Is a Second Urine Specimen Necessary for the Diagnosis of Asymptomatic Bacteriuria?

By use of pulse-field gel electrophoresis, we evaluated the molecular identity of 32 Escherichia coli isolates obtained in 2 consecutive urine cultures from 16 patients as part of a large study of asymptomatic bacteriuria in diabetic women and found different E. coli isolates in 7 of 16 patients, meaning that nearly half (44%) of the patients who had previously classified as having asymptomatic bacteriuria were reinfected with a different strain.

The generally accepted definition of asymptomatic bacteriuria (ASB) is the presence of at least 10⁵ cfu/mL of the same single species in 2 consecutive cultures of clean-voided specimens of midstream urine from an individual without symptoms of a urinary tract infection (UTI) [1–5]. The reason to repeat the culture is to discriminate between true bacteriuria and contamination. Usually, no time frame for taking these cultures is given. In the Infectious Diseases Society of America (IDSA) guidelines, a minimum of 24 h between the consecutive cultures is required [5].

In a 1955 study of patients with pyelonephritis, Kass [6] defined significant bacteriuria as the presence of at least 10⁵ cfu/mL bacterial species, noting that lower counts probably represented contamination. Boshell and Sanford [7] also considered 10⁵ cfu/mL as a reliable cutoff point in their comparison of catheterized and clean-voided urine specimens. The importance of using clean-voided urine samples was substantiated by Kunin et al. [8] in their study of schoolchildren.

In a therapy trial of ASB in elderly women, Nicolle et al. [9] noted that a single specimen was acceptable for the diagnosis of ASB if one organism was isolated with a count of at least 10⁵ cfu/mL. This was supported by an earlier report by the members of the Medical Research Council Bacteriuria Committee [10], which described ASB as the presence of at least 10⁵ cfu/mL of the same bacterial species in only 1 specimen from an individual without symptoms of a UTI.

In reviewing the literature with regard to the definition of ASB, it seems clear why the cutoff point of 10⁵ cfu/mL was chosen and why it is necessary to collect a clean-voided midstream urine specimen. However, it is not clear what the basis is for the current guideline to repeat the culture, and the recommended time period between the 2 cultures remains obscure. Furthermore, with the current techniques, the comparison between the first and second culture results is a comparison at the species level. It is not known whether the same strain persists in the urinary tract. Therefore, we decided to evaluate the molecular identity of strains isolated in 2 consecutive cultures.

We evaluated the molecular identity of strains isolated in 2 consecutive cultures during a large study of ASB in women with diabetes mellitus. Our study group consisted of 417 women with diabetes mellitus. Exclusion criteria for study entry were the presence of underlying genitourinary abnormalities (including the presence of a neurogenic bladder or the use of intermittent catheterization). Two clean-voided midstream urine specimens were collected for the evaluation of ASB at an interval of 2–4 months. Before the urine specimen collection, the women cleaned the labia and periurethral area with cotton wool rinsed with tap water 3 times in a cranial-caudal direction. All urine samples were immediately stored at 4°C and cultured 2 h after collection. Urine culture was performed according to standard procedures. Two colonies of each culture were sampled and stored.

Escherichia coli isolates were cultured overnight in Mueller Hinton broth, embedded in 1% gel (pulse-field–certified agarose), and lysed in situ. The chromosomal DNA was then digested with XhoI (Boehringer, Mannheim, Germany). Pulse-field gel electrophoresis (PFGE) was performed over 20 h at 200 V on a Chef-DR II apparatus (BioRad, Veenendaal, The Netherlands) as described previously [11], with an initial and a final time of 2.2 and 54.2 s, respectively, and a temperature of 14°C. Molecular identity was defined as indistinguishable when the restriction pattern had the same number of bands and the corresponding bands were the same apparent size, and molecular identity was defined as unrelated when <50% of the fragments in the pattern were identical. Molecular identity was defined as closely related when the PFGE pattern differed by changes consistent with a single genetic event, resulting in 2–3 band differences [12].

Two consecutive positive cultures (in 2–4 months) with the same species of microorganism were found in 53 (13%) women, with 1 strain of E. coli being the causative microorganism in 36 (68%). None of these women had diabetic nephropathy. PFGE was conducted on 32 available E. coli isolates from 16 patients. No differences in age, duration and regulation of the diabetes, and history of previous UTIs were found between these 16 patients and the 20 patients whose E. coli isolates were not available for PFGE.

Indistinguishable E. coli isolates were found in 8 (50%) of 16 patients, whereas 1 patient had 2 closely related isolates. Different E. coli isolates were found in 7 (44%) of 16 patients (figure 1). One of the patients with 2 indistinguishable isolates and one of the patients with 2 different isolates had used an-
timicrobial therapy (both amoxicillin) for an upper-airway infection in the time between the 2 cultures.

We showed by use of PFGE that nearly half the patients previously classified as having ASB had different E. coli strains after a 2–4 month period, implying reinfection. On the other hand, 8 of 16 patients had persistence of the same E. coli strain, without the presence of symptoms, during 2–4 months. Because it is interesting to diagnose persistent bacteriuria during a long period without the development of symptoms, we decided to study 2 cultures during a 2–4 month period. With the presence of the new PFGE techniques and these results, it seems that the old definition of ASB has to be reconsidered. We must ask whether a second urine specimen is necessary for the diagnosis of ASB, and if so, what time is best to repeat it?

The reason to culture a second time is to discriminate between true bacteriuria and contamination. In most studies, only the positive cultures are repeated to diagnose ASB [2, 3, 13]. To our knowledge, no other data about comparisons of strains of 2 different cultures by use of PFGE have been published. Kass [14] described a high reproducibility of species of the presence of at least 10^5 cfu/mL after culturing a second urine specimen after a long period (1–12 months) from the same asymptomatic patient; 66 of the 67 women included in the study still had 10^5 cfu/mL in their urine. We suggest that the cutoff point that is the most important to discriminate between true bacteriuria and contamination is 10^5 cfu/mL. In terms of time, most cultures are repeated after 1 week [3, 13] or 2 weeks [2], but intervals of 2–4 months between the 2 cultures have been described [15]. Kass [14] used a long time interval between tests, and in the IDSA guidelines, only a minimum (24 h) and not a maximum period between the consecutive cultures is mentioned [5].

The new PFGE technique makes it necessary to reconsider the old definition of ASB, and it seems unclear why the current guideline demands a second urine culture. One culture of a clean-voided specimen of midstream urine from an individual without symptoms of a UTI with at least 10^5 cfu/mL of the same single bacterial species might be adequate to diagnose ASB. Using a time frame of 2–4 months between consecutive cultures will lead to a misdiagnosis in 50% of patients.

Suzanne E. Geerlings, Ellen C. Brouwer, Wim Gaastra, and Andy I. M. Hoepelman
Departments of *1* Internal Medicine, *Division of Infectious Diseases and AIDS*, and *2* Laboratory of Medical Microbiology, Eijkman-Winkler Institute, University Hospital Utrecht, and *3* Department of Bacteriology, Institute of Infectious Diseases and Immunology, Veterinary Faculty, Utrecht University, Utrecht, The Netherlands

References


Figure 1. Pulse-field gel electrophoresis of DNA from 20 *Escherichia coli* strains isolated from the urine of 10 women with asymptomatic bacteriuria. Strains isolated from the same patient are placed next to each other.