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Bacteriological Survey of Urinary Tract Infection ✿

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Difficulties in the diagnosis of infections of the urinary tract are common, and it is important that satisfactory criteria be established that may prove helpful to the clinician in making such diagnosis. It is well established that urether harbours some pathogenic organisms in its normal flora and these are grown on culturing urine specimens. Therefore the more presence of any given pathogenic bacteria in urine is not an adequate basis for ruling it in or out as a cause of clinical urologic disease. The bacterial count of urine has offered one means of resolving this problem (2,4). Thus quantitative study of the baccterial flora of freshly obtained urine, as an added tool to separate true bacteriuria from contamination that occurs during the collection process

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has received much attention during recent years and the technique of bacterial count is widely practiced in diagnostic laboratories in most European and American countries (6,7).

This concept is quite new in Iran and the number of la oratories performing colonial count during a routine examination of urine are indeed very scarce. Therefore an attempt will be made in this study to introduce this technique and to elucidate the necessity of bacterial count during the examination of urine and also to indicate relationship of quantitative measurement of microbial flora of urine in health and disease among general population under the hygenic conditions and customs prevalent in this country.

Further, etiological agents responsible for urinary tract infection among 801 specimens examined are identified and the antibiotic sensitivity of these are determined so that physicians and surgeons could have a general idea of the present day relation existing between the most important bacteria causing infection of urinary tract and different therapeutic drugs used to eradicate them.

Materials and methods

1251 specimens of urine were examined during the survey. These were obtained from two different sources. One group including 801 specimens came from the general population both adults and children who were referred by the physicians in town to the diagnostic laboratory of the Institute of Public Health Research. They all apparently had some clinicel evidence of infection of urinary tract. Second group included 450 specimens from healthy soldiers of Saltanat Abad barracks. These urines were examined to determine the frequency of pus cells in the wet mount, the existence of pathogenic bacteria and the colonial count in each milliliter of noncentrifuged urine.

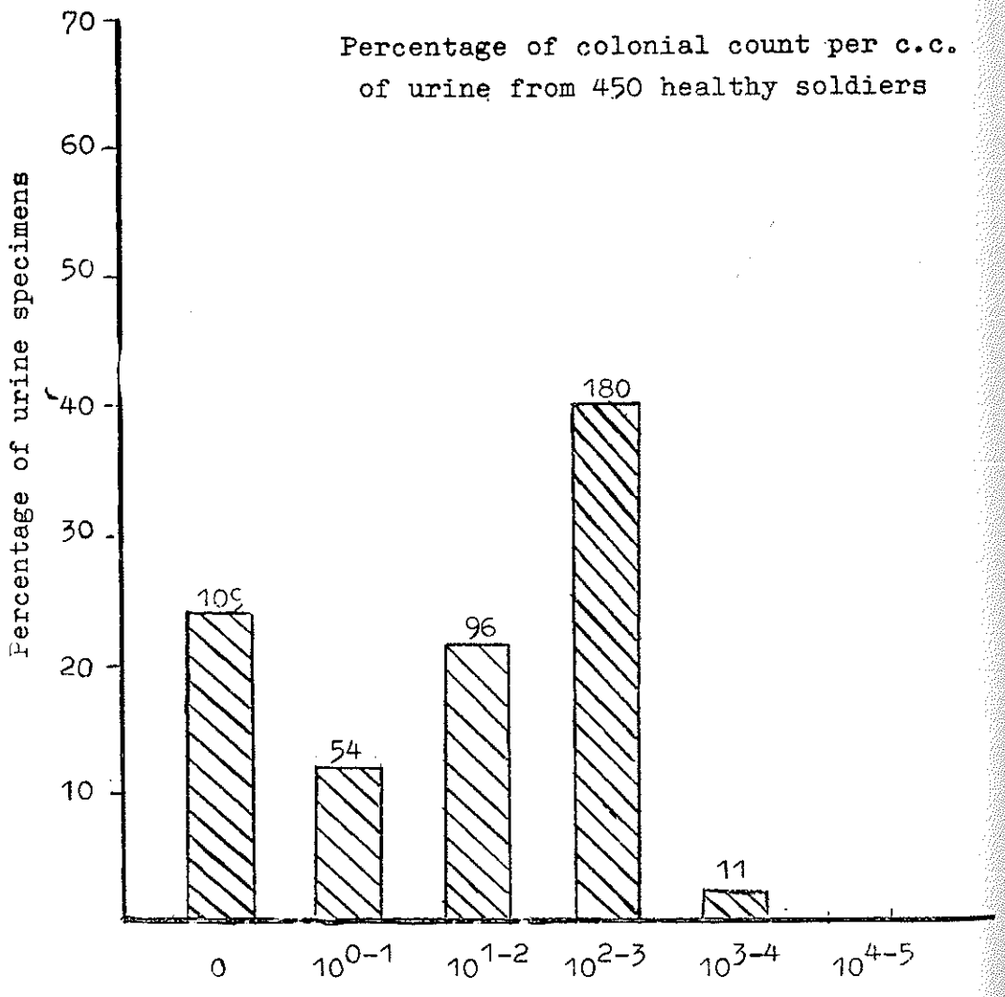
To show the importance of time for obtaining a true colonial count, the number of bacteria in each milliliter of bacteria in each milliliter of 112 urine specimens of the same soldiers were determined twice. One count was made immediately after the delivery and the second one was determined after the urines had remained in the laboratory (32° C) for six hours.

Clean voided specimen: The perinium and vulva in the females, and the prepuce and glans penis in males were washed with hexocholorophene soap and then properly cleaned with water and the urine was obtained by midstream voided technique. Morning specimens were requested when possible. Most of the specimens were given at the Institute and were examined within half hour of delivery. Urines were collected in sterile flasks. Fifteen c.c. of this was transferred to sterile centrifuge tube and centrifuged at 2000 RPM for ten minutes. The supernatant was poured off and the sediment was cultured on Endo and blood agar plates. From the same sediment a wet mount was made and examined under the high power objective. Using the sterile uncentrifuged urine, 0:2 ml was transferred to a test tube containing 1.8 ml of strile broth. This was similarly diluted ten fold to another test tube. Starting from the more diluted, O.1 ml was taken from each test tube and placed into two strile petri dishes marked $\overline{10}^{-1}$ and $\overline{10}^{-2}$ indicating the dilution factor of each. 10 cc of melted nutrient agar which was cooled to 45° C was poured on each plate and urine and agar were mixed by rotary agitation and then left to solidify (8). To calculate the number of organisms per milliliter of urine the colonies were counted in both plates and the figures were multiplied by 100 and 1000 respectively. All plates were incubated at 37° C over_night and the bacteria grown were identified using standard biochemical reactions and cultural characteristics. The antibiotic sensitivity test was performed by spreading 4 - 5 isolated colonies on a blood agar plate, using a bent glass rod. The antibiotic disks from BBL were used and the results were usually readable after 6_7 hours of incubation at 37° C.

Results:

Fig. I

Percentage of colonial count per c.c. of urine from 450 healthy soldiers



We intend to use colonial count to seperate bacterium

From the contamination that occurs during the collection process. A test of this view, however, requires the study of a population group that is considered to be free of infection of urinary tract. From Fig I, we can see that none of the 450 healthy soldiers had 10,000 bacteria per milliliter of iheir urine. The highest number of bacteria obtained per milliliter of urine was 9600. Only 2.4 per cent of urines examined had colonial count between 1,000 to 10,000, 40 per cent contained between 100 to 1000, 21.3 per cent had 10 to 100 colonies and 12 per cent had between 1-10 colonies. No bacteria were found among 24.2 per cent of urines examined. All these individuals gave urine exactly in the same manner as the patients.

As shown in table I, among 112 urine specimens from healthy individuals, there were 14 cases where after remaining for six hours at room temp. (32° C), the colonial count increased to 10,000 or more per c.c. of urine. This shows that delay in examination may cause false positive results.

As seen in table 2, from the point of view of laboratory 470 (58.6%) individuals from 801 cases examined are free of infection but 102 (12.7%) persons who have the three factors present in their urine are considerered to have urinary tract infection.

Those individuals who have one or two positive finding in their urine are considered as suspicious.

In table 3, there is one urine, in which pus cells are present and colonial count is more than 10,000 bacteria per c.c., but pathogenic bacteria is not isolated. The only explanation here for high counts is laboratory contamination. However, presence of pus cells and high counts or presence of pur cells with the isolation of pathogenic bacteria is quite possible as seen in the table.

Table 1

Increase of colonial counts in 14 urine rpecimens after remaining six hours at room temp. (32 C°)

No.	Number of colonies after one hour	Number of colonies after 6 hours
1	400	10,000
2	460	20,000
3	900	10,000
4	1000	10,000
5	1200	24,000
6	2000	20,000
7	2000	96,000
8	2000	20,000
9	2600	12,000
10	5000	20,000
11	8000	40,000
12	8480	56,000
13	9000	20,000
14	9600	12,000

Table 2

The results of bacteriological examination of 801 urine specimens from patientts.

	No. of cases	Percentage
All three factors present	102	12.7
Two factor Present	77	9.6
One factor present	152	18.9
All factors absent	470	58.6

Factors considered:

1. Presence of pus cells in direct examination (more than 4/HPF).
2. Presence of pathogenic bacteria in urine.
3. Prerence of more than 10,000 bacteria per c.c. of urine.

Table 3

Result of examination of 77 urine specimens with two factors present.

	No. of cases	Percentage
Presence of pus cells and more than 10,000 colonies per c.c. of urine	1	1.2
Presence of Pathogenic bacteria and more than 10,000 colonies per c.c. of urine	45	58.4
Presence of pur cells and pathogenic becteria	31	40.2

Table 4

Result of examination of 152 urine specimens with one factor present.

	No. of cases	Percentage
Presence of pus cells	62	40.7
Presence of more than 10,000 colonies per c.c.	2	1.3
Presence of pathogenic bacteria	88	57.8

In table 4, as in the previous table, there are two urine specimens in which only high colonial counts haye been observed. This again can be explained only through laboratory contamination.

In table 4, as in the previous table, there are two Urine Specimens which only high colonial counts have been observed This again can be explained only through laboratory contamination.

The presence of pus cells is indication of some type of infection, but mere isolation of pathogenic bacteria is not significant.

It appears from table 5, that resistance of E. coli strains to drugs shown in the first column are greater than 60 per cent. Whereas for drugs in the second column (except for chloramphenicol and penbritin the resistance is around 40 per cent or less than that.

Table 5

Per cent of resistant strains of E. coli to antibiotics and chemotherapeutic drugs.

Agent	Sensitive	Resistant	Per cent of resistance	Agent	Sensitive	Resistant	Per centof resistance
Erythromycin	0	114	100	Penbritin	8	11	57
Penicillin	1	98	98.9	Chloromycetin	53	64	54.7
Gantrisin	3	41	93	Albamycin	26	19	42
Sulfadiaizne	4	39	90	Ceporan	11	8	42
Streptomycin	22	83	79	Colymycin	22	6	21
Novobiocin	29	87	75	Nalidixic Acid (Neg Gram)	16	4	20
Terramycin	18	50	73	Kanamycin	37	7	15.4
Tetracyclin	30	79	72	Mandelamine	48	7	12.7
Triplesulfa	3	5	62	Furadantin	60	6	9
Aureomycin	24	39	61	Neomycin	104	5	4.5

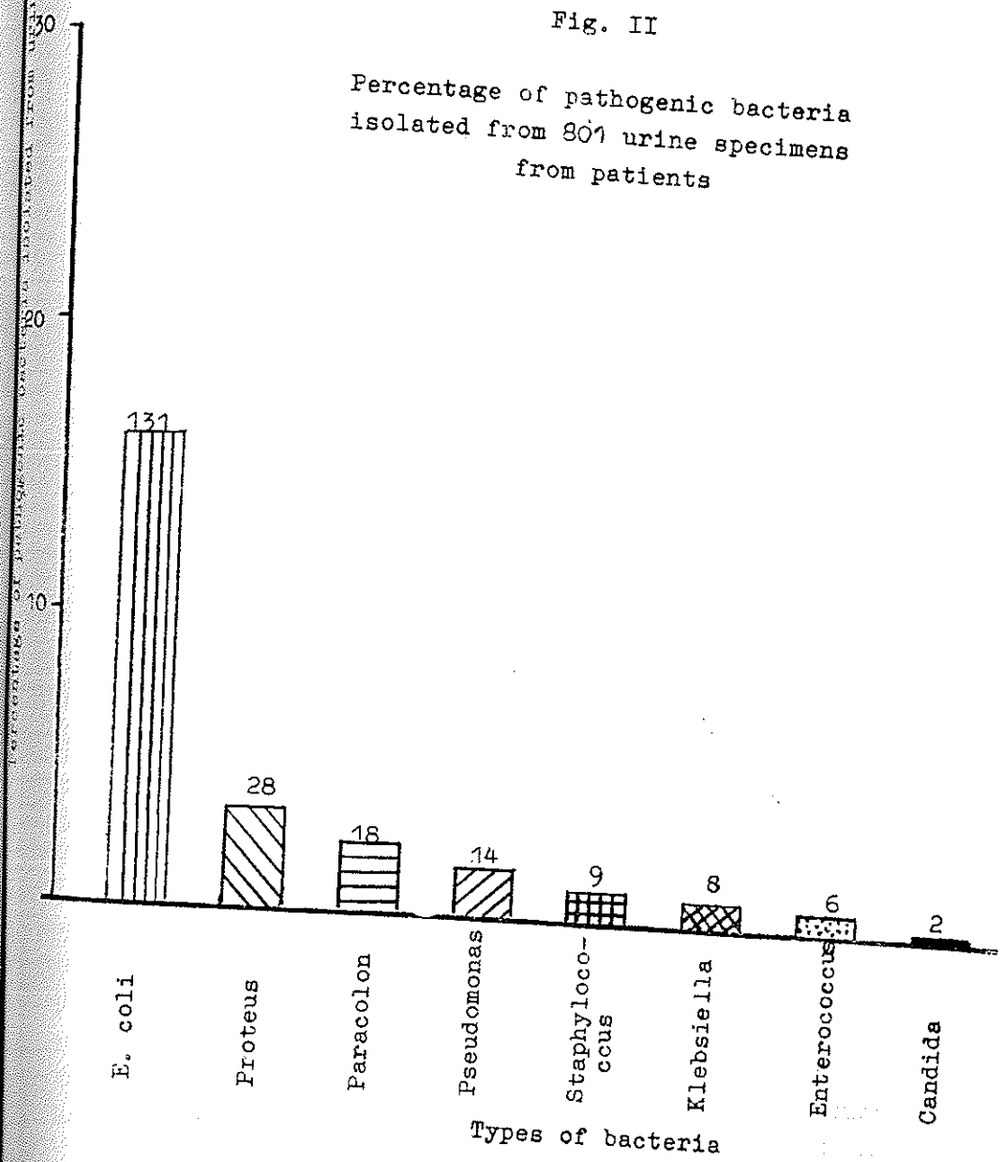


Fig. II

Percentage of pathogenic bacteria isolated from 801 urine specimens from patients

It is seen from Fig II that the frequency of different bacteria as etiological agents of urinary tract infection in this study are as follows: E. coli (60%), Proteus (28%), Paracolon (19%), Pseudomonas (14%), Staphylococcus coagulase positive (9%), Klebsiella (3.9%), Enterococcus (2.7%), Candida (9%).

From this and similar investigatigations, it appears that kidney and urinary tract are as a rule more sensitive to E.coli than to other bacteria (1,2). There is as yet no explanation for this. In fact one would expect the infection of urinary tract to be caused mostly by the bacteria usually present as normal flora of urether. For example Staphylococcus, Streptococcus or even Enterococcus. The per cent of infection caused by E. coli as compared to others is so great that lack of hygien alone during toileting cannot be taken responsible for all thess infections caused by E.coli. The main question to be answered in the future is whether certain types of E.coli have a greater nephropathogenic potentiality than others, in the same manner that few special serotypes of E.coli in contrast to numerous others are endowed with unexplained capacity of producing enteritis in young infants (5).

Discussions

In our experience properly obtained specimens contain either only relatively few colonies per c.c. (up to few hundreds) or many thousends of colonies. The former are more likely to be associated with contamination and the latter with infection. In certain instances, however, the bacterial count may fall below the renge characteristic of infection (6). These exceptional cases which may amount up to 5 per cent may be due to several factors. It is obvious that bacterial counts will not be as great if an antibacterial agent has been introduced into the urine. Similary unless a sufficient incubation time has elapsed in the urinary tract the bacteria will not have multiplied sufficently enough to give high counts. It is on this basis the quantitative approach is that some organisms are relatively fastidious, for example enterococci and therefore will not multiply well in the urine. Staphylococcus on the other hand being an organism that exists in clusters to begin with, naturally is going to have a lower plate count than the gram negative organisms that do not have such clustered

characteristics. We should realize these limitations of colonial count but such circumstances that tend to lower bacterial count in the urine do not operate frequently and as a rule quantitative measurement of bacteria in urine enables us to interpret the results of routine bacteriological examination with greater precision and intelligence. For instance in table II there are one hundred and two cases where pus cells and E. coli are present in urines and also the number of pathogenic bacteria are more than 10,000/ml. From the point of view of laboratory all these cases have infection of urinary tract and we can make such assumption only because of the quantitative measurement of pathogenic bacteria in urine. Likewise in table II there are forty five cases where E. coli is isolated and the number of same bacteria are greater than 10,000 per milliliter of urine. Again the significance of E. coli present in these strains per c.c. of urine are determined. On the other hand in 31 cases (table III) where pus cells are present and pathogenic bacteria are isolated but the colonial count is low, provided that limitation of this technique is taken into consideration as mentioned before, the low bacterial count enables us to disregard the presence of pathogenic bacteria in the urine. Consequently it will give us reason to search for other causes resulting in the presence of pus cells. For instance it is clear that infection by M. tuberculosis, Mycoplasma or Trichomonas could be the cause of pus cells in urine, but none of these agents grow on culture media by ordinary methods. Pus cells in urine may also be due to nonspecific urethritis or sometimes due to infection of prostate, but examination of urine from these of patient will not reveal high counts.

Apart from the points discussed above, colonial count and its correct interpretation also offers the advantage of replacing catheterization when this technique is used soley for the purpose of bacteriological examinations of urine. This is important since during recent years catheteterization has been criticized and taken responsible for introducing infection into the urinary tract (9).

Summary

1251 urines were examined and in each case the three following criteria were studied:

1- Presence of pus cells in wet mount (4 or more HPF).

2- Isolation of pathogenic bacteria.

3- Presence of 10,000 or more bacteria per c.c. of urine.

801 specimens were obtained from patients referred to the diagnastic laboratory of Institute of Public Health Research. and 450 specimens were collected from healthy soldiers in view of obtaining an idea of colonial count in urine specimens from healthy individuals. Further colonial couht uf 112 specimen were measured twice in six hours interval. This was done to show that delay in examination may give false positive result.

Finally the etiological agents of infections were identified and antibiotic sensitivity of E. coli strains isolated were determined.

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