Enhanced quantitative urine culture technique, a slight modification, in detecting under-diagnosed pediatric urinary tract infection

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Research note

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Abstract

Objectives The pediatric urinary tract infection (UTI) often remains under-diagnosed or neglected owing to non-specific clinical presentations, patient failing to describe the actual situation, and of clinical practice in diagnosis—relying upon on in-vitro culture report. The study was aimed to determine the etiologies of UTI in children with enhanced quantitative urine culture technique (EQUC). Results Of 570 urine samples, the significant bacterial growth detected: 16.14% with EQUC, and12.8% with standard urine culture protocol. 20.6% causative isolates to UTI, as detected, with EQUC, missed on standard urine culture (SUC) technique. The age group, in range 1-4 years, was more prone to the infection, where E. coli was the commonest pathogen. EQUC detected the etiologies—MDR, XDR, and ESBL producers; as reported: “no growth” with the SUC technique. 69 E. coli isolates grown with EQUC technique when subjected to susceptibility testing: 46% were ESBL producer, 56.5% were multidrug-resistant (MDR), and 1.4% found extensive drug-resistant (XDR). However, with SUC technique: 40.5% ESBL, 44% MDR but no XDR detected. Hence a simple modification on culture protocol could be a crucial modification for the detection of etiologies contributing UTI, and to reduce inapt antimicrobial burden. Keywords UTI, EQUC, uropathogens, children, ESBL, MDR, XDR

Background

Urinary tract infection is one of the most common infection with a leading cause of morbidity and mortality in children(1). However, in this age-group, the infection often remains under-diagnosed or neglected owing to non-specific clinical presentations, patient failing to describe the actual situation, and of clinical practice of diagnosis(2). Since 1950s, the clinical practice has relied upon SUC protocol as a gold standard in detecting etiologies contributing UTI; nevertheless, continues to be questioned for its precision in both clinical diagnosis(3). Hence, the precise diagnostic protocol is mandatory to reduce the superfluous antimicrobial burden and to truncate the possible adverse consequences, in the pediatric population (1)(3)(2).

Although, the documented incidence of the infection, attending general hospital, ranges from 23.1% to 37.4% in the Nepalese population(4). Incongruously, in Nepal, and most developing countries, the pediatric UTIs cases are treated empirically due to lacking appropriate diagnostic protocol, unavailability of standard therapeutic guidelines, and undocumented resistivity trend of the pathogens in local and regional levels (5)(4)(6). Meanwhile, an accurate diagnosis of etiologies and its resistivity trend against the most preferred antibiotics is crucial for successful clinical management and prophylaxis. With these backdrops, we conducted a study to determine etiology of UTI among children with EQUC, a slight modification on SUC technique, to trace if significant etiology contributing pediatric UTI was missing with SUC.

Methods

Study design and sample population

The cross-sectional study was carried out form April 2017- October 2017 in International Children friendship hospital, Kathmandu, Nepal. The study hospital is a tertiary referral center for children. The totals of 570 urine samples were enrolled in our study. The study populations were infants and children not exceeding 14 years old seeking treatment for presumed UTI.

Inclusion and exclusion criteria

Children enrolled in pediatric outpatient department or admitted to ward with clinical diagnosis as UTI were included. The clinical diagnosis was made by the corresponding unit pediatrician in the presence of fever and/or any of the symptoms suggestive to UTI.

The urine samples which grew more than one type of organism were considered as a contaminant (in those children who had previously known history of antimicrobial therapy within 48 h before attending the hospital) and hence, excluded from the study.

Sample collection and analysis

The urine samples (collected either with urethral catheterization, or supra-pubic aspirations and pediatric urine collection bag for toilet-untrained children, and mid-stream urine for toilet-trained children) were processed semi-quantitatively with SUC and EQUC techniques.

In brief, the SUC protocol used 1 µl of urine, spread quantitatively onto 5% sheep blood (blood agar plate [BAP]) and MacConkey agars (BD BBL Prepared Plated Media; Hi-Media) and incubated aerobically at 35°C for 24 h. The urine samples were then inoculated the corresponding subset of EQUC conditions using three urine volumes (1µl, 10 µl, and 100 µl) and additional plating conditions. Each urine sample was spread quantitatively on to (BAP, chocolate Agar,); chocolate agar plates were incubated in 5% CO2 at 35°C for 48 h; BAP and MacConkey agars were incubated aerobically at 35°C for 48 h. Only confluent growth of a single organism, with a count of $\geq 10^5$colony forming units (CFU)/ml, were presumed as significant growth. For EQUC the significant colony was calculated in reference to volume inoculated as described by BrincatC, et al with a little modification(3). Further, microbial identification was determined using the recommended in-house set of biochemical test and colony characteristics.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of bacterial isolates against different antibiotics was tested by the disk diffusion method [modified Kirby-Bauer method] on Mueller Hinton agar (Hi-Media, India) following standard procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, USA(7). The antimicrobials used were: penicillin [ampicillin (10 µg)], penicillins with β-lactamase inhibitors [ampicillin-sulbactam (10/10 µg), amoxicillin-clavulanic acid (10 µg)], narrow-spectrum cephalosporin [cefazolin (30 µg)], extended-spectrum cephalosporins [ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg)], cephamycin [cefoxitin (30 µg)], anti-pseudomonal penicillins with β-lactamase inhibitors [piperacillin-tazobactam (100/10 µg)], monobactam [aztreonam (30 µg)], carbapenems

[imipenem (10 µg), meropenem (10 µg)], aminoglycosides [gentamicin (10 µg), amikacin (30 µg)], fluoroquinolones [ciprofloxacin (5 µg), ofloxacin (5 µg)], folate pathway inhibitor [co-trimoxazole (25 µg)], and polymyxin [colistin (10 µg)]. The interpretations of antibiotic susceptibility results were made according to the zone size interpretative standards of CLSI(7).

Identification of MDR, XDR and potential ESBL

MDR and XDR isolates were identified in reference to the combined guidelines of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC)(8). The isolate, resistant to at least one antimicrobial from three different groups of first-line drugs tested was regarded as MDR; while the isolates resistant to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remains susceptible to only one or two categories) are termed as XDR(7) (8). For the confirmation of all potential ESBL producers, Combined Disk test (CDT), as recommended by CLSI was performed in all isolates (7).

Data management and statistical analysis

Data obtained (patient's demographics and the results) were entered and managed on Microsoft Excel (version 2010 Microsoft Corporation, USA); relation of variables was calculated in frequencies and percentages.

Results

Patients' demographics

Of 570 urine samples, the significant bacterial growth detected: 92(16.14%) with EQUC, and 73(12.8%) with SUC protocol.

Of 92 UTI cases, the infection was higher in female children, i.e. (n=67) compared to male (n=25). The age group, in range 1-4 years, and the patient admitted to wards were more prone to the infection (Table 1).

EQUC Vs SUC in uropathogens detection

EQUC detected all possible etiologies, contributing UTIs, in the clinically suspected subjects; as reported: "no growth" with the standard urine culture protocol. Of total enrolled cases, 92 significant UTIs cases were detected with EQUC; however, only 73 with SUC technique. 20.6% are being missed with the SUC technique. A statistical outline was drawn with paired t-test (Additional file-1 (a) (b)).

Among the study population, the etiology, *E. coli,* predominantly found as culprits preceding UTIs: 69(75%) with EQUC and 63(68.4%) with SUC technique. The uropathogens i.e. *Candida albicans, Provedencia retegerii,* and *Morganella morganii* were missed, as no pathogen grew, with SUC technique (fig.1).

Resistivity pattern of uropathogens

Most *E. coli* isolates were resistant to ampicillin (77%), followed by ciprofloxacin (65.07%), cotrimoxazole (51%), nitrofurantoin (33.3%), gentamycin (25.3%), cefixime (22.2%) and ceftriaxone (22.2%). Nevertheless, the entire strains revealed high susceptibility (up to 100%) towards colistin and tigecycline (Additional file-2).

MDR, XDR, and ESBL producers

Among total 69 *E. coli* isolates, subjected for antimicrobial susceptibility testing: ESBL n=32 (46%); MDR n=39 (56.5%) and XDR n=1 (1.4%) detected with EQUC. The SUC protocol, however, detects ESBL 28(40.57%), MDR 31(44%) and XDR (nil) (Table-2).

Discussion

However, may incur adverse consequences, the pediatric UTI, cases are often under-diagnosed or neglected due to non-specific clinical presentations and of clinical practice—relying upon in-vitro culture report. Therefore, a precise diagnosis is crucial for clinical management. In this perspective, our study underscores the insufficiencies in SUC protocol in detecting significant etiologies, possibly MDR and XDR isolates, and advocates for a slight modification concerning the sample volume being inoculated.

Among the study population, the incidence of urinary tract infection was 16.14%; where *E. coli* (68.5%) was the commonest pathogen. The analogous rates have been reported earlier from neighboring hospitals(5)(4)(6)and studies of other nations(9)(10). Alongside, significantly more females(72.0%) were found with UTI substantiating with other similar studies(5)(6). The children of the age group 1-4 years were more prone to the infection. Our premise is comparable to findings conducted in a nearby hospital where less than six years were high-risk age categories(6)(4). The immune status, sanitation, and ascending infection with fecal flora possibly are the reasons behind for such upshots in this age group.

The EQUC technique, a simple but effective technique, was embraced to determine the uropathogen and its resistant pattern in the clinically suspected UTI children. The same technique was applied to the women experiencing UTI like symptoms, before(3). As reported “no growth” with the standard urine culture protocol, EQUC detected all possible etiologies, contributing UTIs. Of total 92 detected cases as UTI, 73 were isolated with SUC, conceding 20.6% being missed. Similar findings comparing supremacy to EQUC parallels with our findings; however, the study population was clinically suspected women with UTI.

Among 69 *E. coli* isolated, highest resistance (77% each) were attributed to ampicillin followed by ciprofloxacin (65.07%). Our findings are nearly similar as observed by Parajuli et al.(87%) to ampicillin and (78%) to ciprofloxacin. Likewise, our findings are coherent, regarding resistance trend of the isolate against ampicillin and ciprofloxacin, to that of Ansari et al. (74%) and (77%); the age-group of study subjects was different, however(11). The isolate, *E. coli*, found resistant to cefixime (22.2%) and ceftriaxone (22.2%). Among antimicrobials tested, colistin(100%), imipenem (nearly 99%) were sensitive.

Therefore, a second and third-generation cephalosporin (cefixime and ceftriaxone) could be choices; polymyxin (colistin) and carbapenem (imipenem) could better be opted-in treating childhood UTI.

The etiology, *Staphylococcus aureus,* in pediatric UTI is commonly associated as acquired infection preceding from in-dwelling catheters or other devices(12). Of 7 isolates of *Staphylococcus aureus*, 5 were recovered from the patient after catheterization; 2 of the isolates were resistant to ampicillin and cotrimoxazole; while one each found resistant to ofloxacin, cloxacilline, cefoxitine, cephalexine and nitrofurantoin. The single isolate was Methicillin-resistant *Staphylococcus aureus*(MRSA); as reported by some authors in pediatric population (13)(14).

The uropathogens (*Candida albicans, Provedencia retegerii, and Morganella morganii*) were isolated with EQUC while missed on SUC; although, these pathogen are cited, as the significant etiologies contributing childhood UTI (15)(16)(17)(18). Hence from our study, it can be clinched that each uropathogen, possibly significant causative agent, may have its' own unique threshold bacterial load, concerning the volume to be inoculated on culture media.

Apart from these, our study underscores 5.5% of ESBL, 12.6% MDR, and 1.4% of XDR isolates were about to be missed if only SUC has opted. In this study, MDR and XDR *isolates* were found 56.5% and 1.4% respectively while 46% of uropathogen were found ESBL. Nevertheless, an increasing pattern of resistance trend in uropathogen, along with MDR rates has been reported, among pediatric isolates, from Nepal (19)(6)(5). The level of drug-resistant uropathogen among the children in this study is of serious concern; nevertheless, the exact figures with exact anti-microbial resistance status (that possibly missed with SUC) were not analyzed before.

In most developing countries and Nepal, the higher antimicrobial burden preceding inapt therapeutic guidelines for pediatric patients might be attributable to this intimidating scenario(4)(6)(5). Existing higher rates of ESBL, MDR, and XDR; necessitates the use of carbapenem, colistin, tigecycline, and other mono-antimicrobial therapies (cephamycins, fosfomycin and nitrofurantoin); however, the resistance to these potent therapeutic options may not be stood robust till longer against the emerging MDR strains (11)(20)(21)(22)(23).

Conclusions

EQUC detects uropathogen, possibly MDR, XDR, and ESBL producers, which could be reported: "no growth" with the SUC protocol. Hence a simple modification on SUC protocol could be a crucial modification for detections of childhood UTI.

Limitations

We could not encompass the large sample size with this new modification—EQUC technique. Our study was restricted to phenotypic anti-microbial resistance detection excluding identification of different beta-

lactamases producing isolates. Although, genomic sequencing provides insightful resistance trend due to constricted laboratory resources was not included in our study.

Abbreviations

ASM: American Society for Microbiology; ATCC: American Type Culture Collection; CLSI: Clinical and Laboratory Standard Institute; E.coli: *Escherichia coli*; EQUC: Enhanced Quantitative Urine Culture; ESBL: Extended-Spectrum Beta-Lactamase; MDR: Multiple Drug Resistant; UTI: Urinary tract Infection; XDR: Extensive Drug Resistant.

Declarations

Authors' contributions

PK and JT made the diagnosis, designed the manuscript, reviewed the literature and prepared the article for submission. ST and CG helped for literature reviews gave a concept of research paper and critically reviewed the manuscript. All authors read and approved the final manuscript

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Competing interest

The authors declare that they have no competing interests.

Availability of data and materials

Data generated or analyzed during this study are included in this published article and remaining are available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Ethics approval and consent to participate

This research was approved by the Institutional Review Committee of International Friendship Children's Hospital, Kathmandu, Nepal. A written informed consent was taken from the patients or their parents before participating in the study. Data regarding personal information and infectious disease were coded and kept confidential.

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Tables

Table 1: patients' demographics

Patients demographics	Uropathogen detected	Uropathogen not detected
Gender		
Male	25	226
Female	67	252
Age group		
<1 year	15	146
1 to 4 years	33	44
5 to 9 years	29	116
10 to 14 years	15	172
Patients distribution		
Out-patient	53	301
In-patient	39	177

Table 2- Uro-pathogens detected as ESBL, MDR, and XDR with EQUC and SUC technique

Organism isolated	Growth positivity (%)	ESBL (%)	MDR (%)	XDR (%)
EQUC	92(16.15%)	32(46.0%)	39 (56.5%)	1(1.4%)
Standard	73(12.80%)	28(40.57%)	31(44.0%)	0
Difference	19(3.35%)	4(5.5%)	8(12.6%)	1(1.4%)

Figures

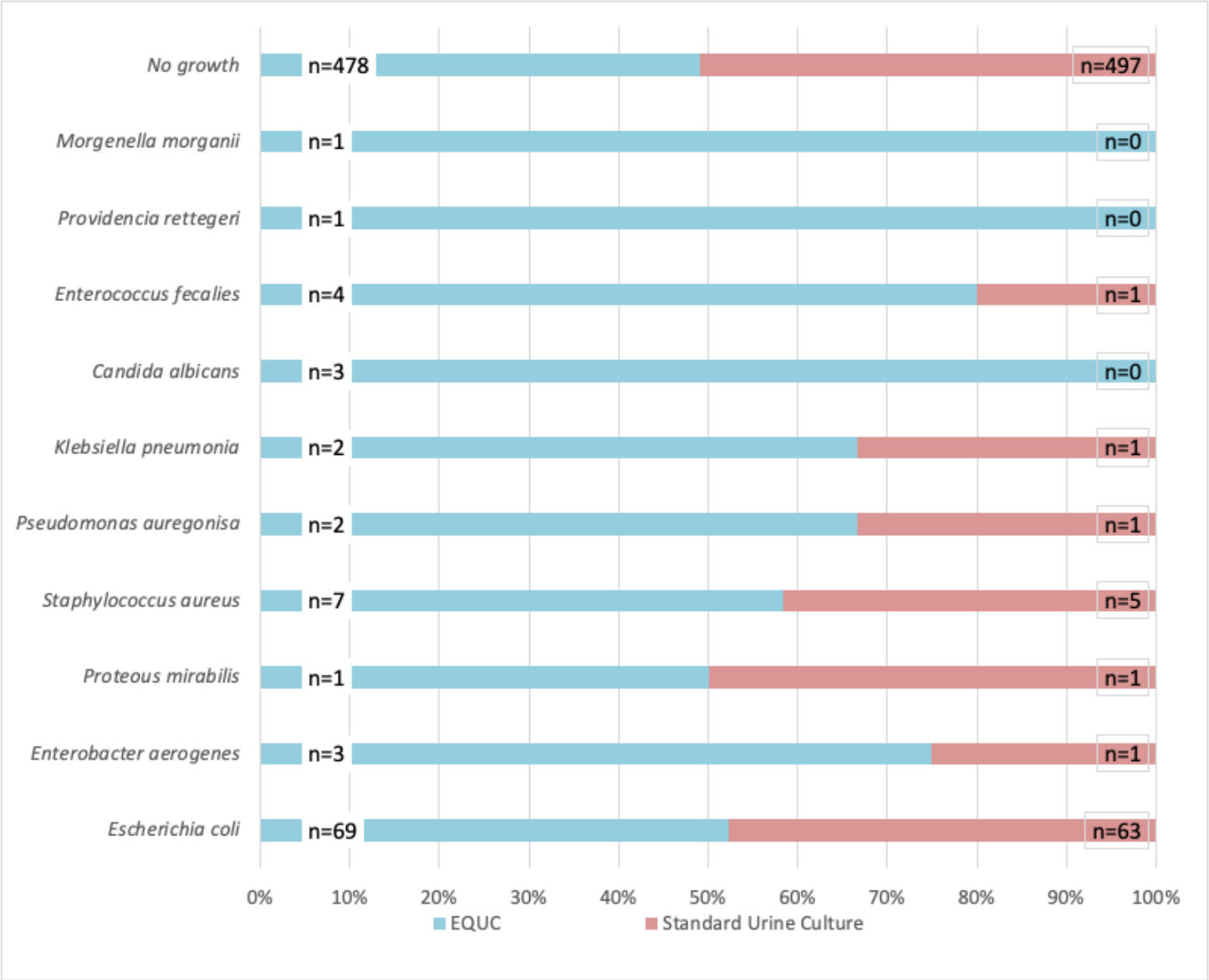

Figure 1

Uropathogens isolated with EQUC and SUC techniques