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Reliability of Clean-Voided Mid-Stream Urine Specimens for the Diagnosis of Significant Bacteriuria in the Female Patient

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QUANTITATIVE evaluation of bacteriuria on "clean" mid-stream specimens of urine has become a widespread means of establishing the diagnosis of urinary tract infection.[1-8] A positive urine culture with 100,000 or more colonies of pathogens per ml. usually suggests the presence of active infection.[4-6, 8] Although colony counts above 10,000 organisms per ml. may at times reflect contamination in the female patient,[4-6] many reports suggest that such counts frequently indicate true bacilluria.[3, 7-13] Thus, only when colony counts are lower than 10,000 per ml. can a positive culture be considered to result from contamination, especially if a pathogenic organism is present in the urine.[3, 7, 10, 12] Nevertheless a physician should become uneasy if, in a given subject, urine cultures repeatedly show the presence of a pathogenic organism in counts lower than 10,000.

The coupling of the clean-voided technique and quantitative bacteriology in the female patient avoids the potential hazard of catheterization.[1, 2, 4, 7, 14, 16] It is generally assumed that distinction between true bacilluria and contamination can readily be made by this technique. However, very little information is available with regard to the incidence of sterile urine cultures in female subjects showing no evidence of urinary tract infection. While direct comments on this point have seldom been made,[2] analysis of some previously reported studies suggests that the incidence of sterile urine in both adult females and young girls without evidence of infection ranges from 25 to 50% when the urine is collected either by catheterization or the clean-voided technique.[2, 4, 7, 8, 17]

It has been recognized that valid interpretation of bacteriological findings in the urine requires a number of essential precautions in the collection and dispatch of urinary specimens.[7, 18] Furthermore, hydration, previous antibacterial therapy and frequent emptying of the bladder may affect bacterial counts.[2, 4, 11] Bladder urine is normally sterile.[11] Apart from faulty handling of specimens, contaminants found in the clean-voided mid-stream urine specimens of normal females originate either from the urethra or external genitalia.[18] In fact, it has repeatedly been stated that urinary specimens obtained by catheterization may be contaminated by the transfer into the bladder of organisms located either in the urethra or on the catheter itself.[11, 14-16] It has even been suggested that whatever the means used to obtain urine specimens, contamination is almost unavoidable and up to 1000 organisms per ml. may be found in normal urine.[14]

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It would appear important to determine with precision the incidence of sterile cultures in females without urinary tract infection using either the clean-voided technique or catheterization of the bladder. The present study indicates that the incidence of sterile urine specimens obtained in females without evidence of urinary tract infection may be as high as 69% with the clean-voided technique when proper precautions are taken. This figure was increased to 87% when highly co-operative young student nurses were studied. Comparison of paired specimens showed 100% sterile cultures obtained by catheterization versus 69% in the clean-voided ones. This observation is at variance with the concept that urethral organisms can be easily introduced in the bladder by catheterization. In another group of female patients with evidence of active urinary tract infection, excellent correlation was found with regard to the type and number of organisms between paired clean-voided specimens and specimens obtained by catheterization.

MATERIALS AND METHODS

Two graduate nurses were trained to collect both clean-voided and catheter specimens. All specimens were collected in the same room by the same two nurses. Both the nurses and patients wore sterile rubber gloves during the entire procedure of urine collection. The perineum was carefully scrubbed with green soap for two to three minutes. The labia majora were then separated and the vulva washed with green soap, using a fresh cotton swab after each downward stroke. The same maneuver was repeated with an aqueous solution of Zephiran (benzalkonium) 1:1000. The subjects were then instructed to void after separating the labia majora. After the stream was well started, a sterile screw-cap jar was placed into the path of the stream and a small sample of urine collected. Subjects in whom paired specimens were obtained were told to stop voiding as soon as the clean-voided specimen was obtained. A sterile rubber French No. 8 catheter lubricated with a small amount of sterile lubricant was then inserted into the urethra and a final specimen collected in another screw-cap jar. The specimens were taken immediately to the bacteriology laboratory where they were processed immediately for bacterial counts, routine bacterial identification and gram stain. Afterwards, the remainder of the specimen was sent to the renal laboratory for microscopic examination of the sediment after centrifugation. Bacteriological studies were performed at frequent intervals to ascertain the sterility of both the catheters and the rubber gloves used during this study.

The urine specimen used for bacteriological studies was divided into two portions. The first was centrifuged at 1800 r.p.m. for 10 minutes and the sediment studied as follows: a loopful of sediment was streaked on an azide blood agar plate and a MacConkey agar plate; another portion of the sediment was gram-stained. Pure strains were cultured on appropriate media and identified on the basis of the usual biochemical fermentation and serological reactions.

The second portion of the urine specimen was diluted (1:1000) with sterile distilled water and 1.0 ml. was pipetted into a sterile Petri dish to which was added 9.0 ml. of melted blood-enriched tryptose agar and mixing was effected by swirling. When the contents of the pour plate solidified, it was incubated at 37° C. for 24 hours and colonies were counted under magnification in a Quebec colony counter. All counts were corrected to 1.0 ml. of urine.

The following groups of female subjects were studied:

Group 1.—This group consisted of 53 healthy student nurses (aged 18 to 23 years, mean 19). All were volunteers. No subject with a previous history of urinary symptoms, bladder catheterization or recent antibiotic therapy was accepted for the study. The first morning clean-voided mid-stream specimen was collected following an overnight fast.

Group 2.—This group consisted of 29 female patients admitted on a general medical female ward service (aged 21 to 53 years, mean 42). Every subject had a benign condition and none showed past or present clinical evidence of urinary tract infection. In these patients both clean-voided and catheterized urine specimens were collected at the same time after an overnight fast. No patient developed any clinical or bacteriological evidence of urinary tract infection up to four weeks after catheterization.

Group 3.—This group consisted of 27 female patients (aged 24 to 54 years, mean 43). They were admitted to either a semiprivate or general medical ward. They all showed evidence of active urinary tract infection including pyuria (urinary sediment showing more than five white blood cells per high-power field), frequency, burning on micturition, dysuria and occasionally pyelographic changes suggestive of chronic infection. In this group of patients, both clean-voided and catheterized specimens were collected at the same time after an overnight fast.

TABLE I.—RESULTS OF URINE CULTURE OBTAINED BY THE CLEAN-VOIDED TECHNIQUE IN 53 HEALTHY STUDENT NURSES (GROUP I)

	No. of cases	
No growth	46 (87%)	
Positive	7 (13%)	
Case No.	*Organism*	*Bacterial counts per ml. of urine*
1	*E. coli*	<1000
2	Enterococci	1000
3	Enterococci	2000
4	Lactobacillus	15,000
5	Lactobacillus	20,000
6	Lactobacillus	<1000
7	Lactobacillus	3000

RESULTS

Group 1.—The clean-voided urines contained no bacteria after 48 hours in 46 (87%) of the 53 subjects (Table I). In the remaining seven subjects, a pathogen was found in only three instances in counts not exceeding 2000, while four subjects showed lactobacillus, a well-known urinary contaminant in the female (Table I).

Group 2.—In this group of subjects the incidence of sterile clean-voided urines was also quite high (69%), while not a single catheterized specimen showed bacterial growth after 48 hours (Table II). In the nine remaining subjects in whom the clean-voided specimen showed bacterial growth, the bacterial count exceeded 5000 colonies per ml. in only two instances, a pathogen *(E. coli)* being found in one instance and a contaminant (sarcinae) in the other. Thus, under the present conditions, the clean-voided specimens showed a high incidence of sterile cultures while the few positive cultures showed a low bacterial count. It is of interest that no catheterized specimen showed any bacterial growth, this observation being at variance with the concept that introduction of organisms from the urethra to the bladder is practically unavoidable during catheterization.[14]

TABLE II.—RESULTS OF URINE CULTURE OBTAINED BY THE CLEAN-VOIDED TECHNIQUE AND CATHETERIZATION IN 29 FEMALE PATIENTS WITHOUT EVIDENCE OF URINARY TRACT INFECTION (GROUP 2)

	Clean-voided		*Catheter*
No growth	20 (69%)		29 (100%)
Positive	9 (31%)		0
Case No.	*Organism*	*Bacterial counts per ml. of urine*	
1	*E. coli*	5000	
2	*E. coli*	<1000	
3	*E. coli*	2000	
4	*E. coli*	10,000	
5	*Proteus mirabilis*	5000	
6	Enterococci	<1000	
7	*Staphylococcus albus*	<1000	
8	*B. subtilis*	<1000	
9	Sarcinae	8000	

TABLE III.—RESULTS OF URINE CULTURE OBTAINED BY THE CLEAN-VOIDED TECHNIQUE AND CATHETERIZATION IN 27 FEMALE PATIENTS SHOWING EVIDENCE OF URINARY TRACT INFECTION (GROUP 3)

		Bacterial counts per ml. of urine	
Case No.	*Organism*	*Clean-voided*	*Catheter*
1	*E. coli*	$>10^5$	$>10^5$
2	*E. coli*	100,000	70,000
3	*E. coli*	$>10^5$	$>10^5$
4	*E. coli*	$>10^5$	$>10^5$
5	*E. coli*	$>10^5$	$>10^5$
6	*E. coli*	$>10^5$	$>10^5$
7	*E. coli*	80,000	80,000
8	*E. coli*	$>10^5$	$>10^5$
9	*E. coli*	$>10^5$	100,000
10	*E. coli*	$>10^5$	$>10^5$
11	*E. coli*	100,000	100,000
12	*E. coli*	$>10^5$	$>10^5$
13	*E. coli*	$>10^5$	$>10^5$
14	*E. coli*	$>10^5$	$>10^5$
15	*E. coli*	$>10^5$	$>10^5$
16	*E. coli*	100,000	80,000
17	*E. coli*	30,000	15,000
18	*A. aerogenes*	$>10^5$	$>10^5$
19	*A. aerogenes*	$>10^5$	$>10^5$
20	*A. aerogenes*	$>10^5$	$>10^5$
21	*Proteus vulgaris*	$>10^5$	$>10^5$
22	*Proteus vulgaris*	$>10^5$	$>10^5$
23	*Proteus mirabilis*	$>10^5$	$>10^5$
24	Enterococci	100,000	80,000
25	Enterococci	$>10^5$	$>10^5$
26	*Pseudomonas aeruginosa*	200,000	60,000
27	*Gaffkya tetragena*	100,000	2000

Group 3.—In this group of patients with evidence of urinary tract infection, a very high degree of correlation was found between clean-voided and catheter specimens with regard to both the type of organism and the bacterial counts (Table III). In most instances, bacterial counts exceeded 100,000 colonies per ml. of urine. No instance of mixed bacterial flora was found in this group of patients. Common urinary pathogens were found in all cases except one (Case 27). In the latter it is significant that *Gaffkya tetragena,* a well-known contaminant, was still found in the catheterized specimen, albeit in very low counts. It should be noted that the gram stain showed organisms in clean-voided or catheter specimens showing more than 30,000 colonies per ml. of urine.

DISCUSSION

The present study clearly indicates that the incidence of sterile clean-voided mid-stream urine specimens in females without evidence of urinary tract infection may be quite high if the proper precautions are taken. Most reported studies indicate that sterile urine specimens obtained either by the clean-voided technique or catheterization in asymptomatic females do not exceed 50%,[2, 4, 7, 8, 17] the latter figure being reported in young girls under 12 years of age.[17] The present figures of 87% in healthy student

nurses and 69% in hospitalized female subjects without evidence of urinary tract infection are of great interest. They show that it is technically possible to obtain sterile urine specimens in the great majority of subjects without urinary tract infection. This of course has the advantage of greatly reducing possible misinterpretation of bacteriological reports showing counts below 100,000 colonies and even below 10,000 organisms per ml. of urine. Urine specimens shown to be sterile on repeated occasions should facilitate exclusion of active urinary tract infection in the great majority of cases.[13]

It is realized that a great deal of trouble was taken to collect urine specimens in the present study. It is believed, however, that all the steps described are essential if results such as those reported are to be expected. In a general hospital of any substantial size it is doubtful that clean-voided specimens collected in a standardized manner by ever-changing professional personnel in various departments are devoid of the usual risks of contamination from the time of collection to that of bacteriological manipulation.[18] In view of the present findings, the question should certainly be raised regarding the advisability of training a permanent group of nurses who would be responsible for collecting all routine clean-voided urine specimens in females throughout the hospital.

The present study demonstrates another important point, namely, that selection of subjects may influence the incidence of sterile cultures in non-infected females. The high incidence of sterile cultures observed in student nurses is not surprising in view of the fact that in terms of co-operation, intellectual behaviour, personal hygiene and negative medical history including pregnancy,[4] this group of subjects was a highly selected one. Nevertheless, the results obtained in the older group of asymptomatic female subjects, most of whom had already experienced at least one pregnancy, are also quite impressive and more representative of a general hospital patient population.

In contrast to previous reports,[11, 15] the present study suggests that atraumatic bladder catheterization performed by trained personnel with utmost precautions will show sterile urine in 100% of subjects showing no evidence of urinary tract infection. Thus, the concept that a catheter will introduce urethral bacteria into the bladder in sufficient numbers to inoculate the urine and result in bacterial growth[14, 16] is not supported by the present study. This finding is in agreement with another recent report.[13] However, in view of the ever present danger of infection inherent in catheterization whatever the precautions used,[1, 2, 4, 7, 14, 16] the present observation should not be taken to justify the use of catheterized urine specimens for cultures in the female patient.

The present findings in patients showing evidence of urinary tract infection are in full agreement with the high bacterial counts reported in various studies.[2-7] Furthermore, the excellent correlation observed between clean-voided and catheter specimens confirms once again the concept that high bacterial counts usually indicate active urinary infection whether clean-voided or catheter specimens are used.[2, 4, 6, 7] Catheter specimens appear to offer no additional advantage in the diagnosis of unequivocal bacteriuria.

The major problem resides in the interpretation of the positive urine culture showing pathogenic organisms in low bacterial counts when the clean-voided technique is used in female patients. The present demonstration of a high incidence of sterile urine in clean-voided specimens in female subjects without infection should be helpful in reducing this difficulty. In fact, by increasing the incidence of sterile urine cultures from some 30% to near 70%, the incidence of positive urine culture with low bacterial counts in asymptomatic female patients should now become less than 20% in contrast to the 60% estimated in other studies.[2, 4, 7, 8, 17]

Results such as those reported in the present study can only be expected if collection of clean-voided specimens is performed under conditions which will ensure the necessary precautions. The latter involve the proper training of professional personnel, adequate co-operation from patients through kind and intelligent instruction, immediate dispatch of urine specimens to the bacteriology laboratory and rapid transfer of urine to a nutrient medium.

Summary The incidence of sterile urine cultures was evaluated in female subjects without evidence of urinary tract infection. All clean-voided mid-stream or catheterized urine specimens were obtained with utmost care by two specially trained graduate nurses. In a group of 53 healthy student nurses (aged 18 to 23 years) the incidence of sterile urine in clean-voided specimens was 87%. In another group of 29 hospitalized female subjects without evidence of urinary tract infection (aged 21 to 53 years) 69% of clean-voided urine cultures were sterile while the positive specimens showed bacterial counts under 10,000 organisms per ml. Paired catheter specimens were 100% sterile. In another group of 27 hospitalized female patients who showed evidence of active urinary tract infection, excellent correlation with regard to the type of organism and bacterial counts was obtained in paired clean-voided and catheter specimens. In this group bacterial

counts usually exceeded 100,000 organisms per ml. The present study demonstrates that if proper precautions are taken, the incidence of sterile urine specimens obtained by the clean-voided technique in non-infected females may be increased to 70% from the 30% in previously reported studies. Such results should help to reduce considerably the difficulty in interpreting bacteriological reports indicating the presence in the urine of a pathogenic organism in low bacterial counts.

Résumé La présente étude a été entreprise afin de déterminer la fréquence avec laquelle on peut obtenir des cultures d'urine complètement stériles en employant la technique de la miction propre chez la femme ne présentant pas de signe d'infection urinaire. Tous les échantillons d'urine ont été recueillis par deux infirmières diplômées soit par la technique de la miction propre soit par cathétérisme en utilisant les plus grandes précautions pour éviter toute contamination. Chez 53 étudiantes infirmières (âgées de 18 à 23 ans), la culture des urines obtenues par miction propre s'est avérée complètement négative dans 87% des cas. Chez 29 femmes hospitalisées (âgées de 21 à 53 ans) qui ne présentaient aucune évidence d'infection urinaire, 69% des urines obtenues par miction propre étaient stériles, les autres échantillons montrant une numération bactérienne inférieure à 10,000 colonies par cm^3. Les échantillons d'urine obtenus au même moment par cathétérisme étaient tous stériles. Une excellente corrélation a été observée quant au type de microorganisme et quant au décompte bactérien entre les échantillons obtenus par miction propre et cathétérisme chez 27 femmes hospitalisées présentant des signes d'infection urinaire active. Dans la plupart des cas, la numération bactérienne dépassait 100,000 colonies par cm^3. La présente étude démontre que les échantillons d'urine obtenus par miction propre chez la femme sans infection urinaire seront stériles dans 70% des cas si toutes les mesures nécessaires pour éviter la contamination sont prises. Ces résultats contrastent avec d'autres études publiées où seulement 30% des échantillons étaient stériles. Les résultats obtenus devraient permettre de réduire considérablement les difficultés diagnostiques des données bactériologiques qui indiquent la présence dans l'urine de microorganismes pathogènes en faible quantité.

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