Observational Study

Medicine®

OPEN

Utility of point-of-care Gram stain by physicians for urinary tract infection in children ≤36 months

Toshifumi Yodoshi, MD[a,b,c,*], Masato Matsushima, MD, PhD, MPH[b], Tomohiro Taniguchi, MD[d], Saori Kinjo, MD[a]

Abstract

Urinary tract infection (UTI) in children requires early diagnosis and treatment to prevent repeated UTI and renal scarring. This study aimed to evaluate the usefulness of the point-of-care Gram stain by physicians for suspected UTI in children at Okinawa Chubu Hospital as a rapid diagnostic test.

A single-center, retrospective study was undertaken between January 2011 and December 2015. Patients aged 36 months or younger who were reviewed had suspected UTI in the emergency room or outpatient clinic. Urine culture, urinalysis, and point-of-care Gram stain were performed on a single specimen. Patients with structural or functional urological defects requiring routine catheterization were excluded. We compared the diagnostic performance among the rapid diagnostic tests (i.e., pyuria, point-of-care Gram stain, or both). Kappa statistics were used to evaluate the agreement between the results of point-of-care Gram stain and morphotypes of urine culture with the 95% CI (bias corrected bootstrap interval). We also analyzed which antibiotics were more susceptible to the bacteria of urine culture results, selected by the results of point-of-care Gram stain or empirical treatment based on the Japanese guidelines by McNemar test.

Of 1594 patients reviewed in the study, 1546 were eligible according to our inclusion criteria. Using urine culture as the gold standard for UTI, the sensitivity and specificity of pyuria were 73.2% and 95.1%, whereas those of the point-of-care Gram stain were 81.4% and 98.2%, respectively. The concordance rate between the morphotypes of bacteria detected by point-of-care Gram stain and those of urine culture was 0.784 (kappa coefficient) (95% CI 0.736–0.831). Furthermore, the proportion of "susceptible" in the minimum inhibitory concentration of pathogen-targeted treatment based on the point-of-care Gram stain was higher than that of empirical therapy (exact McNemar significance probability: .0001).

Our analysis suggests that the point-of-care Gram stain is a useful rapid diagnostic tool for suspected UTI in young children. Pathogen-targeted treatment based on the point-of-care Gram stain would lead to better antibiotic selection compared with empirical therapy.

Abbreviations: 95% CI = 95% confidence interval, LR = likelihood ratio, MIC = minimum inhibitory concentration, NPV = negative predictive value, PPV = positive predictive value, UA = urinalysis, UTI = urinary tract infection.

Keywords: Gram stain, rapid diagnostic test, urinary tract infection

1. Introduction

Urinary tract infection (UTI) represents one of the most significant causes of serious bacterial infection in the pediatric population.[1] A delay in treatment may result in repeated UTI and scarring of the urinary tract, leading to renal failure.[2] Therefore, appropriate use of antibiotics for UTI immediately after diagnosis is imperative. However, diagnosing UTI utilizing medical history or a physical examination alone is difficult because symptoms may not be specific, especially for infants and young children.[3] The American Academy of Pediatrics guidelines suggests that the diagnosis of UTI should be made on the basis of quantitative urine culture results in addition to evidence

Editor: Girish Chandra Bhatt.

This study was supported by a grant for postgraduate students from The Jikei University School of Medicine. The study sponsor had no role in the study design, data collection, analysis and interpretation, writing the report, or the decision to submit the article for publication.

MM is an adviser of the Centre for Family Medicine Development practice-based research network, MM received a lecture fee and travel fee for lecture from the Centre for Family Medicine Development, MM received a grant from the Japan Society for the Promotion of Science, and MM is a Program Director of the Jikei Clinical Research Program for Primary-care. TY was a former trainee of the Jikei Clinical Research Program for Primary-care. None of the other authors has any potential competing interest to be declared.

[a] Department of Pediatrics, Okinawa Chubu Hospital, Okinawa, [b] Division of Clinical Epidemiology, Research Center for Medical Sciences, The Jikei University School of Medicine, Tokyo, Japan, [c] Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [d] Division of General Internal Medicine & Infectious Diseases, Hiroshima Prefectural Hospital, Hiroshima, Japan.

[*] Correspondence: Toshifumi Yodoshi, Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA (e-mail: lucky.toshist@gmail.com).

Medicine (2019) 98:14(e15101)

Received: 30 November 2018 / Received in final form: 4 March 2019 / Accepted: 11 March 2019

http://dx.doi.org/10.1097/MD.0000000000015101

of pyuria, with a threshold of 5 white blood cells per high-power field in microscopic analysis.[4] In contrast, the UK's National Institute for Health and Clinical Excellence guideline[5] and the Japanese Association for Infectious Disease/Japanese Society of Chemotherapy (JAID/JSC) Guide to Clinical Management of Infectious Diseases 2015[6] suggests that excluding UTI is not possible, even if pyuria is not present and emphasizes urine culture as the gold standard for diagnosing UTI. Our research group previously reported that 21% of patients with UTIs did not present with pyuria at the time of diagnosis.[7] Because results of urine cultures take a few days, an accurate rapid test for bed-side diagnosis of UTI can be valuable. A recent meta-analysis for rapid urine tests for UTI in children included 95 clinical studies and 95,703 children (<18 years of age), with urine culture as the gold standard test.[8] Sensitivity and specificity was 91% and 96% for urine Gram stain, 74% and 86% for pyuria, 79% and 87% for leukocyte esterase test, and 49% and 98% for a nitrite test, respectively. While a urine Gram stain is useful, the guidelines do not include the results of Gram stains as part of the diagnosis of UTI[4–6] because its utility compared to pyuria on urinalysis (UA) is still controversial.[9,10] Nevertheless, no clinical studies have been conducted to evaluate point-of-care Gram stains performed by physicians for the diagnosis of UTI in young children.

This study aimed to determine the usefulness of a point-of-care Gram stain by physicians for suspected UTI in children in a large hospital in Japan.[11] The specific aims were to compare the validity of point-of-care Gram stain compared with urine cultures (gold standard test) and to evaluate the appropriateness of the antibiotic selection based on the results of the Gram stain compared with empirical therapy.

2. Materials and methods

2.1. Study design

This was a retrospective study of patients with suspected UTI in a single-center from January 2011 to December 2015.

2.2. Study setting

Our hospital in Okinawa, Japan is a 550-bed acute care general hospital, including 41 pediatric beds. Approximately 20,000 children visit the Emergency Department (ED) annually and nearly 1500 are hospitalized in a pediatric ward. Okinawa Chubu Hospital has a Postgraduate Medical Education Program since 1966 and is affiliated with the University of Hawaii.[12] All physicians perform Gram stain tests in the ED and pediatric outpatient clinic, immediately after urine samples are obtained. They then select antibiotics based on the results of Gram staining.[13,14]

Our practice endorses catheterization of children younger than 36 months old. UA, urine culture, and point-of-care Gram stain were done on a single urine specimen obtained by catheter before administering antibiotics. The Gram stain was performed and interpreted by trained resident physicians as soon as possible after obtaining the urine samples. The technique of Gram staining was regularly instructed and evaluated by the members of infectious disease division in our facility.

2.3. Inclusion criteria and exclusion criteria

We reviewed patients 0 to 36 months of age visiting our hospital between January 2011 and December 2015 with suspected bacterial UTI and all febrile infants younger than 2 months. Because diagnosing UTI at an age younger than 2 months with a medical history or physical examination is difficult, these inclusion criteria were necessary to measure the accuracy of a rapid test for UTI in young children.[3] Patients with structural or functional urological defects requiring routine catheterization were excluded from the study. Those who were not submitted either Gram stain or UA were also excluded.

2.4. Clinical management of patients

In our hospital, antibiotics for UTI were selected after point-of-care Gram stains in the ED or the pediatric outpatient clinic. The antibiotic regimen in our facility was based on Japanese[6] or USA[4] guidelines; the first-line therapy was based on local antibiotic-resistance patterns, updated annually by our hospital's microbiology lab.[13] A second-generation cephalosporin is recommended for Gram-negative rods (Enterobacteriaceae, such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus mirabilis*). Ampicillin and gentamicin are recommended for Gram-positive cocci (*Enterococcus faecalis* and *Streptococcus agalactiae*).

First-line therapy based on the JAID/JSC[6] is ampicillin and gentamicin for neonates and first to third generation cephalosporins for infants and young children.

2.5. Data collection

Medical records were reviewed in order to obtain pertinent demographics, clinical and laboratory information, all bacteria that were isolated, and the prescribed antibiotics. All point-of-care Gram stains were done by physicians.

2.6. Variable definitions

Point-of-care Gram stain, and urine culture were performed from un-centrifuged urine and UA was performed from centrifuged urine on a single urine specimen. Urine culture was considered positive if 10^4 colony forming unit of uropathogens were identified.[6] Urine culture results were used as the gold standard of diagnosis of UTI for comparison. Urine cultures were evaluated by laboratory technicians without clinical information, pyuria on UA or Gram stain results. Pyuria was defined as positive when five leukocytes per oil immersion field were observed by using an automated urine analyzer (Aution Hybrid, Arkray, Kyoto, Japan). Point-of-care Gram stains were performed and interpreted by physicians soon after urine samples were obtained. A point-of-care Gram stain was deemed positive if white blood cells were present, and any organisms were observed. Morphotypes and the presumptive bacteria were as follows: Gram-positive cocci for *E faecalis*, *S agalactiae*, or *Staphylococcus aureus* and Gram-negative rods for *E coli*, *Klebsiella* spp., *Enterobacter* spp., *P mirabilis*, or *Pseudomonas aeruginosa*.

2.7. Statistical analysis

The median and interquartile ranges were used when data were non-normally distributed. Categorical variables were reported as percentages. To compare performance among the rapid diagnostic tests, we calculated sensitivity, specificity, positive likelihood ratio (LR), negative LR, and positive and negative predictive values (PPVs and NPVs) with the 95% confidence interval (95% CI) for each testing method (i.e., pyuria, point-of-care Gram stain, or both). Kappa statistics were used to evaluate the agreement between the results of point-of-care Gram stain and morphotypes of urine culture with the 95% CI (bias corrected bootstrap interval).[15] These were calculated by bootstrap methods with options of 1000 replications and a random-number seed (1234321).

We also analyzed which antibiotics were more susceptible to the bacteria of urine culture results, selected by the results of point-of-care Gram stain or empirical treatment based on the Japanese guidelines 2015[6] by McNemar test. The drug susceptibility test was classified by the minimum inhibitory concentration (MIC). The MIC is the standard of the United States Clinical and Laboratory Standards Institute (CLSI; S: susceptible, I: intermediate, R: resistant). The data were analyzed with Stata software, version 14.2 (Stata Corp., College Station, TX).

2.8. Sample size calculation

According to Cantey et al,[16] when a urine culture test is regarded as the gold standard for diagnosis of UTI, the sensitivity of the Gram stain is 97.3% and specificity 73.8%. Assuming that the predicted value of the sensitivity in the point-of-care Gram stain was 0.973, the expected proportion was 0.027 (= 1 − 0.973), the width of the interval was 0.05, and the confidence level was 95%, the sample size of patients with a positive urine culture needed for sensitivity calculation was 162. Additionally, assuming that the estimated value of specificity in the point-of-care Gram stain was 0.738, the expected proportion was 0.262 (= 1 − 0.738), the width of the interval was 0.05, and the confidence level was 95%, the number of negative urine cultures required was 1189. A total of 1351 people would be required. In our hospital, because 350 urine cultures are annually submitted and approximately 35 patients aged younger than 3 years with UTI are hospitalized, we set the research period as 5 years.

2.9. Ethics

The study was approved by the Institutional Review Board of Okinawa Chubu Hospital (No. 28-70), and the need for written informed consent was waived. This study was conducted in accordance with Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. We were not required to obtain individual informed consent from the patients included in the study. We posted information on our research on the institution's website and a bulletin board at the hospital.

3. Results

Urine cultures of 1594 patients were processed during the study period (Fig. 1). Forty-six (2.9%) patients did not have urine

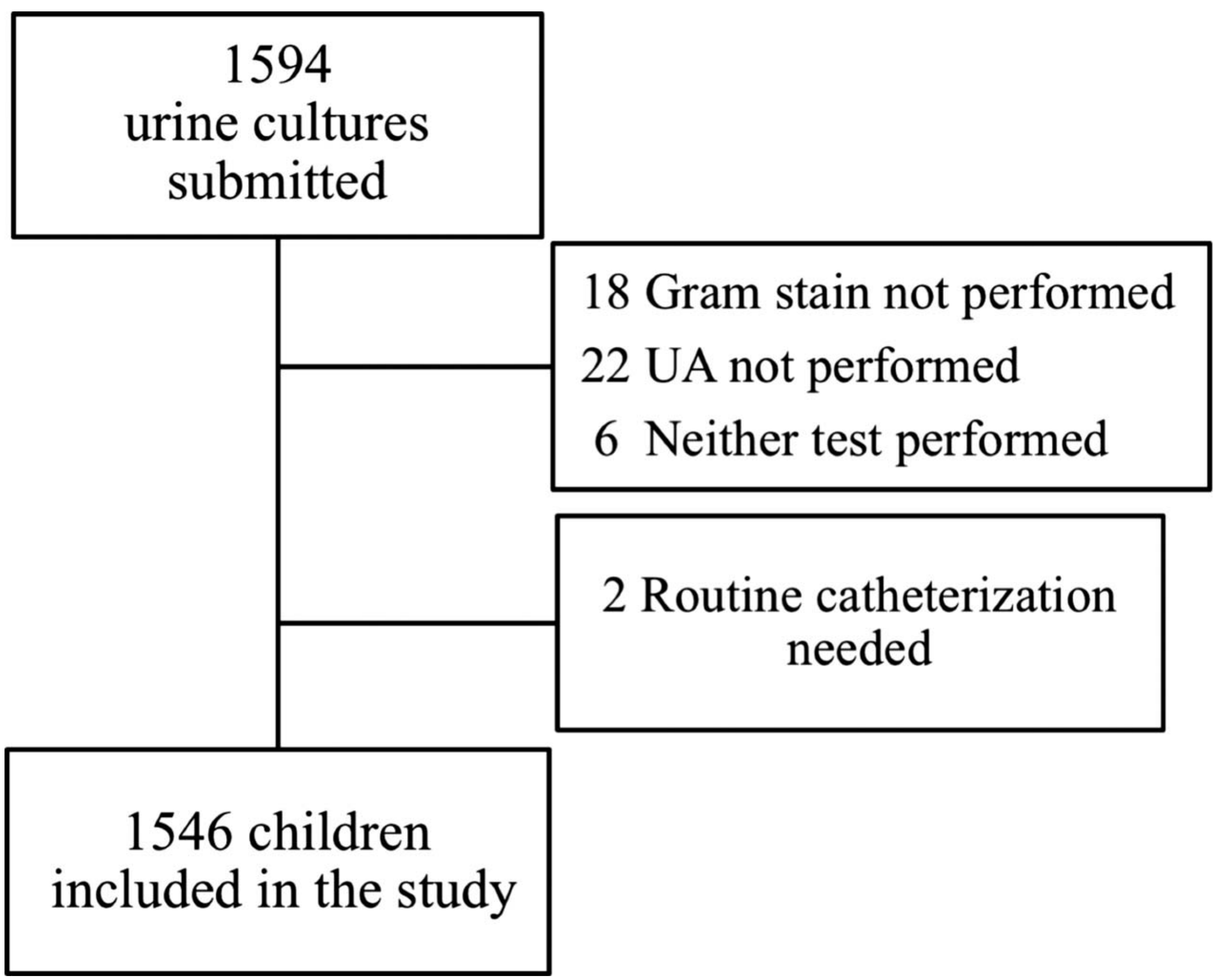

Figure 1. Flowchart for the selection of patients.

Table 1

Clinical and laboratory characteristics of this study.

Variable	All subjects, n=1546
Age; month (IQR)	3 (1, 15)
Female sex; n (%)	696 (45%)
Multiple UTI episodes; n (%)	20 (1.3%)
Positive urine cultures; n (%)	183 (12%)

Data are presented as n (%) for categorical variables, and medians and interquartile for continuous variables.
IQR=interquartile, UTI=urinary tract infection.

cultures, UA, and/or point-of-care Gram stain from a single urine specimen. Two (0.1%) patients were excluded because of routine catheterization. Of the remaining 1546 patients, 696 (45%) were girls and the median age was 3 months (interquartile range, 1–15 months) (Table 1). Of these, 183 (12%) patients had a pathogen detected in urine culture. Among urine culture-positive patients, 63 (34%) were girls and the median age was 3 months (interquartile range, 2–11 months).

We calculated the sensitivity, specificity, PPV, NPV, positive LR, and negative LR of pyuria on UA, point-of-care Gram stain, and combination of pyuria and point-of-care Gram stain, and positive combination of either pyuria or point-of-care Gram stain (Table 2). Urine culture was considered a gold standard test for comparison. The sensitivity and specificity of pyuria were 73.2% and 95.1% respectively, whereas the point-of-care Gram stain had a sensitivity and specificity of 81.4% and 98.2%, respectively. Furthermore, the sensitivity and specificity of combined pyuria and point-of-care Gram stain had a sensitivity and specificity of 70.0% and 99.6% respectively, while the sensitivity and specificity of either pyuria or point-of-care Gram stain had a sensitivity and specificity of 100% and 95.8%, respectively.

Of the 183 positive urine cultures, bacteria isolated from the urine culture were *E coli*, *Klebsiella* spp., *E faecalis*, *P mirabilis*, and multiple identified bacteria, including *E faecalis* (Table 3). Extended-spectrum beta-lactamases were confirmed in 12 (9.5%) cases in *E coli*, 2 (9.1%) cases in *Klebsiella* spp., and 2 (50%) cases in *P mirabilis*.

The kappa coefficient between the morphotypes of bacteria detected by the point-of-care Gram stain, and those detected by urine culture was 0.784 (95% CI 0.736–0.831) (Table 4). For the bacteria detected by urine culture, 87.4% of the pathogen-targeted treatments based on the point-of-care Gram stain were "susceptible" at MIC. In contrast, 79% of the empirical therapy based on the guideline was "susceptible" at MIC (exact McNemar significance probability was .0001). All cultured bacteria could be covered with antibiotics based on the point-of-care Gram stain except multidrug-resistant bacteria, such as extended-spectrum beta-lactamases.

Table 3

Bacteria isolated from urine cultures.

	Number, percentage (%)	Special bacteria
Gram-negative rod		
E coli	126 (68.5%)	ESBL 12 (9.5%)
Klebsiella spp.	22 (12.0%)	ESBL 2 (9.1%)
Enterobacter spp.	4	
P mirabilis	4	ESBL 2 (50%)
P aeruginosa	1	
Gram-positive coccus		
E faecalis	11 (6.0%)	
S agalactiae	2	
Multiple identified bacteria	8	*E. faecalis* 7 (3.8%)
Others	5	MRSA 1

ESBL=extended-spectrum beta-lactamases, *E faecalis*=*Enterococcus faecalis*, *E coli*=*Escherichia coli*, MRSA=methicillin-resistant *Staphylococcus aureus*, *P mirabilis*=*Proteus mirabilis*, *P aeruginosa*=*Pseudomonas aeruginosa*, *S agalactiae*=*Streptococcus agalactiae*.

Table 4

Concordance rate of the point-of-care gram stain and urine culture.

	Urine culture			
	None	GNR	GPC	Multiple-organism
Point-of-care Gram stain				
None	1339	28	4	2
GNR	18	128	1	6
GPC	6	4	10	0
Multiple identified bacteria	0	0	0	0

Kappa coefficient: 0.784 (95% CI 0.736–0.831).
GNR=Gram negative rod, GPC=Gram positive coccus.

4. Discussion

We found that point-of-care Gram stain was superior to pyuria on UA as a rapid diagnostic test for UTI. Specifically, the sensitivity and positive LR were higher with Gram stain. Positive LR was 158.9 when both pyuria and the point-of-care Gram stain were positive, and sensitivity was 100% when either pyuria or the point-of-care Gram stain was positive. The agreement of bacterial morphology between the point-of-care Gram stain by physicians and results of the cultures was substantial by evaluating the kappa coefficient. Furthermore, the proportion of "susceptible" at MIC of the pathogen-targeted treatment based on the point-of-care Gram stain was higher than that of empirical therapy according to current Japanese guidelines.[6]

Table 2

Properties of rapid tests for UTI.

	Sensitivity %, [95%CI]	Specificity %, [95%CI]	PPV %, [95%CI]	NPV %, [95%CI]	Positive LR [95%CI] /Negative LR [95%CI]
Pyuria on UA	73.2% [0.662–0.795]	95.1% [0.938–0.962]	66.7% [0.609–0.720]	96.4% [0.954–0.971]	14.9 [11.6–19.1]/0.28 [0.22–0.36]
Point-of-care Gram stain	81.4% [0.750–0.868]	98.2% [0.974–0.989]	86.1% [0.806–0.903]	97.5% [0.967–0.982]	46.2[30.9–69.2]/0.19[0.14–0.26]
UA (pyuria) and point-of-care Gram stain	70% [0.627–0.765]	99.6% [0.990–0.998]	95.5% [0.905–0.980]	96.1% [0.952–0.969]	158.9[71.1–355.1]/0.30[0.24–0.38]
UA (pyuria) or point-of-care Gram stain	100% [0.980–1.00]	95.8% [0.946–0.968]	76.3% [0.714–0.805]	100%	23.9[18.6–30.8]/0.00

CI=confidence interval, LR=likelihood ratio, NPV=negative predictive value, PPV=positive predictive value, UA=urinalysis, UTI=urinary tract infection.

Our study shows that the point-of-care Gram stain and pyuria on UA had a very high specificity. This resulted in a higher positive LR, hence a positive test makes UTI extremely likely and that moderate sensitivity does not rule out UTI even if each test is negative. Importantly, the NPVs for UTI of Gram stain together with pyuria were 100%. Cantey et al reported that the sensitivity and specificity of the Gram stain using centrifuged urine for UTI were 97.3% and 85%, while those of pyuria were 97.5% and 74%, respectively.[16] This previous report showed a much higher sensitivity than that of our study. This difference is likely because urine was not centrifuged for pyuria and Gram stain in our study. Shaw et al[17] also examined the sensitivity and specificity of the Gram stain using un-centrifuged urine for UTI by laboratory technicians. They found that the sensitivity and specificity were 81% and 97%, respectively.[17] Point-of-care Gram stain in our study had as high a sensitivity as that in the study by Shaw et al[17] using unspun urine for the Gram stain and the gold standard of 10^4 uropathogens in urine culture. The Gram stain is a rapid diagnostic test for UTI, which can be carried quickly at low cost. For clean-catch, unspun urine, the presence of at least one bacteria is likely to indicate a bacterial count of $\geq 10^5$ CFU/mL,[18] and the absence of bacteria in several fields on a Gram stain indicates the probability of fewer than 10^4 bacteria/mL.[18] In our study, we used point-of-care Gram stain with unspun urine, a procedure that took 5-minute.

In our study physicians, not laboratory technicians, performed the Gram stain, and the kappa coefficient suggests that they were accurate in their reading. There have been no reports on the validity of the point-of-care urine Gram stain by physicians. Furthermore, we show that choice of antibiotics based on point-of-care Gram stain was better than recommended empirical therapy. In empirical therapy based on the Japanese guideline or local susceptibility patterns in Japan, the first-line drug of UTI for neonates is ampicillin plus gentamicin, and the first-line antibiotics after infancy are cephalosporins. However, cephalosporins, such as cefixime and cefotaxime, are used in most UTI cases in children living in North America.[19] Except during the neonatal period, such empirical antibiotics do not cover *E faecalis*, which accounts for 9% to 10% of childhood infections.[20–22] In our study, the rate of detection of *E faecalis* in the whole culture was 9.8%. Additionally, antibiotics that were selected based on point-of-care Gram stain were 8.4% more sensitive to culture results than those by empirical therapy based on the Japanese guidelines. These results suggest that the point-of-care Gram stain enabled selection of antibiotics that can cover *E faecalis* more accurately than empirical therapy. Furthermore, in our study, bacteria that were not covered by point-of-care Gram stain would not have been covered by empirical therapy.

Limitations of our study include the retrospective nature of this study. It is also possible that despite clear guidelines in our hospital to do a Gram stain before sending a sample for a UA testing, in some children, the physician knew the results of a pyuria on UA before performing the Gram stain test, possibly resulting in biased reporting. In addition, we used unspun urine for testing, different than the technique used in the lab. However, we report similar sensitivity and specificity of the Gram stain as when performed by laboratory technicians.[17] Finally, there may be limited applicability of this study to all patient populations. Although the recommendation of this study is that physicians perform Gram stains on urine samples, federal regulatory standards in some countries, such as the United States, may restrict physicians from carrying out urine Gram stains in non-certified settings.

In conclusion, our analysis suggests that the point-of-care Gram stain is a useful rapid diagnostic tool for suspected UTI in children less than 36 months of age. Antibiotic selection based on point-of-care Gram stain is preferable compared to empirical therapy recommendations.

Acknowledgments

We would like to thank all staff members of the Okinawa Chubu Hospital who performed Gram staining to determine appropriate therapy for our patients. We also thank Professor Ran D Goldman (University of British Columbia) and Dr Thomas L. Hurt (Mary Bridge Children's Hospital) for their review of this manuscript.

Author contributions

Conceptualization: Toshifumi Yodoshi, Tomohiro Taniguchi.
Data curation: Toshifumi Yodoshi, Masato Matsushima, Saori Kinjo.
Formal analysis: Toshifumi Yodoshi, Masato Matsushima.
Funding acquisition: Toshifumi Yodoshi.
Investigation: Toshifumi Yodoshi, Masato Matsushima, Tomohiro Taniguchi, Saori Kinjo.
Methodology: Toshifumi Yodoshi, Masato Matsushima, Tomohiro Taniguchi, Saori Kinjo.
Project administration: Toshifumi Yodoshi.
Resources: Toshifumi Yodoshi, Masato Matsushima, Tomohiro Taniguchi, Saori Kinjo.
Software: Toshifumi Yodoshi, Masato Matsushima.
Supervision: Masato Matsushima, Tomohiro Taniguchi, Saori Kinjo.
Validation: Masato Matsushima, Tomohiro Taniguchi, Saori Kinjo.
Visualization: Toshifumi Yodoshi, Masato Matsushima.
Writing – original draft: Toshifumi Yodoshi.
Writing – review & editing: Masato Matsushima, Tomohiro Taniguchi, Saori Kinjo.
Toshifumi Yodoshi orcid: 0000-0001-7260-731X.

References

[1] Montini G, Tullus K, Hewitt I. Febrile urinary tract infections in children. N Engl J Med 2011;365:239–50.
[2] Coulthard MG, Lambert HJ, Vernon SJ, et al. Does prompt treatment of urinary tract infection in preschool children prevent renal scarring: mixed retrospective and prospective audits. Arch Dis Child 2014;99: 342–7.
[3] Simões e Silva AC, Oliveira EA. Update on the approach of urinary tract infection in childhood. J Pediatr 2015;91:S2–10.
[4] Roberts KB. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 2011;128:595–610.
[5] Baumer JH, Jones RW. Urinary tract infection in children, National Institute for Health and Clinical Excellence. Arch Dis Child Educ Pract Ed 2007;92:189–92.
[6] Yamamoto S, Ishikawa K, Hayami H, et al. JAID/JSC Guidelines for Clinical Management of Infectious Disease 2015 - Urinary tract infection/male genital infection. J Infect Chemother 2017;23: 733–51.
[7] Toyoura M, Yoshimura H, Ashimine K, et al. The report of clinical overview of upper tract infection (UTI) in children in Japanese single center [in Japanese]. J Pediatr Nephrol 2001;14:121–8.
[8] Williams GJ, Macaskill P, Chan SF, et al. Absolute and relative accuracy of rapid urine tests for urinary tract infection in children: a meta-analysis. Lancet Infect Dis 2010;10:240–50.

[9] Waisman Y, Zerem E, Amir L, et al. The validity of the uriscreen test for early detection of urinary tract infection in children. Pediatrics 1999;104:E41.

[10] Lockhart GR, Lewwander WJ, Cimini DM, et al. Use of urinary gram stain for detection of urinary tract infection in infants. Ann Emerg Med 1995;25:31–5.

[11] Iwata K. Gram staining by physicians: an invaluable practice still seen in East Asia. Clin Infect Dis 2004;39:1742–3.

[12] Maeshiro M, Izutsu S, Connolly KK. A history of the University of Hawaii Postgraduate Medical Education Program at Okinawa Chubu Hospital, 1966–2012. Hawaii J Med Public Health 2014;73:191–4.

[13] Taniguchi T, Tsuha S, Shiiki S, et al. Gram-stain-based antimicrobial selection reduces cost and overuse compared with Japanese guidelines. BMC Infect Dis 2015;15:458.

[14] Fukuyama H, Yamashiro S, Kinjo K, et al. Validation of sputum Gram stain for treatment of community-acquired pneumonia and healthcare-associated pneumonia: a prospective observational study. BMC Infect Dis 2014;14:534.

[15] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159–74.

[16] Cantey JB, Gaviria-Agudelo C, McElvania TeKippe E, et al. Lack of clinical utility of urine gram stain for suspected urinary tract infection in pediatric patients. J Clin Microbiol 2015;53:1282–5.

[17] Shaw KN, McGowan KL, Gorelick MH, et al. Screening for urinary tract infection in the emergency department: which test is best? Pediatrics 1998;101:e1.

[18] Sobel JD, Kaye D. Bennett JE, Dolin R, Blaser MJ. Urinary Tract InfectionsMandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Philadelphia: Saunders imp Elsevier; 2014;886–913.

[19] Walton JM. Antibiotics for acute pyelonephritis in children. Paediatr Child Health 2015;20:349–50.

[20] Biyikli NK, Alpay H, Ozek E, et al. Neonatal urinary tract infections: analysis of the patients and recurrences. Pediatr Int 2004;46: 21–5.

[21] Burbige KA, Retik AB, Colodny AH, et al. Urinary tract infection in boys. J Urol 1984;132:541–2.

[22] Hummers-Pradier E, Koch M, Ohse AM, et al. Antibiotic resistance of urinary pathogens in female general practice patients. Scand J Infect Dis 2005;37:256–61.