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CORRELATION OF URINE CULTURE WITH URINE MICROSCOPY, LEUCOCYTE ESTERASE AND DIPSTICK NITRITE IN DETECTION OF UTI.

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ABSTRACT

OBJECTIVE: To correlate urine culture with urine microscopy, leucocyte esterase and dipstick nitrite in detection of UTI.

MATERIAL AND METHODS:STUDY SITE - OPD & IPD of Ekta Institute Of Child Health, Shanti Nagar, Raipur (C.G.) STUDY POPULATION – Pediatric age group between 1month to 12 year with clinical suspicion of UTI in OPD & IPD at EKTA INSTITUTE OF CHILD HEALTH, RAIPUR STUDY DESIGN – A Prospective observational study. Sample size :All the children between 1month and 12 year with clinical suspicion of UTI in OPD and IPD of Ekta institute of child health during the study period and whose parents give consent. Period of study:October 2014 to September 2016. Inclusion criteria 1. Children of age 1 month to 12 year with clinical suspicion of UTI. Exclusion criteria 1. Prior administration of antibiotic therapy in past 72hrs

RESULTS: Based on the detailed history, clinical findings, laboratory data, along with inclusion, exclusion criteria, and with consent of parents, 76 children were eligible for study. Urine sample was collected as per feasibility and requirement for study from these children. All samples were subjected to microscopy, dipstick leucocyte esterase and nitrite. Among these, 38

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turned out to be culture positive and 38 were culture negative. Organisms isolated through culture were in order of E.coli(31), Pseudomonas(4) and Klebsiella(3) according to the frequency. In our study in detection of UTI, urine microscopy alone found to have Sensitivity- 97.37%, Specificity- 18.42%, PPV- 54.41%, NPV97.5%, urine dipstick leucocyte esterase alone had Sensitivity- 76.32%, Specificity- 63.16%, PPV- 67.44%, NPV- 72.73%, urine dipstick nitrite alone had Sensitivity- 39.47%, Specificity- 100%, PPV- 100%, NPV62.3%. When anyone of the three screening tests were considered as positive, sensitivity and negative predictive value increased to 100%. A figure of 100 % could be due to small sample size. Even then by considering anyone of screening test positive as significant, sensitivity increased than each screening test using alone.

CONCLUSIONS: Our study emphasizes that considering dipstick esterase and nitrite as screening tests along with microscopy will reduce the chance of missing a case of UTI. Delay in diagnosing a case of UTI may lead to complications, further renal scarring and renal failure according to severity.

INTRODUCTION

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Urinary tract infection is common bacterial infection in children. UTIs have been considered a risk factor for the development of renal insufficiency or end-stage renal disease in children, although some have questioned the importance of UTI as an isolated risk factor, because only 2% of children with renal insufficiency[(1)] report a history of UTI. This paradox may be secondary to better recognition of the risks of UTI and prompt diagnosis and therapy. Furthermore, many children receive antibiotics for fever without a specific diagnosis (e.g., treating a questionable otitis media) resulting in a partially treated UTI. Diagnosis of Urinary tract infection is primarily based on symptoms and signs but the definitive diagnosis depends on positive bacterial culture of urine (gold standard). Urine culture is expensive and is also time consuming because the result usually takes 48 -72 hours to be available for the clinician. The quality of urine sample will affect the ability to detect bacteria and confirm the diagnosis of urinary tract infection[(2).]

There are few other tests which can be used for screening of UTI like dipstick leucocyte esterase and nitrite.

The leukocyte esterase is a semi quantitative test that detects the neutrophil specific esterase activity released from degraded white blood cells. The nitrite reduction test detects nitrite produced by urinary bacterial pathogens. Nitrites are not found in urine normally and usually result when urinary bacteria reduce nitrates to nitrites. Many gram negative and gram positive bacteria are capable to do so.[(3)]

A positive dipstick nitrite test indicates that those organisms are present in significant numbers (more than 100,000 per ml).[(4)]

Results of urinalysis, particularly the leukocyte esterase and nitrite tests, often are used to determine whether treatment is needed or not. A culture will be performed in cases of suspected urinary tract infection. Many clinicians interpret positive results in these tests as indicators of

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probable infection and use the results to guide patient treatment. However, there is disagreement about the quality of urinalysis as a screening test for urinary tract infections.

In this study we tried to compare the performance of the dipstick (leukocytes esterase, nitrite) testing, urine microscopy and urine culture in detection of urinary tract infection in children between 1month to 12 year. Improving the diagnostic tests for detection of urinary tract infection helps to avoid unnecessary antibiotics, which are contributing in the growing problem of antibiotic resistance.

AIMS & OBJECTIVES:

To correlate urine culture with urine microscopy, leucocyte esterase and dipstick nitrite in detection of UTI.

MATERIAL & METHODS:

STUDY SITE -
OPD & IPD of Ekta Institute Of Child Health, Shanti Nagar, Raipur (C.G.)

STUDY POPULATION –

Pediatric age group between 1month to 12 year with clinical suspicion of UTI in OPD & IPD at EKTA INSTITUTE OF CHILD HEALTH, RAIPUR

STUDY DESIGN –
A Prospective observational study

Sample size-

All the children between 1month and 12 year with clinical suspicion of UTI in OPD and IPD of Ekta institute of child health during the study period and whose parents give consent.

Period of study-

October 2014 to September 2016.

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Inclusion criteria

1. Children of age 1 month to 12 year with clinical suspicion of UTI.

Exclusion criteria

1. Prior administration of antibiotic therapy in past 72hrs.

METHODOLOGY –

A. METHOD OF COLLECTION OF DATA

Primary diagnosis will be determined at the time of opd visit in patients with fever, frequent micturition, dysuria/baby crying during micturition, vomiting, anorexia, etc.

Urine sample collected either midstream catch, catheterized, or supra pubic aspiration[13] as feasible as possible. Along with them other lab parameters for infection will also be done. Specimens containing squamous epithelial cells were not excluded from analysis because other investigators have found that the presence of squamous cells does not affect the diagnostic accuracy of the test[7,9].

Urine sample is subjected to urine microscopy, dipstick leucocyte esterase, nitrite and urine culture. Dip stick reports and urine microscopy collected and followed for culture report and collected and analyzed.

LAB METHODS:-

Urine routine microscopy: Urine sample collected as explained above is subjected to centrifugation. After centrifugation, sediment at the base is collected and made a slide with cover slip on the sample and observed in high power field. Microscopy of the urine is recommended in textbooks for the diagnosis of urinary tract infections[11]. Urinary microscopy is considered as suggestive of UTI if,

1) pus cells are >10 in uncentrifuged sample

2) pus cells are >5/hpf in centrifuged sample.

Hoberman and Wald reported that the positive predictive value of pyuria (10 white blood cells/mm^3) and bacteriuria is as high as 84.6%. Because of the low sensitivity, negative urine microscopy does not rule out UTI.

Urine leucocyte esterase : Leucocyte esterase is a reliable indicator of leucocytes in urine. A positive reaction (small or greater) at less than 2 minute reading time may be regarded as positive indication of leucocytes in urine. Granulocytic leukocytes contain esterases that catalyze

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the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy- 5-pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple product.
Ingredients- 0.4% w/w derivated pyrrole amino acid ester; 0.2%w/w diazonium salt; 40.9% w/w buffer; 58.5% w/w nonreactive ingredients.

Urine dipstick nitrite : Dipstick nitrite is specific for nitrite and will not react with any other substance normally excreted in urine. Nitrite concentration increases with the length of time the urine specimen is retained in the bladder prior to collection. A minimum of 4 hrs of bladder incubation significantly increases the likelihood of obtaining a positive result. This test depends upon the conversion of nitrate to nitrite by the action of gram negative bacteria in the urine. At the acid pH of the reagent area, nitrite in the urine reacts with p-arsanilic acid to form a diazonium compound. This diazonium compound in turn couples with 1,2,3,4-tetrahydrobenzo(h)quinolin-3-ol to produce a pink color.
Ingredients- 1.4% w/w p-arsanilic acid; 1.3%w/w 1,2,3,4,-tetrahydrobenzo(h)quinolin-3-ol; 10.8% w/w buffer; 86.5% w/w nonreactive ingredients.

Urine culture : Hicrome UTI agar is used which is a differential medium recommended for presumptive identification of microorganisms mainly causing urinary tract infections. Suspend 56.8 grams in 1000 ml distilled water. Heat up to boiling point to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121^0 c) for 15 min. Cool to 50^0 c. mix well and pour into sterile Petri plates. Cultural characteristics observed after incubation at 35-37^0 c for 18-24 hrs.
Ingredients- Peptic digest of animal tissue 15gms/l; Chromogenic mixture 26.8gms/l; Agar 15gms/l; Final pH(at 25^0c) is 6.8. Cut off for significant bacteriuria was taken as 10^5 cfu/ml[12].

Statistical methods:

Calculation of sensitivity, specificity, and predictive values

Sensitivity, specificity, and predictive values were calculated for leukocyte esterase, nitrite, or blood on dipstick and for RBCs, WBCs, or bacteria on microscopic urinalyses. Sensitivity, specificity, and predictive values were calculated as follows [10]:

Sensitivity=True positive/(True positive+False negative)

Specificity=True negative/(True negative+False positive)

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Positive Predictive Value= True positive/(True positive + False positive)

Negative Predictive Value= True negative/ (True negative + False negative)

RESULTS AND DISCUSSION:

This is a prospective observational study which was performed to correlate urine culture with urine microscopy, leucocyte esterase and dipstick nitrite in detection of UTI.

In present study, out of 76 children studied, 47% were male and 53% female. 18% of the children in study were infants and 82% were between 1 and 12 yr.

Table-1

Sex Distribution Of Children Studied

Sex	No.	%
Male	36	47
Female	40	53
Total	76	100

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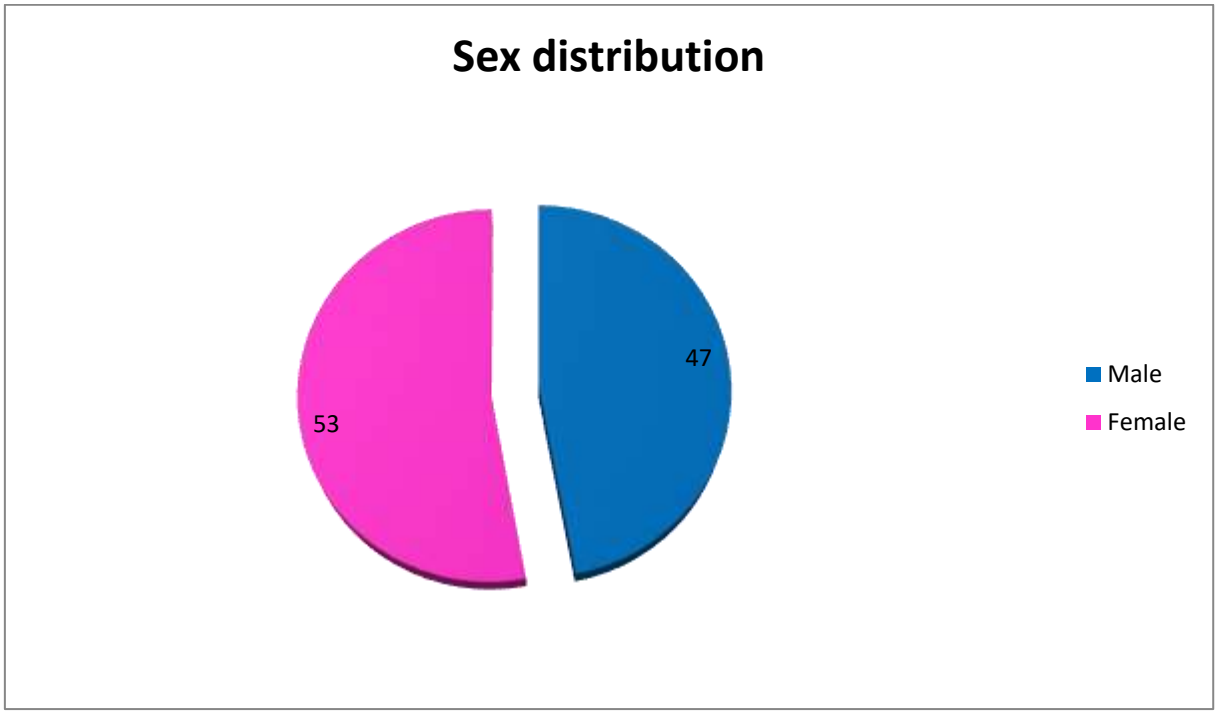

Table-2

Distribution Of children studied as per age

Out of total children 14 (18%) were infants and 62 (82%) were 1 to 12 yr.

Age	No.	%
1month - 1yr	14	18
1yr - 12 yr	62	82
total	76	100

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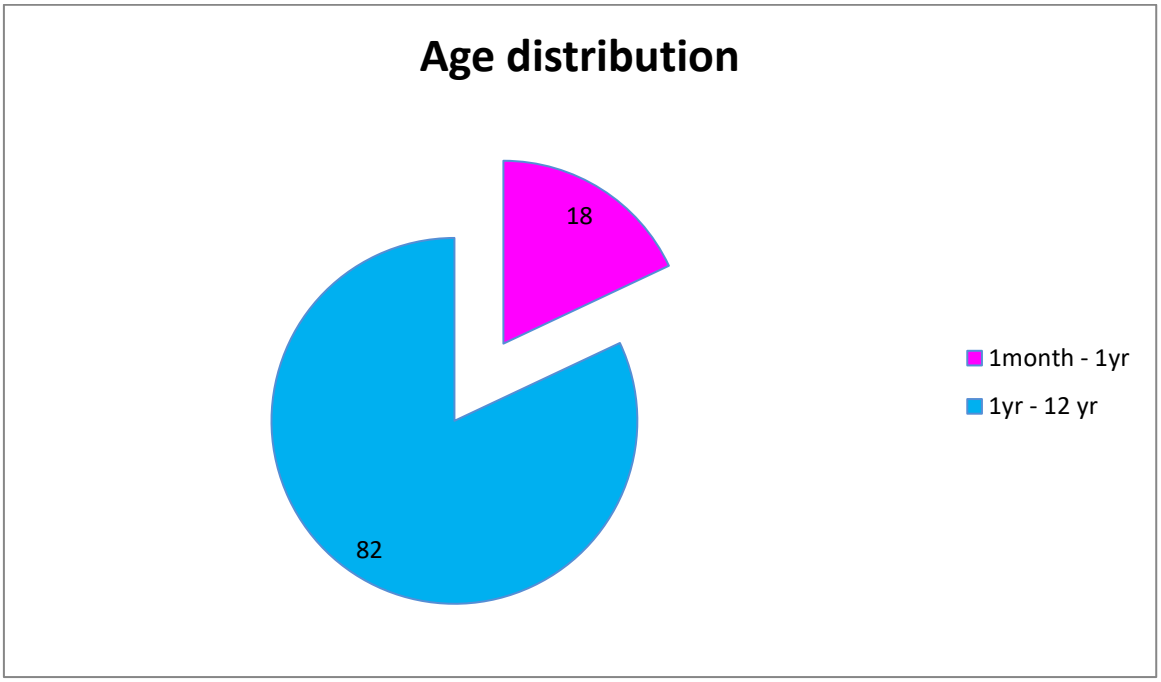

Table-3

Distribution of samples based on Urine Microscopy

Out of 76 samples, 68 turned out to be positive for microscopy and 8 were negative.

Urine Microscopy	No	%
Positive	68	89
Negative	8	11
Total	76	100

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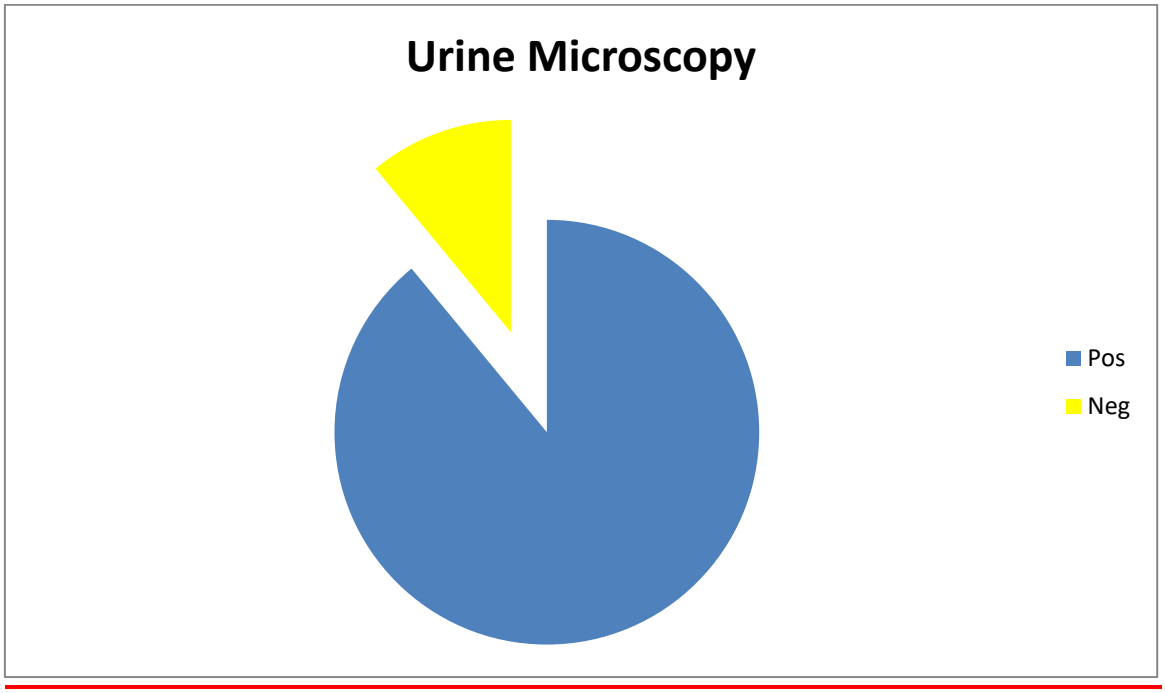

Table-4

Distribution of sample based on Leucocyte Esterase

Out of 76 samples, 43 were positive for leucocyte esterase and 33 were negative.

Leucocyte esterase	No	%
Positive	43	56
Negative	33	44
Total	76	100

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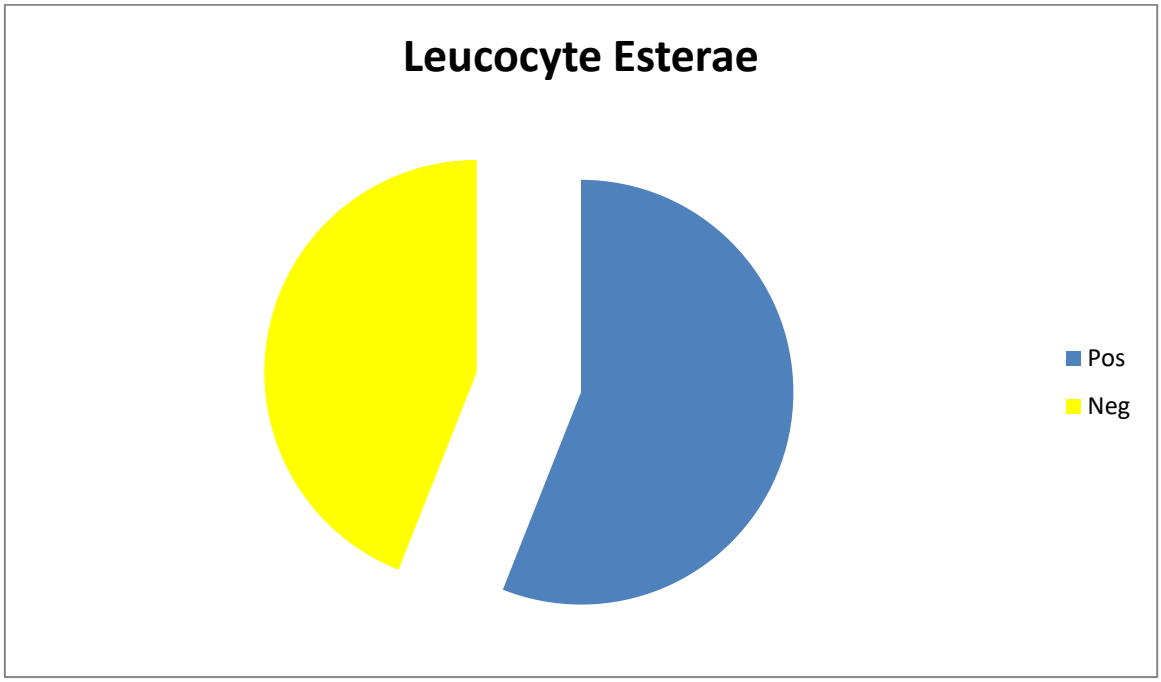

Table-5

Distribution of sample based on Nitrite

Among 76 samples, 15 samples were positive for nitrite and 61 were negative.

Nitrite	No	%
Positive	15	20
Negative	61	80
Total	76	100

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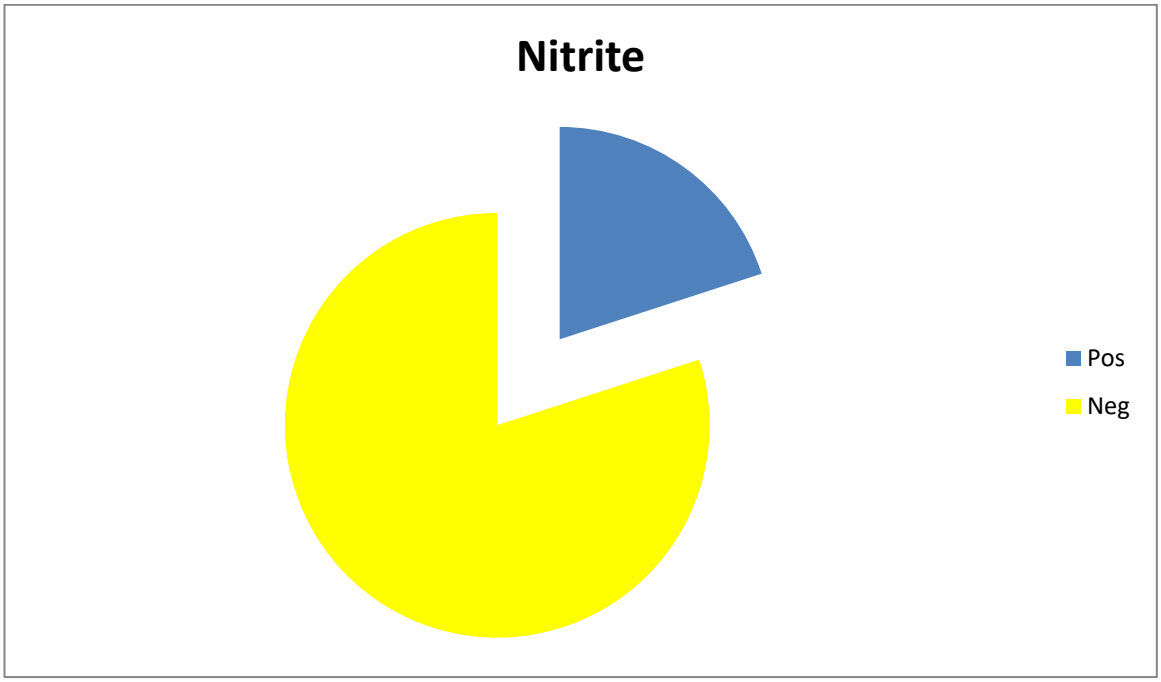

Table-6

Distribution of samples based on culture

Out of 76 samples cultured, 38 (50%) were positive and 38 (50%) were negative.

Urine culture	No.	%
Positive(yield growth)	38	50
Negative(no growth)	38	50
Total	76	100

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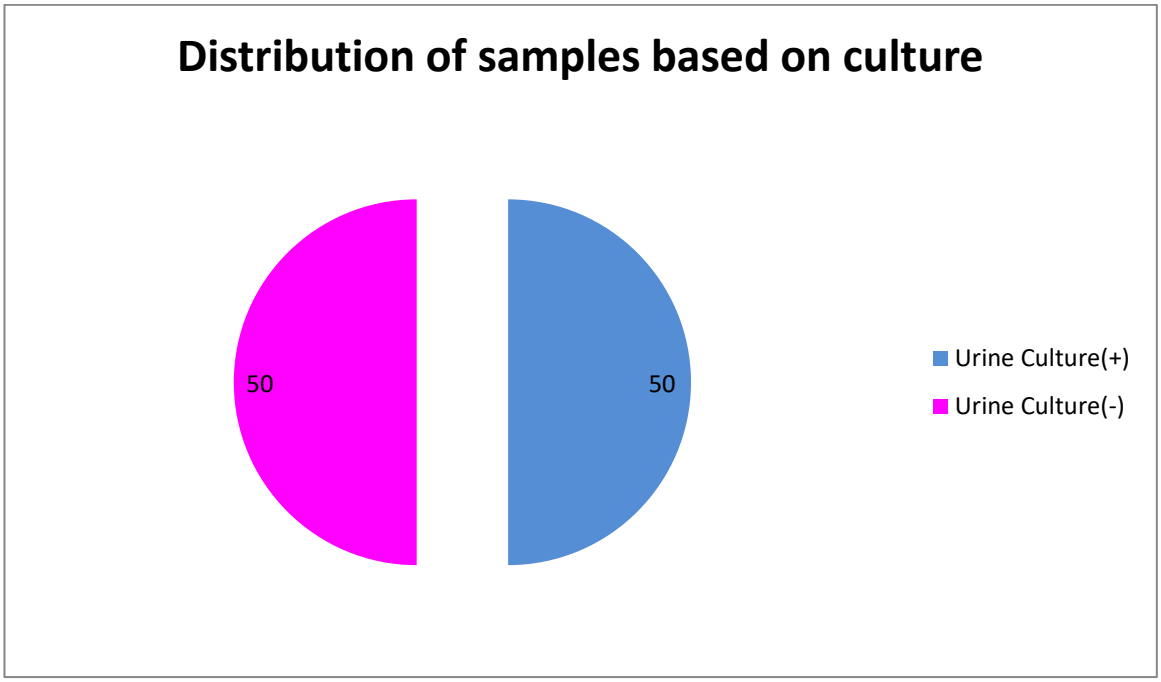

Table-7

Distribution of isolated organism on urine culture

Isolated organism	No
E.coli	31
Pseudomonas	4
Klebsiella	3
Total	38

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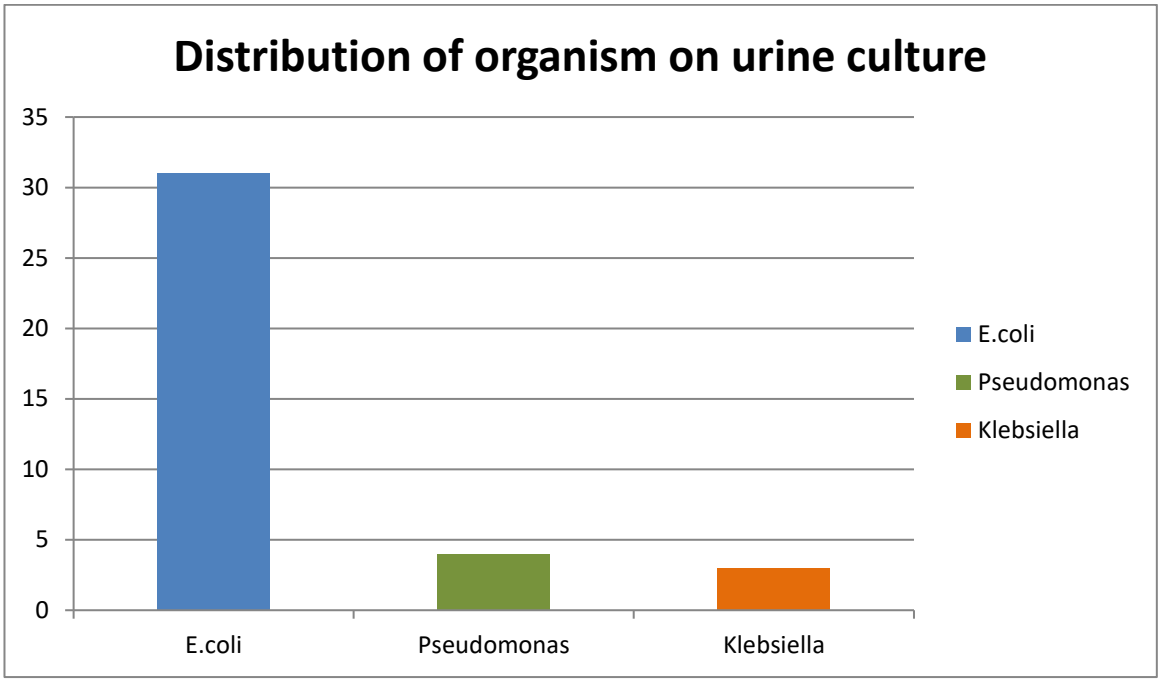

Organisms isolated through culture were in order of E.coli(31), Pseudomonas[14](4) and Klebsiella(3) according to the frequency.

In our study, positive urine culture with significant bacteriuria (10^5) was found in 38 (50%) out of 76.

DR. S.I. ADELEKE.[6] et al study had 19 (29%) positive out of 65 urine sample cultured.

Ramazan memisogullan[5], Hatice yuksel, et al study had 89 (35.6%) positive out of 250 urine sample cultured.

N Taneja[8], SS Chaterjee, et al study had 98 (21.8%) positive out of 450 urine sample cultured.

This variability in percentage of positivity of culture could be due to mode of collection of sample which has been done according to feasibility in all studies like collection of urine through sterile bags in infants in some studies. More positivity in our study can be due to strict clinical criteria utilization.

Table-8

Sensitivity, Specificity and Predictive values of Microscopy With Culture As Standard

	Culture				Total	$\chi 2$
	+	N	–	n		

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Microscopy	Positive	True Positive	37	False Positive	31	68	5.02 Yates Correction 3.52 p<.051
	Negative	False Negative	01	True negative	07	08	
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
Microscopy	97.37%	18.42%	54.41%	97.50%

- Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.
- 37 cases were positive for microscopy (true positive) and 1 case was negative (false negative) when culture was positive. 7 cases with negative culture were negative for microscopy (true negative), while 31 cases in spite of no growth showed positive microscopy (false positive).
- Sensitivity of microscopy showed that positive microscopic results can correctly identify 97.37% of cases with UTI when culture is positive.
- Specificity of microscopy showed that negative microscopic results can correctly identify 18.42% of cases without UTI when culture is negative.
- PPV of microscopy showed 54.41% probability that subjects with a positive test truly have UTI.
- NPV of microscopy showed 97.50% probability that subjects with a negative test do not have UTI.
- A significant association was observed between microscopy and culture
- **DR. S.I. ADELEKE.[6] et al** study had Sensitivity- 43%, PPV- 37.1% for urine microscopy.
- **Ramazan memisogullan[5], Hatice yuksel, et al** study had Sensitivity- 91%, Specificity- 68%, PPV- 61%, NPV- 93% for urine microscopy.
- **U.S.Nayak[7] et al**, study had Sensitivity- 63.5% for urine microscopy.
- **N Taneja[8], SS Chaterjee, et al** study had Sensitivity- 68.4%, Specificity- 60.8%, PPV- 32.7%, NPV- 87.3% for urine microscopy.
- More sensitivity and low specificity in our study can be due to small sample size and also can be due to observational variation by pathologists.

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Table-9

Sensitivity, Specificity and Predictive values of Leucocyte esterase

		Culture				Total	$\chi 2$
		+	N	–	N		
LET	Positive	True Positive	29	False Positive	14	43	12.05 p<.00005
	Negative	False Negative	09	True negative	24	33	
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
LET	76.32%	63.16%	67.44%	72.73%

- Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.
- 29 cases were positive for leucocyte esterase (true positive) and 9 cases were negative (false negative) when culture was positive. 24 cases with negative culture were negative for leucocyte esterase (true negative), while 14 cases in spite of no growth showed positive leucocyte esterase (false positive).
- Sensitivity of leucocyte esterase showed that positive leucocyte esterase results can correctly identify 76.32% of cases with UTI when culture is positive.
- Specificity of leucocyte esterase showed that negative leucocyte esterase results can correctly identify 63.16% of cases without UTI when culture is negative.
- PPV of leucocyte esterase showed 67.44% probability that subjects with a positive test truly have UTI.
- NPV of leucocyte esterase showed 72.73% probability that subjects with a negative test do not have UTI.
- A significant association was observed between leucocyte esterase and culture.
- **DR. S.I. ADELEKE.[6] et al** study had Sensitivity- 74%, PPV- 87.2% for urine dipstick leucocyte esterase.
- **Ramazan memisogullan[5], Hatice yuksel, et al** study had Sensitivity- 80%, Specificity- 60%, PPV- 52%, NPV- 84% for urine dipstick leucocyte esterase.
- **U.S.Nayak[7] et al**, study had Sensitivity- 61% for urine dipstick leucocyte esterase.
- **N Taneja[8], SS Chaterjee, et al** study had Sensitivity- 73.5%, Specificity- 58.5%, PPV- 33%, NPV- 88.8% for urine dipstick leucocyte esterase.
- Statistics for leucocyte esterase in our study correlate with other studies.

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Table-10

Sensitivity, Specificity and Predictive values of Nitrite

		Culture				Total	$\chi 2$
		+	N	–	n		
NT	Positive	True Positive	15	False Positive	00	15	18.68 Yates Correction 16.28 p<.00005
	Negative	False Negative	23	True negative	38	61	
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
NT	39.47%	100%	100%	62.30%

- Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.
- 15 cases were positive for nitrite (true positive) and 23 cases were negative (false negative) when culture was positive. 38 cases with negative culture were negative for nitrite (true negative), while no cases in spite of no growth showed positive nitrite (false positive).
- Sensitivity of nitrite showed that positive nitrite results can correctly identify 39.47% of cases with UTI when culture is positive.
- Specificity of nitrite showed that negative nitrite results can correctly identify 100% of cases without UTI when culture is negative.
- PPV of nitrite showed 100% probability that subjects with a positive test truly have UTI.
- NPV of nitrite showed 62.3% probability that subjects with a negative test do not have UTI.
- A significant association was observed between nitrite and culture.
- **Ramazan memisogullan[5], Hatice yuksel, et al** study had Sensitivity- 80%, Specificity- 60%, PPV- 52%, NPV- 84% for urine dipstick nitrite.
- **U.S.Nayak[7] et al**, study had Sensitivity- 50% for urine dipstick nitrite.
- **N Taneja[8], SS Chaterjee, et al** study had Sensitivity- 73.5%, Specificity- 58.5%, PPV- 33%, NPV- 88.8% for urine dipstick nitrite.
- Variation in sensitivity could be due to difference in duration of stasis of urine before sample collection i.e. children less than 3-4 yrs cannot hold urine for long time. In our

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study we had to collect some samples even in day time because of noncompliant patients. 100% specificity can be because of small sample size.

Table-11

Sensitivity, Specificity and Predictive values of Either Leucocyte esterase or nitrite positive

		Culture				Total	$\chi 2$
		+	N	–	n		
LET/NT	Positive	True Positive	33	False Positive	14	47	20.12 $p < 0.000007$
	Negative	False Negative	05	True negative	24	29	
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
LET/NT	86.84%	63.16%	70.21%	82.76%

- Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.
- 33 cases were positive if either of esterase or nitrite considered positive (true positive) and 5 cases were negative (false negative) when culture is positive. 24 cases with negative culture were negative when either of esterase or nitrite considered positive (true negative), while 14 cases in spite of no growth were positive when either of esterase or nitrite considered positive (false positive).
- Sensitivity when either of esterase or nitrite considered positive showed that positive results when either of esterase or nitrite considered positive can correctly identify 86.84% of cases with UTI when culture is positive.
- Specificity when either of esterase or nitrite considered positive showed that negative results when either of esterase or nitrite considered positive can correctly identify 63.16% of cases without UTI when culture is negative.
- PPV when either of esterase or nitrite considered positive showed 70.21% probability that subjects with a positive test truly have UTI.
- NPV when either of esterase or nitrite considered positive showed 82.76% probability that subjects with a negative test do not have UTI.
- **U.S.Nayak[7] et al**, study had Sensitivity- 68% when considered urine dipstick leucocyte esterase or nitrite as positive.
- **N Taneja[8], SS Chaterjee, et al** study had Sensitivity- 79.6%, Specificity- 56.5%, PPV- 33.8%, NPV- 90.9% when considered urine dipstick leucocyte esterase or nitrite as positive.

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- Statistics for this combination in our study correlate with other studies.

Table-12

Sensitivity, Specificity and Predictive values of any of Microscopy, Leucocyte esterase, Nitrite Positive

		Culture				Total	$\chi 2$
		+	N	–	N		
Micro /LET/ NT	Positive	True Positive	38	False Positive	32	70	6.51 Yates Correction 4.53 p<.0331
	Negative	False Negative	00	True negative	06	06	
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
Microscopy/LET/NT	100%	15.79%	54.29%	100%

- Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.
- 38 cases were positive when any of microscopy, esterase, nitrite considered positive (true positive) and no cases were negative (false negative) while culture is positive. 6 cases with negative culture were negative when any of microscopy, esterase, nitrite considered positive (true negative), while 32 cases in spite of no growth were positive when any of microscopy, esterase, nitrite considered positive (false positive).
- Sensitivity when any of microscopy, esterase, nitrite considered positive showed that positive results when any of microscopy, esterase, nitrite considered positive can correctly identify 100% of cases with UTI while culture is positive.
- Specificity when any of microscopy, esterase, nitrite considered positive showed that negative results when any of microscopy, esterase, nitrite considered positive can correctly identify 15.79% of cases without UTI when culture is negative.
- PPV when any of microscopy, esterase, nitrite considered positive showed 54.29% probability that subjects with a positive test truly have UTI.
- NPV when any of microscopy, esterase, nitrite considered positive showed 100% probability that subjects with a negative test do not have UTI.
- **U.S.Nayak[(7)] et al**, study had Sensitivity- 75% when considered any one of urine microscopy, dipstick leucocyte esterase, nitrite as positive.

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- **N Taneja[8], SS Chaterjee, et al** study had Sensitivity- 95.9%, Specificity- 52.3%, PPV- 35.9%, NPV- 97.9% when considered any one of urine microscopy, dipstick leucocyte esterase, nitrite as positive.
- High sensitivity and low specificity in this combination in our study again probably due to small sample size.

Table-13

Sensitivity, Specificity, Predictive values and Accuracy of Microscopy, LET, NT test

Test	Sensitivity (95% CI)	Specificity (95% CI)	Predictive vales (95% CI)		Accuracy (%)
			Positive	Negative	
Microscopy	97.37 (86.19;99.93)	18.42 7.74; 34.33	54.41 41.88;66.55	87.50 47.35;99.68	57.89
LET	76.32 59.76;88.56	63.16 45.99;78.19	67.44 51.46;80.92	72.73 54.48;86.70	69.73
NT	39.47 24.04-56.61	100 90.75-100	100 78.20-100	62.30 48.96;74.39	56.57
LET/NT	86.84 71.91;95.59	63.16 45.99;78.19	70.21 55.11;82.66	82.76 64.23;94.15	75.00
Micro/ LET/NT	100 90.75;100.00	15.99 6.02;31.25	54.29 41.94;66.26	100 54.07;100	57.89

Conclusion:

Our study emphasizes that considering dipstick esterase and nitrite as screening tests along with microscopy will reduce the chance of missing a case of UTI. Delay in diagnosing a case of UTI may lead to complications, further renal scarring and renal failure according to severity.

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ABBREVIATION

UTI Urinary Tract Infection

LET Leucocyte Esterase Test

NT Nitrite

PPV Positive Predictive Value

NPV Negative Predictive Value

DOR Diagnostic Odds Ratio

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SC South Carolina

OPD Out Patient Department

IPD In Patient Department

E.coli Escherichia coli

CI Confidence Interval