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Evaluation of Bacteriuria in Urine Samples Using Nitrite Test and Leucocyte Esterase Test Versus Urine Culture Among Undergraduate Students in Owerri, Nigeria

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Abstract

Background: Analysis of urine is regarded as the foremost medical test heralding the beginning of laboratory medicine. The aim of this study was to show the utility of simple urinalysis in the detection of bacteria in the urine of healthy subjects, using nitrites assay and leucocyte esterase in the detection of bacteriuria among university students.

Materials and Methods: A cross-sectional survey was carried out among students in a federal tertiary educational institution, using the random sampling technique.

Results: There was a total of 456 respondents with 351 (76.97%) females and 105 males (23.03%). Majority of respondents were within the age ranges of 20-24 years (231=50.66%) and 25-29 years (126=27.63%). The urine samples of 90 (19.7%) respondents were nitrite positive, while the leucocyte esterase test was positive in 60 (13.2%) respondents. The sensitivity of urine nitrite test was 30.9%, while the specificity was 86.6%. The sensitivity of leucocyte esterase test was 18.2%, while the specificity was 89.7%.

Conclusion: The sensitivity of urine nitrite test was higher than that of leucocyte esterase test, and hence has higher value in detecting the presence of urinary tract infection than leucocyte esterase test, although the latter demonstrated relatively higher specificity.

Keywords: ***Urine analysis; Urine nitrite; Urine Leucocyte Esterase test; Urine culture; FUTO; Owerri; Nigeria***

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1. Introduction

Analysis of urine is regarded as the foremost medical test heralding the beginning of laboratory medicine [1]. Although cheap, easy-to-do, with minimal technology, urine evaluation is very informative in patient care, in ascertaining healthy state, infective disease, metabolic disease, or mitotic disease conditions. Ancient Sumerian and Babylonian texts, as far back as 6000 years ago, hold the record of the beginning of analysis of urine in the evaluation of patients [2]. However, microscopic examination of urine was first attempted at the beginning of 16th century and then by the 17th century there was emergence of compound microscopes by Hans and Zacharias Jansen [2]. Urine, the first fluid to be examined in the body, in modern-day can be analyzed using cutting-edge molecular technology. It can give insight into both metabolic and non-metabolic diseases conditions in the body, and use of quick point-of-care urine dipstick has come to stay with its merits and demerits [3]. In the Netherlands, excessive conduct of urinalysis among Emergency Department patients and possibly over-treatment of asymptomatic bacteriuria has been criticized, with a call for use of dipstick as a screening tool for decision on need for detailed analysis [4]. Additionally, a researcher found out that the outcome of urinalysis requested in pediatric patients according to byelaws were often not critically utilized or followed up with further tests [5]. Microsatellite analysis of urine has been demonstrated to be useful in detecting the presence of organ-confined cancer of the kidney [6]. Erythrocyturia, pyuria, proteinuria, and pyelocaliceal dilation all of inflammatory origin have been reported in patients with acute appendicitis, and should not mislead the managing team from adopting a surgical option [7]. Similarly, the findings of positive urine ketone bodies and nitrate have been found to be predictive of perforation in children with acute appendicitis [8].

In Nigeria, apart from carrying out blood film for malaria parasite, bilirubinuria, urobilinogenuria, proteinuria and hematuria have been found to be useful in the diagnosis of patients with severe malaria – using simple urinalysis [9]. The use of this simple test as a routine screening tool has been recommended due to its importance in disease surveillance [10]. Micro-hematuria alone was found to be 52% sensitivity and 91.67% specificity in the diagnosis of urinary schistosomiasis, and hence useful in resource-poor setting as a screening tool among others [11]. The utility of urinalysis is even more apt in communities or organizations where facilities for diagnosis of simple medical conditions such as urinary tract infection is not available or the expertise needed to man it is in short supply. A simple medical condition such as urinary tract infection can pose serious health threat if not discovered on time. Organizations, schools, industries with sick bay may have to send their sick folks to a distant health facility, sometimes at high cost, at other times when the pathology has worsened. Even when the necessary hard wares for diagnostic services are available, trained professionals may be in short supply. The aim of this study was to show the utility of simple urinalysis in the detection of bacteria in the urine of healthy subjects, using nitrites assay and leucocyte esterase in the detection of bacteriuria among university students.

2. Materials and Methods

2.1 Research design

This research utilized cross-sectional survey design.

2.2 Study area

This study was carried out between January and February 2022 among university students of Federal University of Technology Owerri (FUTO). FUTO is the foremost university of technology established in the South East Nigeria in the year 1980. It is a

tertiary institution situated in Owerri West Local Government Area of Imo State bounded by four communities namely Ihiagwa, Ezi-Obodo, Obinze and Umuagwo. There are twelve (12) faculties in it, and the School of Agriculture and Agricultural Technology (SAAT), School of Basic Medical Sciences, School of Biological Sciences (SOBs), School of Engineering (SSET), School of Environmental Sciences (SOES), School of Health Technology (SOHT), School of Information and Communication Technology (SICT), School of Management Technology (SMAT), School of Physical Sciences (SOPS), School of Postgraduate Studies (SGPS) and Directorate of General Studies. Each of these facilities has a minimum of five Departments. Students study respective courses in these departments. The students are over 22,000 in population. The university provides accommodation within the campus for students, but a large number of students live off-campus in the neighbouring communities. Sick students are treated in the university health centre located within the institution.

2.3 Study sites

Faculties and Departments of the Federal University of Technology Owerri were the study sites.

2.4 Study instrument

Self-administered questionnaire was used for the study.

2.5 Variables

Age, sex, symptoms of urinary tract infection (for exclusion), antibiotic treatment (for exclusion), urinalysis (including urine nitrite and leucocyte esterase test), urine microscopy, and urine culture.

2.6 Bias

The study population was limited to apparently normal individuals, not real patients.

2.7 Study population/participants

Students (apparently normal individuals) of Federal University of Technology Owerri were the participants used for this study.

Exclusion criteria: Students excluded from this study include those who voluntarily opted out of the study, students who had taken antibiotics medication in the two weeks preceding the sample collection and females who were menstruating. Students who reported symptoms and signs of urinary tract infection such as dysuria, incontinence, frequency, urgency, suprapubic pain or flank pain were also excluded from the study.

2.8 Sample collection

Sterile universal bottles were issued to each student for collection of clean catch midstream urine specimens. The students were educated on the need to collect urine with as little contamination as possible. Female students were taught to cleanse their vulva and around their urethral opening with clean water, and part their vulva so as to get clean catch urine. The containers were

labelled by numbering and gender. Names were not written to avoid bias and ensure confidentiality. The corresponding questionnaires from the respective students were also tagged with the same numbers labelled on their sterile bottles.

2.9 Processing of urine samples

The collected clean catch mid-stream urine samples were examined macroscopically, microscopically, and chemically. Culture was done in three different media for identification and isolation of bacteria. The materials used were Bunsen burner, wire loop, blood agar, chocolate agar, MacConkey agar, face mask, latex gloves. The universal bottle containing midstream urine was mixed by inverting the bottle. It was passed through the Bunsen flame three times to reduce the load of micro-organisms on the lid. The lid was opened and a loopful of urine was collected using the wire loop. The loopful was inoculated in the culture medium to make primary inoculum. The culture medium was then placed in the incubation chamber at 37ºC for 24 to 48 hours for the growth of micro-organism. Urine culture was considered significant bacteriuria when colony forming units ≥ 10^5 / ml of voided urine was obtained. A single pure colony suspended in nutrient 6 with and subculture in machinery plate and blood agar plate incubated at 37ºC for 24 hours. Isolates were identified using colony morphology and biochemical test. The antimicrobial sensitivity was determined using sensitivity disk by Kirby-Bauer method.

2.10 Sample size determination

With total population of students in FUTO estimated to be 25,000.00, the formula for survey developed by Yaro Yamen was used to determine the minimum sample size as follows: $n = \frac{N}{1+Ne^2}$ where n=minimum sample size, *N*=Estimated Total population of students, and e=desired precision/level of significance, usually 5% (0.05) at 95% Confidence Interval (CI). Hence, n=25,000/ 1+25,000 X 0.05^2=400.

2.11 Sampling method

A total of five hundred (500) students were selected for the study via random sampling technique. Multi staged sampling was done. In the first stage, the twelve faculties were sampled randomly and five of the facilities chosen namely School of Biological Sciences (SOBS), School of Information and Communication technology (SICT), School of Physical Science (SOPS), School of Environmental Sciences (SOES) and School of Agriculture and Agricultural Technology (SAAT). In the second stage, five departments were randomly selected from each of the five faculties un the final of sampling twenty (20) students from each department were selected giving a total of 100 students in each faculty. The researchers had a meeting with the respective department heads and student representatives who made the students available on agreed dates. The respective students were selected in the final stage via balloting each of the selected students were given and structured questionnaire.

2.12 Validity/Reliability of instrument

The questionnaire was pretested prior to the study to ensure accuracy, appropriateness and to ascertain it was understandable. The collected data were checked for consistency and accuracy. The sterling of the prepared culture was checked by incubating 5% of the prepared culture randomly selected for 24 hours at 37ºC. Laboratory identification procedures such as inoculation of culture media, colony characterization and measuring of antibiotic susceptibility were checked.

2.13 Data analysis

Data was analyzed using SPSS version 25. All analysis was done at 95% confidence interval and a p-value of less than 0.05 was considered significant. Data was presented in frequencies and percentages as appropriate. The association of bacteria and demographic characteristics was assessed using the chi-square statistic.

3. Results

TABLE 1 shows the demographic characteristics of respondents. A total of 456 respondents were involved in the study. There were 351 (76.97%) females and 105 males (23.03%), and majority were within the age ranges of 20-24 years (231=50.66%) and 25-29 years (126=27.63%).

TABLE 1. **Demographic Characteristics (n=456).**

	Number	**Percent (%)**
Gender		
Male	105	23.03
Female	351	76.97
Age - Groups (Years)		
15-19	57	12.50
20-24	231	50.66
25-29	126	27.63
30-34	33	7.24
35-39	9	1.97

FIG. 1 shows the result of urinalysis using nitrite evaluation. Three hundred and sixty-six (80.3%) respondents tested negative for nitrite in their urine (absence of bacteriuria), while 90 (19.7%) were nitrite positive (presence of bacteriuria).

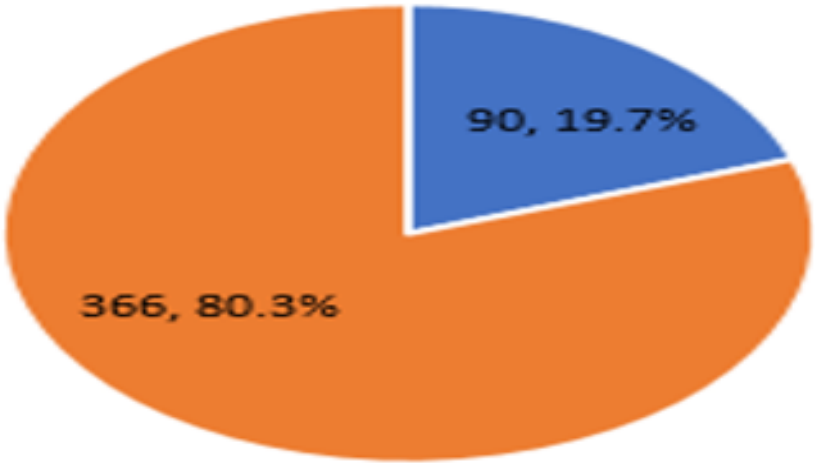

FIG. 1. **Pie chart showing percentage of respondents that tested positive for nitrite Orange Color = Nitrite Negative; Blue Color = Nitrite Positive.**

FIG. 2 shows the result of urine analysis evaluated with leucocyte esterase test. Three hundred and ninety-six (86.8%) respondents had negative leucocyte esterase test (absence of bacteriuria), while 60 (13.2%) had positive leucocyte esterase test (presence of bacteriuria).

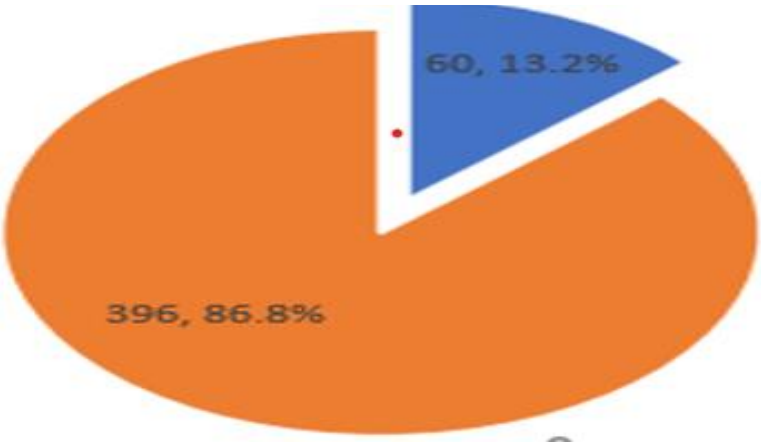

FIG. 2. **Pie chart showing percentage of respondents that tested positive for leucocyte esterase Orange Color = Leucocyte Esterase Negative; Blue Color = Leucocyte Esterase Positive.**

Summary of urine variables is shown in TABLE 2. There was no protein, glucose, ketone, ascorbic acid, blood, red blood cells, epithelial cells, or casts, and normal urobilinogen values, in the urinalysis carried out on respondents.

TABLE 2. **Summary of Urinalysis Variables (n=456).**

Urinalysis	Present n (%)	Not Present n (%)	Total n (%)
Protein	0 (0.0)	456 (100.0)	456 (100.0)
Glucose	0 (0.0)	456 (100.0)	456 (100.0)
Urobilinogen	Normal 456 (100.0)	0(0.0)	456 (100.0)
Ketone	0 (0.0)	456 (100.0)	456 (100.0)
Ascorbic acid	0 (0.0)	456 (100.0)	456 (100.0)
Blood	0 (0.0)	456 (100.0)	456 (100.0)
RBC	0 (0.0)	456 (100.0)	456 (100.0)
Epithelia cells	0 (0.0)	456 (100.0)	456 (100.0)
Casts	0 (0.0)	456 (100.0)	456 (100.0)

TABLE 3 shows the number and percentage of white blood cells found per high-power field in the study. There were no white blood cells per high power field in the urine samples of 243 (53.29%) respondents. One hundred and twenty respondents (26%) had at least 5 white blood cells per high power field.

TABLE 3. **Urine White Blood Cell Readings.**

Value/HPF	Number	Percentage (%)
1	36	7.89
2	24	5.26
3	3	0.66
4	30	6.59
5	3	0.66
6	18	3.95
8	69	15.13
10	3	0.66
11-12	6	1.32
15	18	3.95
16-20	3	0.33
Nil	243	53.29
Total	456	100.0%

TABLE 4 shows the specific gravity of the urine of respondents. All had specific gravity of <1.010.

TABLE 4. **Urine Specific Gravity.**

Specific Gravity	Number	Percent
<1.010	456	100

TABLE 5 shows the result of evaluation of nitrite test versus urine culture. There were 90 respondents in which the urine samples were nitrite positive, out of which the urine culture was positive (the true positive) in 51 (56.7%) respondents, and negative (false positive) in 39 (43.3%). Also, 366 respondents had urine nitrite negative result, out of which 114 were culture positive (false negative), and 252 had negative culture result (true negative). The sensitivity of urine nitrite test was 30.9%, while the specificity was 86.6%.

TABLE 5. **Evaluation of Nitrite Test vs Culture.**

Nitrite	Culture		Total
	Positive	Negative	
Positive	51 (TP)	39 (FP)	90
Negative	114 (FN)	252 (TN)	366
Total	165	291	456

TP: True positive, FP: False positive, FN: False negative, TN: True Negative

Sensitivity = TP/(TP + FN) x 100 = 51/(51 + 114) x 100 = 30.9%

Specificity = TN/(TN+ FP) x 100 = 252/(252 + 39) x 100 = 86.6%

TABLE 6 shows the result of evaluation of leucocyte esterase test versus culture. There were 60 respondents whose urine samples showed positive result for leucocyte esterase test, out of which 30 (50%) had positive culture result (true positive) and 30 (50%) had negative leucocyte esterase test. The leucocyte esterase test was negative in the samples of 396 respondents, out of which urine culture was positive in 135 (true positive=34.1%), and negative culture in 261 (true negative=65.9%) respondents. The sensitivity of leucocyte esterase test was 18.2%, while the specificity was 89.7%.

TABLE 6. **Evaluation of Leucocyte esterase test vs Culture.**

Leucocyte esterase	**Culture**		**Total**
	Positive	**Negative**	
Positive	30 (TP)	30 (FP)	60
Negative	135 (FN)	261 (TN)	396
Total	165	291	456

4. Discussion

The tertiary institution is a place of learning dominated by apparently healthy individuals. We therefore reason that if the urine nitrite and leucocyte esterase tests are able to detect the presence of some concentrations of bacteria in the urine of these apparently healthy individuals (asymptomatic persons), it could hold some hope in the detection of higher concentrations of bacteria in urine in real patients. There were more females (twice), and majority were within 20 and 29 years of age. This implies that there were probably more females undergoing tertiary school education as compared with males. This observation and reasoning align with the findings of another study carried out in Owerri - Imo State were higher number of girls were found to be successful in Biology at Secondary School Certificate Examination [12]. However, another study reported in 2017 in the same institution revealed more male involvement in information technology education and higher number of females with excellent grades [13]. The dominant age range (20-29 years) in this study is expected, and is similar to that reported in previous studies [13,14].

Ninety (19.7%) respondents tested positive for nitrite suggesting the presence of bacteria in urine, compared with leucocyte esterase test which were positive in only 60 (13.2%) respondents. Nitrite test therefore detected more respondents who appeared to have bacteria in their urine. A normal urine should not show any trace of nitrite. The nitrite test indirectly measures presence of nitrate-reducing bacteria granted that the urine contains sufficient dietary nitrates. This reduction can only occur if the urine stays in the bladder longer than 4 hrs. Most bacterial species causing urinary tract infection reduce nitrate in the urine to nitrite. Examples of nitrate-reducing bacteria are the Enterobacteriaceae and most of the non-fermenters. Candida and Streptococci including Enterococci do not reduce nitrates. Our study shows that more than 19% of apparently healthy students had bacteria in their urine.

The presence of bacteria in the urine enables conversion of nitrate to nitrite, and also mobilisation of white blood cells which yield the coagulase used for the coagulase test. Leucocytes in the urine could be a pointer to infection in the urine. High levels indicate that the immune system is trying to fight off bacterial or parasitic infection such as a fungus. If not due to contamination,

the repeated presence, in urine, of 3 to 5 leukocytes per field suggests a low urinary tract (bladder and urethra) infection. In the presence of bacteria or a positive nitrite test in a symptomatic patient, the presence of leukocytes confirms a urinary tract infection.

The respondents used for this study, as earlier indicated, were apparently normal individuals, and those who had symptoms of urinary tract infection were excluded. There is little wonder therefore, when the results of urine analysis for other variables (except nitrite and leucocyte esterase test) showed absence of protein, glucose, casts, ketone, ascorbic acid, blood, red blood cells, epithelial cells, and normal value for urobilinogen. However, we noticed the presence of leucocytes in 213 (48.71%) respondents. Repeated presence in urine, of 5 or more leucocytes (120 or 26% respondents) per field suggests a low urinary tract (bladder and urethra) infection. In the presence of bacteria or a positive nitrite test in a symptomatic patient, the presence of leukocytes confirms a urinary tract infection. The specific gravity was less than 1.010 in all the respondents. This is also indicative of the fact that the respondents were drawn from normal individuals and not patients.

Urine nitrite test demonstrated higher sensitivity for bacteriuria among normal individuals (30.9%), compared with a sensitivity of 18.2% for urine leucocyte esterase test. The findings of this study are different from another carried out among hospital patients in India which showed urine nitrite sensitivity of 23.31% and leucocyte esterase test of 48.5% [15]. This latter study emphasized the unreliability of lone use of these two tests without urine culture. Similar conclusion was also reach in another independent study in Karachi, in Pakistan [16]. However, in another study, leucocyte esterase test was reported to have demonstrated 100% sensitivity [17]. Urine nitrite test has been reported to be more sensitive in adults than in children less than 2 years, and has a 99% likelihood of being indicative of urinary tract infection [18]. Children less than 2 years old are not capable of holding urine in their urinary bladder for up to 4 hrs required to convert nitrates to nitrites by nitrate converting *Enterobacteriaceae*.

Similarly, urine specimens in our study were collected from healthy adults, not early morning urine that may have been retained for at least 4 hrs. Could the results of these tests be environment or population dependent? This is yet to be ascertained. Additionally, urine leucocyte esterase test showed a relatively higher specificity (89.7%), as against a value of 86.6% for urine nitrite test. A similar study reported 76% specificity for leucocyte esterase test [17].

5. Limitations

This study is limited in that the study population was apparently healthy individuals. Another study is needed in our environment of practice to be carried out among patients with symptoms of urinary tract infection, where these tests could be done and compared with culture results.

6. Conclusion

The sensitivity of urine nitrite test was higher than that of leucocyte esterase test, and hence has higher value in detecting the presence of urinary tract infection than leucocyte esterase test, although the latter is demonstrated relatively higher specificity. Urine nitrite test may therefore be used as a screening tool in resource-poor setting where urine culture facility is not readily available.

7. Acknowledgement

We acknowledge the input of our field officers who assisted in the collection and examination of urine samples used for this study.

8. Statement on Ethics

Research Ethics approval was obtained from the ethical research committee of Federal University of Technology Owerri. Informed consent was obtained from students after detailed description of the purpose of the study. Enrolment in the study was voluntary. Students who opted out of the study were allowed to do so without any indictment.

9. Source of Funding

The study was self-funded by the authors.

10. Conflict of Interest

None declared

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