False-Positive Chlamydiazyme Results during Urine Sediment Analysis Due to Bacterial Urinary Tract Infections

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Our study examined whether urinary tract infections (UTIs) would cause false-positive results when urine sediment was tested with the Chlamydiazyme (CZ) system. Thirty-six infected urine samples and fifteen controls were studied. All controls were negative. Forty-seven percent of *Escherichia coli* UTIs (*n* = 30) and 100% of *Klebsiella pneumoniae* UTIs (*n* = 4) were positive on CZ testing of urine sediment. Nine *E. coli* UTIs positive by CZ were negative by direct fluorescent-antibody staining. When suspensions of the pure cultures were analyzed, 47% of *E. coli* and 100% of *K. pneumoniae* samples were CZ positive. False-positive results were not related to organism biotype or urine characteristics, including pH, specific gravity, and leukocyte count. We conclude that the presence of a UTI and also bacterial contamination must be ruled out prior to urine sediment testing.

Since cultures of *Chlamydia trachomatis* are expensive and time-consuming, a wide variety of antigen detection techniques have been developed. These techniques include direct fluorescent-antibody (DFA) staining and the enzyme immunosorbent assay (5–7). Though enzyme immunosorbent assay systems normally rely solely on samples obtained by swabs, the Food and Drug Administration has recently approved the testing of urine sediment from males with the Chlamydiazyme assay (CZ; Abbott Laboratories, North Chicago, Ill.). A possible source of false-positive results when this new technique is used is bacterial contamination of urine. The manufacturer states in the product insert that bacteria do not cross-react when the CZ is used. However, several investigators have found evidence of cross-reactivity to a wide variety of bacterial species. Saikku et al. (4) reported cross-reactivity between *C. trachomatis* and *Acinetobacter calcoaceticus* in the CZ. Taylor-Robinson et al. (8) noted that pure cultures of *Escherichia coli, Gardnerella vaginalis, Neisseria gonorrheae*, and group B streptococci gave positive CZ results at concentrations of 10^5 to 10^7/ml. This cross-reactivity has been of particular concern in swabs obtained from children. Several cases of false-positive CZ results obtained during evaluation of children for sexual abuse were described by Hammerschlag et al. (2). Porder et al. (3) noted similar cases and showed that group A streptococci were capable of cross-reacting with the CZ test.

Because of prior reports concerning cross-reactivity between *C. trachomatis* and a variety of bacteria, we undertook a study to determine if bacterial urinary tract infections (UTIs) would cause false-positive CZ results when urine sediment was tested. It is probable that in the near future urine sediment testing will replace the use of swabs in males. By clarifying one possible source of false-positives, the specificity of this rapid, noninvasive procedure would be increased.

MATERIALS AND METHODS

Specimen collection. All urine samples from males and females (*n* = 620) submitted for analysis at the 27th Medical Group Hospital were considered for study. The urine samples were tested by microscopic examination for leukocytes (WBCs) and bacteria. If a urine sample was positive on microscopic examination (>5 WBC per high-power field or positive for bacteria), it was cultured on blood-MacConkey media, and a calibrated loop was used to obtain a colony count (per milliliter). A sample of the urine was also processed as described below and stored as a frozen pellet at −20°C. All bacterium biotype codes were defined by using MicroScan Dry Panels (Baxter Healthcare Corp., W. Sacramento, Calif.). If the urine culture was positive (>10,000 organisms per ml), both the culture and its respective frozen pellet were entered into the analysis part of the study. All culture-negative frozen pellets were discarded.

Specimen processing. A 7-ml aliquot of each sample to be tested was placed in a sterile test tube and centrifuged for 5 min at 2,500 to 3,000 rpm (1,200 to 1,500 × *g*). The supernatant was discarded. The pellet was stored at −20°C for a period not exceeding 5 days prior to testing. Prior to CZ testing, the frozen pellets were suspended in 1 ml of specimen diluent buffer. CZ analysis was performed per manufacturer instructions. The CZ procedure used has been described elsewhere (1, 5).

Examination of bacterial cultures. In order to ensure that positive CZ values were not due to the presence of chlamydial antigens, the pure bacterial cultures were examined. Five to six colonies of the pure culture were suspended in 7 ml of phosphate-buffered saline (PBS; pH 7.2). These samples were processed as described above.

Mock-up specimens. In order to ensure that positive results were not unique to urine from females, clean urine samples from males were obtained. All samples were from asymptomatic males who had urine collected during routine military physical exams. These urines were WBC esterase negative and had no WBCs or bacteria seen on microscopic analysis. Two colonies from one of the pure cultures were inoculated into a 7-ml aliquot from one of the selected clean male urine samples. The inoculated urine was incubated at 35 to 37°C for 4 h. The sample was processed as described above.

Controls. Control urine samples (15 total, 9 from males, 6 from females) were analyzed. All controls were negative for WBC esterase and had no WBCs or bacteria seen on

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TABLE 1. Reactivity of CZ with bacterially infected urine

Organism	No. of samples with >10,000 bacteria	No. of CZ-positive strains (%)		
		Urine	Pure culture in PBS	Inoculated urine[a]
Escherichia coli	30	14 (47)	14 (47)	18 (60)
Klebsiella pneumoniae	4	4 (100)	4 (100)	4 (100)
Citrobacter freundii	1	0 (0)	1 (100)	1 (100)
Proteus sp.	1	0 (0)	0 (0)	0 (0)

[a] Pure culture inoculated in clean urine.

microscopic analysis. A 7-ml aliquot from each control sample was processed as described above.

Fluorescent-antibody testing. Nine *E. coli*-infected urine samples which were CZ positive were also examined by DFA staining with a fluorescein-conjugated monoclonal antibody against *C. trachomatis* outer membrane (MicroTrak; Syva Co.). A 7-ml aliquot of the infected urine was centrifuged at 2,500 rpm (1,200 × g). The supernatant was discarded. A swab was dropped into the pellet, smeared onto a slide, stained, and examined according to the instructions of the manufacturer.

Concentration study. In order to determine the effects of bacterial concentrations on the false-positive rate, a pure culture of *E. coli* which had previously been positive on urine CZ testing was selected. A suspension of this pure culture in PBS was examined under a hemacytometer to determine bacterial concentration. This suspension was used as a stock solution. Nine clean urine samples from males were collected. By serially diluting the stock solution into four 7-ml aliquots of each urine sample, concentrations of 10^5 to 10^8 *E. coli* were obtained. The 7-ml aliquots were processed as described above.

RESULTS

A total of 620 urine samples were considered for study. Of these samples, 168 met the necessary criteria on microscopic analysis and were cultured. Thirty-six of the cultured samples proved to be UTIs (bacterial count, >10,000 organisms per ml) and were utilized for CZ analysis. Thirty-three of these UTIs were in females and three were in males. Fifteen noninfected controls were also tested by using the CZ method. All controls were negative.

Table 1 shows the total number of each species identified from the infected urine samples, as well as the number of times each species gave a positive CZ result on either the initial urine sediment or a suspension of the pure culture in PBS. Each sample of bacteria which had been isolated by culture was inoculated into a clean urine sample from a male. The number of times each species gave a positive result after inoculation is also noted in Table 1.

At our facility, 12% of females presenting with sexually transmitted disease symptoms are positive for *C. trachomatis* infection (1). The percentages which were positive on testing *E. coli*- and *Klebsiella pneumoniae*-infected urine samples with CZ were 47 and 100%, respectively. These percentages are significantly higher than those expected in our symptomatic population with a $P < 0.05$ for *E. coli*.

Three of the UTIs occurred in males. Two of these were positive by CZ.

When suspensions from the pure cultures were tested, 47% of the *E. coli* were positive. This is the same percentage
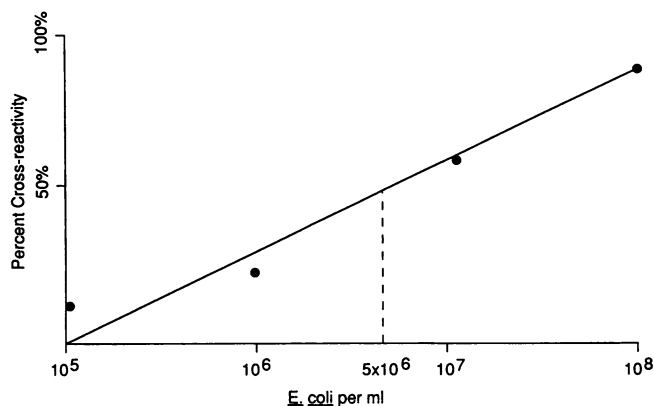

FIG. 1. Percentage of *E. coli* suspensions at various concentrations producing false-positive values with the CZ test.

of samples as seen during the testing of the infected urine samples. However, the infected urine samples and the pure culture samples which tested positive were frequently not matching pairs. Only seven pairs were positive for both the infected urine samples and the pure culture samples.

In order to ensure that CZ-positive infected urine samples were true false-positives, nine *E. coli*-infected urine samples which had been positive by CZ were also tested by DFA staining. All nine of these specimens were read negative by DFA staining for chlamydia antigen.

Specific gravity, pH, and the presence of WBCs or epithelial cells did not correlate with false-positive results. Eighteen biotypes of *E. coli* and four biotypes of *K. pneumoniae* were represented in the samples. There was no correlation between biotype and cross-reactivity.

One *E. coli* culture previously positive on urine CZ testing was selected in order to examine the effects of bacterial concentration on cross-reactivity. Nine sets of suspensions of this *E. coli* at concentrations of 10^5, 10^6, 10^7, and 10^8 organisms per ml were tested. The percentages of suspensions positive at each concentration are indicated in Fig. 1. Fifty percent of *E. coli* suspensions with a concentration of 5×10^6/ml would be expected to give a false-positive result.

DISCUSSION

As several investigators have noted, a wide variety of bacteria are capable of causing false-positive results with CZ. Our study yielded positive CZ rates in infected urine samples of 47% for *E. coli* and 100% for *K. pneumoniae*. We believe for two reasons that most of these positive results were actually false-positives. First, the 47% positive rate for *E. coli* is much higher than the expected baseline positive rate (12%) in our symptomatic female population ($P < 0.05$). Second, nine *E. coli*-infected urine samples which had been positive by CZ were negative by DFA staining. Our study indicates that UTIs will lead to false-positive results when urine sediment is tested with CZ.

A probable mechanism for the false-positive results is the cross-reaction of bacterial components with the polyclonal antibodies used in the CZ method. However, other mechanisms are not excluded by this study.

Urine sediment testing is currently approved only for males. Though most (33 of 36) of the UTIs tested were in females, we believe that the results of our study are equally applicable to CZ analysis in males for two reasons. First,

three of the initial urine samples collected were from males, and two of these were positive by CZ. Second, all bacterial strains collected were inoculated into clean urine from males. These inoculated urine samples from males showed a high rate of cross-reactivity. The higher rate in the inoculated samples is presumably due to increased bacterial concentrations.

There was no relationship between the specific biotype of bacteria and cross-reactivity with CZ. Identical biotypes of *E. coli* produced both positive and negative results on testing. Urine characteristics such as specific gravity, pH, and presence of WBCs were also unrelated to false-positive results. The only significant factor we could identify affecting cross-reactivity with *E. coli* was concentration. When bacterial concentration exceeded 5×10^6/ml, a 50% rate of cross-reactivity occurred. This value is in close agreement with the results of Taylor-Robinson et al. (8), who found consistent cross-reactivity between 10^5 and 10^7 organisms per ml. It should be noted that a high rate of cross-reactivity occurs at bacterial concentrations easily approached during UTIs.

Though UTIs are uncommon in males, they must be ruled out prior to testing urine sediment for chlamydia. We believe that all urine samples submitted for CZ analysis should be examined for evidence of infection. If there is a positive WBC esterase or presence of increased WBCs or bacteria on microscopic analysis, a bacterial culture should be performed. In the face of a positive culture for bacteria, the results of CZ testing must be viewed with skepticism. Our study also emphasizes the importance of proper handling of urine specimens prior to testing. When bacteria were inoculated into clean urine samples and allowed to grow, an extremely high false-positive rate was obtained. Adequate refrigeration, as recommended by the manufacturer, is absolutely essential to prevent bacterial overgrowth in specimens and concomitant false-positive results.

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