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Two Methods of Collecting Clean-voided Urine Specimens

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TWO METHODS OF COLLECTING CLEAN-VOIDED URINE SPECIMENS

by

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Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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CHAPTER I

THE PROBLEM AND DEFINITIONS OF TERMS USED

Authorities agree that catheterization, as a method of collecting urine specimens, is responsible for an increase in urinary tract infections. Studies show that bacteria which lie within the urethral channel cannot be removed by cleansing the genitalia. When these bacteria are pushed into the bladder upon catheterization, they contribute to urinary tract infection and introduce contaminants into the urine specimens.

Studies also show that the clean-voided method of collecting urine specimens has been found to be a reliable method of collection for detection and evaluation of urinary tract infections in women. It has the advantage over catheterizations in that bacteria are not introduced into the bladder.

No study was found that evaluated the comparison of clean-voided specimens taken from women whose genitalia were cleansed as compared to clean-voided specimens taken from the same group of women where their genitalia had not been cleansed. This study was done to find out if there was a need to cleanse the genitalia in women before collecting a clean-voided urine specimen.

I. THE PROBLEM

Statement of the Problem

The problem of this study was to determine the difference between the number of organisms in a clean-voided urine specimen collected

from women whose genitalia had not been cleansed as compared with a clean-voided urine specimen collected from the same women after the genitalia had been cleansed.

Need for the Study

There should be continuity in the procedure of collecting urine specimens so that the results are consistent. Therefore, there is a need to provide information which will assist in devising a simplified nursing procedure for the collection of clean-voided urine specimens.

Hypothesis

The hypothesis of the study was that there is no difference in the number of organisms found in a clean-voided urine specimen collected from women whose genitalia are not cleansed as compared with a clean-voided urine specimen collected where the genitalia are cleansed. This hypothesis was based on the theory that the first ejection of urine in the collection of clean-voided urine specimens washes away the majority of bacteria found inside and around the meatus of the urethra.

Assumptions

It was assumed in this study that:

1. All clean-voided urine specimens were collected by a uniform and proper procedure.
2. The laboratory reports were correct.
3. A large number of bacteria inhabit the female genitalia.
4. Because skin cannot be sterilized, some bacteria may be found inside or around the meatus of the urethra after any cleansing technique.

5. Holding the labia away from the meatus of the urethra reduces the possibility of contamination of the clean-voided urine specimen.
6. After sufficiently holding the labia apart, the bacteria found inside and around the meatus of the urethra are the primary source of contamination in the collection of clean-voided urine specimens.

Limitations

The limitations of this study were that:

1. There was a possible variation in the location of the meatus of the urethra which could have contributed to contamination of the clean-voided urine specimen of some women.
2. The only antiseptic used in cleansing the meatus of the urethra was Virac.
3. The population was limited to nursing students in a selected school of nursing.
4. All women who had taken antibiotics within six weeks prior to the study, who were known to have a urinary tract infection, or who were known to have diabetes were not included in the study.

II. METHOD OF STUDY

After a careful review of literature the experimental method was selected as the method of study. The experimental method is defined by

Greenwood as follows: "The proof (test) of an hypothesis which seeks to hook up two factors into a causal relationship through the study of contrasting situations."[1] The major purpose of the experimental method given by Rummel "is to describe the effect of certain treatments upon some characteristic of a group or population and to test some hypothesis about this effect."[2] The literature review furnished not only an orientation to the subject but background of a need for a more simplified procedure for collecting clean-voided urine specimens.

Permission was granted to conduct the study from the hospital administrator, director of the school of nursing, and the head of the laboratory facilities.

The data were obtained by taking two clean-voided urine specimens from twenty-six nursing students in a selected school of nursing. The first specimen was taken where the genitalia had not been cleansed and the second specimen was taken approximately twenty-four hours later where the genitalia had been cleansed. To assure comparable findings, a specific and standardized procedure for the collection of urine specimens was developed in collaboration with a microbiologist.

A pilot study was done to refine the collection procedure.

III. DEFINITION OF TERMS

For clarity of meaning, terms used in this study are defined as

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[1]Carter V. Good and Douglas E. Skates, Methods of Research, New York: Appleton-Century-Crofts, Inc., 1954, p. 698.

[2]J. Francis Rummel, An Introduction to Research Procedures in Education, New York: Harper and Brothers, 1958, p. 203.

follows:

Bacilluria: presence of bacillary bacteria in the urine.

Bacteriuria: presence of any type of bacteria in the urine.

Clean-voided Urine Specimen: the urine specimen taken while the labia are held away from the urethral meatus after ejection of approximately half of the urine in the bladder.

Mid-stream Urine Specimen: a clean-voided urine specimen. The urine specimen taken while the labia are held away from the urethral meatus after the ejection of approximately one-third of the urine in the bladder. The second one-third of the urine is collected as the specimen and the remaining one-third is discarded.

Virac: a trade name for a germicidal agent. "Virac is a water miscible, lyophilic sol consisting essentially of elemental iodine combined with a cationic, surface-active, quaternary ammonium base given the generic name UNDECOYLIUM Chloride-Iodine."[3]

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[3]Ruson Laboratories, Inc., The Virac Story, Portland, Oregon: Ruson Laboratories, Inc., September 1, 1959, p. 1.

CHAPTER II

REVIEW OF THE LITERATURE

A review of literature was done in order to obtain a background of knowledge in the areas of urinary tract infections, methods of collecting clean-voided urine specimens, and procedures in analyzing the specimens. Studies showed various ways of obtaining clean-voided specimens using different kinds of cleansing agents. However, there were no studies found where clean-voided urine specimens had been taken from women whose genitalia had not been cleansed and compared with a second clean-voided urine specimen taken from those same women where the genitalia had been cleansed.

The need for a simple yet reliable method of collecting urine specimens without infecting the urinary tract was shown in the review of literature.

I. INFECTIONS OF THE URINARY TRACT

"Infections of the urinary tract are second in frequency only to infections of the respiratory tract."[1] "Of all chronic bacterial diseases, urinary tract infections are among the most difficult, the most perplexing and the most disturbing."[2] Furthermore, "satisfactory information on the incidence, pathogenesis and natural history of pyelonephritis and related infections of the urinary tract is surprisingly

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[1]Edward H. Kass, "Asymptomatic infections of the Urinary Tract," Transactions of the Association of American Physicians," 69:56, 1956.

[2]Chester Scott Keefer, "Pyelonephritis--Its Natural History and Course," Bulletin of the Johns Hopkins Hospital, 100:130, 1957.

incomplete."[3] Therefore, it was the intent of this paper to bring out certain aspects which according to Kass are well established.[4]

Pathways of Infection

The pathways by which infection enters the urinary tract has been a subject of controversy for decades.[5] Though it is not possible in most cases to establish the route of bacterial invasion of the kidney, it is possible that bacteria may enter the urinary tract by more than one route.[6] Davis cites the following ways in which bacteria may reach the interior of the urinary tract:

1. By way of the blood stream (hematogenous infection).
2. By way of the lymphatic system (lymphogenous infection).
3. By way of instruments or other objects inserted through the urethra.
4. Through wounds in the urinary tract, resulting from accidental penetrations, wounds with firearms or other weapons, or surgical incisions, accidental or intentional.
5. By direct extension from other organs as, for example, in the rupture of a pelvic abscess into the bladder.
6. By surface extension from the exterior along the mucosal surface of the urethra.
7. Through congenital abnormal openings as, for example, congenital recto-urethral fistula.[7]

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[3]Edward H. Kass, "Chemotherapeutic and Antibiotic Drugs in the Management of Infections of the Urinary Tract," American Journal of Medicine, 18:764, May, 1955.

[4]Ibid.

[5]Harry A. Derow, "Management of Pyelonephritis," New England Journal of Medicine, 255:338, August 16, 1956.

[6]Ibid.

[7]David M. Davis, Mechanisms of Urologic Disease, Philadelphia: W. B. Saunders Company, 1953, p. 22.

Catheterization as a Source of Infection

Catheterization as a source of urinary tract infections was the only route of infection considered for the purpose of this study.

It has been stated by Merritt and Sanford that it is widely appreciated that catheterization includes the hazard of introducing organisms into the bladder which may produce urinary tract infections.[8] Guze and Beeson felt that there was no practical, harmless way to destroy the bacteria that may lie within the urethral channel. Also, that during the passage of the catheter, these bacteria may be transferred into the bladder which could be the origin of bacterial growth in the culture.[9]

In order to find out if bacteria were in the urethras of women, Guze and Beeson carried out a study of thirteen patients undergoing gynecologic surgery. These women were catheterized in the operating room under anesthesia and under apparently optimal aseptic and antiseptic conditions. The aim was to culture the tips of catheters which had been inserted into the urethra of these patients, the tips were swirled around in two milliliters of sterile saline solution and cultured. The result of these thirteen tests showed a growth in six of the cases. A pure growth of Escherichia coli was found in four, Esch. coli and Staphylococcus aureus in one and Aerobacter aerogenes in one.[10]

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[8] A. Donald Merritt and Jay P. Sanford, "Sterile-Voided Urine Culture," Journal of Laboratory and Clinical Medicine, 52:463, September, 1958.

[9] Lucien B. Guze and Paul B. Beeson, "Observations on Reliability and Safety of Bladder Catheterization for Bacteriologic Study of Urine," New England Journal of Medicine, 255:474, September 6, 1956.

[10] Ibid.

In order to further establish that bacteria can be introduced into the bladder urine during the procedure of catheterization, specimens from a bladder aspiration during a laparotomy were compared to catheterized specimens which were obtained shortly afterward. These urine specimens were taken from twelve patients. All twelve specimens were taken from twelve patients. All twelve specimens aspirated from the bladder during the laparotomy were sterile. Though eight catheterized specimens showed no growth, three specimens showed the presence of Esch. coli and one showed the presence of Pseudomonas aeruginosa.[11]

In a course of studies done by Kass, it was found that after a single catheterization, the likelihood of inducing bacteriuria is 2 to 4 percent.[12]

Schwartz placed the chance of producing infection by a single catheterization even higher, or between 3 to 6 percent. However, he states that catheterization is necessary in spite of the risk of introducing bacteria into the bladder when:

1. Urinary retention exists.
2. Repeated clean-voided specimens yield borderline results.
3. Immediate antimicrobial therapy is needed in an acutely ill patient, and there is not time for multiple specimens to be obtained.
4. The patient is unable or unwilling to cooperate.[13]

At Boston City Hospital, patients with indwelling catheters were

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[11] Ibid.

[12] Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:711, 1957.

[13] Bradford B. Schwartz, "Urinary Tract Infections in Children," Virginia Medical Monthly, 89:164, March, 1962.

studied. Using one hundred thousand bacteria per milliliter of urine as the dividing line between contamination and infection, it was found that 95 percent of the patients with indwelling catheters developed bacilluria within ninety-six hours, most of them within twenty-four hours.[14]

Guze and Beeson felt that "studies support the view that catheterization, no matter how carefully done, carries with it some danger of introducing infection into the bladder cavity."[15]

Organisms Indicating Infection of the Urinary Tract

Bailey and Scott have pointed out that "the bacterial flora of normal urine differs from that of infected specimens."[16] Below is listed those organisms which are most frequently encountered in normal urine:

- Staphylococci, coagulase-negative
- Diphtheroid bacilli
- Coliform bacilli, including intermediate forms
- Enterococci
- Proteus species
- Alpha hemolytic and beta hemolytic streptococci
- Saprophytic yeasts
- Bacillus species[17]

As a rule, one or more of the following will be included in the flora of infected urine:

- Coliform bacilli, including intermediate forms
- Proteus species
- Pseudomonas aeruginosa and

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[14]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:60, 1956.

[15]Guze and Beeson, op. cit., p. 475.

[16]Robert W. Bailey and Elvyn G. Scott, Diagnostic Microbiology, Saint Louis: The C. V. Mosby Company, 1962, p. 88.

[17]Ibid.

other Pseudomonas species
Enterococci
Staphylococci, coagulase-positive and coagulase-negative
Alcaligenes species
Herellea species
Haemophilus species (probably Haemophilus vaginalis)
Candida albicans
Beta hemolytic streptococci, usually Groups B and D
Gonococcus
Mycobacterium tuberculosis
Salmonella and Shigella species[18]

According to Keefer, the organisms most commonly isolated from single-type infections are Escherichia coli and Staphylococcus aureus. "Common Gram-negative organisms that cause pyelonephritis in declining frequency are Aerobacter aerogenes, Pseudomonas aeruginosa, and Proteus vulgaris."[19] The most common Gram-positive microorganisms found in the urine, aside from staphylococci, are enterococci.[20]

It is further stated that there is general agreement that:

> E. coli and Staph. aureus are the two organisms most often present in acute urinary tract infections, and that Proteus, Pseudomonas and Enterococcus are more common in chronic and anatomically complicated infections than in uncomplicated infections. Enterococci are found most often in women following catheterization. Cultures containing more than one species are usually indicative of chronic or complicated infections.[21]

On the whole, Kass tends to agree with Keefer by saying that Escherichia coli and related bacteria "are the most common bacteria found in most series of acute and chronic pyelonephritis."[22] In chronic

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[18] Ibid.

[19] Keefer, op. cit., p. 109.

[20] Ibid.

[21] Ibid., p. 111.

[22] Edward H. Kass, "Chemotherapeutic and Antibiotic Drugs in the Management of Infections of the Urinary Tract," American Journal of Medicine, 18:766, May, 1955.

and complicated infections of the urinary tract the percentages of staphylococci, enterococci, and members of the genera Proteus, Aerobacter, and Pseudomonas increase. This is considered particularly so if the infections have occurred following instrumentation, catheterization or prior to antibiotic therapy.[23]

Significant Bacteriuria as an Indication of Infection

Attempts have been made to distinguish between true bacteriuria and contamination but "most of these attempts have not received widespread acceptance because the criteria for distinguishing between contamination and bacteriuria were usually arbitrary."[24] Kass defined true bacteriuria as the "actual residence of bacteria within the urine of the urinary tract,"[25] and contamination as "the adventitious entry of bacteria into the urine during the collection of the specimen."[26]

In support of his views, Kass has pointed out that it has been generally accepted that the urinary tract was normally free of bacteria and that true bacteriuria was a pathologic finding. Evidence showed that bacteria usually multiplies to numbers exceeding 10^5 (100,000) bacterial colonies per milliliter of urine when they are discharged from the kidney or has entered the urinary tract in another way. However, if the urine is contaminated by passing through the urethra or

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[23] Ibid.

[24] Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:709, 1957.

[25] Ibid.

[26] Ibid.

surrounding structures, "the numbers of colonies obtained from the urine usually do not exceed 10^4 (10,000) per milliliter."[27]

Kaitz and Williams have supported Kass in saying that "the correlations between specimens obtained by needle aspiration and those obtained by voiding or catheterization are excellent if about 10^5 bacteria per milliliter are used to distinguish bacteriuria from contamination."[28,29]

Others agreed with Kass that 100,000 bacteria per milliliter of urine would be indicative of untreated infection.[30,31] However, opinions varied in the number of organisms felt to be indicative of infections of the urinary tract. Switzer, Windom and others felt that 50,000 to 100,000 organisms per milliliter in a clean-voided urine specimen would indicate a urinary tract infection.[32,33] Merritt and

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[27]Edward H. Kass, "Bacteriuria and the Pathogenesis of Pyelonephritis," Laboratory Investigation, 9:111, 1960.

[28]Alan L. Kaitz and Elizabeth J. Williams, "Bacteriuria and Urinary-Tract Infections in Hospitalized Patients," New England Journal of Medicine, 262:425, March 3, 1960.

[29]Edward H. Kass, "Bacteriuria and the Pathogenesis of Pyelonephritis," Laboratory Investigation, 9:111, 1960.

[30]George G. Jackson and others, "Urinary Findings Diagnostic of Pyelonephritis," Journal of the American Medical Association, 166:17,

[31]Charles V. Pryles and Nina L. Steg, "Specimens of Urine Obtained From Young Girls by Catheter Versus Voiding," Pediatrics, 23: 451, March, 1959.

[32]Sam Switzer, "The Clean-Voided Urine Culture in Surveying Populations for Urinary Tract Infection," Journal of Laboratory and Clinical Medicine, 55:563, April, 1960.

[33]Robert E. Windom and others, "Routine Urine Cultures in Private Practice," American Journal of the Medical Sciences, 241:16, January, 1961.

Sanford concluded that 50,000 bacteria per milliliter was the point at which contamination could be differentiated from significant bacteriuria in a clean-voided urine specimen.[34]

In a catheterized urine specimen, Boshell and Sanford, Kass, and Merritt and Sanford agreed that significant bacteriuria was over ten thousand colonies of bacteria per milliliter.[35,36,37]

Asymptomatic Urinary Tract Infection

Asymptomatic bacteriuria may be defined as:

> That condition in which more than one hundred thousand colonies of bacteria are isolated from one milliliter of aseptically collected urine from someone who has no overt signs or symptoms of urinary tract infection.[38]

It was found that the incidence of asymptomatic urinary tract infections in a sampled medical outpatient population was 6 percent. The incidence in diabetic women was 18 percent and in diabetic men, 5 percent. In women with cystoceles, the incidence was 23 percent and 11 percent in pregnant women at term.[39] Kass has stated that in a popu-

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[34]Merritt and Sanford, op. cit., p. 466.

[35]Buris R. Boshell and Jay P. Sanford, "A Screening Method for the Evaluation of Urinary Tract Infections in Female Patients Without Catheterization," Annals of Internal Medicine, 48:1043, May, 1958.

[36]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:58, 1956.

[37]Merritt and Sanford, loc. cit.

[38]Leon G. Smith and James Schmidt, "Evaluation of Three Screening Tests for Patients with Significant Bacteriuria," Journal of the American Medical Association, 181, 431, August 4, 1962.

[39]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:60, 1956.

lation where a relatively high incidence of asymptomatic bacteriuria and pyelonephritis has been studied, "bacteriuria is found to be present for weeks or months before the development of frank symptoms."[40]

The frequency in the occurrence of asymptomatic bacteriuria in the population at large is great enough to be an important problem in public health and in preventive medicine. "Asymptomatic bacteriuria is a disease state that plays a key role in the pathogenesis of pyelonephritis"[41] which is the number one infectious disease problem due to bacteria.[42] Therefore, in order to provide protection against pyelonephritis, bacteriuria must be eliminated.[43]

Factors Influencing Infections of the Urinary Tract

Literature revealed many factors which contribute to infections of the urinary tract. Some of these factors are mentioned under the headings of clinical states, sex, and age.

Clinical states. Varying degrees of evidence shows that infections of the urinary tract are associated with clinical states such as hypertension, malignant nephrosclerosis, toxemias of pregnancy, and diabetes mellitus.[44]

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[40]Edward H. Kass, Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:712, 1957.

[41]Edward L. Quinn and Edward H. Kass (ed.), Biology of Pyelonephritis, Boston, Massachusetts: Little, Brown and Company, 1960, p. 399.

[42]Keefer, op. cit., p. 107.

[43]Quinn and Kass (ed.), op. cit., p. 411.

[44]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:56, 1956.

In autopsy, it was found that pyelonephritis occurs about four times as often in diabetics as in non-diabetics.[45] In a study of one hundred diabetic patients at autopsy, it was found that 30 percent had suffered from significant infection.[46] From 859 necropsies of diabetic patients, there were 107, or 12.4 percent, where infection of the kidneys was felt to have caused or contributed to death.[47]

Sex. In a series of studies done by Kass, it was found that females had a higher incidence of bacteriuria.[48] In one study it was found that the occurrence of bacteriuria on the medical wards at the Boston City Hospital was 30 percent in seventy-six female patients and in 12 percent of the male patients.[49] Barnard and others stated that out of fifty-two diabetic patients referred for treatment of urinary tract infections, fifty-one were women.[50]

Kunin and others carried out a survey of infections of the urinary tract in 3,057 school children. The children were in the grades

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[45]Edward H. Kass, "Chemotherapeutic and Antibiotic Drugs in the Management of Infections of the Urinary Tract," American Journal of Medicine, 18:764, May, 1955.

[46]Donald M. Barnard and others, "Urinary-Tract Infections in Diabetic Women," New England Journal of Medicine, 248:136, January 22, 1953.

[47]Hugh A. Edmondson and others, "Necrosis of Renal Papillae and Acute Pyelonephritis in Diabetes Mellitus," Archives of Internal Medicine, 79:152, 1947.

[48]Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:711, 1957.

[49]Quinn and Kass (ed.), op. cit., p. 406.

[50]Barnard and others, loc. cit.

ranging from the first through the twelfth. It was found that from 1,647 boys, there were no infections encountered. But there were fifteen infections found among the 1,410 girls.[51] This is attributed to the short urethra of the female which becomes contaminated with feces.[52,53]

Age. Though pyelonephritis may be observed at all ages, Keefer states that there are three major peaks of incidence.[54]

The first peak of incidence is in infancy and childhood. In this age group, it is suggested that congenital abnormalities may be the major cause.[55]

The second peak of incidence is during the child-bearing period. In this age group the following factors are listed as contributing factors:

> In young adults, important predisposing factors are catheterization, spinal anesthesia with urinary retention followed by catheterization, obstruction of the urethra, pregnancy, stone and late congenital defects such as congenital cystic kidneys, congenital and familial interstitial nephritis, and hypoplastic kidneys.[56]

The third peak of incidence is in the elderly. In this group

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[51]Calvin M. Kunin and others, "Epidemiology of Urinary-Tract Infections," New England Journal of Medicine, 263:823, October 27, 1960.

[52]Harry A. Derow, "Management of Pyelonephritis," New England Journal of Medicine, 255:339, August 16, 1956.

[53]Robert E. Desantels and J. Hartwell Harrison, "The Mismanagement of the Urethral Catheter," Medical Clinics of North America, 43: 1574, November, 1959.

[54]Keefer, op. cit., p. 112.

[55]Ibid.

[56]Ibid.

the common predisposing factors are "benign prostatic hypertrophy and cancer of the prostate in males and diabetes and cystitis in females."[57]

Kalliomaki and Kasanen found that in one hundred adult patients the average age of women patients with pyelonephritis was fifty-one and the average age of the men was fifty-five years of age. In another study of 300 adult patients with pyelonephritis, the average age of the women was fifty-two and the average age of the men was fifty-one.[58]

Antibacterial Therapy

Keefer has stated that 90 percent of the acute uncomplicated urinary tract infections of short duration where there is a single strain of microorganism susceptible to antibiotics or antiinfective agents, can be cured.[59] However, control of the chronic and complicated cases may not be greater than 10 percent.[60,61] The bacteriologic count is generally about ten million or more in the patients with chronic infections. Even if they cannot be cured with antimicrobial agents, the bacteriologic count may be lowered to one thousand to ten thousand which often affords symptomatic relief.[62]

Derow has pointed out the danger of prophylactic antimicrobial

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[57] Ibid.

[58] J. K. Kalliomaki and A. Kasanen, "Pyelonephritis," Annales Medicinae Internae Fenniae, 49:48, 1960.

[59] Keefer, op. cit., p. 127.

[60] Ibid., p. 128.

[61] Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:712, 1957.

[62] Ibid.

therapy. It may not prevent infection of the urinary tract but "may be responsible for a greater incidence."[63] Though these agents may temporarily reduce pyuria, at the same time they may be replacing susceptible bacteria with resistant bacteria.[64] Keefer stated that there was a common occurrence of reinfection with drug-resistant strains of bacteria.[65]

III. THE CLEAN-VOIDED URINE SPECIMEN

The feasibility of using the "sterile-voided," mid-stream method of collecting urine combined with some quantitative bacterial count in the screening of either male or female patients for asymptomatic bacteriuria has been demonstrated both in hospitalized patients and clinic out-patients. Since two to three percent of female patients develop infection after urethral catheterizations, such techniques are essential if routine screening cultures are to be performed.[66]

Procedure of Collecting Mid-stream Urine Specimens

In conjunction with an investigation conducted by Dr. Paul Beeson on the pathogenesis of pyelonephritis, Hart and Magee were asked to develop a method of collecting mid-stream urine specimens which would provide safe and satisfactory specimens. After experimenting with

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[63]Harry A. Derow, "Management of Pyelonephritis," New England Journal of Medicine, 255:382, August 23, 1956.

[64]Ibid.

[65]Keefer, loc. cit.

[66]Windom and others, op. cit., p. 14.

several different cleansing methods, a method of collecting the specimens was devised.[67]

When the patient expressed a desire to void, she was placed on a bedpan and draped as for a catheterization. After putting on sterile gloves, the nurse separated the labia so that the urethral meatus was exposed. Two gauze sponges were immersed in Phisohex and were used to cleanse the meatus using single downward strokes, cleansing both chemically and mechanically. Thirty cubic centimeters of normal saline was then poured over the cleansed area. Without permitting the labia to close over the meatus, the nurse instructed the patient to forcibly void a stream of urine. This initial stream of urine cleansed the urethral canal of organisms. The nurse caught the subsequent stream in a sterile container without touching any part of the vulva. The nurse permitted the labia to close after the specimen was obtained. The patient finished voiding into the bedpan or toilet.[68]

In testing this procedure, ten specimens of urine were collected. The bacteriological culture showed no growth in eight of these, but showed growth in two specimens where the pathogens were indicative of an active infection for which the patients were treated.[69]

In further studies, laboratory data were kept on ninety-one specimens gathered from eleven units of the hospital. Thirty-one specimens

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[67]Elizabeth L. Hart and Margaret J. Magee, "Collecting Urine Specimens," American Journal of Nursing, 57:1323, October, 1957.

[68]Ibid.

[69]Ibid., p. 1324.

were collected on the units where the personnel had received formal demonstration and instruction in using the technique for the collection of mid-stream urine specimens. Sixty specimens were taken from patients on units where the personnel were given only oral direction by a physician which included emphasis on cleansing and keeping the labia separated, and avoiding contact of the sterile container with the vulva while collecting the specimen. Where detailed instruction was given, twenty-eight out of thirty-one specimens collected yielded satisfactory results. In those specimens collected after only an explanation of precautions, satisfactory results were found in fifty-one out of sixty cases. Satisfactory results were defined as those specimens which showed no growth on culture.[70]

It was felt that the patient's cooperation is vital to the success of the procedure. Therefore, one of the nurse's major roles is to help the patient overcome the feeling of stress by telling her why each step of the procedure is done.[71]

Dr. H. M. Leather, at Bristol Royal Infirmary, devised a simple method of obtaining mid-stream urine specimens from female patients. This method made use of a glass tube which is approximately one-half inch in diameter and six inches in length, and is bell-shaped at one end.[72]

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[70] Ibid.

[71] Ibid.

[72] Mary T. Hutchings, "Mid-stream Specimens of Urine," Nursing Times, 57:1317, October 13, 1961.

Before the use of the bell-shaped tube for the collection of mid-stream urine specimens, the external genitalia was washed with soap and water and then dried. The labia minora was then separated with the first and second fingers of the left hand to expose the urethral orifice. The area was cleansed thoroughly using cotton balls soaked in a .5 percent solution of Cetavlon and dried carefully with gauze.[73]

After a thorough cleansing of the urethral meatus, the labia minora was separated and the bell-shaped end of the tube was placed gently but firmly over the meatus letting the labia minora close around the tube. The patient then voided through the tube and the mid-stream was caught in a sterile container.[74]

Hutchings carried out a study using the bell-shaped tube to collect mid-stream urine specimens on twenty female patients without renal disease. The results showed that all twenty specimens were sterile. Only sixteen mid-stream urine specimens were found to be sterile after collecting the specimens without the use of the tube. In another study, five student nurses collected urine specimens on twenty-four patients using the bell-shaped tube and all the specimens were sterile on culture.[75]

Hutchings felt that the advantages of the tube method were:

1. Contact of urine with the vulva is avoided and bacterial contamination is eliminated.
2. It is a simpler and cleaner method as the urine stream is controlled.

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[73] Ibid., p. 1318.

[74] Ibid.

[75] Ibid.

3. There is less embarrassment for the patient, as the labia are not held apart during the act of micturition.
4. Specimens are easily obtained during menstration.[76]

Cleansed Versus Uncleansed Mid-stream Urine Specimens

Boshell and Sanford demonstrated the effectiveness of the cleansing procedure "by collecting several specimens for culture without prior cleansing."[77] It was found that all the specimens contained more than ten thousand organisms per milliliter of urine.[78]

Mid-stream urine specimens were taken from fifty young girls on a pediatric service. Different members of the nursing staff collected these specimens without cleansing the genitalia of these girls.[79] Forty-seven of the fifty girls (94 percent) were found to be within the range of one to one hundred thousand organisms per milliliter of urine.[80] Even though Pryles and Steg felt that one hundred thousand colonies per milliliter of urine indicated infection, they stated that "clean-voided specimens are valid only if the patient is prepared before collection of the specimen as carefully as for catheterization."[81]

Reliability of the Mid-stream Urine Specimen

Pryles and Steg conducted another study on a pediatric service with fifty-eight young girls ranging in age from three to twelve years.

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[76] Ibid.

[77] Boshell and Sanford, loc. cit.

[78] Ibid.

[79] Pryles and Steg, op. cit., p. 442.

[80] Ibid., pp. 445-447.

[81] Ibid., p. 451.

Paired catheter and clean-voided urine specimens were collected within one hour of each other. The same graduate nurse collected all the clean-voided specimens. However, other nurses collected the catheterized specimens.[82] The results of this study showed that there was a 96.5 percent positive correlation between the catheterized and clean-voided specimens.[83]

Wood and others collected forty mid-stream urine specimens from antepartum, partum, and puerperal patients. Only six had a positive culture when the paired catheter specimen was sterile. However, five of the contaminated mid-stream specimens were collected in the first six attempts.[84] There were no contaminated mid-stream specimens after the first attempt of each nurse and after the technique had been established.[85] The remaining thirty-four mid-stream specimens were comparable to the specimen taken by catheter with thirty-one showing no abnormalities. These authors concluded that it is possible to obtain a mid-stream specimen comparable to that obtained by the catheter in the majority of female patients.[86]

Kaitz and Williams stated that a single clean-voided specimen containing over one hundred thousand pathogenic bacteria per milliliter will agree with a catheter specimen in about 80 to 90 percent of the

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[82] Ibid., p. 442.

[83] Ibid., p. 451.

[84] E. C. Wood and others, "Mid-stream Urine from Women," British Medical Journal, 1:961, March 26, 1960.

[85] Ibid., p. 962.

[86] Ibid., p. 961.

cases. Clean-voided and catheterized specimens will agree more than 95 percent for two high count clean-voided specimens.[87]

Kass stated that "correlation between bacterial counts over one hundred thousand in clean-voided and in catheterized specimens from the same person is over 95 percent."[88] However, he stated further that before the presence of bacteriuria can be reliably established using the voided specimens, two counts of different voided specimens should be taken from the same person.[89]

Windom and others carried out a study to determine the feasibility of doing sterile-voided urine cultures in a practice limited to internal medicine.[90] A group of one hundred ambulatory, non-indigent female patients were used in this unselected practice.[91] Seventy-eight per cent of these patients were over forty years of age and twelve had a history of urinary tract disease. According to Windom and others, previous studies with hospitalized patients having a similar age distribution were found to have an incidence of asymptomatic bacteriuria in 14 to 15 percent.[92]

The result of this study showed that significant bacteriuria, which was defined as a sterile-voided urine specimen containing greater

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[87]Kaitz and Williams, loc. cit.

[88]Edward H. Kass, "Bacteriuria and the Diagnosis of the Infections of the Urinary Tract," Archives of Internal Medicine, 100:711, 1957.

[89]Ibid.

[90]Windom and others, loc. cit.

[91]Ibid., p. 15.

[92]Ibid., p. 16.

than fifty thousand to one hundred thousand bacteria per milliliter of urine, was demonstrated in only one patient.[93]

It was concluded by Windom and others that the sterile-voided urine collections from female patients combined with a quantitative culture technique, "is as feasible in a medical practice as has been demonstrated in hospitalized patients or in out-patient clinics associated with teaching centers."[94]

III. ANALYSIS OF THE URINE SPECIMEN

In order to satisfy the purposes of this study, the following information was reviewed so that the urine specimens collected would be more valid for culture and so that the proper laboratory procedure could be employed.

Influence of Chemotherapy

According to Dubos, the widespread use of antibiotics and sulfonamides created new problems for the diagnostic laboratory. The specimens taken from patients who had taken one or more of these drugs had very little diagnostic validity with regard to the infective organism.[95]

Kass stated that the bacterial counts of urine may fall below the range that indicates infection even in the presence of pyelonephritis

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[93] Ibid., p. 15.

[94] Ibid., p. 16.

[95] Rene J. Dubos, Bacterial and Mycotic Infections of Man, Philadelphia: J. B. Lippincott Company, 1958, p. 770.

when a bacteriostatic agent is present in the urine.[96]

Sanford and others reported that "the difficulty in determining the significance of organisms found is compounded by antimicrobial therapy which may cause shifts in bacterial flora and the appearance of resistant bacterial strains or species."[97]

Virac as a Cleansing Agent

Iodine, as a germicidal agent, has been known and used with good results for years. Gershenfeld, as stated by Harris and others, cited the merits of iodine as follows:

1. It is bactericidal rather than bacteriostatic.
2. Action is rapid and achieved in comparatively low concentration.
3. Unlike most germicides, iodine is essentially equipotent against all bacteria.
4. In addition to its bactericidal properties, iodine has well-known activity against spores, fungi, and viruses.
5. Its tissue toxicity is comparatively low, the toxicity index being less than that of benzalkonium chloride, mercurie chloride, or phenol.[98]

Frisch and others stated that "iodine exhibits the broadest spectrum against pathogens of any antiseptic known."[99] It has been accepted that iodine has lethal action against "bacilli, cocci, spores,

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[96]Edward H. Kass, "Chemotherapeutic and Antibiotic Drugs in the Management of Infections of the Urinary Tract," American Journal of Medicine, 18:765, May, 1955.

[97]Jay P. Sanford and others, "Evaluation of the 'Positive' Urine Culture," American Journal of Medicine, 20:88, January, 1956.

[98]John E. Harris and others, "The Adaptation of Virac, a New Iodophore, to Clinical Use," Archives of Opthalmology, 60:206, August, 1958.

[99]Arthur W. Frisch and others, "Skin Degerming Agents With Special Reference to a New Cationic Iodophore," Surgery, Gynecology and Obstetrics, 107:445, October, 1958.

spirillae, fungi, yeasts, viruses, rickettsias, protozoa, and helminths."[100]

However, iodine has been said to have certain disadvantages which limits its application in clinical medicine. First iodine stains the skin and is somewhat caustic; second, there has been a fairly high incidence of sensitivity to iodine; and third, it is generally more irritating to the mucous membranes.[101]

On the other hand, associates have indicated that "some of the objectionable properties of iodine have been removed by the preparation of so-called iodophores, or iodine carriers."[102]

Shortly before 1958, Virac, a trade name for a new iodophore, was made available. It is "a complex of elemental iodine and a quaternary ammonium base (N-methylheptylcolaminoformylmethylpuridinium chloride)."[103] The ammonium base in Virac is said to be a strong detergent and its germicidal activity is reported to be superior to benzalkonium chloride (Zephiran).[104] Virac is also superior to hexachlorophene and has been found to be "nine times more efficient as a degerming agent than soap and water."[105]

Virac has the advantages of providing an iodine product "which

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[100]Harris and others, loc. cit.

[101]Ibid.

[102]Ibid.

[103]Ibid.

[104]Ibid.

[105]Frisch and others, loc. cit.

does not stain the skin, has a low iodine vapor pressure, and is not caustic."[106] Furthermore, "studies with Virac thus far have indicated the product to be an efficient germicide and to have the clinical usefulness."[107] Frisch and others have stated "that the iodophore Virac is a promising new germicide."[108]

Residual Effect of Germicide on Bacteria in Urine After Collection

Virac contains the germicidal properties of iodine and quaternary ammonium.[109] "Quaternary ammonium germicides belong to a group of compounds known as 'surface-active' agents or detergents."[110] Several investigators have found that the agar in test media will absorb Quats which leads to erroneous results.[111] Therefore, the increased use of quaternary ammonium in germicides presented a need for a substance which would inactivate this group of compounds. The neutralizing substance needed was one which would (1) inactivate the bactericidal and bacteriostatic properties of the compound for a prolonged period of time, and (2) would have no antibacterial action itself and little

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[106]Harris and others, loc. cit.

[107]Ibid.

[108]Frisch and others, op. cit., p. 446.

[109]Harris and others, loc. cit.

[110]C. A. Lawrence, "Inactivation of the Germicidal Action of Quaternary Ammonium Compounds," Journal of the American Pharmaceutical Association, 37:57, 1948.

[111]George E. Reddish (ed.), Antiseptics, Disinfectants, Fungicides, and Chemical and Physical Sterilization, Philadelphia: Lea and Febiger, 1957, pp. 593-594.

growth-promoting properties upon microorganisms.[112]

Letheen broth has been recommended as a suitable substance which contained an inhibitor for quaternary ammonium compounds and thus give optimal growth of the test organism.[113] The formula for Letheen broth contains beef extract (Difco), peptone (Armour), sodium chloride, lecithin, tween 80, and distilled water. In this formula it is the lecithin which acts as the chief neutralizer "while the tween acts as a solubilizing and dispersing agent for the lecithin."[114]

The germicidal properties of iodine found in Virac are neutralized by several organic and inorganic agents. Among the inorganic substances which have been used are sodium thiosulfate, metallic mercury and ammonia water. Among the organic compounds which neutralize the effects of iodine are serum, glycerin, syrup, feces, ascitic fluid, egg, milk, sputum and urine.[115]

Urine Specimens for Bacteriological Examination

"It was Pasteur who first directed attention to the fact that urine was an excellent culture media for bacteria."[116] Kass also stated that "urine is usually an excellent culture medium for the common path-

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[112]Lawrence, loc. cit.

[113]John A. Kolmer and others, Approved Laboratory Technic, New York: Appleton-Century-Crofts, Inc., 1951, p. 602.

[114]Quisno and others, "A Neutralizing Medium for Evaluating the Germicidal Potency of the Quaternary Ammonium Salts," American Journal of Pharmacy, 118:321, September, 1946.

[115]Reddish, op. cit., p. 229.

[116]Keefer, op. cit., p. 127.

ogens of the urinary tract;[117] and that if urine is kept at room temperature for two hours or longer, there would be a distinct increase in the number of bacteria.[118] Derow and Kass have indicated that urines may be stored at room temperature for one hour, or at refrigerator temperature for forty-eight hours without significant changes in the bacterial population.[119,120] According to Davidsohn and Wells, the refrigeration temperature should be at 4 degrees centigrade until the urine specimen is cultured.[121]

Bacterial Counts

Bailey and Scott felt that in order to evaluate the clinical significance of a positive culture, there must be a means of estimating the number of organisms which may be present in the specimen.[122] Kass and MacDonald and others stated that by the quantitation of the numbers of bacteria in the urine, it was possible to distinguish contamination

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[117]Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:710, 1957.

[118]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:60, 1956.

[119]Harry A. Derow, "Management of Pyelonephritis," New England Journal of Medicine, 255:380, August 23, 1956.

[120]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:60, 1956.

[121]Israel Davidsohn and Benjamin B. Wells, Clinical Diagnosis by Laboratory Methods, Philadelphia: W. B. Saunders Company, 1962, p. 731.

[122]Bailey and Scott, loc. cit.

from true bacilluria.[123,124] According to Jackson and Grieble, Merritt and Sanford, and Riley, the quantitative bacterial counts were a suitable means of detecting significant bacteriuria in female voided urine specimens. Furthermore, these studies recommended that this procedure be used in screening for urinary tract infections.[125,126,127]

As stated by Derow, "the advantages of the quantitative bacterial counts on urine specimens using the agar pour-plate technic was emphasized by Marple in 1940."[128] According to Sanford and others, the quantitative bacterial counts using the agar pour-plate technic offered the advantages of rational management of urinary tract infection, and permitted a more reliable evaluation of therapy.[129]

Smith and Martin described the pour-plate agar technic as follows:

> In pour plates the bacteria are dispersed through the medium when liquid, and the medium then is allowed to cool

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[123]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:63, 1956.

[124]Richard A. MacDonald and others, "Relation Between Pyelonephritis and Bacterial Counts in Urine," New England Journal of Medicine, 256:922, May 16, 1957.

[125]George G. Jackson and others, loc. cit.

[126]Merritt and Sanford, op. cit., p. 463.

[127]Harris D. Riley, Jr., "Evaluation of a Method for Detecting and Following Urinary Tract Infection in Females Without Catheterization, Journal of Laboratory and Clinical Medicine, 52:847, December, 1958.

[128]Harry A. Derow, "Management of Pyelonephritis," New England Journal of Medicine, 255:380, August 23, 1956.

[129]Sanford and others, loc. cit.

> The discrete "colonies" which develop from the single isolated microorganism, are descendants of single organisms.
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> The actual "pouring" of plates usually is preceded by the preparation of three graded dilutions of the material to be examined.
>
> The success of the pour plate method is dependent to a great extent, upon the number of colonies which develop in the plate. If the inoculum contains too many bacteria, the resulting colonies are so minute that differential morphologic characteristics cannot be determined.
>
> The chief advantage of the pour plate is that the effects of the bacterial metabolites in the surrounding medium are much more intense than they are on the surface of the medium.
>
> During incubation the plates are kept inverted to prevent the condensation water, squeezed out of the agar during the hardening process, from collecting on their surfaces and forming channels for the diffusion of bacteria.
>
> If incubated at 37.5 degrees centigrade, the colonies usually develop in eighteen to twenty-four hours.[130]

Kass stated that there were not many limitations in the quantitative approach to the diagnosing of significant bacilluria.[131] However, there were certain instances where the urine showed a bacterial count below the range that was characteristic of infections and these instances were outlined by Kass:

1. If a bacteriostatic agent is present in urine;
2. If the rate of urine flow is rapid, the numbers of bacteria discharged from the kidney small, and pooling of urine in the bladder for a time sufficient to permit multiplication of bacteria to maximal numbers has not occurred;
3. If the microorganism involved is fastidious in its

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[130]David T. Smith and Donald S. Martin, Zinsser's Textbook of Bacteriology, New York: Appleton-Century-Crofts, Inc., 1948, p. 925.

[131]Edward H. Kass, "Chemotherapeutic and Antibiotic Drugs in the Management of Infections of the Urinary Tract," American Journal of Medicine, 18:765, May, 1955.

growth requirements and grows poorly in urine (this has been a rare occurrence, and thus far has been limited to organisms not commonly found in the urinary tract);

4. If there is obstruction of the ureter, thus interfering with the discharge of bacteria into the bladder;
5. If the infection is limited to areas of the kidney not directly accessible to renal tubules.[132]

V. SUMMARY

Literature was reviewed in three areas: infections of the urinary tract, clean-voided urine specimens, and analysis of the urine specimen.

It was found that urinary tract infections occur in frequency second only to infections of the respiratory tract. Though information on the incidence, pathogenesis, and natural history of these infections is incomplete, it is known that bacteria may enter the urinary tract by more than one route. Catheterization as a source of urinary tract infection was the route of infection considered for the purpose of this study. The organisms present most often in acute urinary tract infections are Escherichia coli and Staphylococcus aureus. In the chronic and complicated infections, the percentages of the staphylococci, enterococci, and members of the genera Proteus, Aerobacter, and Pseudomonas increase. Most authors agreed that the point at which true bacteriuria could be distinguished from contamination was if the number of organisms found in the urine specimen exceeded 100,000 bacterial colonies per milliliter of urine. Asymptomatic bacteriuria is a disease

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[132]Edward H. Kass, "Asymptomatic Infections of the Urinary Tract," Transactions of the Association of American Physicians, 69:62-63, 1956.

state that plays a key role in the pathogenesis of pyelonephritis and is a condition where 100,000 colonies of bacteria per milliliter of urine is found in aseptically collected urine from someone without overt signs or symptoms of urinary tract infections. The frequency in the occurrence of asymptomatic bacteriuria in the population at large is great enough to be an important problem in public health and in preventive medicine. Literature revealed that there are factors which contribute to urinary tract infections. Evidence has shown that some clinical states contribute to infections of the urinary tract. Also it has been found that women have a higher incidence of bacteriuria. Though infections of the urinary tract occur at all ages, it was felt that there are three major peaks of incidence: (1) in infancy and childhood, (2) during the child-bearing period, and (3) in the elderly. While 90 percent of the acute uncomplicated urinary tract infections may be cured with antibiotics or antiinfective agents, only about 10 percent of the chronic and complicated cases can be cured.

Several methods of collecting clean-voided urine specimens were revealed to be a reliable method of screening female patients for bacteriuria. The clean-voided method has the advantage over catheterization in that an instrument carrying bacteria is not introduced into the urinary tract. The correlation between bacterial counts over one hundred thousand per milliliter of urine in clean-voided and in catheterized specimens from the same person has been found to be over 95 percent.

The patient receiving antibiotics or sulfonamides may cause shifts in the bacterial flora. Therefore, the analysis of the urine

specimen would have very little diagnostic validity with regard to the infective organism. Virac as a germicide is considered superior to benzalkonium chloride, hexachloraphene, and nine times more efficient than soap and water. Though the iodine in Virac is neutralized by the presence of urine, the quaternary ammonium present has been found by investigators to be absorbed by the agar in text media which leads to erroneous results. Letheen broth was recommended as a suitable substance which inhibited the quaternary compounds to give optimal growth of the test organism. Because urine is an excellent culture medium for the common pathogens of the urinary tract, urine specimens should not be kept at room temperature for more than one hour. However, it may be kept forty-eight hours at refrigerated temperature of 4 degrees centigrade without significant changes in the bacterial population. The quantitative bacterial count using the agar pour-plate technic permits an evaluation of the clinical significance of the positive culture.

CHAPTER III

METHOD OF STUDY

The study was conducted to find the difference in the number of organisms in two clean-voided urine specimens. One specimen was collected from women whose genitalia had not been cleansed and compared with a clean-voided urine specimen collected from the same women after the genitalia had been cleansed.

I. METHOD OF APPROACH

The experimental method was used for this study. Greenwood interprets this method to mean "the proof (test) of an hypothesis which seeks to hook up two factors into a causal relationship through the study of contrasting situations."[1] The major purpose of the experimental method given by Rummel "is to describe the effect of certain treatments upon some characteristic of a group or population and to test some hypothesis about this effect."[2]

Literature reviewed in the areas of infections of the urinary tract, methods of collecting clean-voided urine specimens, and procedures in analyzing the specimens served as background for developing the tool and assisted in interpretation of the data. There were no studies found in which organisms were compared in urine specimens taken

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[1]Good and Skates, loc. cit.

[2]Rummel, loc. cit.

from the same group of women before their genitalia had been cleansed and after it had been cleansed.

The study was conducted in the laboratory of a selected hospital. Sophomore nursing students from the school of nursing of this same hospital were chosen as the population. Permission was granted to conduct the study from the hospital administrator, director of the school of nursing and the head of the laboratory facilities.

Selection of Subjects

A population was needed with subjects who were free of bacteriuria. A subject with bacteriuria would have a great number of organisms making it difficult to count the number correctly in many cases. For the purpose of the study it was necessary to be able to count the organisms. Otherwise, a comparison of organisms could not be made in the two urine specimens taken from each woman. Also it was necessary that this population was not taking antibiotics which would contribute to a reduction in the number of organisms. An available population that met these requirements was a group of nursing students who were beginning their first quarter at the selected school of nursing.

In order to gain cooperation of the group, they were told the reason for the study and what would be expected of them during their participation. They were assured privacy during participation and that detailed instructions would be given to them at the time they gave their urine specimen.

Of this class of thirty-five students, one was a male. Of the remaining thirty-four female students, three were deleted after telling

the group that those who knew that they had a urinary tract infection or who had taken antibiotics within six weeks prior to the collection of the urine specimens could not be included in the study. Two specimens were taken from five students for the pilot study and the remaining twenty-six students each gave the two specimens necessary for the study. These specimens for the study were collected over a period of three weeks. Three groups of these students met according to their availability at the time of collection.

Selection and Development of the Procedure

So that all clean-voided urine specimens collected could be considered reliable, there was a need to be consistent in collecting the specimens. Therefore, a standard procedure was developed. After consulting with a microbiologist, it was thought that the procedure established should be practical for clinical purposes. It was therefore decided that each subject would collect her own specimen preceded by instruction from the researcher. A pilot study was done to evaluate the procedure.

Pilot Study

A pilot study was conducted using five subjects. An uncleansed and cleansed mid-stream urine specimen was obtained from each of these subjects in order to evaluate the procedure as to its practicality and adequacy for the purpose of the study. The specimens were collected in the mid-day.

When each subject arrived in the laboratory to give the uncleansed specimen she was told that a mid-stream urine specimen was needed. This

was explained to her by telling her that she was to void approximately one-third of the urine in her bladder. Then she was told to stop the flow of urine and take about one-third of the urine in the bladder for the specimen and discard the remaining one-third into the toilet. The subject was shown a diagram of the female genitalia. This was to familiarize her with the relationship of the urethra to the vagina and the necessity of holding the labia minora, as well as the labia majora, away from the urethra. She was then shown and told how to use the first and second fingers to hold back these structures before giving the specimen. After an opportunity was given to clarify any questions, the subject was shown how to hold the sterile specimen bottle so as not to touch the top of the bottle to her body in order to not contaminate the specimen. The subject was then shown to the lavatory facilities where the researcher opened the specimen bottle. Again opportunity was given for clarification.

On the following day when the subject returned to give the cleansed specimen, the above instruction was repeated. The subject was told that the only difference in collecting this specimen and the previous one was that the genitalia would be cleansed. After showing the subject to the lavatory, the researcher poured the germicide Virac into a sterile medicine glass which contained three sterile cotton balls. The subject was then told that after holding the labia apart with the first and second fingers, she was to take the first cotton ball and cleanse once from front to back on one side of the urethral meatus. The second cotton ball was to be used in cleansing the opposite side, and the third was to cleanse the center area passing the cotton ball

directly over the urethral meatus. The subject was told that the cotton ball was to be discarded into the toilet after passing over each area once, from front to back. The subject was told that after completing the cleansing, she was ready to void approximately one-third of the urine in her bladder. Then after stopping the urine stream she was to collect the middle one-third of urine for the specimen and discard the remaining one-third of urine. Opportunity was then given for clarification.

Each specimen was carefully capped and refrigerated immediately to prevent multiplication of bacteria. Agar pour-plates were made of each specimen on the same afternoon as collected. Three pour-plates in dilutions of 1:100, 1:1,000, and 1:10,000 were made on each specimen and were incubated at 37 degrees centigrade for forty-eight hours. This was done in order to determine the number of organisms in each specimen. The organisms were counted with the use of the Quebec colony counter by the investigator.

After completing the pilot study, it was felt that there were three changes that needed to be made in the procedure before the data for the study were collected. First there was the addition of hand-washing with soap and water before the specimen was taken. It was thought that cleansed hands were less likely to introduce bacteria into the specimen if the fingers accidently came in contact with the urine stream. Secondly, another clean-voided method of collecting urine specimens was substituted for the pilot mid-stream method. The procedure for the clean-voided method chosen was one in which the subject was told to void approximately one-half of the urine in the bladder

into the toilet and to void the remaining urine into the specimen bottle. For the purpose of the study, it was felt that this change in the method of collecting a urine specimen simplified the procedure. Also the subjects expressed difficulty with the mid-stream method. They felt it was difficult to estimate the first third of urine for ejection, then stop the urine stream and give the specimen, then stop a second time and still have enough urine left in the bladder for a third ejection of urine to be discarded. Furthermore, it was not felt necessary to collect mid-stream urine specimens in order to obtain a suitable clean-voided specimen. Kass has pointed out that it has been generally accepted that the urinary tract is normally free of bacteria and that true bacteriuria is a pathologic finding.[3] He defined contamination as "the adventitious entry of bacteria into the urine during the collection of the specimen."[4] It was further suggested that contamination of the specimen may take place as the urine passes through the urethra or surrounding structures.[5] Therefore, the microbiologist agreed that the clean-voided method chosen was more practical for the purpose of the study. Third, sterile Letheen broth was used in preparing all dilutions before culturing to neutralize the quaternary ammonium which might be present from the cleansing procedure. It was felt that there was a possibility that some of the cleansing agent may have been intro-

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[3]Edward H. Kass, "Bacteriuria and the Pathogenesis of Pyelonephritis," Laboratory Investigation, 9:111, 1960.

[4]Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:709, 1957.

[5]Edward H. Kass, "Bacteriuria and the Pathogenesis of Pyelonephritis," Laboratory Investigation, 9:111, 1960.

duced into the specimen as the cleansed urine specimen was collected directly after the cleansing of the genitalia had taken place. The need for neutralizing the quaternary ammonium can be found in Chapter II. The data obtained from the pilot study were not included in the study.

Collection of Data

All specimens for the study were collected in the mid-day. The uncleansed specimen was taken first from each subject followed by the cleansed specimen taken the following day. The reason for taking the uncleansed specimen first was because of the possibility that some of the subjects might not have cleansed their genitalia for several days. By eliminating the advantage of cleansing the day before, the uncleansed specimen could be considered more reliable.

When each subject arrived in the laboratory to give the uncleansed specimen she was told that a clean-voided specimen was needed. This was explained to her by telling her that she was to void about half of what she had in her bladder into the toilet and to void the remaining urine into the specimen bottle. The subject was then shown a diagram of the female genitalia. This was to familiarize her with the relationship of the urethra to the vagina and the necessity of holding the labia minora, as well as the labia majora, away from the urethra. She was then shown how to use the first and second fingers to hold back these structures before giving the specimen. After an opportunity was given to clarify any questions, the subject was told how to hold the sterile specimen bottle so as not to touch the top of the bottle to her body and cause

contamination of the specimen. The subject was then shown to the lavatory facilities where the researcher opened the specimen bottle. Again opportunity was given for clarification. The subject was instructed to wash her hands before proceding.

On the following day when the subject returned to give the cleansed specimen, the above instruction was repeated. The subject was told that the only difference in collecting this specimen and the previous one was that the genitalia would be cleansed. After showing the subject to the lavatory, the researcher prepared the germicide to be used. This consisted of a sterile medicine glass which contained three sterile cotton balls. The germicide was poured into the glass saturating the cotton balls. The subject was then told that after holding her labia apart with the first and second fingers, she was to take the first cotton ball and cleanse once from front to back on one side of the urethral meatus. The second cotton ball was to be used in cleansing the opposite side, and the third was to cleanse the center area passing the cotton ball directly over the urethral meatus. The subject was told that the cotton ball was to be discarded into the toilet after passing the cotton ball over each area once from front to back. After completing the cleansing, she was ready to void approximately half of the urine in her bladder into the toilet and catch the remaining part into the sterile container. After given opportunity for clarification, the subject was instructed to wash her hands before proceding.

Virac was the agent used to cleanse the genitalia. It has the germicidal properties of iodine and quaternary ammonium.[8] When quater-

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[8]Harris and others, loc. cit.

nary ammonium is introduced on the agar in the test media, it is absorbed to produce erroneous results.[9] Therefore, sterile Letheen broth was used in the specimen which permitted optimal growth of the test organism by inhibiting the action of the quaternary ammonium.[10] The germicidal properties of iodine was neutralized by the presence of urine.[11] Therefore, immediately after the cleansed urine specimen was taken, a sterile pipette was used by the researcher to transfer one centimeter of urine into nine centimeters of sterile Letheen broth making a dilution of 1:10. From this 1:10 dilution, the 1:100, 1:1,000, and 1:10,000 dilutions were made and incubated on the agar pour-plates.

Each of the uncleansed and cleansed urine specimens was carefully capped and refrigerated immediately following its collection in order to prevent multiplication of bacteria. Agar pour-plates were made of each specimen on the same afternoon as collected. Three pour-plates in dilutions of 1:100, 1:1,000, and 1:10,000 were made on each specimen and were incubated at 37 degrees centigrade for forty-eight hours. This was done in order to determine the number of organisms in each specimen. The organisms were counted with the use of the Quebec colony counter by the researcher.

II. SUMMARY

The experimental method was used to find the difference in the

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[9]Reddish, op. cit., pp. 593, 594.

[10]Kolmer and others, loc. cit.

[11]Reddish, op. cit., p. 229.

number of organisms in two clean-voided urine specimens taken from the same woman. The population was twenty-six nursing students who were free of bacteriuria and who had not taken antibiotics within six weeks prior to the collection of the urine specimens. An uncleansed clean-voided specimen was taken from each woman followed by a cleansed clean-voided specimen taken the following day where the genitalia had been cleansed with a germicidal substance. Each specimen was carefully capped and refrigerated immediately after collection. Agar pour-plates were made on each specimen in three dilutions and incubated at 37 degrees centigrade for forty-eight hours. The organisms were counted in order to determine the number of organisms in each specimen.

CHAPTER IV

ANALYSIS AND INTERPRETATION OF DATA

The purpose of this chapter is to show the analysis and interpretation of the data obtained by taking two urine specimens from the same woman. Twenty-six women were used for the study with an uncleansed and cleansed clean-voided urine specimen being taken from each subject. With the assistance of a statistician the data was interpreted with the use of the median test.

I. ANALYSIS AND INTERPRETATION OF THE DIFFERENCE IN THE NUMBER OF ORGANISMS IN TWO CLEAN-VOIDED SPECIMENS

Statistical Analysis and Interpretation of the Data

Table I shows the number of organisms found in the urine specimen of each subject in the uncleansed clean-voided specimen. The number of organisms found in the cleansed clean-voided specimens are found in Table II. Both tables show the number of organisms in each dilution of urine. In order to determine the number of organisms in the specimen, an average count was determined from the three dilutions, except in the cases where the organisms were too numerous to count (TNC) on certain plates. In the specimens where the organisms on one plate were felt to be too numerous so that a correct count could not be made, an average was made in the two remaining dilutions. However, there were two cases among the uncleansed specimens where it was possible to count the organisms only in the 1:10,000 dilutions, making it necessary to accept this count as the probable number of organisms found in each. In the

TABLE I

ORGANISMS PER MILLILITER OF URINE TAKEN FROM WOMEN WHOSE GENITALIA HAD NOT BEEN CLEANSED

Subjects	Dilution 1:100	Dilution 1:1,000	Dilution 1:10,000	Total Average of Dilutions to Nearest One Hundred
1	TNC	17,000	0	8,500
2	0	11,000	0	3,700
3	400	0	0	100
4	16,400	22,000	30,000	22,800
5	0	0	0	0
6	24,700	16,000	20,000	20,200
7	16,000	19,000	20,000	18,300
8	3,900	8,000	40,000	17,300
9	200	0	0	100
10	TNC	16,000	40,000	28,000
11	1,900	1,000	0	1,000
12	400	2,000	0	800
13	TNC	TNC	450,000	450,000
14	15,700	17,000	10,000	14,200
15	43,500	23,000	30,000	32,200
16	40,100	52,000	80,000	57,400
17	TNC	TNC	540,000	540,000
18	25,200	20,000	60,000	35,100
19	200	7,000	0	2,400
20	13,600	57,000	40,000	36,900
21	300	0	0	100
22	TNC	74,000	90,000	82,000
23	11,400	9,000	10,000	10,100
24	700	5,000	40,000	15,200
25	3,700	4,000	0	2,600
26	26,000	23,000	0	16,300
Total number of organisms				1,415,300

TNC - Too Numerous to Count

TABLE II

ORGANISMS PER MILLILITER OF URINE TAKEN FROM WOMEN WHOSE GENITALIA HAD BEEN CLEANSED

Subjects	Dilution 1:100	Dilution 1:1,000	Dilution 1:10,000	Total Average of Dilutions to Nearest One Hundred
1	100	1,000	0	400
2	100	1,000	0	400
3	0	5,000	0	1,700
4	3,400	2,000	10,000	5,100
5	200	0	0	100
6	20,700	20,000	20,000	20,200
7	57,700	55,000	40,000	50,900
8	200	1,000	0	400
9	0	1,000	0	300
10	0	3,000	0	1,000
11	2,000	2,000	0	1,300
12	0	0	0	0
13	15,700	7,000	10,000	10,900
14	0	1,000	0	300
15	1,400	0	20,000	7,100
16	400	0	0	100
17	TNC	484,000	690,000	587,000
18	300	0	10,000	3,400
19	4,200	2,000	0	2,100
20	6,800	1,000	0	2,600
21	0	0	0	0
22	7,400	7,000	20,000	11,500
23	7,200	11,000	10,000	9,400
24	TNC	59,000	60,000	59,500
25	65,400	58,000	90,000	71,100
26	3,700	0	30,000	11,200
Total number of organisms				858,000

TNC - Too Numerous to Count

uncleansed clean-voided specimens from the twenty-six women, a mean of 54,438 organisms per milliliter of urine was found. From the twenty-six samples of cleansed clean-voided specimens, a mean of 33,000 organisms per milliliter of urine was found. The mean of the organisms from both procedures were well below the 100,000 organisms per milliliter of urine designated by investigators as the point at which an infection of the urinary tract may be suspected. Therefore, for the purpose of being practical and for this study, the difference in the two means of 21,438 organisms per milliliter of urine was considered negligible. However, this difference in the two means was not considered as evidence to substantiate a null hypothesis.

In order to show the significant difference in the uncleansed and cleansed clean-voided specimens taken from twenty-six women, the median test using the chi-square test of significance was done. This method was used to test the data since a non-parametric test was indicated because the samples did not show a symmetrical curve as in a normal curve. The level used to denote significance was 3.841 at the .05 level. This level indicates that due to chance there is a 95 percent probability that the results would show as great a difference if the experiment was repeated. Therefore, the null hypothesis would be accepted concluding that there was no difference in the number of organisms in the clean-voided urine specimens collected by the two methods. The results of the test were found to be 3.769. Since the results were only slightly below the .05 level which was the chosen level of significance, it was decided with the assistance of a statistician that the null hypothesis could be accepted on a 95 percent probability that by

chance the results would show as great a difference if the experiment was repeated. At the same time, because the results were slightly below the .05 level, they were not considered conclusive evidence to support the hypothesis of this experiment.

Two subjects were found to have over 100,000 organisms per milliliter of urine in their specimens which indicated a possible urinary tract infection. One subject was found to have over 500,000 organisms per milliliter of urine in both the uncleansed and cleansed specimens. After an examination by her physician, she was diagnosed and treated for an infection of the urinary tract. The other subject was found to have 450,000 organisms per milliliter of urine in the uncleansed urine specimen but only had 10,900 organisms per milliliter of urine in the cleansed urine specimen. It was felt that the cleansing of the genitalia would not have made that great a difference in the number of organisms in the two specimens if the first procedure had been done correctly. Therefore, the number of organisms found in the uncleansed specimen were contributed to either inexperience or a lack of understanding on the part of the subject in the collection procedure as the uncleansed specimen was collected before the cleansed specimen. The results of this subject's specimens substantiate the statement made by Kass that before the presence of bacteriuria can be reliably established using voided specimens, the counts of two different voided specimens should be taken from the same person[1] (preferably without therapy).

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[1]Edward H. Kass, "Bacteriuria and the Diagnosis of Infections of the Urinary Tract," Archives of Internal Medicine, 100:711, 1957.

Of the twenty-six clean-voided specimens, there were seventeen specimens in which the genitalia had not been cleansed that had a higher organism count per milliliter of urine than the specimen in which the genitalia had been cleansed. However, there were eight specimens in which the genitalia had been cleansed where the organism count per milliliter of urine was greater. The organism count of one subject was the same in both specimens.

Possible Factors Influencing the Samples

It was felt that there were factors which may have altered the outcome of the study. These factors were: (1) the samples were obtained from a select group, (2) all uncleansed specimens were taken as the first sample, (3) the cleansed specimen was taken after the subject had become acquainted with the clean-voided method of collecting urine specimens the previous day by giving the uncleansed specimen, and (4) each subject was allowed to perform the procedure in collecting her own urine specimen.

II. SUMMARY

Data were obtained by taking an uncleansed and cleansed clean-voided urine specimen from twenty-six women. In analyzing and interpreting the data, an average of the three dilutions was made on each specimen and was accepted as the number of organisms in each specimen. Dilutions in which the organisms were felt to be too numerous to count (TNC) were not included in the average. For the group of uncleansed specimens, the mean was 54,438 organisms per milliliter of urine. In the group of cleansed specimens, there was a mean of 33,000 organisms

per milliliter of urine. Though the difference of 21,458 organisms per milliliter of urine in the two means was felt to be negligible for the purpose of this study, this difference was not considered as evidence to substantiate a null hypothesis. In order to show the significant difference in the two methods of collecting urine specimens, the median test using the chi-square test of significance was done. The level used to denote significance was 3.841 at the .05 level. The results of the test were found to be 3.769. Since the results were only slightly below the level of significance, the null hypothesis was accepted on a 95 percent probability that by chance the results would show as great a difference if the experiment was repeated. However, since the results were below the .05 level, they could not be considered as conclusive.

It was found that one subject had a count of over 500,000 organisms per milliliter of urine in both the uncleansed and cleansed specimens and was later diagnosed and treated for an infection of the urinary tract. A second subject had 450,000 organisms per milliliter of urine in the uncleansed specimen but was found to have only 10,900 organisms per milliliter of urine in the cleansed urine specimen.

Of the twenty-six clean-voided specimens, there were seventeen specimens in which the genitalia had not been cleansed that had a higher organism count per milliliter of urine than the specimen in which the genitalia had been cleansed. However, there were eight specimens in which the genitalia had been cleansed where the organism count per milliliter of urine was greater. The organism count of one subject was the same in both specimens.

Factors which may have altered the outcome of the study were:

(1) the samples were obtained from a select group, (2) all uncleansed specimens were taken as the first sample, (3) the cleansed specimen was taken after the subject had become acquainted with the clean-voided method of collecting urine specimens the previous day by giving the uncleansed specimen, and (4) each subject was allowed to perform the procedure in collecting her own urine specimens.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

I. SUMMARY

Catheterization, as a method of collecting urine specimens, contributes to urinary tract infections. Therefore, the clean-voided method has been considered a more suitable means of collecting urine specimens. The purpose of this study was to find out if there was a need to cleanse the genitalia of women before collecting a clean-voided urine specimen. It was believed that finding the difference between the number of organisms in uncleansed and cleansed clean-voided urine specimens would assist in devising a simplified nursing procedure for the collection of clean-voided urine specimens.

A review of literature revealed that urinary tract infections occur in frequency second only to infections of the respiratory tract. It is known that bacteria may enter the urinary tract by more than one route. The organisms found most often in acute urinary tract infections are Escherichia coli and Staphylococcus aureus. In the chronic and complicated infections, staphylococci, enterococci, and members of the genera Proteus, Aerobacter, and Pseudomonas are found to be present in increasing numbers. Most authors agreed that when more than 100,000 colonies of bacteria per milliliter of urine was found in the urine specimen, it was indicative of a urinary tract infection or true bacteriuria. When more than 100,000 colonies of bacteria per milliliter of urine was found in the urine specimen without overt signs or symptoms,

the subject was said to have asymptomatic bacteriuria. The frequency in the occurence of asymptomatic bacteriuria in the population at large is great enough to be an important problem in public health and in preventive medicine. According to several authors, the clean-voided urine specimen was revealed to be reliable in screening women for urinary tract infections. At the same time, the clean-voided method was found to have the advantage over catheterizations in that bacteria are not introduced into the bladder. The quantitative bacterial count using the agar pour-plate technic permits a reliable evaluation of the clinical significance of the positive culture.

The experimental method was used to carry out the study. Twenty-six female nursing students from a selected school of nursing were the population. An uncleansed clean-voided urine specimen was taken from each woman followed by a clean-voided urine specimen taken the following day where the genitalia had been cleansed with a germicidal substance. Agar pour-plates were made on each specimen in three dilutions and incubated at 37 degrees centigrade for forty-eight hours. In order to determine the number of organisms in each dilution of urine, the organisms were counted with the use of the Quebec colony counter.

In the twenty-six uncleansed urine specimens, there was a mean of 54,438 organisms per milliliter of urine. The twenty-six cleansed urine specimens had a mean of 33,000 organisms per milliliter of urine. The median test using the chi-square test of significance was done. The level used to denote significance was 3.841 at the .05 level. The results of the test were found to be 3.769. Even though the results were slightly below the level of significance, the null hypothesis was

accepted on a 95 percent probability that by chance the results would show as great a difference if the experiment was repeated. However, since the results were slightly below the .05 level, they could not be considered as conclusive.

One subject was found to have a count of over 500,000 organisms per milliliter of urine in both urine specimens. She was later diagnosed and treated for an infection of the urinary tract. A second subject was found to have 450,000 organisms per milliliter of urine in the uncleansed specimen but was found to have only 10,900 organisms per milliliter of urine in the cleansed urine specimen.

Of the twenty-six clean-voided specimens, there were seventeen specimens in which the genitalia had not been cleansed that had a higher organism count per milliliter of urine than the specimen in which the genitalia had been cleansed. However, there were eight specimens in which the genitalia had been cleansed where the organism count per milliliter of urine was greater. The organism count of one subject was the same in both specimens.

Factors which may have altered the outcome of the study were: (1) the samples were obtained from a select group, (2) all uncleansed specimens were taken as the first sample, (3) the cleansed specimen was taken after the subject had become acquainted with the clean-voided method of collecting urine specimens the previous day be giving the uncleansed specimen, and (4) each subject was allowed to perform the procedure in collecting her own urine specimens.

II. CONCLUSIONS

Within the limits of this study, the clean-voided urine specimen collected from a woman whose genitalia had not been cleansed can be considered as reliable as the urine specimen collected from the same woman after the genitalia had been cleansed. It may also be concluded that the instructions given by the researcher were adequate and that the first ejection of urine was sufficient to wash away the majority of organisms from around the urethral meatus.

III. RECOMMENDATIONS

Recommendations based on the findings of this study were:

1. That a larger sample be used for a similar study.
2. That a similar study be done using a cross-section of clinic patients.
3. That a similar study be done with the cleansed specimen being collected first.
4. That a similar study be done with the researcher collecting both specimens by performing the procedure.
5. That a similar study be done using pictures to illustrate the instructions in carrying out the procedure in place of verbal instructions by the researcher.

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LOMA LINDA UNIVERSITY

Graduate School

TWO METHODS OF COLLECTING CLEAN-VOIDED URINE SPECIMENS

by

Jo Helen Butner

An Abstract of a Thesis

in Partial Fulfillment of the Requirements

for the Degree Master of Science

in the Field of Nursing

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ABSTRACT

The purpose of this study was to find out if there was a need to cleanse the genitalia in women before obtaining a clean-voided urine specimen.

The experimental method was chosen as the method of study. Two clean-voided urine specimens were obtained from twenty-six nursing students in a selected school of nursing. One clean-voided urine specimen was obtained from each subject where the genitalia had not been cleansed and compared with a second clean-voided urine specimen obtained the following day from the same subject where the genitalia had been cleansed.

It was found that there was a mean of 54,438 organisms per milliliter of urine for the group of samples where the subjects had not cleansed the genitalia. From the group of samples where the subjects had cleansed their genitalia, there was found to be a mean of 33,000 organisms per milliliter of urine. In order to show the significant difference in the two groups of samples, the median test using the chi-square test of significance was done. The results of the test were found to be 3.769 which is slightly below 3.841, the .05 level which was selected to denote significance. Since the results were only slightly below the .05 level, it was decided that the null hypothesis could be accepted on a 95 percent probability that by chance the results would show as great a difference if the experiment was repeated. However, because the results were slightly below the .05 level, they were not considered conclusive evidence to support the hypothesis of

the experiment.

One subject was found to have a urinary tract infection having a count of over 500,000 organisms per milliliter of urine in both samples. A second subject was found to have 450,000 organisms per milliliter of urine in the uncleansed clean-voided urine specimen but only 10,900 organisms per milliliter of urine in the clean-voided urine specimen where the genitalia had been cleansed. Because the uncleansed specimen was obtained first, the results from the second subject were attributed to either inexperience or a lack of understanding on the part of the subject as to the collection procedure.

Of the twenty-six clean-voided specimens, there were seventeen specimens in which the genitalia had not been cleansed that had a higher organism count per milliliter of urine than the specimen in which the genitalia had been cleansed. However, there were eight specimens in which the genitalia had been cleansed where the organism count per milliliter of urine was greater. The organism count of one subject was the same in both specimens.

Within the limitations of this study, it was concluded that a clean-voided urine specimen taken from a woman where the genitalia had not been cleansed can be considered as reliable as the clean-voided urine specimen taken where the genitalia had been cleansed when such a specimen is secured under similar circumstances as those used in the study.