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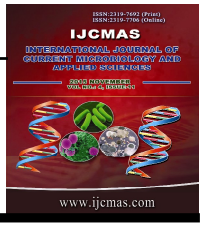

Original Research Article

A Comparative Study of Aerobic Bacteriological Profile of Urinary Tract Infection in Children by Standard Loop and Dip Slide Semi - Quantitative Culture Methods and Their Antibiogram

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Keywords

UTI, Calibrated standard loop, Dip slide culture method

A B S T R A C T

Urinary tract infections (UTIs) are common bacterial infections associated with considerable morbidity in children. We have conducted a study of aerobic bacteriological profile of UTIs in children of 0 to 14years. A total of 108 urine samples were cultured by calibrated standard loop and dip slide culture methods as per standard procedures. Any growth on the media was identified by appropriate biochemical tests. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method following CLSI guidelines. Of 108 cases, 87 were culture positive, and all (100%) were positive by standard loop where as 11.50% were positive by dip slide culture method. *Escherichia coli* (47.91%), and Coagulase negative *Staphylococcus* (13.54%) were the commonest isolates. Most of the isolates were found to be more susceptible to ceftriaxone, piperacillin tazobactam, imipenem, and nitrofurantoin. In our study UTI was more commonly seen in female children with ratio (M:F) of 1.6:1. *Escherichia coli* was the commonest isolated Gram negative organism. Urine culture by calibrated standard loop remains the gold standard method.

Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases seen in the community (Eshwarappa *et al.*, 2011). It represents one of the most common diseases which are encountered in the medical practice today, with an estimated 150 million cases per annum world-wide (Mukharjee *et al.*, 2013). UTIs are associated with considerable morbidity and health care cost, with varied clinical spectrum of severity ranging from asymptomatic bacteriuria to cystitis and pyelonephritis to septic shock with multiorgan system failure(Agarwal, 2012). Most UTIs in children result from ascending infections, although haematogenous spread may be more common in the first 12 weeks of life. Most UTIs in children are monomicrobial often caused by *Escherichia coli* (60–80% of cases), *Proteus* (more common in boys and in children with renal stones), *Klebsiella*, *Enterococcus* and

coagulase negative *Staphylococci* (Brian *et al.*, 2005).

Paediatric urinary tract infections are associated with high morbidity and long term complications like renal scarring, hypertension and chronic renal failure (Taneja, 2010). Hence the present study has been undertaken for the first time in our institution. Our aim was to study the aerobic bacteriological profile of urinary tract infections in children by comparing standard loop and dip slide semi - quantitative culture methods and their antibiogram.

Materials and Methods

The present study was carried out over a period of one year from Jan 2012 to Dec 2012. Clinically suspected UTI in children of 0 – 14 years with symptoms of fever with or without chills, burning micturition, frequency, dysuria, suprapubic pain were included & those on antibiotic therapy were excluded. The parents were instructed to collect mid-stream urine sample from their children in sterile wide mouth containers as per standard guidelines. After sample collection, transported immediately to the laboratory for further processing.

Urine sample is examined for the following parameters appearance, colour, pH, and odour. Wet mount preparation and Gram stain was done for all urine samples following the procedures explained in practical text book of Mackie & McCartney. In urine sediments white blood cells (WBC) are usually reported as follows (Monica Cheesebrough, 2009):

Few: Up to 10 WBCs/HPF (High Power Field, i.e., using 40X objective)
Moderate Number: 11–40/HPF
Many: More than 40WBCs/HPF

For our convenience we graded few as 1+, moderate as 2+ and many as 3+.

Inoculation on blood agar and MacConkey agar was done using calibrated standard loop. Culture is also done by dip slide method. Dip slides were prepared following Mackie and Sandys (1965, 1966) method (Naylor, 1967). The urine should be mixed thoroughly before plating (Kieran *et al.*, 2010). Calibrated loop designated to deliver a known volume, either 0.01 or 0.001 ml of urine was used (Mackie & McCartney). Reporting of bacterial count was done following the table (Table 1).

If 25 *E. coli* colonies are counted and a 1/500 ml loop was used, the approximate number of CFU per ml of urine: 500×25 = 12, 500, such a count would be reported as:

10000 – 100 000 *E.coli* /ml.

The number of colonies on the dip slide is directly proportional to the viable bacterial count of the fluid in which the slide has been dipped. After isolation, Gram stain, and biochemical reactions were done according to the procedures described in Mackie and McCartney practical microbiology and Konemen text book of diagnostic microbiology. Antimicrobial susceptibility testing was done on Mueller Hinton agar using Kirby- Bauer disk diffusion method.

The following antibiotics were used as per CLSI guideline

Norfloxacin(Nx) - 10μg, Nitrofurantoin (NIT) – 300 μg, Penicillin(P) – 10 units, Cotrimoxazole (COT): Trimethoprim / sulfamethoxazole - 1.25 μg/23.75 μg, Nalidixicacid (NA) - 30 μg, Cefoxitin(CX) - 30 μg, Erythromycin (E) - 15μg, Clidamycin (CD) - 2 μg, Linezolid (LZ) - 30 μg, Vancomycin (VA) - 30 μg, Ofloxacin (OF) - 5 μg, Chloramphenicol (C) - 30 μg, Amikacin (AK) - 30 μg, Cefuroxime(CXM)

- 30 μg, Imipenem(I) - 10μg, Ceftriaxone(CTR) - 30 μg.

Results and Discussion

From total of 108 cases, 87 cases were positive for urine culture with significant bacteriuria. Distribution of cases according to gender is shown in the table 2

Females were higher in number as compared to males with a ratio of 1.6:1. For all samples culture was done by both standard loop and dip slide culture methods. Of 87 culture positive urine samples only 10 were positive by Dip slide culture method. Before doing urine culture, urine microscopy was performed to look for the presence of pus cells and bacteria. The dip slide culture method showed growth when wet mount microscopy of urine sample showed pyuria with grade 3+, the dip slide gave scanty growth.

Antibiogram of Gram negative organisms is shown in table 3. Antibiogram of Gram positive organisms is shown in the table 4.

In the present study, the culture positive cases were higher in females (62.03%) than the males showing the ratio of 1.6:1. Similar to our results, a study by Sharma *et al.* (2011) showed that at least 1% of boys and 3% of girls develop urinary tract infection during first ten years of life.

Our results were comparable with the observations made by Dennis Guttman, their clinical trials showed that all specimens containing > 10^5 organisms per ml gave dip slide counts of 200 or more colonies, and specimens with > 10^4 organisms per ml produced counts above 40 colonies (Dennis *et al.*, 1967).

William A, CRAIG and Calvin MK performed a comparative bacterial counts of 1,000 clinical specimens using the pour plate and dip slide method and observed that dip inoculum detected all but 19 of 258 specimens (92.6%) with 100,000 or greater colonies per ml. And also found that this simple, convenient method should allow more extensive use of quantitative urine culture in the diagnosis and follow up of patients with urinary tract infections in office practice. It should not be considered a substitute for the more definitive pour plate method or for standard methods for characterization of bacteriological species when more exact information is required. J Robson, N Lurie and J Tudor Hart had done a study on dip slide urine culture in children under 5 years old. Out of 567 registered children dip slide urine was obtained from 158 (27.9%). Out of 123 positive dip slides 91 (73.7%) yielded a significant pure growth, five (4.7%) a significant mixed growth and 27 (21.9%) yielded an unidentified bacterial count of ≥ 10^5 organisms per ml (Robson *et al.*, 1979). Our observations were comparable with the observations made by Jeana *et al.* (1995) observed that dip slide culture method and other screening methods use has been limited by the lack of sensitivity when pathogens are present in low numbers (Jeana *et al.*, 1995).

In present study, majority of the isolates were Gram negative organisms accounting for 83.33%. Among Gram negative organisms *E. coli* was the commonest isolate (57.5%) of the 87 culture positive urine samples. 95.67% of isolated *E. coli* were susceptibility to imipenem, 93.47% to piperacillin tazobactam. The antibiotic pattern of *E. coli* in present study is in agreement with the study conducted by Eliana *et al.* (2008). About 21.7% and 39.3% of *E. coli* were shown to be sensitive to cefuroxime and amikacin respectively.

Study conducted by Mansour *et al.* (2009) observed that all Gram negative organisms were sensitive to amikacin (90.5 - 100%). Similarly a higher proportion of *E. coli* were isolated by Ji *et al.* (2011) (81.4%), Mohammed *et al.* (2007) (61%), Andrew *et al.* (2013) (63%), Eileen *et al.* (2011) (70%), (Gupta *et al.*, 2012), Kumar *et al.* (2003) (32.8%), Hadiza *et al.* (2003) (55.6%), Gvl *et al.* (47.6%), Eliana *et al.* (2008) (77%), Mansour *et al.* (2009) (59%), Tanja *et al.* (2011) (67.7%).

Table.1 Reporting of bacterial count

(CFU) per ml of urine	Report the bacterial count
< 10 000 organisms/ml (10^4ml/)	Not significant
10 000 – 100 000/ml (10^4 – 10^5/ml)	Doubtful (repeat specimen)
>100 000/ml (10^5/ml)	Significant bacteriuria

Table.2 Distribution of cases according to gender

	Clinically suspected		Culture Positive		Culture Negative	
Gender	**Number**	**%**	**Number**	**%**	**Number**	**%**
Male	41.00	37.96	30.00	34.48	11.00	52.38
Female	67.00	62.03	57.00	65.52	10.00	47.61
Total	108.00	100.00	87.00	100.00	21.00	100.00

Table.3 Antibiogram of Gram negative organisms

Antibiotics	***E. coli* (%)**	***C. freundii* (%)**	***K. pneumoniae* (%)**	***P. aeruginosa* (%)**	***Proteus vulgaris* (%)**
Gentamicin	41.30	33.34	33.60	25.00	NT*
Piperacillin tazobactam	93.47	83.34	88.50	75.00	66.66
Amikacin	39.13	55.56	44.30	0.00	0.00
Cefuroxime	21.73	38.90	22.40	0.00	0.00
Ceftriaxone	69.56	66.70	66.70	75.00	66.67
Cefoxitin	45.65	38.90	44.25	25.00	33.33
Ofloxacin	54.34	61.12	66.70	75.00	66.00
Imipenem	95.67	88.80	88.50	100.00	100.00
Norfloxacin	67.39	72.30	66.70	75.00	33.33
Nalidixic acid	47.82	55.55	44.25	0.00	100.00
Nitrofurantoin	84.72	78.00	77.78	25.00	100.00
Ciprofloxasin	47.82	50.00	44.25	50.00	33.33

Table.4 Antibiogram of Gram positive organisms

Antibiotics	*S. aureus (%)*	*CONS (%)*	*Enterococcus* spp*(%)*
Penicillin	0.00	15.38	0.00
Linezolid	100.00	100.00	50.00
Vancomycin	100.00	100.00	100.00
Erythromycin	100.00	53.84	50.00
Cefoxitin	100.00	NT*	NT*
Ofloxacin	100.00	61.53	50.00
Norfloxacin	100.00	53.84	0.00
Nitrofurantoin	100.00	84.61	50.00
Nalidixic acid	0.00	38.46	0.00
Cotrimoxazole	0.00	69.23	0.00
Ciprofloxacin	100.00	69.23	0.00
Piperacillin tazobactam	100.00	100.00	100.00

In present study 16.6% of Gram positive organisms were isolated, coagulase negative *Staphylococcus* was the most common isolate. And other isolates were *Enterococcus* spp and *S. aureus*. All the Gram positive isolates showed 100% sensitive to vancomycin, piperacillin tazobactam and were resistant to penicillin.

In conclusion, Gram negative bacilli were predominantly associated with UTI compared to Gram positive cocci. *Escherichia coli* being the most common organism cultured in urine of these children, followed by *Citrobacter freundii* and *Klebsiella pneumoniae*.

Compared to calibrated standard loop, dip slide culture method showed growth only when there was significant bacteriuria with pyuria Grade 3+. Hence urine culture by calibrated standard loop remains the ideal method for diagnosing UTI. Most of the isolated Gram negative organisms showed sensitive to ceftriaxone, piperacillin tazobactam, imipenem, and nitrofurantoin. And isolated Gram positive cocci were sensitive to ofloxacin, linezolid, vancomycin, nitrofurantoin and piperacillin tazobactam.

The urine of all children who are suspected as having UTI should be cultured by standard loop to avoid diagnostic error, for proper antibiotic selection, and to prevent drug resistance at primary level. Dip slide is less sensitive when compared to Standard loop method.

References

Andrew, J.R., David, M.G., Margaret, M., Ephraim, O.T., Mohan, N., Andrew, P.S. *et al.* 2013. Microbiology of urinary tract infections in Gaborone, Botswana. *PLoS ONE,* 8(3): 57776.

Blondeau, J.M., Yaschuk, Y., Galenzoski, D., Hrabok, D., Isaacson, M., Lee, L. *et al.* 1995. Evaluation of the cult-dip plus dip slide method for urinary tract infection. *J. Clin. Pathol.*, 48: 710–713.

Brian, S.A., Sarah, H., Curry, 2005. Urinary tract infection in children. *Am. Acad. Family Physicians,* 72(12): 2483–2488.

Collee, J.G., Duguid, J.P., Fraser, A.G., Marmion, B.P., Simmons, A. Laboratory strategy in the diagnosis of Infective syndromes. In: Collee, G., Fraser, A.G., Simmons, A., Marmion, B.P. (Eds), Mackie and McCartney practical medical microbiology, 14th edn. Churchill Livingston, New York. Pp. 84–89.

Dennis, G., Naylor, G.R.E. 1967. Dip slide: An aid to quantitative urine culture in General practice. *Brit. Med. J.*, 3: 343–345.

Eileen, M.B., Kehl, K.S. 2011. A critical appraisal of the clinical microbiology laboratory in the diagnosis of urinary tract infections. *J. Clin. Microbiol.*, 49(9 Suppl.): S34–S38.

Eliana, B.M.G., Eitan, N.B., Stanley, N., Nataly, A.S., Vanda, B., Julio, T. 2008. Antibiotic resistance pattern of pediatric community acquired urinary infections. *Braz. J. Infect. Dis.*, 12(4): 321–323.

Eshwarappa, M., Dosegowda, R., Vrithmani, A.I., Khan, M.W., Shivakumar, P., Kempegowda, P. 2011. Clinico microbiological profile of urinary tract infection in South India. *Indian J. Nephrol.*, 21(1): 30–36.

Foxman, B. 2003. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis. Mon.*, 49(2): 53–70.

Gupta, V., Yadav, A., Joshi, R.M. 2002. Antibiotic resistance pattern in uropathogens. *Indian J. Med. Microbiol.*, 20(2): 96–98.

Hadiza, H.L., Didier, M., Antoine, T. 2003. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in Bangui, Central African Republic. *J. Antimicrob. Chemother.*, 51: 192–194.

Jeana, F., Judy, T., Miller, J.M. 1995. Evaluation of a new, semi quantitative screening culture device for urine specimens. *J. Clin. Microbiol.*, 33(5): 1351–1353.

Ji, E.Y., Wun, K.K., Jin, S.L., Kyeong, S.S., Tae-Sun, H. 2011. Antibiotic susceptibility and imaging findings of the causative microorganisms responsible for acute urinary tract infection in children: A five - year single center study. *Korean J. Pediatric*, 54(2): 79–85.

Kieran, M.K., Liam, G.G., Brendan, D. 2010. A survey of the management of urinary tract infection in children in primary care and comparison with the NICE guidelines. *BMC Fam. Pract.*, 11: 6.

Kumar, C.S.V., Jairam, A., Chetan, S., Sudesh, P., Kapur, I., Srikaramallya, 2002. Asymptomatic bacteriuria in school going children. *Indian J. Med. Microbiol.*, 20(1): 29–32.

Mansour, A., Manijeh, M., Zohreh, P. 2009. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishpur J. Microbiol.*, 2(3): 118–123.

Mohammed, A., Mohammed, S., Asad, U.K. 2007. Etiology and antibiotic resistance patterns of community – acquired urinary tract infections in JNMC Hospital Aligarh, India. *Ann. Clin. Microbiol. Antimicrob.*, 6(4): 1–7.

Monica Cheesebrough, 2009. Examination of urine In: District laboratory practice in tropical countries, Part 2, 2nd edn, Cambridge University Press, Cambridge. Pp. 105 – 115

Mukharjee, M., Shreya, B., Sandip, K.M., Monalisa, M. 2013. Multi drug resistance and extended spectrum beta lactamase production in uropathogenic *E. coli* which are isolated from hospitalized patients in Kolkatta India. *J. Clin. Diagn. Res.*, 7(3): 449–453.

Nader, S., Natalia, E.M., James, E.B., Max, H.F. 2008. Prevalence of Urinary Tract infection in childhood: a meta – analysis. *Pediatric Infect. Dis. J.*, 27(4): 302–308.

Naylor, G.R.E. 1967. The Dip – slide: A modified dip inoculum transport medium for the laboratory diagnosis of infections of the urinary tract. *J. Hyg. Camb.*, 65: 367–371.

Robson, J., Lurie, N., Hart, J.T. 1979. Ten years experience in general practice of dip slide urine culture in children under five years old. *J. Royal College Gen. Practioners*, 29: 658–661.

Sharma, A., Shrestha, S., Upadhyay, S., Rijal, P. 2011. Clinical and Bacteriological Profile of urinary tract infection in children at Nepal Medical College Teaching Hospital. *Nepal Med. Coll. J.*, 13(1): 24–26.

Taneja, N., Chatterjee, S., Singh, M., Singh, S., Sharma, S. 2010. Paediatric urinary tract infections in a tertiary care centre from North India. *Indian J. Med. Res.*, 131: 101–105.

Tanja, I., Sanda, G., Adela, A., Vesna, C., Mirna, S., Marijan, S. 2011. Changes in bacterial resistance patterns in children with urinary tract infections on antimicrobial prophylaxis at University Hospital in split. *Med. Sci. Monit.*, 17(7): 355–361.

Washington CW, Stephen DA, William MJ, Elmer WK, Gary WP, Paul CS *et al.*, 2006 Introduction to Microbiology, Part II: Guidelines for the collection, transport, processing, analysis, and reporting of cultures from specific specimen sources. In: Koneman's Color atlas and Textbook of Diagnostic Microbiology, 6th edn. Lippincott Williams & Wilkins, Philadelphia. Pp. 82–87.