RESEARCH PAPER

Identification of Probable Urinary Tract Infection in Children Using Low Bacterial Count Thresholds in Urine Culture

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Received: Aug 7, 2022;
Initial review: Oct 05, 2022;
Accepted: Dec 26, 2022.

Objectives: To assess the proportion of children, symptomatic for urinary tract infection (UTI), with urine culture showing single bacterial species >10^4 CFU/mL, and to compare patient and disease characteristics between children having low counts (from >10^4-10^5 CFU/mL) and those with counts >10^5 CFU/mL. **Methods:** Prospective observational study, enrolling symptomatic children aged 1 month to 12 years. Mid-stream clean-void or catheter collected urine were cultured. Children with single species >10^4 CFU/mL were scheduled for imaging studies, following age criteria of Indian Society of Pediatric Nephrology guidelines. The main outcome was proportion with single bacterial species >10^4 CFU/mL in urine culture. **Results:** Of 216 children (132 males) with median (IQR) age of 24 (12, 48) months, 38 (17.6%) showed single species growth >10^4 CFU/mL. Of these, 29 (13.4%) were diagnosed as UTI at cutoff >10^5 CFU/mL, and an additional 9 (4.2%) were found to have 'probable low-count UTI' (from >10^4 to 10^5 CFU/mL). One child in the latter group had bilateral hydroureteronephrosis, vesico-ureteral reflux and renal scarring. There was largely no difference in parameters between children with low counts and those with counts >10^5 CFU/mL. **Conclusion**: An additional proportion of symptomatic children with probable urinary tract infection and possible underlying urological abnormalities may be identified by lowering bacterial colony count cutoff to >10^4 CFU/mL, in clean-voided and catheter-based urine samples.

Keywords: *Bacterial colony, Diagnosis, Urological abnormalities.*

Published online: Feb 09, 2023; **PII**: S097475591600488

A reliable diagnosis of urinary tract infection (UTI) in a child is often challenging, because it is difficult to differentiate between contaminants and true bacterial infection [1]. The traditional definition of significant bacteriuria of >10^5 colony-forming units (CFU)/mL in clean-voided urine samples [2,3] has been extrapolated from studies done in adult females nearly 60 years ago [4]. Similarly, the recommended cutoff of >50,000 CFU/mL in transurethral catheter specimen [2,5] was derived in a study nearly 3 decades ago [6]. The accuracy of these cutoffs has been questioned [7], suggesting a possibility of true UTI in children even with lower colony counts [8,9].

There is lack of evidence regarding low bacterial colony count UTI in children, especially in clean-voided or catheter samples [10,11], resulting in variation across different guidelines [2,3,5,12,13]. Our study aimed to identify the proportion of symptomatic children who show growth of a single bacterial species with colony counts >10^4 CFU/mL in urine culture, and to compare the clinical characteristics, bacteriological profile and imaging findings between children having 'probable low bacterial count UTI' (from >10^4CFU/mL up to 10^5CFU/mL) and those diagnosed with UTI at conventional bacterial colony count cutoff (>10^5 CFU/mL).

METHODS

This prospective observational study, in a tertiary care hospital, was conducted from 1 October, 2020 to 30 September, 2021. Following approval by the institutional ethics committee, consecutive children from 1 month to 12 years of age, having clinical features suggestive of UTI, as described in the table of signs/symptoms in infants and children suggestive of UTI, provided by NICE (National Institute for Health and Clinical Excellence, 2018) UTI guidelines [14], were screened for eligibility. Children already on antibiotics, those with indwelling bladder catheter, and those in which the clinical features were better explained by a diagnosis other than UTI, were excluded.

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After taking informed consent, clinical details were recorded and urine samples were collected in sterile containers for urinalysis and culture. While a mid-stream, clean-voided sample was collected from toilet trained

children, a catheter sample was taken from non-toilet trained children. Centrifuged specimen was used for urinalysis but was used neat to plate for culture. A semi quantitative culture technique was used. Using a loop of 0.002 mL, urine was plated on CLED (Cysteine-Lactose-Electrolyte Deficient) agar. The number of colonies grown per microliter of urine gave the estimate of bacterial growth. One microliter of urine growing 100 colonies, meant growth of 10^5 CFU/mL of urine. Thus, for a growth of 10^4 CFU/mL of urine, 1 microliter grew at least 10 colonies.

All symptomatic children who showed growth of single bacterial species in urine culture with colony counts $>10^4$ CFU/mL were scheduled for radiological investigations following the age criteria as in ISPN 2011 guidelines [2]. Those children with bacterial counts $>10^5$ CFU/mL in urine culture were provided standard treatment with or without antibiotic prophylaxis [2]. However, administering antibiotics to children with low bacterial colony counts ($>10^4$ - 10^5 CFU/mL) in urine culture, was at the discretion of the treating physician, depending upon the sickness and clinical features.

For this study, "probable UTI" was identified if: *a*) clinical features consistent with UTI (as per NICE guidelines 2018 [14], table of symptoms and signs in infants and children suggestive of urinary tract infection), and *b*) urine culture showing growth of a single bacterial species with colony counts ranging from $>10^4$ to 10^5 CFU/mL, with either mid-stream or catheterized sample.

Taking prevalence of UTI of 7-10% in symptomatic children, at conventional colony count cutoffs [15], and expecting 15% [11] would be identified at threshold of $>10^4$ CFU/mL, with a precision of 0.05 and 95% CI, we got a sample size of 196.

Statistical analysis: Mean (SD) or median (IQR) were compared between groups by independent sample *t* test or Mann Whitney *U* test, respectively. Proportions were compared by chi-square test. Analysis was done using SPSS 21 software, taking statistical significance at $P<0.05$.

RESULTS

Of the 850 children assessed for eligibility, 216 children (61.1% males), with median age of 24 (IQR12, 48) months, met the inclusion criteria. The baseline characteristics of the children are shown in **Table I**. Most (*n*=195) of them presented with the "most common symptoms" category of the NICE table [14]. Six children had prior documented history of UTI: two of them having UTI 1 month and 5 months ago, had not been evaluated earlier radiologically. Remaining four had previous UTI between 3-24 months ago. All four had posterior urethral valve (PUV), three of them having hydronephrosis and vesico-ureteral reflux (VUR).

Table I Demographic and Clinical Characteristics of Children With Suspected Urinary Tract Infection (*N*=216)

Parameters	*Value*
Age (mo)[a]	24 (12, 48)
Girls	84 (38.8)
Weight (kg)[b]	12.3 (6)
Height (cm)[b]	87.5 (20.2)
Previous UTI	6 (2.8)
Known CAKUT[c]	7 (3.2)
Duration of symptoms (d)[a]	3 (2,5)
Fever	161 (74.5)
Vomiting	61 (28.2)
Irritability	11 (5.1)
Lethargy	3 (1.4)
Poor feeding	6 (2.8)
Loin tenderness	1 (0.5)
Dysuria	34 (15.7)
Frequency	10 (4.6)
Cloudy urine	3 (1.4)

Values are in n (%), [a]median (IQR) or [b]mean (SD). UTI:urinary tract infection; CAKUT:congenital anomalies of kidney and urinary tract; [c]children with posterior urethral valve and/or vesico-ureteral reflux.

Urine specimens were collected by catheter and by clean-voided method from 123 and 93 children, respectively.

Urine culture results showed 51/216 (23.6%) children having bacterial growth of a single species, 19/216 (8.8%) having mixed growth while rest 146/216 (67.6%) had no growth (**Fig. 1**). Of the 51 pure growths, 38 samples (17.6% of all enrolled symptomatic children) showed colony counts $>10^4$ CFU/mL. While 29 (13.4%) children were diagnosed with UTI at conventional colony count cutoff ($>10^5$ CFU/mL), an additional 9 (4.2 %) were found to have 'probable low-count UTI' (from $>10^4$ to 10^5 CFU/mL). Of the six children having previous history of UTI, two had sterile urine cultures, 3 had UTI at conventional counts and one had low-count UTI. *E. coli* was the most common organism isolated in 31 (81.6%), followed by *Klebsiella*

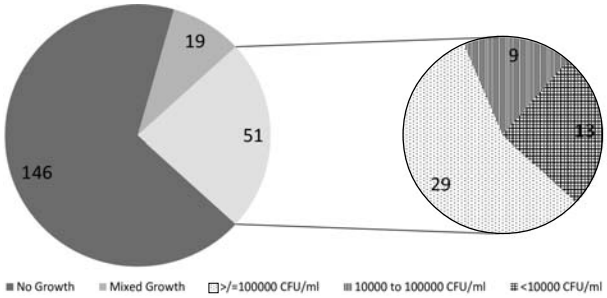

Fig. 1 Results of urine culture of symptomatic children with suspected urinary tract infection (*n*=216).

pneumoniae, *Enterococcus faecium*, *Proteus mirabilis* and *Staphylococcus aureus*.

Radiological investigations revealed abnormalities in one 31-month boy, among the nine children with 'probable low-count UTI.' He was found to have bilateral hydroureteronephrosis on ultrasonography, bilateral VUR (Grade III right kidney, Grade II left kidney) in MCU, and multiple scars in the right kidney in DMSA scan. Ultrasonography was done in 37 children, with eight (21.6%) showing abnormal findings, seven with hydronephrosis (including the boy with 'probable low-count UTI'), and one child with small contracted kidneys. Of the 15 children in whom MCU was indicated, eight (including two with low-counts) underwent the procedure, five (62.5%) of them showing abnormalities. Of the 31 children (eight with low-counts) who were required to undergo DMSA renal scan, 17 children (5 with low counts) could avail it, and four (23.5%) of them showed scars in DMSA, one of them being a child with 'probable low-count UTI.

Comparison of parameters (**Table II)** between children with UTI diagnosed at conventional colony count cutoff and those with 'probable low-count UTI' showed no significant difference in terms of age, gender, method of collection of urine, nor frequency of abnormal imaging findings. While all the children diagnosed with UTI at conventional colony counts received antibiotics as per guidelines, four children with 'probable low-count UTI' were prescribed antibiotics by the treating physician.

DISCUSSION

Our study showed that by lowering the threshold of significance to a bacterial colony count of $>10^4$ CFU/mL, 17.6% children with symptoms could be identified with such bacterial growths. While at conventional colony count cutoff, only 13.4% children would have been diagnosed with UTI, an additional 4.2 % were found with 'probable low-count UTI.'

Taking a cutoff of $\geq 10^4$ CFU/ml, Primack, et al. [16], diagnosed an additional 1.8% symptomatic children with UTI aged 2 months to 6 years. Low bacterial count $<10^5$ CFU/mL in suprapubic urine samples have been reported earlier [10]. Moreover, in catheterized samples, authors have demonstrated that about 9-10% of children with UTI would be missed if the colony count cutoff was not decreased below 50,000 CFU/mL [11].

In our study, a higher proportion of children with 'probable low-count UTI' was below 2 years of age, compared to the conventional-count UTI group. The difference; though, not statistically significant, does reiterate the argument in favor of using low-count cutoffs in infants and young children, who are unlikely to hold urine long enough to allow bacterial growth to reach counts beyond 50,000 or 100,000 CFU/mL. The two children with low counts identified by Primack, et al. [16], were aged 8 months and >2 years, respectively. Another study reported that the number of children with low-counts decreased with increasing age of the children [11].

Table II Characteristics of Children With UTI Diagnosed at Conventional Bacterial Colony Count Cutoff ($>10^5$ CFU/mL) and Probable Low-Count UTI ($>10^4$-10^5 CFU/mL)

Parameters	*Conventional -count UTI* [b] *(n=29)*	*Probable low-count UTI*[c] *(n=9)*	*OR (95% CI)*	*P value*
Age (mo)[a]	29 (13,90)	24 (16,39)	-	0.436
Male sex	13 (44.8)	7 (77.8)	0.58 (0.33 to 0.98)	0.084
Urine collection by catheter	17 (58.6)	5 (55.6)	1.13 (0.25 to 5.12)	0.871
NICE 'most common symptoms'	29 (100)	8 (88.9)	1.12 (0.89 to 1.42)	0.069
Fever	20 (69)	8 (88.9)	0.77 (0.55 to 1.09)	0.236
Vomiting	8 (27.6)	2 (22.2)	1.24 (0.32 to 4.82)	0.750
Dysuria	12 (41.4)	0	1.70 (1.25 to 2.31)	0.020
Antibiotics given	29 (100)	4 (44.4)	2.20 (1.08 to 4.67)	<0.001
Urea (mg/dL) [a]	19 (15.5,28.7)	20 (14, 34.5)	-	0.976
Creatinine (mg/dL) (*n*=90)[a]	0.27 (0.19, 0.52)	0.25 (0.15,0.36)	-	0.377
Abnormal DMSA (*n*=17)	3/12 (25)	1/5 (20)	1.25 (0.17 to 9.31)	0.825
Abnormal MCU (*n*=8)	4/6 (66.7)	1/2 (50)	1.33 (0.29 to 5.95)	0.673
Abnormal USG (*n*=37)	7/28 (25)	1/9 (11.1)	2.20 (0.32 to 15.9)	0.379
E. coli growth	26 (89.7)	5 (55.6)	6.94 (1.17 to 41.67)	0.021

Values are represented as no. (%) or [a]median (IQR). [b]colony count $>10^5$ CFU/mL; [c]colony count $>10^4$ to 10^5 CFU/mL. UTI:urinary tract infection, USG:ultrasonography; MCU:micturating cystourethrogram, NICE:National Institute for Heath and Clinical Excellence (2018) UTI guidelines [14].

WHAT THIS STUDY ADDS

- By lowering bacterial colony count cutoff to >10^4 CFU/mL, in clean-voided and catheter-based urine samples, an additional proportion of symptomatic children with probable urinary tract infection and possible underlying urological abnormalities may be identified.

While most of the symptoms were similar between the groups, as reported earlier [11], symptoms of dysuria were seen exclusively in the conventional-count UTI group, probably because this group had more children of older ages compared to the low-count group.

One of the nine children with 'probable low-count UTI' had underlying urologic malformations and VUR. Prevalence of scars in kidneys or underlying anatomical abnormalities has hardly been studied in children having UTI with low bacterial counts. Our findings somewhat match with those of Kallenopoulos, et al. [11], who reported similar prevalence of reflux and urologic malformations in children with low and high colony counts. The possibility that such children harboring underlying significant urinary tract abnormalities, may be missed if they are not evaluated further, considering their low-count growths as insignificant.

The relatively small number of children in the low-count group may be a limitation for extrapolating this data to the population. Not all children where radiological investigations were indicated could undergo evaluation. The strengths of our study include patients of a wide age-range, and urine samples collected by clean-voiding or catheter.

This study shows that lowering the threshold of significance for bacterial colony count to >10^4 CFU/mL for both clean-voided and transurethral catheter-based specimens of urine, may help in detection of an additional proportion of symptomatic children with probable low-count UTI, over and above those diagnosed with UTI at conventional colony counts. Such children, even with low bacterial colony counts in urine culture, have a possibility of underlying urologic malformations, and hence, may be considered for further evaluation.

Ethics clearance: Institutional Ethics Committee, CNBC, No. F.1/ IEC/CNBC/ 11/07/ 2020/Protocol no. 76/9670, dated Sep 29, 2020.

Contributors: KM: conceived and designed the study, supervised data acquisition and did the data analysis, made the primary draft of the manuscript for publication; RN: helped in data acquisition, compilation and drafting the manuscript; MK: gave critical inputs in designing the study, data analysis and revised the manuscript critically; KS: was involved in designing the study and supervised all laboratory investigations and approved all results. All authors approved the final version before submission and agree to be accountable for all aspects of the work.

Funding: None; *Competing interests*: None stated.

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