Journal of Chromatography B, 935 (2013) 32–35

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Rapid detection of bacteria in urine samples by the "three-plug-injection" method using capillary electrophoresis

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ARTICLE INFO

Article history:
Received 24 March 2013
Accepted 16 July 2013
Available online 25 July 2013

Keywords:
Urine
Capillary electrophoresis
Bacteria
Detection

ABSTRACT

This study explored a method that can rapidly detect bacteria in urine samples for the auxiliary determination of urinary tract infections (UTIs). Urine samples from patients with UTIs (230 cases) were obtained using aseptic technique. The urine biochemical assay was then carried out using an automated urine analyzer for all the urine samples. Bacterial species were identified by a combination of bacterial culture technique, morphological observation and the BACT-IST microbial identification/susceptibility analysis system. The most common seven species of bacteria in the study included *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Bacterial samples were suspended in sample buffer solutions and separated by the "three-plug-injection" method using capillary electrophoresis (CE). Each species of bacteria appeared as a bacterial peak. The mixture of the seven species also provided only one peak. Further analysis showed that the concentration limit for the "three-plug-injection" method is 10^6 colony forming units (CFU)/mL, and there is a good linear relationship between the peak height and bacterial concentration ($R^2 = 0.99$). The effect of urine composition on CE results was also investigated. The results showed that urine composition, i.e., proteins, white blood cells (WBCs) and red blood cells (RBCs), affected the peak retention time but could not affect the separation of bacteria. The results demonstrated that the bacteria in urine samples can be detected within 10 min by the "three-plug-injection" method using CE. The "three-plug-injection" method is therefore suitable for the rapid detection of organisms in clinical urine samples from UTIs.

1. Introduction

Confirming the presence or absence of pathogenic microorganisms in urine samples is one of the most important diagnostic indicators of urinary tract infections (UTIs). Pathogenic microorganisms in UTIs involve bacteria, mycoplasma and fungi. According to previous reports [1,2], bacterial infections account for approximately 90% of all UTIs. Many methods are currently employed to detect bacteria in urine samples, including the urine bacteriological culture technique, automated urinalysis instruments, direct bacterial counts and other methods. Currently, the most widely used method of clinical diagnosis of UTIs is the urine bacteriological culture technique. The degree of infection is determined by bacterial colony counts. This technique is reliable and accurate. However, it is time-consuming, and the results can be affected if the patients take antibiotics. Other approaches such as hybridization [3], amplification [4,5], and immunoassay [6] require considerably shorter analysis times. These techniques are all quite specific and allow for the identification of bacteria at the species level in most cases. The time needed for these tests in most instances is only several hours, considerably less than that for culture-based methods. These tests are selective for specific microorganisms. However, UTIs involve a wide range of pathogens, and even each pathogen may have a variety of genotypes and antigen types. Hence, confirmation of the pathogens of UTIs using these molecular techniques may be difficult.

In the past 10 years, capillary electrophoresis (CE) has been explored as a technique for the separation and identification of microorganisms. Early work in microbial analysis by CE was performed in 1987 by Hjerten et al. who demonstrated that *Tobacco*

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http://dx.doi.org/10.1016/j.jchromb.2013.07.011

mosaic virus and *Lactobacillius casei* were transported similarly close to the electroosmotic flow (EOF) [7]. Later studies found that the capillaries used were too long, and the bacterial peaks were too broad. The main factor identified was the adhesion between the bacteria and the capillary and the aggregation of bacteria in the capillary [8]. In 1999, Armstrong and coworkers published a revolutionary concept of covering the silanol groups with polyethylene oxide (PEO, M_n = 600,000) [9]. Good peak shapes and very high apparent efficiencies were observed. Different species of bacteria have different peak times and peak shape profiles. Later, this method was used to identify the causative pathogen for UTIs rapidly by direct analysis of a urine sample with no pretreatment [10]. In another paper [11], the authors analyzed microorganisms in consumer products and could also tell live cells from dead cells. Recently, Armstrong and coworkers [12–14] reported that the "three-plug-injection" method can be applied to provide a quick answer regarding the presence or absence of microorganisms.

Rapid detection of pathogenic microorganisms in urine samples is critical and necessary for the clinical diagnosis and treatment of UTIs. CE has some advantages in the detection of bacteria. The CE analysis time is short, and the technique can be implemented easily in the clinic. However, a method that can simultaneously address different species of bacteria in one experiment is also needed. The operation of the test may be affected by components in urine, e.g., biochemical components, salts, cells and so on.

We studied the CE profiles of seven species of bacteria in patients with UTIs by the "three-plug-injection" method and determined the sensitivity of this method. We wanted to show one method that could rapidly detect bacteria in urine samples.

2. Experimental

2.1. Materials

All urine samples (male 105, female 125) were collected from March 2011 to June 2011 at the second hospital of Tianjin Medical University in China. All patients presented with at least one of the following clinical symptoms of UTIs: frequent urination, painful urination, hematuria or cloudy urine. A midstream urine sample (40 mL) was collected at the hospital under the supervision of a nurse. Then, the sample was divided into two parts, one for urine biochemical assay and the other for bacteriological culture initiated immediately after collection.

Based on the established etiology and results of antimicrobial susceptibility testing, patients were informed which antimicrobial agent should be used for treatment. All patients were informed orally and in writing in Chinese. After the information was given, patients signed an approved consent form. The study protocols were approved by the ethical committee at the second hospital of Tianjin Medical University. No patients refused to participate.

2.2. Buffer solutions

Tris(hydroxymethyl)aminomethane (TRIS), citric acid, sodium hydroxide, hydrochloric acid, cetyltrimethylammonium bromide (CTAB) and caprylyl sulfobetaine (SB3-10) were obtained from Aladdin Reagent (Shanghai Co., Ltd., China). Two buffer solutions were available: the sample buffer solution (solution A) and the working buffer solution (solution B). The former buffer solution (1 mM TRIS, 0.33 mM citric acid, pH 7) is used for dissolving samples and spacer segments. The latter buffer solution (1 mM TRIS, 0.33 mM citric acid, 1 mg/mL CTAB, pH 7) is used for CE. The blocking solution contains SB3-10 at the concentration of 5 mg/mL in solution B adjusted to pH = 7.

2.3. Identification of different bacterial species

Morning midstream urine samples (10 mL) were obtained using aseptic technique, and cultured immediately after collection. Urine samples (100 μL) were inoculated on the blood plate medium, MacConkey medium, and chocolate medium, respectively, cultured at 37 °C for 24 h. Single microbe colonies were then selected and identified using the BACT-IST microbial identification/susceptibility analysis system (Dark Horse Medical Instrument Co., Ltd., Zhuhai, China). The bacterial species that were identified were plated on agar plates and stored under refrigeration until needed.

2.4. Sample preparation of different bacterial concentrations

When used for experiments, a single colony was taken from the agar plate and grown again in brain–heart-infusion (BHI) broth for 12 h at 37 °C. The microorganisms were centrifuged, the excess broth was removed, and the microorganisms were washed once with solution A, then re-centrifuged and decanted. The suspension was diluted with solution A to give a concentration of 0.5 of the optical density measured at 600 nm which is equivalent to $\sim 2.0 \times 10^8$ colony forming units (CFU)/mL using an ultraviolet and visible (UV/vis) spectrometer. Other bacterial concentrations (10^7, 10^6 CFU/mL) were approximated by serial dilutions. These samples were then used for analysis. This procedure was followed each day to produce new samples.

2.5. Sample preparation of biochemical components with *Escherichia coli*

Urine samples (5 mL) were analyzed using a UriTek-151 urine analyzer (Kang Dataike Co., China) for the biochemical assay. Urine samples that were rich in one of the following biochemical indexes (urinary protein content (+++), red blood cells (RBC, +++) and white blood cells (WBC, +++)) were selected for follow-up experiments. Another 5 mL of urine sample was centrifuged at 3400 rpm for 5 min to get the urine sediment in pellet form. The supernatant was removed carefully, and the pellet was re-suspended in 1 mL sample buffer by vortexing for 1 min. Then, the suspension was centrifuged again for 5 min. The sediment was vortexed with *E. coli* suspension at a concentration of 10^8 CFU/mL for 1 min, then the suspension was centrifuged again for 5 min and finally re-suspended in 1 mL sample buffer. Then, 1 mL urine samples containing different composition (protein, WBC and RBC) and *E. coli* at the concentration of 10^8 CFU/mL were prepared for analysis.

2.6. Capillary electrophoresis

CE experiments were carried out with an HPE 100-CE system (Bio-Rad, USA) equipped with a UV detector. The bare silica capillaries used in this experiment were from Hebei Yongnian County Reafine Chromatography Equipment Co., Ltd., China. The capillaries were 33 cm long (26 cm to the detector), with an i.d. of 75 μm and an o.d. of 365 μm. Samples were detected at 214 nm. New capillaries were initially conditioned with the following rinses: 1 M NaOH, deionized water, 1 M HCl, and running buffer each for 5 min. Between runs, the capillaries were washed with 1 M NaOH, deionized water, and running buffer for 3 min each. After all wash cycles, the capillary was filled with running buffer containing CTAB. Three injections were made prior to the run: (1) sample plug consisting of microorganisms; (2) spacer plug consisting of running buffer; and (3) plug containing blocking agent. These injections were done using the electric injection method. Unless otherwise noted, sample injections were made for 10 s, spacer injections for 5 s and blocker injections for 5 s. All experiments were repeated at least 3 times