Urinary tract infections (UTIs) are a common occurrence in children. The management and laboratory diagnosis of these infections pose unique challenges that are not encountered in adults. Important factors, such as specimen collection, urinalysis interpretation, culture thresholds, and antimicrobial susceptibility testing, require special consideration in children and will be discussed in detail in the following review.

Urinary tract infections (UTIs) are frequent in childhood and may have significant adverse consequences, especially for young children. The importance of UTIs is reflected not only by their frequency but also by the range of clinical severity that may occur, from asymptomatic to mild or moderate symptomatic lower UTI to bacteremia and septic shock. In addition, it has been shown that UTIs with fever in young children increase the probability of kidney involvement and are associated with an increased risk of underlying nephrourologic abnormalities and consequent renal scarring (1). Kidney scarring is considered to cause long-term morbidity (hypertension, chronic renal disease, preeclampsia), though much of this has now been shown to be caused by preexisting intrinsic renal disease (1).

Thus, it is clear that an accurate, reliable diagnosis of UTI in children is critical. Underdiagnosis may cause immediate or long-term harm while overdiagnosis subjects healthy children to unnecessary treatment and potentially invasive diagnostic testing. We know that in children less than 2 years of age, the clinical presentation may be nonspecific and also that the threshold established in adults for a clinically significant concentration of bacteria in the urine is not appropriate for this age group (2).

In this review, we will present a discussion of issues relevant to the diagnosis of UTIs in children, particularly as they differ from those in adults. We will review the literature to provide a framework for determining optimal laboratory testing for UTIs in children, from birth to adulthood, and will use the available evidence to explore controversial areas in diagnostic testing.

EPIDEMIOLOGY AND ETIOLOGY

Febrile UTIs are most common among boys and girls who are 2 to 24 months of age and occur in about 5% of children (3). Neonates (≤2 months of age) appear to have similar or higher rates of UTI with fever (4.6% to 7.5%) compared to older infants, with even higher rates of up to 20% in infants with low birth weights, predominantly males (2, 4). In a study of pediatric oncology patients with fever and neutropenia, the rate of UTI was 8.6%. None of the children with UTIs had symptoms referable to the urinary tract, despite a median age of 8 years, or concomitant bacteremia. UTI occurred as frequently as bacteremia in this population (5). UTIs occur at a higher rate in girls than in boys over the first 8 years of life (7% to 8% versus 2%, respectively), but nonfebrile UTIs are most frequent in girls who are older than 3 years of age (1).

With respect to the evaluation of young children with fever, Shaw et al. observed that 64% of young children with UTIs who were assessed in the emergency department were thought by the examining physician to have other sources of fever, i.e., upper respiratory tract (including otitis media) or gastrointestinal infection (6). Thus, until the age of about 5 years, the nonspecificity of symptoms in children dictates that front-line laboratory testing to diagnose UTI, i.e., urinalysis (UA) and urine culture, should provide the highest possible negative predictive value (NPV) and positive predictive value (PPV).

The most common cause of UTI in all age groups is *Escherichia coli* (65% to 75%). Other agents include *Klebsiella* species, usually *Klebsiella pneumoniae* (23%), *Proteus mirabilis* (7%), other Enterobacteriaceae, *Enterococcus* species, *Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus* (1% to 4%) (7, 8). *S. saprophyticus* is known to be an important cause of UTIs in adolescent, sexually active females but has also been shown to cause symptomatic UTIs in younger boys and girls. A prospective study by Abrahamsson et al. showed that of 59 *S. saprophyticus* infections in children under 16 years of age, 25% occurred in boys, 64% of whom were less than 13 years of age (9). *Candida* species most commonly cause UTIs in preterm neonates but may also, on occasion, be responsible for infection in otherwise healthy older children.

SPECIMEN TYPES

Febrile infants, children who present in shock, and all children who have urgent clinical indications to start antibiotics should be catheterized if they cannot provide a voided specimen unless there is gross infection of the genital area, labial adhesions in females, or failure to visualize the urethral opening in uncircumcised males. A midstream or clean catch sample is the optimal specimen for toilet-trained and older children without any obvious infection or abnormality of the external genitalia. In school-aged children, cleansing is not required unless there is gross contamination of the genitalia (10). Suprapubic aspirate (SPA) is carried out rarely but is reserved for diapeded, uncircumcised boys whose urethral opening cannot be visualized and those infants/children who cannot be catheterized or who cannot produce an uncontaminated midstream sample (11). A recent systematic review and meta-
analysis of preanalytic practices affecting the contamination and accuracy of urine cultures concluded that, for children, the methodologic difficulties related to small sample size and heterogeneity in positivity thresholds and the inability to generate hierarchical summary receiver operating characteristic (HSROC) curves made it impossible to determine the method of noninvasive urine collection that is most accurate for the diagnosis of urinary tract infection in children using this methodology. However, multiple studies over many years support the use of straight catheterization for infants and midstream or clean catch urine without cleansing for older children as the best methods for obtaining a good urine specimen for culture.

Bag urine samples, though easy to obtain, are unsatisfactory specimens, and their use is strongly discouraged due to their very high false positive rate (63%) compared to catheter (9%) and the unnecessary and potentially harmful treatment and investigations that may ensue. Al-Orifi et al. observed that 2% of contaminated urine samples obtained by bag culture resulted in one or more adverse clinical outcomes (compared to specimens obtained by catheter), including unnecessary recall (adjusted odds ratio [OR], 4.9), delayed diagnosis and treatment (infinite OR), unnecessary treatment (OR, 4.8), unnecessary prolonged treatment (OR, 15.6), unnecessary radiologic investigation (OR, 4.1), and unnecessary hospital admission (OR, 12.4) (12). The only utility of this method is that a negative bag urine sample tested by dipstick and culture effectively rules out UTI. The problem is that clinicians find it hard to ignore a “positive” culture when it returns, thus initiating a treatment and investigation cycle that is usually unwarranted.

**URINALYSIS**

Urinalysis has been shown to be an important addition to urine culture in the detection of UTI in children and adults, as the assessment of inflammation by way of pyuria can aid in the determination of contamination/colonization or asymptomatic bacteriuria versus infection. Fairley and Barracough showed that the use of leukocyte excretion rate was a highly reproducible, though impractical, method that clearly distinguished clinically infected patients from uninfected patients or those with asymptomatic bacteriuria (13). The only method of assessment of pyuria that correlates tightly with the gold standard leukocyte excretion rate is the presence of >10 white blood cells (WBCs)/mm³ as detected by hemocytometer analysis of an uncentrifuged urine specimen (14). The “standard” method using a centrifuged urine sample (with a threshold of 5 WBCs per high-power field [HPF] or approximately 25 WBCs/μl) is not standardized with respect to the centrifugation parameters or the pellet and resuspension volumes and, therefore, shows poorer correlation with the leukocyte excretion rate and a poor predictive value. Applying the hemocytometer WBC method to the evaluation of screening tests for the diagnosis of UTI in children 2 to 24 months of age, Hoberman found that an enhanced urinalysis, combining >10 WBC/mm³ or Gram stain detection of any bacteria per 10 oil immersion fields on uncentrifuged urine, gave a sensitivity of 96% and a specificity of 93% (47). This method showed better performance characteristics than the standard microscopic urinalysis (83% sensitivity and 87% specificity) or the dipstick analysis (leukocyte esterase [LE] or nitrite positive, 67% and 79% specificity, respectively) (15).

Although it has been shown that pyuria can occur in the absence of UTI, as in fever from other infections and conditions such as Kawasaki disease or after vigorous exercise, it is rare that it is absent in true UTIs (3). The American Academy of Pediatrics (AAP) clinical guideline suggests that when pyuria is absent in a true UTI, it is usually either the method or the definition of pyuria that is at fault (3).

An exception to the value of the detection of pyuria in the diagnosis of UTI is in febrile neutropenic children. Sandoval et al. found a sensitivity of only 40% for the detection of pyuria by microscopy in 1/5 febrile neutropenic children with a positive urine culture (≥10⁶ CFU/ml of a known urinary pathogen) (3). Klaassen et al. determined that only 4% of 54 episodes of UTI in febrile neutropenic children with UTI (≥10⁵ CFU/ml) had detectable pyuria as determined by urine microscopy (16). Urine nitrite testing may be useful in this population in addition to the microscopic detection of bacteria, as neither would be affected by the absence of pyuria.

The urinary nitrite test requires about 4 h for a uropathogen to convert dietary nitrates into nitrites in the bladder to yield a positive test. With the rapid bladder emptying found in infants and children, especially those with inflammation associated with UTIs, this test may be falsely negative. Other causes of false-negative tests include uropathogens that do not reduce nitrate to nitrite, i.e., *Enterococcus spp.*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, and *Candida spp.*; antibiotics that inhibit bacterial metabolism; and the competitive effect of ascorbic acid in the urine. Thus, although it has high specificity for UTIs, as a single test, the sensitivity is low.

According to the AAP 2011 clinical practice guideline, once a clinician has decided that the pretest likelihood of the risk of UTI merits obtaining a urine culture, establishing the diagnosis of UTI in this age group requires “both urinalysis results that suggest infection (pyuria and/or bacteriuria) and the presence of at least 5 × 10⁴ CFU/ml of a single uropathogen cultured from a urine specimen obtained through catheterization or suprapubic aspiration” (sensitivity, 91.2%; PPV, 96.5%) (2). It is important to note that SI units (le Système international d’unités, which is an enhanced version of the metric system) are used for clinical laboratory reporting in most major countries except the United States. The SI requires the reporting of volume in CFU per liter; thus, the AAP threshold for significant bacteriuria in SI units would be 50 × 10⁴ CFU/liter, which has the unfortunate effect of appearing very high to clinicians who read American UTI literature (reported in CFU/ml). Given the additional resources required for either a Gram stain or a hemocytometer WBC count in addition to culture, most microbiology laboratories do not perform either as part of routine UTI diagnostics, even in dedicated pediatric laboratories (Table 1) (S. E. Richardson, unpublished data). As a compromise, if it is not possible for a laboratory to include a Gram stain or hemocytometer WBC count, some laboratories move the reporting threshold for a significant bacterial countdown to ≥10⁴ CFU/ml (≥10⁵ CFU/liter in SI units) or even lower. This practice is supported by a recent study (17) showing that, in infants with bacteremic UTI (not neutropenic), which by definition excludes infants with contaminated urine cultures or asymptomatic bacteriuria, either the leukocyte esterase (LE) test or the nitrite test had a sensitivity of 97.6% and a specificity of 93.9% in detecting UTI, which is much higher than those values previously reported in this population. Standard microscopic detection of pyuria showed a similar sensitivity, but the specificity was considerably lower (around 65%), which probably reflects the fact that various sub-
TABLE 1  Survey of pediatric urine culture practices in North America

| Institution | Country | Culture threshold by specimen type (CFU/ml) | Reflex testing? | Microscopic analysis performed by microbiology? | Microscopic analysis affect testing/reporting? | Clean catch | Catheter | Suprapubic Bag urine 
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<td>/H11022 10 &lt; 10, /H11022 10 &lt; 10, /H11022 10 &lt; 10</td>
<td>No</td>
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<td>No</td>
<td>USA</td>
<td>Canada</td>
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<td>No</td>
<td>No</td>
<td>Yes if automated IRIS in hematology lab indicates infection</td>
<td>No for urine samples submitted directly to microbiology for culture</td>
<td>USA</td>
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<td>No</td>
<td>On request only</td>
<td>No</td>
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<td>No</td>
<td>On request only</td>
<td>Yes, extent of ID/AST determined according to WBC/mm³, i.e., positive pyuria determines ID/AST for probable pathogens 10⁴ to 5 x 10⁵ CFU/ml.</td>
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<td>Yes, extent of ID/AST determined according to WBC/mm³, i.e., positive pyuria determines ID/AST for probable pathogens 10⁴ to 5 x 10⁵ CFU/ml.</td>
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aID/AST, identification and antimicrobial susceptibility testing.
optimal definitions using centrifuged urine samples were used. If validated in other age groups of children, the microscopic detection of pyuria might be rendered unnecessary by the much simpler dipstick test. This would be a boon to laboratories, as it is time-consuming and its interpretation continues to suffer from the lack of a standardized methodology (spun versus unspun, number of cells/mm³ versus number of cells/HPF, centrifuge speed and time). Evidence to support the detection of the inflammatory component of a UTI by dipstick or automated detection of pyuria across all age groups would be very helpful, as it would provide a quick confirmation of the clinical significance of positive urine culture results in most cases.

Automated urine screening for UTI, with determinations of quantitative bacteriuria and pyuria, is becoming more common in laboratories that serve predominantly adult populations. The advantage of this approach is that if negative urine cultures can be reliably identified by rapid screening, urine culture may theoretically be eliminated in up to 80% of specimens. However, the difference between the commonly employed thresholds for significant bacteriuria in adults compared to children, i.e., ≥10³ CFU/ml versus ≥50 × 10⁴ CFU/ml or lower, respectively, means that the available technologies must be evaluated for children at a lower threshold. Currently available systems use flow cytometry followed by fluorescent staining or digital imagery analysis for recognition of bacteria, WBCs, red blood cells (RBCs), and other particles (18, 19). Most published studies to date are in predominantly adult populations, and some have reported good negative predictive values for the diagnosis of UTI.

Data regarding the utility of these systems in diagnosing UTI or in ruling out UTI in children are limited at present (20–22). Two of these studies used the Iris iQ200 Elite or Iris iQ Elite systems (digital imagery followed by Auto-Particle Recognition software analysis; Iris Diagnostics) (19, 20). In the study by Shah et al. (20), automated urinalysis (UA) for pyuria and bacteriuria was compared to “enhanced UA” (≥10 WBCs/mm² and any bacteria per 10 oil immersion fields on Gram stain) using a threshold of ≥5 × 10⁴ CFU/ml of a single uropathogen as a positive urine culture (20). Although the sensitivity and the positive predictive value (PPV) of automated pyuria detection (79.5% and 37.5%, respectively) were lower than those for the microscopic detection of pyuria (≥10 WBCs/mm³, 83.6% and 59.4%, respectively) when either method was combined with urine Gram stain (not automated detection of bacteriuria), the sensitivity and PPV were comparable for the detection of a positive urine culture (77.5% and 84.4% for enhanced UA versus 75.5% and 84% for automated pyuria detection and Gram stain). Note that the retention of the resource-intensive Gram stain was necessary for acceptable sensitivity and PPV, as the automated detection of bacteriuria did not provide the same discrimination, even when coupled with automatic pyuria detection.

Cantey et al. compared the results of automated UA (positive = nitrite or leukocyte esterase positive or ≥10 WBCs per oil immersion field [note that microscopy was performed from spun urine if automated UA was positive]) and Gram stain (any organisms detected) to the growth of ≥5 × 10⁵ CFU/ml of a uropathogen by conventional culture (21). They found that automated UA alone had a sensitivity, PPV, and NPV of 97.4%, 49.4%, and 99.6%, respectively, compared to those of Gram stain alone (97.3%, 33.6%, and 99.5%, respectively). Combining automated UA with Gram stain produced a sensitivity of 97.5%, a PPV of 50.6%, and a NPV of 99.6%. The authors concluded that automated UA does not need to be supplemented with Gram stain; however, they stop short of stating that urine specimens with negative automated UA can be reported as negative without conventional culture.

A third pediatric study (22) used the Sysmex UF-1000i automated urine particle analyzer (Sysmex America), which uses flow cytometry to determine cell counts, and the Siemens Clinitek 500 urine chemistry analyzer (Bayer Corporation) for analyzing UA test strips against manual dipsticks (Siemens Multistix 10 SG; Siemens Corporation), using ≥5 × 10⁵ CFU/ml as the definition of a positive urine culture (22). They found that the manual dipstick (LE or nitrite positive) showed a sensitivity of 95%, a specificity of 98%, a positive likelihood ratio (LR+) of 57.1, and a negative likelihood ratio (LR−) of 0.05; the automated UA for WBCs (≥100 cells/µl) had a sensitivity of 81%, a specificity of 98%, a LR+ of 42.9, and a LR− of 0.15; and the automated UA for bacteria (≥250 cells/µl) had a sensitivity and specificity of 98%, a LR+ of 48.8, and a LR− of 0.02. The authors concluded that automated bacterial counts showed the best ability to diagnose UTIs but that both automated WBC counts and manual dipstick testing showed good and comparable diagnostic performance and could probably be used instead of automated bacterial counts. They also found that combinations of automated WBC and bacterial counts did not outperform bacterial counts alone.

Thus, the limited experience of automated urinalysis in pediatric UTIs to date indicates that there is variability in the predictive value of the detection of automated WBCs and bacteria, depending on the system used. Furthermore, combined with the data from Schroeder et al. (17), which showed the excellent performance of the dipstick in predicting bacteremic UTIs in infants less than 3 months of age, these studies suggest that microscopic methods, such as Gram stain and hemocytometer WBC counts, may be supplanted by either manual or automated methods for the detection of pyuria or bacteriuria in children. Whether these findings hold true if a lower threshold for significant bacteriuria is used in children, i.e., 10⁴ CFU/ml, and how different automated testing systems compare to one another at different bacterial thresholds remain to be studied. The results of these studies will determine whether it will be possible to rule out UTI in children using a nonmicroscopic screening test and will, therefore, eliminate the need to culture every urine sample from a child with a suspected UTI.

**CULTURE-BASED DIAGNOSIS**

Accurate culture-based diagnosis of UTIs is dependent on the utilization of appropriate growth thresholds to distinguish infection from colonization. Culture-based definitions are complicated by asymptomatic bacteriuria, which can result in quantities of growth that resemble infection. A number of factors may influence where a threshold should be set, including specimen type, patient age, and perhaps even organism type. As mentioned above, most laboratories that provide services to adult-only populations use a threshold of ≥10⁵ CFU/ml to define significance (23). Although outside the scope of this review, it is worth noting that there is good evidence to suggest that the commonly used adult threshold (≥10⁴ CFU/ml) is too high, particularly in women with urethritis and cystitis (24). Conversely, there is no consensus as to what threshold should be used to diagnose UTIs in various pediatric populations. This is reflected in the variation seen across
Currently existing recommendations (3, 15, 25–27). Interestingly, the European Association of Urology/European Society for Pediatric Urology guidelines state that any amount of growth from SPA specimens is suggestive of a UTI. This is contrary to what was demonstrated by Pryles et al., who showed that SPA-collected urine may yield low-level contamination (2/42, 4.8%) (28). These findings are supported by those of Karacan et al., who identified contamination in 1 of 11 (9.1%) SPA urine cultures (29).

A recent informal survey of microbiology laboratory directors of North American laboratories serving pediatric populations showed that no two laboratories were using the same urine culture interpretive criteria (Table 1) (Richardson, unpublished data). Responses were elicited from 24 pediatric microbiologists at individual institutions via the ASM ClinicMicroNet listserv (17 American and 7 Canadian), resulting in 11/24 (46%) responses (7 American and 4 Canadian). Not only do institutions differ with respect to the thresholds used for catheter, clean catch, and suprapubic urine specimens, but laboratories also differ as to the acceptability of bag urine samples for culture. Most pediatric laboratories use a threshold varying from 10^4 to 10^5 CFU/ml for clean catch/midstream urine samples and catheter urine samples. However, some laboratories use a threshold as low as 10^3 or 10^4 CFU/ml for clean catch and catheter specimens, for which there is little evidence as will be discussed. The approach to suprapubic urine samples is quite diverse, with thresholds varying from “any count” to >10^5, 10^6, or 10^7 CFU/ml. This lack of consensus is due in part to the fact that the peer-reviewed literature varies considerably in what it suggests the correct threshold should be. A number of independent studies have attempted to identify the bacterial counts that most accurately define urinary tract infection in children. However, these studies use heterogeneous gold standards, culture methods, and patient populations, making it difficult to confidently establish thresholds. Nonetheless, semiquantitative urine culture is the standard of practice in pediatric microbiology, and as a result, laboratories must choose a threshold to guide their practice. The following review will discuss the most relevant literature with respect to establishing urine culture thresholds in pediatric patients.

Before discussing the literature regarding pediatric urine culture thresholds, we want to briefly address the limitations of semiquantitative culture. The standard practice in clinical microbiology is to interpret urine culture results in terms of absolute organism concentration (i.e., 50,000 CFU/ml), which correlates with a threshold that defines clinical significance. However, it has been shown that the inherent inaccuracy of the sampling loop (10 μl or 1 μl) may result in more than a ±50% error rate, and inoculation angle can also have a significant impact on the reported bacterial count. Despite these methodological limitations, laboratories must select absolute thresholds with which to interpret their urine culture results, though clinicians should be aware of the existing inexactitude of the measurement. Accordingly, there is no rationale for reporting urine culture results in multiples of 10,000, for instance, between 10^4 and 10^5 CFU/ml. Although we also recognize the effect this inherent variability has had on reported thresholds in the published literature, we feel that combining results from multiple studies provides stronger evidence for a specific threshold and minimizes the error rate. For the purpose of this review, we will consider the values in each study to be absolute and comparable, as a detailed discussion of individual study methodologies is outside the scope.

Several variables should be considered when evaluating urine culture thresholds; these include patient age, patient status, specimen type, and causative organism. The diagnosis of UTI in children aged 2 to 24 months was addressed by the 2011 AAP clinical practice guideline (3). Although it is important for laboratory protocols to consider distinct age ranges, the reality is that most laboratories apply a single threshold to all age groups. Therefore, it is likely that the AAP recommendation for 2- to 24-month-old children (or any age-specific recommendation) will be extended to all age groups. The AAP document recommends a urine culture threshold of 5 × 10^5 CFU/ml based largely on a 1994 study published by Hobberman and colleagues (2). Of 2,181 catheter-obtained urine specimens from febrile children who were less than 2 years of age (including neonates), there were 110 patients with ≥10^6 CFU/ml uropathogens detected; the majority of specimens (84%) had ≥10^5 CFU/ml, another 9% had 5 × 10^3 to 9.9 × 10^4 CFU/ml, and 7% had 10^4 to 4.9 × 10^5 CFU/ml. Ninety-three of 102 patients (91%) with ≥5 × 10^4 CFU/ml had significant pyuria (≥10 leukocytes/mm^3 in uncentrifuged urine). In contrast, cultures with 10^4 to 4.9 × 10^5 CFU/ml were more likely to grow mixed or nonpathogenic Gram-positive cocci, and were less likely to have significant pyuria (33%). Although it is clear that a number of true UTIs occur between 10^4 and 4.9 × 10^5 CFU/ml in children, the authors conclude that ≥5 × 10^4 CFU/ml is the threshold that will capture the majority of true infections while minimizing false-positive results.

The threshold recommended by the AAP was based on a requirement to also include a valid method for detecting pyuria in the clinical determination of probable UTI, and, as such, they chose a higher threshold than a number of other studies presented below. Some of the earliest work was performed by Pryles et al., who published two elegant 1959 studies that demonstrated near-perfect diagnostic correlation between SPA, catheter-obtained specimens (COS), and clean-voided urine (CVU) (11, 28). In their December 1959 study, patients undergoing elective surgery without symptoms of UTI were enrolled and had both SPA and COS cultured (28). Within this low-risk population for UTI, they found that nearly all growth was below a 10^5 CFU/ml threshold; in fact, most cultures were negative (40/41, 98%) or grew ≤10^4 CFU/ml. In their March 1959 publication, they compared growth from COS to CVU samples in girls aged 2 to 12 years with and without UTI and found a 98% diagnostic correlation between specimens (11). In the same study, they found that urine culture fell into two categories: those without UTI symptoms were negative or showed growth of <10^5 CFU/ml, and those with UTIs grew ≥10^5 CFU/ml. Of the 17 infected patients in this study, three had colony counts that were between 10^3 and 10^5 CFU/ml. However, on repeat culturing, all three patient specimens grew ≥10^5 CFU/ml, leading the authors to conclude that colony counts between 10^3 and 10^5 CFU/ml represent an intermediate result that should be confirmed with a second culture. They concluded that monomicrobial growth of ≥10^5 CFU/ml from patients with UTI symptoms should be considered highly suggestive of UTI.

A number of other studies suggest using a lower threshold for defining pediatric UTI at ≥10^4 CFU/ml. One of the more recent publications to arrive at this conclusion was that of Swerkersson and colleagues, who conducted a population-based retrospective investigation of 430 children who were <1 year of age without urogenital anomalies and who had symptoms of UTI (30). In the study, they correlated clinical and laboratory results as well as...
findings on cystourethrography and $^{99m}$technetium dimercapto-
succinic acid scintigraphy with urine bacterial counts at the initial
presentation. Over the 10 year study, they found that 19% ($n = 83$) of patients had UTIs with colony counts of $<10^5$ CFU/ml.
Importantly, they found that there was no difference in vesi-
coureteral reflux, kidney damage, or recurrence in those who pre-
mitted spillover into the urine from the bacteremia. This study
results (1 case of GBS had microscopic pyuria and all 7 were LE
30–32 months of age with bacteremic UTI, 19/283 (6.7%) chil-
dents with lower counts, i.e., $10^4$ to $10^5$ CFU/ml. Al-
who a second urine culture was obtained within 2 h of the first.
Using the first culture only, they found 100% sensitivity and 93% specificity in diagnosing UTI and concluded that the 7% false-
positive rate resulting from using this threshold was unacceptably high. They recommend instead a threshold of $\geq 10^6$ CFU/ml, to
increase the specificity to 95%, while still maintaining a sensitivity of 100% (33). Importantly, they also show that $\geq 10^5$ CFU/ml of heavy mixed growth was never indicative of infection and should be considered contamination. A significant problem with this study is that urine samples that cultured $<10^5$ CFU/ml of a single uropathogen were considered insignificant and excluded from the analysis. This represented 21/69 or 30.4% of urine samples with
pure growth of a uropathogen. There was no attempt to determine whether or not these were clinically significant, i.e., by urinalysis or clinical chart review. Excluding low-count urine samples with-
out further study may have resulted in a cleaner analysis but prob-
ably also resulted in significant undiagnosis, which should be
as great a concern as overdiagnosis.

There are a number of studies that can be referenced to support thresholds of $\geq 10^4$, $\geq 5 \times 10^4$, $\geq 10^5$, and $\geq 10^6$ CFU/ml. Of course, diagnostic specificity will be maximized with the higher thresholds but at the expense of sensitivity. As laboratories establish their own protocols, they should balance these two important criteria and consider that following thresholds of $\geq 5 \times 10^4$
CFU/ml may miss from 7% to 20% of UTIs, depending on the threshold used. Thus, it is our opinion that the body of literature
supports a threshold of $\geq 10^4$ CFU/ml, which is more conservative than that recommended by the AAP ($\geq 5 \times 10^4$ CFU/ml). The evidence is not strong enough to suggest using a threshold of $<10^4$
CFU/ml.

In addition, this review of the literature did not identify
enough evidence to support generating age-specific thresholds, although some studies did show that low-burden infection was
particularly prevalent in those $<24$ months of age. A limitation of
this review, and of the literature, is that only a small number of
cases from the adolescent population were captured for analysis,
making it difficult to know whether thresholds for this group
ought to be different than for younger children.

It is clear that pediatric UTI is a complex disease with a spec-
trum of presentations. For example, conditions such as reduced
bladder incubation time, dilution due to hyperhydration, antibi-
otic therapy, and organism type can all lead to true urinary tract
infection presenting as low-burden infection. In most patients,
the presence of pyuria should be considered in conjunction with
coli in order to establish the clinical significance of cul-
ture results and to reduce the possibility of a false-positive diag-
nosis. The presence of significant pyuria may be particularly valu-
able in distinguishing asymptomatic bacteriuria from infection or
in distinguishing contamination from infection, especially in
those cultures with lower counts, i.e., $10^4$ to $10^5$ CFU/ml. Al-
though the hemocytometer WBC counting method is regarded as
the most reliable, standard microscopy may also be helpful. Given
the recent evidence for the utility of the LE test in infants (17), it
may not be necessary for the pediatric microbiology laboratory to
perform microscopy for pyuria and bacteriuria (especially the re-
source-intensive hemocytometer WBC count). Adjunctive rapid
urinalysis testing for LE and nitrates at point of care or in the core
or microbiology laboratory (manual or automated) may be the
only necessary additional test to urine culture in determining the
clinical significance of urine culture results with high degrees of
sensitivity and specificity. It is important to keep in mind, however, that neutropenic patients are unable to mount a significant polymorphonuclear response to infection, and thus, in this setting, a threshold of ≥10^4 CFU/ml will be the main criterion for infection. The addition of a nitrite test and microscopic detection of bacteria may aid in determining the significance of a low colony count in this population.

** SUSCEPTIBILITY TESTING IN PEDIATRIC URINARY TRACT INFECTION **

It is generally accepted that the successful management of an acute UTI requires the initiation of antibiotic treatment before culture and antimicrobial susceptibility results are available (34). As such, the AAP recommends the empirical use of amoxicillin-clavulanate, trimethoprim-sulfamethoxazole (TMP-SMX), cephalixin, and cefixime among other oral cephalosporins, though clinicians should be guided by their local antibiogram profiles for uropathogens, as agents like TMP-SMX are becoming less acceptable for empirical therapy (3). These recommendations are similar to those of the European Association of Urology (25). In fact, many antibiotic prescriptions are ordered even before urinalysis results have been completed (21). It is therefore critical that health care providers understand the probability of isolating a given pathogen as well as the likely antibiotic susceptibility profile of that pathogen. As in adult UTIs, *E. coli* is the most common cause of infection in children (34). However, children with underlying renal disease are at greater risk for non-*E. coli* infections as are hospitalized patients (34). The antibiogram of urinary tract pathogens varies by patient population and by institution. Beetz and Westenfelder surveyed the resistance rates of *E. coli* isolates that were collected from children with UTIs from several European regions (34). As an example, rates of resistance to amoxicillin-clavulanic acid (AMC) ranged from 7% (France) to 43% (Turkey). Similarly, 0.9% of isolates from London were resistant to cefuroxime while 19% were reported to be resistant in Turkey (34).

The type and duration of empirical antimicrobial treatment a patient receives are dictated, not only by the likely organism and its antibiogram, but also by patient characteristics (age, underlying condition, history of UTI, and the type of infection [cystitis versus pyelonephritis]). It has been shown that oral antibiotics are as effective as parenteral therapy in the treatment of children 0 to 18 years of age (2, 35). Certain clinical circumstances warrant the admission of parenteral therapy, including the presence of sepsis syndrome, immune-compromising conditions, the inability to take oral medication, underlying urological abnormality, infection due to antibiotic resistant bacteria, and family psychosocial issues. Antimicrobial susceptibility testing (AST) may play an important role in guiding antibiotic selection for the treatment of these infections, whether it is for initial drug selection or for the alteration of empirical therapy. Surprisingly though, there is little literature demonstrating the clinical relevance of AST in children with uncomplicated UTI. The Clinical and Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and other organizations provide guidance documents for AST, but the majority of their recommendations focus on systemic infections (relevant to complicated UTI) and not uncomplicated UTI (36–38). Because many commonly used UTI antibiotics achieve high concentrations in the urine, it is unlikely that breakpoints derived for systemic infections would accurately predict clinical outcome in uncomplicated UTI. For some specific antibiotics, CLSI and EUCAST provide guidance specifically for uncomplicated UTI due to *Enterobacteriaceae*.

The CLSI approach utilizes cephalothin or preferably cefazolin to predict results for oral cephalosporins (cefalexin, cefdine, cefpodoxime, cefprozil, cefuroxime, cephalaxin, and loracarbef). However, there is evidence that some AST systems overestimate cephalothin resistance and, therefore, eliminate important antibiotics for the treatment of pediatric UTI (39). CLSI does not indicate UTI-specific breakpoints for amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, or ciprofloxacin, commonly recommended as oral agents for UTI treatment in children, although it does for nalidixic acid, fosfomycin, and amoxicillin, which are uncommonly used antibiotics for pediatric UTI (3).

In contrast, EUCAST provides UTI-specific breakpoints for a broader range of individual drugs, including amoxicillin-clavulanic acid, amoxicillin, individual cephalosporins (cefadroxil, cephalaxin, cefpodoxime, cefitobuten, and cefuroxime), fosfomycin, nitrofurantoin, and trimethoprim alone. Despite the loss of activity of amoxicillin and TMP-SMX as first-line agents for UTI, amoxicillin-clavulanic acid (AMC) remains a commonly recommended and used antibiotic in the treatment of uncomplicated pediatric UTI and, thus, clinically relevant testing remains important to guide its use (21). A large U.S. study of outpatient urinary isolates of *E. coli* (n = 759,749, largely adults) showed a rise in resistance to amoxicillin-clavulanate of only 0.3% between 2000 and 2010 (40). Similarly, a large Irish study showed a 0.06% reduction in *E. coli* susceptibility to AMC in community UTIs (including children) between 1999 and 2009 (41).

It is important to remember that CLSI amoxicillin-clavulanic acid breakpoints for *Enterobacteriaceae* (susceptible [S] ≤ 8/4; intermediate [I] = 16/8; resistant [R] ≥ 32/16) have been developed for systemic infections compared to the uncomplicated UTI-specific EUCAST breakpoints (S ≤ 32; R ≥ 32), which take into account the high concentration of this drug in urine. Thus, laboratories using CLSI breakpoints for AMC are likely overcalling resistance, resulting in the loss of an important, inexpensive, well-tolerated drug for use in children (and adults). This is illustrated by examining the data from a large multicenter European study of uncomplicated UTI in women, which shows that by using the systemic breakpoint for the susceptibility of AMC against *E. coli* (S ≤ 8), 82.5% of isolates are susceptible, whereas if the UTI-specific EUCAST breakpoints are applied, 99% of isolates would be considered susceptible (42).

In terms of susceptibility testing in patients with uncomplicated UTIs, there are few data to suggest that AST provides clinically relevant information in any patient population, including children. McNulty et al. performed a prospective study in adult women with uncomplicated UTIs that assessed the clinical relevance of *in vitro* trimethoprim resistance (43). They found that patients with resistant isolates had worse outcomes as defined by longer symptom duration, higher rates of reconsultation, more subsequent antibiotic usage, and higher rates of significant bacteriuria at 1 month. Although one would expect that a UTI due to an extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* would result in treatment failure using noncarbapenem β-lactam agents, this was refuted in several studies (44–46) in which children with UTIs due to ESBL-producing strains all had
favorable clinical and microbiological outcomes despite being treated with β-lactam agents that were categorized as resistant by in vitro AST.

Given the frequency with which UTIs occur in children and the vast amount of AST that is performed on urine culture isolates, there is a surprisingly small body of literature supporting its routine use. A review of the literature only identified three studies that evaluated the clinical relevance of AST in children with UTIs, and all three concluded that in vitro testing did not accurately predict outcome. One might conclude from this that routine AST is not useful and therefore should not be done. However, the trend toward developing UTI-specific breakpoints (EUCAST and CLSI) will hopefully play a role in establishing more robust interpretive AST criteria for uncomplicated UTIs in children and adults. Despite the apparent inability of AST to predict outcome in uncomplicated UTI, there are a few reasons that laboratories continue to perform such testing. First, routine testing allows laboratories to assess ongoing changes in susceptibility, which aids in the development of antibioticograms as well as in public health monitoring for the emergence of antibiotic resistance. Second, although the literature suggests that recurrence in the setting of inappropriately treated infection is rare, situations will undoubtedly arise where patients fail to respond, and the selection of additional therapeutic options will be guided by AST. Lastly, it is often difficult for the laboratory to identify those patients who have an uncomplicated UTI (and may not require AST) from those patients with underlying disease or pyelonephritis who may benefit from AST.

CONCLUSIONS AND RECOMMENDATIONS

Optimal diagnosis of urinary tract infection in children hinges around the determination of an appropriate threshold of bacterial growth that correlates with clinical disease, which is a balance between the increased sensitivity of lower thresholds versus the increased specificity of higher thresholds. In our opinion, the body of evidence suggests that a threshold of 10^4 CFU/ml optimizes sensitivity while providing acceptable specificity. The addition of pyuria detection by one of several methods (hemocytometer WBC count or LE test, in particular) can aid in the interpretation of the clinical significance of urine samples with lower bacterial counts, especially those between 10^4 and 5 x 10^4 CFU/ml, and even at higher counts. The detection of significant pyuria thereby increases the specificity of the threshold-based culture method. This balance is reflected in the 2011 AAP guidelines, which suggest that UTIs in 2- to 24-month-old children should only be diagnosed in the presence of pyuria and a culture yielding ≥5 x 10^4 CFU/ml of a uropathogen.

In contrast to the AAP guidelines, we feel that there is good evidence from multiple studies providing compelling evidence for the significance of counts between 10^3 and 5 x 10^4 CFU/ml in a significant proportion of children with UTIs (7% to 10%) (2, 11, 17, 30–32). To capture these true UTIs and manage them appropriately without compensatory overdiagnosis, it is necessary to lower the diagnostic threshold to ≥10^5 CFU/ml and to accompany this analysis with a rapid, simple, and reliable detector of significant pyuria. Recent evidence suggests that the LE test is more reliable in infants than has been previously suggested (17). This test can be done at point of care and would obviate microscopy in the microbiology or core laboratory. This would be an important adjunct to establishing the clinical significance of urine culture because of its utility in distinguishing true infection with an inflammatory response from contamination or asymptomatic bacteriuria. Lowering the diagnostic threshold for children to ≥10^4 CFU/ml in a well-collected urine specimen that is promptly transported to the laboratory or refrigerated until delivery will produce clinically valuable results with optimal sensitivity and specificity, especially when combined with a reliable test of inflammation in the urine.

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