

Gas-Chromatographic Detection of Urinary Tract Infections Caused by *Escherichia coli* and *Klebsiella* sp.

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A gas-chromatographic method for the diagnosis of urinary tract infections caused by *Escherichia coli* and *Klebsiella* sp. is described. A total of 96 urine samples from individuals with clinical symptoms of urinary tract infection or pyrexia of unknown origin were tested by conventional and gas-chromatographic methods for bacteriurea. The gas-chromatographic method showed complete agreement with the conventional method in diagnosing all of 16 cases of bacteriurea caused by *E. coli* and 4 cases caused by *Klebsiella* sp. The remaining two cases of bacteriurea, caused by other bacteria, were not detected by the gas-chromatographic method.

Escherichia coli is the organism that is most commonly responsible for urinary tract infections (UTIs) (18, 21); the next most common is *Klebsiella* sp. The source of infection is considered to be endogenous in origin. Several bacteriological and chemical methods of detecting bacteriurea have been advocated (11, 16, 20, 22). Cecchini and O'Brien (7) and Newman and O'Brien (19) have described a gas-chromatographic (GC) method of identification of *E. coli*. The method is based on the concept of Eijkman, and *E. coli* is detected by the presence of metabolically produced ethanol in the medium. We have recently described a GC method for the identification of *Klebsiella* sp. (14a). In this paper, we report the application of both of these techniques to the diagnosis of UTIs caused by *E. coli* and *Klebsiella* sp.

MATERIALS AND METHODS

Conventional method. Mid-stream urine specimens were collected from 96 patients at JA Group Hospital, Gwalior, India, who had complaints of UTIs or pyrexia of unknown origin. The samples were cultured on blood agar and MacConkey agar for routine diagnosis of significant bacteriurea (8).

GC method. For the detection of *E. coli* in urine, samples were cultured in M-9 salt mixture medium (M-9L) as described previously (19) and incubated at 44°C for 5 h. M-9A, the medium used for the detection of *Klebsiella* sp., was essentially the same as M-9L, but lactose was replaced by adonitol and carbenicillin (Lyka Labs, Bombay, India) at 50 µg/ml. Samples in M-9A medium were incubated at 37°C. All of the 96 samples of urine were cultured in both media. The appearance of metabolically produced ethanol in the former medium indicates the presence of *E. coli*, and its appearance in the latter medium indicates the presence of *Klebsiella* sp. GC analysis of ethanol in

culture tubes was done with gas chromatograph model ROL 4 (Toshniwal Brothers, New Delhi, India). A stainless-steel GC column (180 by 0.31 cm [inner diameter]) was packed with 15% ethylene glycol succinate on Chromosorb W (high performance) 80/100 mesh. The temperatures of the column, injection port, and flame ionization detector were 110, 150, and 170°C, respectively. Portions (5 µl) of the aqueous spent medium of 5-h cultures of 96 urine samples in M-9L and M-9A media were injected into the column. The peak height recorded for ethanol in each medium was measured. Blank samples from each urine specimen and 15 lots of control culture media were also injected to determine any background peak. Standard ethanol was also injected to record the peak for ethanol.

RESULTS

The experiment was standardized in the initial stage by using laboratory cultures of 10^4 to 10^5 *E. coli* or *Klebsiella* sp. organisms per ml of Millipore membrane-sterilized urine from healthy individuals. Seven cultures of *E. coli* and five cultures of *Klebsiella* sp. were used in the control experiments. Standard peaks were recorded for ethanol in various concentrations ranging from 50 to 900 ppm (µg/liter) in the medium. Samples containing 10^5 *E. coli* or *Klebsiella* sp. organisms consistently produced an amount of ethanol in cultures that was capable of giving a peak height of 45 mm in 5 h. A peak height of 45 mm corresponds to 80 ppm (µg) of ethanol in the medium. Samples containing 10^4 organisms did not produce an amount of ethanol in 5 h that could be recorded by GC.

Of the 96 urine samples, significant bacteriurea was confirmed in 22 cases. Bacteriurea was caused by *E. coli* in 16 cases and by *Klebsiella* sp. in four cases. The causative organisms in the remaining two cases were *Staphylococcus aure-*

us and *Pseudomonas aeruginosa*. There were no cases of mixed infections. The results of GC analysis of cultures indicated that all 16 samples having significant *E. coli* bacteriurea had an amount of ethanol sufficient to produce a peak height ranging from 48 to 239 mm in the medium designed for the detection of *E. coli*. Gas chromatograms of a few of the positive and negative urine cultures and control media are shown in Fig. 1. In the medium designed for the detection of *Klebsiella* sp., all of the four samples had ethanol, giving a peak height of 58 to 195 mm. The 96 blank samples of urine and 15 control media lots had insignificant peak heights (<5 mm), as did samples having bacteriurea caused by *S. aureus* or *P. aeruginosa*. The ethanol peak appeared in 48 s, and there was no other peak even after 10 min. Hence, the samples were injected at 2-min intervals.

DISCUSSION

GC procedures with diagnostic significance in clinical microbiology have been reported previously (4-6, 17). Application of GC and mass spectroscopy has also been done in some cases (1, 14). These techniques, apart from being faster, can replace conventional methods in specific cases. Hayward and Jeavons (12) have used headspace GC techniques for the detection of UTIs caused by *E. coli* and *Proteus* sp. They stressed the need for more extensive trials of the method in other laboratories. The application of GC techniques for the detection of UTIs caused by a variety of other organisms is also lacking. Our report of a GC method of identification of *Klebsiella* sp. is based on the organism's property of fermenting adonitol to yield ethanol (10) and its resistance to carbenicillin (14). This property has been used in the conventional selective medium for the primary identification of *Klebsiella* sp. (3, 9).

In earlier studies on a GC presumptive test for coliforms in water, Newman and O'Brien (19) have observed other peaks, including peaks for acetic acid and ethanol. They concluded that the peak for acetic acid and other peaks are of no significance for the identification of *E. coli*. In our study, the chromatographic conditions were optimized to yield only the ethanol peak.

Bacteriurea in a concentration of 10^5 organisms per ml is described as true urinary infection (13) which correlates very well with the clinical symptoms of UTI. *E. coli* and *Klebsiella* sp. in concentrations of 10^5 organisms per ml produce ethanol in M-9L and M-9A media, respectively, in 5 h. Ethanol at 80 ppm (μg) in the medium, giving a peak height of 45 mm, is considered significant. By these criteria, all 16 strains of *E. coli* and 4 strains of *Klebsiella* sp. causing bacteriurea were correctly detected by

the GC method. Although there have been reports of occasional strains of *Klebsiella* which can grow at 44°C (2, 15, 19), none of these four isolates showed any significant growth at 44°C. None of the 74 urine specimens with no bacteriurea or insignificant bacteriurea were mistaken for specimens with significant bacteriurea. Hence, this GC method can be used as valuable aid for the rapid detection of significant bacteriurea caused by *E. coli* and *Klebsiella* sp.

It is evident from the data presented that the GC technique as described above can easily be adapted for rapid diagnosis of significant bacteriurea caused by *E. coli* and *Klebsiella* sp. The test can be extended to apply to direct sensitivity testing of urine samples from patients with UTIs, and the appropriate antibiotic therapy can be started within 6 h. In this study we did not come across any mixed infection by *E. coli* and *Klebsiella* sp. or any other organisms associated with any one of these. For the detection of mixed infection by *E. coli* and *Klebsiella* sp., the technique can easily be applied. Although the

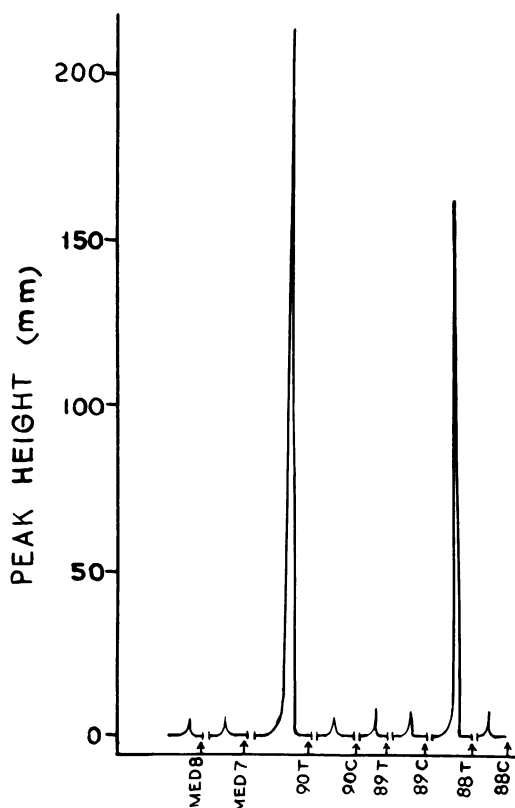


FIG. 1. Gas chromatogram of spent culture medium of *E. coli* grown for 5 h in M-9 medium. Numbers indicate the serial numbers of the urine samples. Abbreviations: C, medium with urine sample serving as control; T, same sample after 5 h of growth at 44°C; Med7 and Med8, different lots of culture medium.

occurrence of mixed infection is rare, we are actively engaged in perfecting the technique for the detection of mixed infection by other bacteria and by a wide range of bacteria associated with UTIs.

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LITERATURE CITED

- Abbey, L. E., A. K. Highsmith, T. F. Moran, and E. J. Reiner. 1981. Differentiation and characterization of *Klebsiella pneumoniae* strains by pyrolysis gas-liquid chromatography and mass spectroscopy. *J. Clin. Microbiol.* **13**:313-319.
- Bagley, S. T., and R. J. Seidler. 1977. Significance of fecal-coliform-positive *Klebsiella*. *Appl. Environ. Microbiol.* **33**:1141-1148.
- Bagley, S. T., and R. J. Seidler. 1978. Primary *Klebsiella* identification with MacConkey-inositol-carbenicillin agar. *Appl. Environ. Microbiol.* **36**:536-538.
- Brooks, J. B., D. C. Edman, C. C. Alley, R. B. Craven, and N. I. Girgis. 1981. Frequency-pulsed electron-capture gas-liquid chromatography and tryptophan color test for rapid diagnosis of tuberculous and other forms of lymphocytic meningitis. *J. Clin. Microbiol.* **12**:208-215.
- Brooks, J. B., D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley. 1980. Rapid differentiation of the major causative agents of bacterial meningitis by use of frequency-pulsed electron-capture gas-liquid chromatography: analysis of acids. *J. Clin. Microbiol.* **11**:45-51.
- Brooks, J. B., D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley. 1980. Rapid differentiation of the major causative agents of bacterial meningitis by use of frequency-pulsed electron-capture gas-liquid chromatography: analysis of amines. *J. Clin. Microbiol.* **11**:52-58.
- Cecchini, G. L., and R. T. O'Brien. 1968. Detection of *Escherichia coli* by gas chromatography. *J. Bacteriol.* **95**:1205-1206.
- Cruickshank, R. 1965. *Medical microbiology*, 10th ed. E. S. Livingstone Ltd. London.
- Davis, T. J., and J. M. Matsen. 1974. Prevalence and characteristics of *Klebsiella* species. Relation to association with a hospital environment. *J. Infect. Dis.* **130**:402-405.
- Edwards, P. R., and W. H. Ewing. 1962. Identification of enterobacteriaceae. Burgess Publishing Co., Minneapolis, Minn.
- Guttmann, D., and E. J. Strokes. 1963. Diagnosis of urinary infection. *Br. Med. J.* **1**:1384-1386.
- Hayward, N. J., and T. H. Jeavons. 1977. Assessment of technique for rapid detection of *Escherichia coli* and *Proteus* in urine by headspace gas-liquid chromatography. *J. Clin. Microbiol.* **6**:202-208.
- Kass, E. H. 1956. Asymptomatic infections of urinary tract. *Trans. Assoc. Am. Physicians* **69**:56.
- Labows, J. N., K. J. McGinley, G. F. Webster, and J. J. Leyden. 1980. Headspace analysis of volatile metabolites of *Pseudomonas aeruginosa* and related species by gas chromatography-mass spectroscopy. *J. Clin. Microbiol.* **12**:521-526.
- Manja, K. S., and K. M. Rao. 1982. Rapid identification of *Klebsiella* by gas chromatography. *J. Chromatogr. Biomed. Appl.* **232**:160-164.
- Matsen, J. M., J. A. Spindler, and R. O. Blosser. 1974. Characterization of *Klebsiella* isolates from natural receiving waters and comparison with human isolates. *Appl. Microbiol.* **28**:272-278.
- Meynell, G. G., and E. Meynell. 1970. Theory and practice in experimental bacteriology. Cambridge University Press, London.
- Mitruka, B. M. 1975. Rapid automated identification of microorganisms in clinical specimens by gas chromatography, p. 123-154. *In* C. G. Hed'en and T. Ille'eni (ed.), *New approaches to the identification of microorganisms*. John Wiley & Sons, Inc., New York.
- Mond, N. C., A. Percival, T. D. Williams, and W. Brumfit. 1965. Presentation, diagnosis and treatment of urinary infection in general practice. *Lancet* **i**:514-516.
- Newman, J. S., and R. T. O'Brien. 1975. Gas-chromatographic presumptive test for coliform bacteria in water. *Appl. Microbiol.* **30**:584-588.
- Simmons, N. A., and J. D. William. 1962. Simple test for significant bacteriuria. *Lancet* **ii**:1377-1378.
- Spencer, A. G., D. Mulcahy, R. A. Shooter, F. W. O'Grady, K. A. Bettelheim, and J. Taylor. 1968. *Escherichia coli* serotype in urinary tract infection in a medical ward. *Lancet* **ii**:839-842.
- Williams, R. E. O., and R. E. Trotman. 1969. Automation in diagnostic bacteriology. *J. Clin. Pathol.* **22**(Suppl.):3.