(^7\)

**Preparation of Culture Suspensions for EIA**

Parental gonococci and their corresponding L-forms were separately suspended in PVP-broth, and the turbidity of each organism suspension was adjusted to optical density (OD) 0.07 at 620 nm initial suspension (IS) in a spectrophotometer (Gilford model Stasar III\(^\circ\)), which yielded an average of 2 \(\times\)10\(^7\) parental cells per milliliter. Each suspension was further diluted 1:100, 1:1,000, and 1:10,000 in PVP-broth with corresponding colony-forming units (CFUs) of 2 \(\times\)10\(^3\), 3 \(\times\)10\(^4\), and 2 \(\times\)10\(^5\) parental cells per milliliter, respectively. The resultant suspensions were used in the EIA in conjunction with the swabs provided with the EIA (Gonozyme) kits. Subsequent treatment of the swabs moistened with test culture suspensions were carried out according to the manufacturer’s protocol with appropriate negative and positive controls. Tested
also were L-form suspensions of four randomly selected strains at antigen levels equivalent to those found in $2 \times 10^5$ and $3 \times 10^6$ parental cells per milliliters, respectively, which had been previously admixed with the heterologous organism mixture containing each $1 \times 10^7$ cells per milliliter each of \textit{A. calcoaceticus var. anitratus}, \textit{C. hofmannii}, \textit{E. coli}, \textit{K. oxytoca}, \textit{P. mirabilis}, \textit{S. epidermidis}, \textit{S. faecalis}, and \textit{C. albicans} (mixture 1) or $1 \times 10^7$ cells per milliliter each of \textit{M. osloensis}, \textit{H. influenzae}, and \textit{A. calcoaceticus} species (mixture 2). Culture suspensions of the heterologous organism mixture 1 or 2 alone were also used as negative controls for the EIA.

It is assumed that the sensitivity of the EIA (Gonozyme) is based on a presence of a minimal, total antigen concentration in particulate and soluble form. Quantitative plating of test L-form suspensions produced CFUs that did not correlate with dilution factors of the IS because of apparent lysis of a large fraction of the L-forms in the PVP-broth. Preliminary studies, however, showed that the sensitivity of the EIA for L-forms and parental cells was the same, lying between 1:1,000 and 1:10,00 dilutions of the IS (0.07 OD equivalent to $2 \times 10^5$ parental cells per mL) of each cell preparation. This indicates that the total antigen concentration between the two cell preparations is roughly equivalent. Therefore, the term, "antigen levels equivalent to those found in ‘n’ parental cells per mL,” was used as an indirect measure of the unknown soluble and particulate antigen concentrations derived from the lysed, nonviable, and the intact, viable L-forms.

Results

Of the 38 strains of \textit{N. gonorrhoeae} tested, 24 strains were induced to L-forms on PVP-L-medium. Of these, 15 yielded L-forms within three to five days of induction. The L-form induction rates for these 15 strains and the remaining nine strains of \textit{N. gonorrhoeae} ranged from 14% to 16% and less than 1%, respectively. The latter nine strains were not tested by the EIA (Gonozyme) because the EIA (Gonozyme) seems to lie between 1 X 103 and 1 X 104 cells per milliliter (1:10,000 dilution of the IS of parental cells and L-forms), the sensitivity of the EIA (Gonozyme) appears uniformly light-grayish, circular colonies with a light, irregular periphery, thus imparting a typical fried-egg appearance to the colonies (Fig. 1). The respective parental colonies showed uniformly light-grayish, circular colonies without discernible periphery (Fig. 2). When subjected to the Dienes stain, the L-form colonies stained blue and remained stained, while the parental colonies, which initially stained blue, lost this coloration with time. Smears of L-form colonies showed numerous, gram-negative, highly amorphous, and round forms measuring 2 μm to 5 μm in diameter. No typical gram-negative diplococci, as observed in the smears of parental colonies, were present. Because of the downward growth into the agar, the L-form colonies were difficult to scrape off the agar surface, whereas the parental colonies were removed with ease with the bacteriologic loop.

All of the pure culture suspensions that were prepared with 15 strains of parental gonococci at $2 \times 10^5$ and $3 \times 10^6$ cells per milliliter and their respective L-form preparations exhibited positive EIA (Gonozyme) results. At $2 \times 10^3$ cells per milliliter (1:10,000 dilution of the IS of parental cells and L-forms), three parental and two of their corresponding L-forms yielded positive results. Similarly, the EIA (Gonozyme) was positive on all mixed culture suspensions that contained L-forms and either one of the heterologous organism mixtures. When tested alone, however, heterologous organism mixtures consistently rendered negative EIA (Gonozyme) results.

Discussion

Gonococcal L-forms have been implicated in a variety of disease processes. However, because of the technical difficulties involved in their isolation as well as in the rarity of the cases, L-forms of \textit{N. gonorrhoeae} are not routinely sought in clinical microbiology laboratories. It was thought, therefore, that the newly developed EIA system for detection of gonococcal antigen (Gonozyme), which does not rely on viable organisms, might also be useful in recognizing L-forms of \textit{N. gonorrhoeae} from clinical specimens. In this study, such a possibility was investigated with the use of culture suspensions containing L-forms of \textit{N. gonorrhoeae} alone or admixed with heterologous organisms.

The present study shows that all of the L-form suspensions at antigen levels equivalent to those found in $2 \times 10^5$ and $3 \times 10^6$ parental cells per milliliter, either alone or with heterologous organisms, exhibited positive EIA results, as were those with the parental gonococci at the same concentrations. Because three parental and two of their respective L-form preparations were only EIA-positive at $2 \times 10^3$ cells per milliliter (1:10,000 dilution of the IS of parental cells and L-forms), the sensitivity of the EIA (Gonozyme) seems to lie between $1 \times 10^3$ and $1 \times 10^4$ cells per milliliter, as shown by the results of the

FIG. 1. L-form colonies of \textit{N. gonorrhoeae} grown on PVP-L-medium for five days at 37 °C under 10% CO$_2$ showing typical fried-egg morphology (X200).

FIG. 2. Parental colonies of \textit{N. gonorrhoeae} grown on PVP-L-medium without penicillin G for 24 hours at 37 °C under 10% CO$_2$ showing uniformly light-grayish circular morphology without discernible periphery (X200).
preliminary studies, with no difference in test sensitivity for the detection of L-form and parental gonococci. This latter fact, together with lack of correlation between CFUs and dilution factors of the IS of L-forms, strongly suggests that the EIA (Gonozyme) detects both intact, viable, and soluble and/or particulate antigens derived from lysed, nonviable L-forms.

In light of the results of the present study, it is tempting to speculate whether, in addition to recognizing antigens (soluble and/or particulate) from lysed, dead gonococci, any of the culture-negative, EIA- (Gonozyme)-positive (the so-called false positive EIA) cases in earlier studies\(^1,10-12\) might indeed have identified cases of asymptomatic gonococcal L-form infection. Culture-negative but EIA-positive results might also represent immunologic cross-reaction with antigens from other organisms. None, however, were detected in this study with other Neisseria species or with ten other diverse microbial species. Further studies to include testing of clinical specimens for both L-form and parental cell isolation and by EIA (Gonozyme), as well as clinical follow-up of positive EIA (Gonozyme) cases, are needed to determine the reliability of the Gonozyme test for diagnosis of gonococcal L-form infection.

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Comparison of Visual and Photometric
Bac-T-Screen® Results

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The Bac-T-Screen® (Marion Laboratories, Kansas City, MO) was used to screen 826 urine specimens. Of these, 85 either pigmented or clogged the Bac-T-Screen filter and could not be evaluated. Results for the remaining 741 specimens were examined both visually and photometrically by a newly developed photometric card reader. The results were then compared. Screening results for all urines containing \(\geq 10^5\) pathogens/mL were equivalent for both methods, with sensitivity and predictive negative values of \(\geq 98\%\) and \( \geq 99\% \), respectively. The predictive values of positive tests were also equivalent at \(57.5\%\) for visual and \(59.6\%\) by photometry. The overall agreement varied with the card reader value used because the photometric card-reader procedure allows the user to select desired sensitivity and specificity levels. (Key words: Bac-T-Screen®; Urine screen; Bacteriuria) Am J Clin Pathol 1986; 85: 622–625

THE LARGE NUMBER of bacteriologically negative urine specimens received for examination by microbiology laboratories has prompted the introduction of numerous urine-screening approaches.\(^4,6-12\) Based on the concept of significant bacteriuria, most rapid screening systems are designed to discriminate among urine specimens that

References