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Diagnostic Performance of Urine Nitrite Testing for Bacterial Urinary Tract Infection Compared to Culture at Enugu State University Teaching Hospital, Enugu, Nigeria.

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Obieze Vivian Amarachi[1], Aneke Christian Chinedu[1], Ugwu Luke Jideofor[1]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Science, Enugu State University of Science and Technology, Nigeria

Correspondence: Obieze Vivian Amarachi, eduaneke042@gmail.com

Abstract

Background: Urine point-of-care testing, using dipstick technology for nitrite is important for empirical therapy, more especially in a resource limited setting, but analytical pathogen specific sensitivity and specificity performance data from Nigeria are limited.

Methods: a cross-sectional study was conducted on 100 urine samples from patients attending Outpatient's Clinic of the Enugu State University Teaching Hospital, Nigeria, from February to April, 2026. Nitrite was assessed using Medit-test Combi 11 [Macherey-Nagel, Germany] in accordance with the manufacturer instructions. Positive nitrite was described as clear pink dis-colouration on the dipstick test strip. Quantitative urine culture was used as reference standards, and significant bacteriuria was defined as >10 bacterial colonies of a pure/predominant isolate on CLED agar, corresponding to $\geq 10^4$ CFU/mL. Sensitivity, specificity, predictive values, and likelihood ratios with 95% confidence intervals were calculated using SPSS v26.

Results: UTI prevalence was 77% [77/100]. Nitrite demonstrated sensitivity 19.4% [95% CI: 11.1–30.5%], specificity 100% [85.2–100%], positive predictive value 100%, negative predictive value 29.9%, positive likelihood ratio ∞, and negative likelihood ratio 0.81 for culture-confirmed UTI. Among 13

nitrite-positive samples, 10 [76.9%] were *Escherichia coli*, 2 [15.4%] *Staphylococcus aureus*, and 1 [7.7%] *Klebsiella spp*. Nitrite missed 34/44 [77.3%] *E. coli* and 23/25 [92.0%] *S. aureus* UTIs. **Conclusions:** In this study, nitrite is a highly specific surrogate marker for *E. coli* UTI but lacks sensitivity for all-cause UTI due to high prevalence of nitrite-negative *S. aureus* and *Enterococcus spp*. Negative nitrite cannot exclude UTI. With negative nitrite, empiric therapy should consider Gram-positive uropathogens.

Keywords: Urinary Tract Infection, Nitrite, Pyuria, Diagnostic Test Accuracy, *Staphylococcus Aureus*, Nigeria

INTRODUCTION

Urinary tract infection (UTI) accounts for over 150 million cases annually and is a leading cause of antibiotic prescription in sub-Saharan Africa [1][2], In Nigeria, in most cases, empiric therapy is often initiated based on point-of-care (POCT) urine dipstick results due to limited access to timely culture [3]. Urine dipstick nitrite testing detects nitrate reduction, which indicates the presence of Enterobacteriaceae in the urine sample. Clinical guidelines frequently interpret nitrite positivity as confirmation of Gram-negative UTI [4], undermining the fact that pathogen distribution varies geographically. Studies from Nigeria report *Staphylococcus aureus* in 20–35% of community UTIs [5] [6], far higher than in Europe or North America where *Escherichia coli* causes over 80% of UTI. Since *S. aureus* and *Enterococcus spp*. lack nitrate reductase and will yield negative nitrite despite the presence of significant bacteriuria, therefore, sure reliance on nitrite as a diagnostic marker of UTI, may result in missed diagnoses and inappropriate empiric therapy in regions with high Gram-positive uropathogen prevalence**.**[7]**,** and where in most cases, the majority of medications for UTI in resource limited settings is empirical therapy [3].

Data evaluating dipstick sensitivity and specificity graded by organisms in Nigeria are scarce, thus the need for this research. In this study, the diagnostic accuracy and flaws of nitrite testing for UTI were evaluated, with respect to pathogen specificity.

MATERIALS AND METHODS

Study design

This was a cross-sectional study on the diagnostic performance of urine dipstick technology, conducted at the Enugu State University Teaching Hospital [ESUTH], a tertiary hospital in Enugu, Enugu State, South-east Nigeria. The study period was from February 3, 2026 to April 2, 2026. Testing procedures and reporting were done in accordance with provisional standards.

Adult non-pregnant outpatients, who were not on urinary catheter, and who were showing at least 2 clinical symptoms of UTI (dysuria, frequency, urgency, or suprapubic pain) from the questionnaire, and who were not or had not taken antibiotics at least 3-4 weeks prior to the study time were included in the study. Prior to sample collection, participants were sensitized on the need to participate in the study and as well, educated on how to collect clean catch mid-stream urine for culture and dipstick testing. Participants were then provided with facilities within the hospital for sample collection and materials for good sampling and patients comforts were provided as well. Sterile universal containers were labelled with the patients' age, sex, and sample number, and each participant collected two urine samples: one for culture, and the other for dipstick urine testing. Samples were submitted by patients immediately after collection, and these were taken to the microbiology laboratory for testing. Samples received were tested within 1 hour 30 minutes of

collection, without refrigeration, but those that did not meet up with the required standards were rejected, including those with volumes <10 ml and, or visibly contaminated with faces

Test methods

All samples underwent dipstick testing using Combi-11 [Analyticon Biotechnologies, Germany]. Nitrite was recorded as negative, trace, or positive per manufacturer colour chart. Trace nitrite was classified as negative for primary analysis per manufacturer instructions. Dipstick testing was performed within 1 hour of sample receipt. The time from sample collection to testing was less than 1-hour 3o minutes.

Reference standard

Quantitative urine culture was performed on Cysteine Lactose Electrolyte Deficient [CLED] agar and 5 % Sheep blood chocolate agar, using a calibrated 0.001 ml loop. Plates were incubated aerobically at 35–37°C for 18–24 hours. Significant bacteriuria was defined as > 10 discreate colonies of a single predominant organism (corresponding to $\geq 10^4$ CFU/ml). This was consistent with CLSI guidelines on urine culture [8]. Organisms were identified using standard biochemical tests: catalase, coagulase, indole, citrate, urease, coagulase and triple sugar iron agar.

Statistical analysis

Data were analysed using IBM SPSS Statistics v26. Diagnostic 2x2 tables were constructed. Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated with 95% confidence intervals using the Wilson score method. Analyses were stratified by *E. coli*, *S. aureus*, and other uropathogens

Results

Of 100 urine samples, 77 (77.0%) met the culture definition for UTI. *E. coli* was isolated in 44 (57.1%), *S. aureus* in 25 (2.5%), *Enterococcus spp.* in 4 (5.2%), *Klebsiella spp*. in 2 (2.6%), and *Proteus spp*. in 2 (2.6%).

Table 1. Diagnostic performance of dipstick tests for culture-confirmed UTI [n=100]

Test	TP	FP	FN	TN	Sensitivity %	Specificity %	PPV %	NPV %	LR+	LR-
Nitrite	13	0	64	23	16.9 (9.3-27.1)	100 (85.2-100)	100 ([75.3-100)	26.4(17 .6-37.0)	∞ (LR+ >10)	0.83 (0.75-0.92)

Abbreviations:

TP: true positive; FP: false positive; FN: false negative; TN: true negative; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio. PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio. 95% CI calculated using Wilson score method.

Table 1 demonstrates that in this study, nitrite testing had a sensitivity of 16.9% [95% CI 9.3-27.1%] for culture-confirmed UTI, indicating 83.1% of true infections were missed. While specificity and PPV were 100% with no false-positives observed, the NPV was only 26.4%, meaning 73.6% of nitrite-negative

patients actually had UTI. The negative likelihood ratio of 0.83 confirms nitrite provides minimal rule-out value in ESUTH.

Table 2. Sensitivity of nitrite stratified by uropathogen [n=77 culture-positive]

Uropathogen	n with UTI	Nitrite positive	Sensitivity %	95% CI	False Negative Rate
All-cause UTI	77	13	16.9	9.3-27.1	83.1
E. coli UTI	44	10	22.7	11.5 - 37.8	77.3
S. aureus UTI	25	2	8.0	1.0 - 26.0	92.0
Enterococcus spp.	2	0	0	0-84.2	100
Others	6	1	16.7	0.4-64.1	83.3

Table 2. shows nitrite sensitivity was lowest for *S. aureus* UTI [8.0%] and highest, though still poor, for *E. coli* [22.7%]. Nitrite missed 34 of 44 [77.3%] *E. coli* UTIs and 23 of 25 [92.0%] *S. aureus* UTIs. Given that *E. coli* and *S. aureus* together caused 69/77 [89.6%] of all UTIs. 83.1% of all culture-confirmed UTIs were missed by nitrite. In this study, reliance on nitrite would fail to detect the vast majority of clinically significant infections at ESUTH.

Table 3. Specificity of nitrite dipstick stratified by uropathogen comparison [n=100]

Uropathogen comparison	n without UTI	Nitrite negative	Specificity % (95 % CI)	95 % CI	False-positive Rate %
All-cause UTI	23	23	100	85.2-100	0
E. coli vs no *E. coli*	56	56	100	93.6-100	0
S. aureus vs *no S. aureus*	75	75	100	95.2-100	0
Enterococcus vs no Enterococcus	98	98	100	96.3-100	0
Others vs no Others	94	94	100	96.1-100	0

Table 3 demonstrates that nitrite testing had 100% specificity for all uropathogen comparisons, with no false-positive results observed in this cohort. This indicates that a positive nitrite result is highly predictive of bacterial UTI at ESUTH, with PPV of 100% [95% CI 75.3-100%]. However, the clinical utility of this high specificity is substantially limited by the low sensitivity shown in Table 2, particularly for the predominant pathogens *E. coli* and *S. aureus*

Table 4. Uropathogen distribution stratified by nitrite result [n=77 culture-positive]

Nitrite Result	**E. *coli* n**	***S. aureus* n**	***Enterococcus* n**	**Others n**	**Total**

Positive	10 (76.9%)	2 (15.4%)	0 (%)	1 (7.7%)	13
Negative	34 (65.4%)	23 (44.2%)	2 (3.8 %)	3 (5.8%)	62

Table 4 shows that *E. coli* remained the predominant organism in both nitrite-positive [76.9%] and nitrite-negative [65.4%] UTIs. Critically, 34/44 [77.3%] of all *E. coli* and 23/25 [92.0%] of all *S. aureus* infections were nitrite-negative. The complete absence of nitrite positivity among *Enterococcus spp* isolates is expected, as this species lacks nitrate reductase. Two samples with trace nitrite (both *Enterococcus spp*), were classified as negative per manufacturer instructions.

DISCUSSION

This study identified that nitrite dipstick testing had poor sensitivity 16.9%, 9.3-27.1), but perfect specificity (100%, 85.2–100%) for UTI which were confirmed by culture. Sensitivity remained low when stratified by organisms and was considerably reduced for *Staphylococcus aureus* UTI (8.0%, 1.0–26.0%) compared with *Escherichia coli* UTI [22.7%, 11.5–37.8%]. This overall nitrite sensitivity (16.9%,) is lower than the 45–60% commonly reported in meta-analyses from high-income settings [7]. This disparity could be attributed to differences in uropathogen distribution. In this study, *S. aureus* accounted for 32.5% of infections, whereas *E. coli* comprised 57.1%. Nitrite production involves the conversion of nitrates to nitrites by the enzyme, nitrate reductase. *E. coli* and other Enterobacteriaceae possess this enzyme, unlike *S. aureus* which does not. As a result of this, 23 of 25 *S. aureus* UTIs were nitrite-negative. In this study, of little concern is the 2 (23 of 25) *Staph aureus* that seemed to have produced nitrite, as *Staph aureus* is a known non-nitrite producer. This may be due to undetected mixed infection with nitrate-reducing Enterobacteriaceae below our 10^4 CFU/mL reporting threshold, as culture protocols prioritize the predominant organism. Alternatively, this may be due to dietary nitrate, or medication interferents, which account for the 5–10% false-positive rate reported for nitrite pads in sterile urine [8]. The 8.0% positivity does not indicate nitrate reductase activity by *S. aureus*, and a similar disparity finding has been reported [9].

Going by these findings, clinicians relying on nitrite dipstick results to guide empirical treatment in settings with high *S. aureus* prevalence risk missing a substantial proportion of infections**.** These findings show serious concern, as our study demonstrates women population dominance: *S. aureus* accounted for 32.5% of UTIs and that 62% of participants were female, a group at high risk for complications from delayed or untreated UTI [10].

The 100% specificity of nitrite dipstick observed aligns with previous studies and reinforces that a positive nitrite is highly predictive of UTI [PPV 100%]. However, the negative predictive value was only 29.9%, confirming that a negative nitrite cannot exclude infection. This finding is clinically important in low-resource health centres where in most cases, the majority of medications for UTI is empirical therapy [3].

LIMITATIONS

First, this was a single-centre study with a modest sample size [n=100], limiting precision of subgroup estimates, particularly for *S. aureus* where the 95% CI was wide [1.0–26.0%]. Second, we did not record patient hydration status or dietary nitrate intake, all of which can affect nitrite results. Third, we defined significant bacteriuria as ≥10^4 CFU/mL per Kass criteria; using a ≥10^5 CFU/mL threshold would lower

UTI prevalence and likely improve nitrite sensitivity, but would also miss clinically relevant low-count infections.[5]

CONCLUSIONS

Nitrite dipstick testing is insufficiently sensitive to rule out UTI in this Nigerian hospital population, particularly for *S. aureus* infections. While a positive result remains highly specific, negative nitrite results require confirmatory culture or microscopy. Antibiotic stewardship programs in similar settings should emphasize that nitrite-negative urine does not exclude UTI and that empirical therapy decisions should integrate clinical assessment and additional laboratory tests where feasible.

RECOMMENDATIONS

- For Clinical Practice in Nigeria and Similar Low-Resource Settings
- Do not use nitrite as a standalone rule-out test: Given 19.4% sensitivity and 29.9% NPV, a negative nitrite result should not exclude UTI. Patients with symptoms require urine microscopy or culture regardless of nitrite result.

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