Evaluation of urine sampling technique: bacterial contamination of samples from women students

ANDERS BAERHEIM

ASBJØRN DIGRANES

STEINAR HUNSKAAR

SUMMARY. A urine specimen is traditionally sampled using the clean-catch midstream technique, but this technique is poorly documented. The aim of this study was to evaluate the bacterial content of urine samples taken using individual steps of the clean-catch midstream technique, or combinations of steps. One hundred and ten women students provided a total of 864 early morning urine samples, using a different sampling technique each morning. Holding the labia apart during sampling more than halved the percentage of samples containing 10^5 colony-forming units per ml or more, and increased the percentage of uncontaminated samples, compared with passing urine without employing any of the precautions. Cleaning the perineum or using the midstream technique resulted in percentages no different from urination without employing any precautions. It is therefore recommended that women should be instructed to carry out the simplest effective urine sampling technique: holding the labia apart.

Keywords: urine samples; specimen handling; screening accuracy.

Introduction

THE clean-catch midstream technique is usually recommended when urine samples are required from women.[1] It is performed by holding the labia apart, cleaning the perineum, and then obtaining a midstream sample. The technique was adopted in the early 1950s, as an alternative to catheterization.[2] However, in spite of its widespread use, its clinical documentation is sparse.[2]

Several authors have questioned the validity of the clean-catch midstream technique.[2-4] It has also been shown that the technique may be difficult to understand and perform, even after repeated instruction.[5]

In a recent retrospective study, it was found that the bacterial content of urine samples was not significantly altered if the woman cleaned the perineum or used the midstream technique, while the bacterial content was significantly reduced when the woman held the labia apart during the micturition.[6] A prospective, experimental study, was thus designed to elucidate how the different steps of the clean-catch midstream sampling technique, alone or in combination, influenced the bacterial content of a urine sample.

A Baerheim, MD, assistant professor and S Hunskaar, MD, assistant professor, Department of Public Health and Primary Health Care, University of Bergen, Norway. A Digranes, MD, chief physician, Department of Microbiology and Immunity, Haukeland Hospital, Bergen, Norway.

Method

The study subjects were healthy women college students studying nursing or medical laboratory technology. Whole classes of students were included but those who were in any way ill were told not to participate. The study was carried out over an eight month period beginning in August 1989.

The clean-catch sampling technique was defined by its three steps: (A) The labia majora and minora should be kept apart with one hand. (B) The perineum should be washed from the front to the back with five cotton swabs moistened in tap water, one at a time. (C) A small portion of the urine should be voided in the lavatory, before collecting from the middle portion, the remainder to be passed uncollected.

The study design required eight consecutive early morning urine samples from each woman, following a new written instruction each day in the following order: voiding without employing any of the precautions, A, B, C, A + B, A + C, B + C, A + B + C. Half of the women received the instructions in the reverse order.

The urine samples were taken at home, and dipslides were inoculated by the women immediately after urination and delivered later to the college campus. They were analysed at the Department of Microbiology and Immunity, Haukeland Hospital, Bergen, using standard techniques. Samples were regarded as uncontaminated if they contained less than 10^4 colony-forming units per ml of urine. The laboratory personnel were blind to the identity of the woman and the sampling methods used for each sample.

On the day of the first sample all of the women completed a questionnaire asking about their weight, height, use of contraception, menstrual status and history of urinary tract infections.

Statistics

Differences between groups were tested using the chi square test and the level of significance was set at 5%. Means are given with 95% confidence intervals.

Results

A total of 111 healthy women students, aged 19 to 40 years (mean 22.6 years, 95% confidence interval (CI) 22.0 to 23.2) were included in the study. Ninety two of the women were studying nursing and 19 physiochemistry. One woman was later excluded as she provided only two samples. The remaining 110 women provided 864 urine samples (98.2% of the possible 880). Demographic data for the women are shown in Table 1.

Of the 864 samples 210 (24.3%) contained 10^5 colony-forming units per ml or more. Most of these samples (160, 76.2%) contained Gram-positive cocci, mainly coagulase-negative staphylococci (68 samples) and enterococci (58 samples).

A total of 106 samples were collected without employing any of the precautions, 31.1% of which contained 10^5 colony-forming units per ml or more (Table 2). When holding the labia apart during micturition, only 13.0% of 108 samples showed 10^5 colony-forming units per ml or more, a reduction of 58.2% ($P<0.01$). The proportion of the samples containing 10^5 colony-forming units per ml or more was not significantly reduced by

Table 1. Demographic data of the 110 women on day one of the study.

Characteristic		95% confidence interval
Mean age (years)	22.6	(22.0 to 23.2)
Mean height (cm)	167.8	(166.8 to 168.9)
Mean weight (kg)	61.3	(59.8 to 62.8)
Contraceptive use (% of women)		
None	52.7	(43.4 to 62.1)
Condom	15.5	(8.7 to 22.2)
Oral contraceptive pill	30.0	(21.4 to 38.6)
Intrauterine device	1.8	(0.2 to 6.4)
Diaphragm	0	
Menstrual status[a] (% of women)		
0–6 days	24.3	(16.2 to 32.4)
7–13 days	17.8	(10.5 to 25.0)
14–20 days	31.8	(23.0 to 40.6)
21–28 days	19.6	(12.1 to 27.2)
>28 days	6.5	(2.7 to 13.0)
Mean no. of episodes of UTI		
Ever	1.8	(1.3 to 2.3)
In last 3 months	0.04	(0.00 to 0.08)

[a]Time since first day of last menstrual period. UTI = urinary tract infection.

Table 2. Bacterial content of 864 early morning urine samples taken using different steps of the clean-catch midstream sampling technique.

	% of samples (95% confidence interval) with:	
	<10^4 cfu ml^{-1}	≥10^5 cfu ml^{-1}
No precautions (n = 106)	14.2 (7.5 to 20.8)	31.1 (22.3 to 39.9)
Cleaning the perineum (n = 106)	11.3 (5.3 to 17.4)	30.2 (21.4 to 38.9)
Midstream technique (n = 109)	14.7 (8.0 to 21.3)	23.9 (15.9 to 31.9)
Holding labia apart (n = 108)	23.1 (15.2 to 31.1)	13.0**(6.6 to 19.3)
Cleaning and midstream technique (n = 108)	16.7 (9.6 to 23.7)	20.4 (12.8 to 28.0)
Cleaning and holding labia apart (n = 110)	15.5 (8.7 to 22.2)	24.5 (16.5 to 32.6)
Midstream technique and holding labia apart (n = 109)	26.6*(18.3 to 34.9)	21.1 (13.4 to 28.8)
Cleaning, midstream technique and holding labia apart (n = 108)	23.1 (15.2 to 31.1)	30.6 (21.9 to 39.2)

cfu = colony-forming units. n = number of women. Versus the specimen taken with no precautions: *P<0.05; **P<0.01.

use of the midstream technique, cleaning the perineum or combinations of steps.

Only 14.2% of the samples collected without employing any of the precautions were not contaminated (Table 2). Holding the labia apart increased the proportion of samples that were uncontaminated to 23.1% (62.7% increase). When the technique of holding the labia apart was combined with the midstream technique, the proportion of uncontaminated samples was further increased to 26.6% (87.3% increase overall, P<0.05). The results obtained for cleaning the perineum or use of the midstream technique were not significantly different from those obtained when taking no precautions.

Of the 435 urine samples taken following an instruction which included holding the labia apart, 22.0% were uncontaminated compared with only 14.2% of the 429 samples where the women were not instructed to hold the labia apart (P<0.01).

The pattern of results was the same when different groups of bacteria were analysed individually. The pattern was also the same whether the women started with no precautions and ended with all three steps or vice versa, and when the women were divided into subgroups according to type of contraceptive used, height/weight, menstrual status, and history of urinary tract infection.

Discussion

The subjects in this study were healthy, young students studying nursing and medical laboratory technology. During their studies they had been taught how to collect a urine sample by the clean-catch midstream technique. In addition, they received written instruction on the different steps and combinations of steps of this technique. However, almost a quarter of the samples contained 10^5 colony-forming units per ml or more. This proportion is similar to that found by other researchers.[7] The majority of these samples contained Gram-positive cocci, especially coagulase-negative staphylococci, similar to the aerobic periurethral flora of healthy women.[8] It is possible that bacteria multiply in the warm, moist vulvar environment to such a degree during the night that they often contaminate the urine to the level of 10^5 colony-forming units per ml or more in early morning samples.

In a previous retrospective study we found that holding the labia apart was the only step of the clean-catch midstream method which significantly reduced the bacterial content of urine samples.[6] This study has retested this experimentally, and has obtained the same result: the bacterial content of urine samples is only significantly reduced by holding the labia apart during urination.

Urine leaves the female urethral orifice in a broad stream, douching the adjacent structures. As a result, vaginal squamous cells, and even hairs, may be found in urine samples. Holding the labia apart minimizes the contact between the urine and the vulva, thereby reducing the chance of contamination. Turner, and later Morris, found that neither cleaning the perineum nor the midstream technique had any effect on the contamination rate.[3,4] In this study cleaning the perineum scored no better than urinating without employing any precautions, while the results were somewhat better when the midstream technique was added to holding the labia apart. Thus, there may be an extra effect by letting the first portion of urine 'wash' the urethra and adjacent vulva, and subsequently avoiding the last portion of urine including afterdrip from the vulva.

The full clean-catch midstream technique is difficult to understand and perform,[5] and consequently often fails to reduce the contamination rate below what is found when the urine sample is collected without employing any precautions. Fisher found, however, that the contamination rate could be lowered by repeating and intensifying the instructions, making them computer-based.[5] This may not be feasible in daily practice.

The results of this study suggest that contamination may be reduced by simplifying the instruction to one step only: holding the labia apart when urine is collected.

References

1. Stamey TA. The diagnosis, localization, and classification of urinary tract infections. In: Stamey TA (ed). *Pathogenesis and treatment of urinary tract infection*. Baltimore (MD): Williams and Wilkins, 1980: 1-51.
2. Immergut MA, Gilbert EC, Frensilli FJ, Goble M. The myth of the clean catch midstream specimen. *Urology* 1981; **17:** 339-340.
3. Morris RW, Watts MR, Reeves DS. Perineal cleansing before midstream urine, a necessary ritual? *Lancet* 1979; **2:** 158-159.
4. Turner GC. Bacilluria in pregnancy. *Lancet* 1961; **2:** 1062-1064.
5. Fisher LA, Johnson TS, Porter D, *et al.* Collection of a clean voided urine specimen: a comparison among spoken, written and computer-based instructions. *Am J Public Health* 1977; **67:** 640-644.
6. Baerheim A, Digranes A, Hunskaar S, Laerum E. Bacteriological findings in urine specimens from women: association with urinary tract symptoms and sampling methods. *Scan J Urol Nephrol* 1991; **25:** 125-127.
7. Platt R. Quantitative definition of bacteriuria. *Am J Med* 1983; **2b:** 44-52.
8. Pfau A, Sacks T. The bacterial flora of the vaginal vestibule, urethra and vagina in the normal premenopausal woman. *J Urol* 1977; **118:** 292-295.

Acknowledgements

Address for correspondence

Anders Baerheim, Division for General Practice, University of Bergen, Ulriksdal 8C, N-5009 Bergen, Norway.