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Monitoring Antimicrobial Susceptibility in Bacterial Isolates Causing Urinary Tract Infections in a Tertiary Hospital in Kathmandu

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Abstract

Urinary tract infection is the most common infection in females worldwide. One in three women experiences at least one episode of urinary tract infection during their lifetime. The objective of this study was to determine the etiology and antimicrobial profile of urinary tract infection. A cross-sectional study was conducted at a tertiary care hospital in Nepal. Thirteen hundred clean catch mid-stream urine samples were tested through standard microbiological techniques. The isolates from urine samples were identified from biochemical tests. Antibiotic susceptibility testing was performed through the Kirby-Bauer disc diffusion technique following CLSI guidelines. The prevalence of urinary tract infection was found at 24.23%. Escherichia Coli was a predominant etiological agent followed by Staphylococcus aureus. The majority of the infection was found between the age group 21-40, with females mostly infected. Most of the microorganisms were isolated from emtergency, obstetrics-gynecology, and nephrology wards. Most of the isolates were resistant to ampicillin, whereas the majority of the gram-positive isolates were resistant to penicillin.A large number of isolates were found to be sensitive to Gentamycin and nitrofurantoin. Routine antimicrobial susceptibility testing of the isolates causing urinary tract infection is recommended to reduce antimicrobial resistance and for the proper treatment of infection.

Keywords

Urinary tract infection, antimicrobial resistance, Nepal

Introduction

Infection of the urinary tract is the most common non-intestinal infection among the female population globally (Dielubanza 2011). It is estimated that about one-third of adult females experience an episode of cystitis (infection of the bladder) at least once, and these episodes can reoccur. Serious consequences like kidney damage and renal failure may occur if the predisposing factors are not kept in check(Minardi et al, 2011). The aetiology of urinary tract infection (UTI) is consistent worldwide. The pathogenesis of UTI involves ascending infection of the urinary tract in susceptible women. The common organisms involved are the coliform bacteria that colonizes the perineum (Proteus species and other Gram-positive rods cause 80-90% Escherichia coli, 5-10% Staphylococcus saprophyticus, and the remainder) (ZalmanoviciTrestioreanu et al, 2010).

The prevalence of UTI is higher in female than in the male. The most crucial factor leading to an increased risk of UTI in a female is pregnancy. During pregnancy, the gravid uterus exerts pressure on the ureters and slows down the flow of urine. Besides, immunological imbalance at the time pregnancy contributes to the likeliness of UTI.

Bacteria multiplying at the opening of the

urethra travels up to the bladder and leads to about 95% cases of UTI. The patients are also affected by the microorganisms that induce inflammation within the male genital tract and urinary tract (Tazebew et al, 2013). In the patient with a urinary catheter, fecal bacteria move to the bladder through the urethra and results in urinary tract infection. An essential step of pathogenesis in UTI in a female is the colonization in the mucosa of the vaginal introitus. Following the microbial colonization of the introitus, bacteria gain entrance into the bladder either through sexual intercourse or through urethral manipulation (Sobel 2009).

Relapse is common in women with deformed micturition that leads to incomplete voiding leading to urine retention. Hence, the factors that could trigger the recurrence of UTI must be taken into consideration. The fecal-perineal-urethral hypothesis states that the uropathogens are residing as the rectal flora act as a reservoir for ascending UTI in a female. Several anatomical and psychosocial factors can be responsible for the recurrence of UTI (Minardi et al, 2011).

Nepal is a resources constrained country, and because of this, most of the people are still not getting the primary health facilities. Due to poor hygiene and a lack of health education, a large number of people suffer from urinary tract infections every year. A proper understanding of the aetiology of UTI is essential for the containment of UTI through the recommendation of the correct therapeutic agent. Therefore, this study was designed to identify the pathogens causing urinary tract infection and to determine the antibiotic susceptibility pattern of the isolates.

Methods

Study design

A cross-sectional study was conducted in collaboration with the Central Department of Microbiology, Tribhuvan University, Kirtipur, and KIST Medical College Teaching Hospital, Imadole-6, Lalitpur, Nepal, from 2013 to 2014. The study was conducted with the samples collected in the hospital for routine care. Not any kind of direct involvement was made with any patient, hence informed consent was not taken from the patients.

Urine specimen collection

Thirteen hundred mid-stream urine samples were collected from clinically UTI suspected patients. The patients were given sterile, dry, wide-necked, leak-proof Hi-Media containers of 100 mL capacity for collection of 10-20 mL of clean-catch mid-stream urine (CC-MSU). The patients were given instructions for the collection of CC-MSU.

Isolation and identification of the isolates

The urine samples were cultured on Mc Conkey agar and Blood agar medium by the semi-quantitative culture technique using a standard loop. The Mac Conkey and Blood agar plates were incubated aerobically at 37◦C overnight. Approximate numbers of colonies were counted, and the number of bacteria, i.e., cfu/mL in urine, was estimated by the volume of urine inoculated previously. For example, 100 colonies on inoculating 0.001 mL of urine would correspond to 10^5cfu/mL.

The bacterial count was reported as follows; less than 10^4/mL organisms not significant; 10^4-10^5/mL organisms, doubtful (suggest repeat specimen); more than 10^5/mL organisms, significant bacteriuria. However, if the culture indicated the appearance of $\geq$3 different types of organisms with no predominating organism, it was interpreted as due to possible contamination of the specimen and asked for another specimen.

The identification of significant isolates was made using standard microbiological techniques based on morphological characteristics and biochemical properties. Gram-positive organisms were identified primarily based on their response to Gram's staining, Catalase, Oxidase, Coagulase, and Bile-Esculin hydrolysis tests. The biochemical tests used for the identification of Gram-negative bacteria included the Catalase test, Oxidase test, Indole test, Methyl Red test, Voges-Proskauer test, Citrate Utilization test, Oxidation-Fermentation test, Triple Sugar Iron Agar (TSIA) test, Motility test, and Gas-Production test.

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion test method following CLSI guidelines (Wayne 2013). Antimicrobial agents tested for susceptibility were

ampicillin, cefazolin, ceftriaxone, cotrimoxazole, nalidixic acid, Ciprofloxacin, norfloxacin, nitrofurantoin, Gentamycin, penicillin, and cefoxitin.

Results and Discussion

From 1300 samples processed, 315 (24.23%) samples showed significant bacteriuria (i.e.>10^5cfu/mL) whereas 985 (75.77%) samples showed insignificant growth (i.e. ≤10^5cfu/mL), growth of contaminants or no growth. Out of 315 isolates, 261 (82.86%) were Gram-negative bacteria, 49 (15.56%) Gram-positive bacteria and the remaining 5 (1.58%) were Candida albicans and non-albicans Candida species (Table 1).

Sixteen different types of bacteria and yeasts were isolated, of which Escherichia coli was the aetiology in a majority (63.49%) of urinary tract infections. The prevalence of UTI in this study was similar to the study of Khadka et al., (2012), which reported 23.2% significant bacteria, while Raja et al., (2011) reported it as 19.7%. The percent of Gram-positive and Gram-negative bacterial isolates in this study was analogous to the study of Khadka et al., (2012) with 84.48% gram-negative and 15.56% gram-positive isolates.

E. coli isolates in this study were 63.49%, which was higher than the study of Sah et al., 2016); Dhungana and Shakya (2015) but lower than that of Raja et al., (2011). K. pneumonia and Enterobacter species isolated in this study were lower than the study of Raja et al., (2011), whereas P. aeruginosa isolates were higher than that of Raja et al., (2011). Acinetobacter species isolated in this study was higher than that in Sah et al., (2016) while lower than the study of Raja et al., (2011). Sah et al., (2016) and Dhungana and Shakya (2015) reported a higher percentage of S. aureus than this study, whereas Raja et al., (2011) found only 0.2% S. aureus causing urinary tract infection. Percentage of Streptococcus species was found lower than the study of Dhunganaand Shakya (2015), and Enterococcus species was lower than that reported in the study of Sah et al., (2016). A similar percent of C. Albicans was found in the study of Raja et al., (2011) with this study. The microorganisms isolated were distributed into 11 age-groups (Table 2).

The majority of the infection was observed in the age group 21-30 years, which is the sexually active age-group. A similar kind of infection rate was found in a study of Dhungana and Shakya (2015) in the age groups 21-30, 31-40, 41-50

Table 1. Microorganisms isolated from urine samples

Isolates	Number	Percentage
Escherichia coli	200	63.49
Klebsiella pneumonia	21	6.67
Klebsiellaoxytoca	1	0.32
Proteus mirabilis	6	1.91
Proteus Vulgaris	5	1.59
Citrobacterkoseri	3	0.95
Citrobacterfreundii	3	0.95
Enterobacter species	1	0.32
Pseudomonas aeruginosa	6	1.91
Acinetobacter species	15	4.76
Staphylococcus aureus	22	6.98
Coagulase-negative *Staphylococcus aureus* (CoNS)	10	3.18
Streptococcus species	7	2.22
Enterococcus species	10	3.18
Candida albicans	2	0.64
Non-albicans*Candida* species	3	0.95
Total	315	100

Table 2. Distributionof isolates in the different age group of patient

Isolates	Age group(years)										Total (%)
	<1 (%)	1-10 (%)	11-20 (%)	21-30 (%)	31-40 (%)	41-50 (%)	51-60 (%)	61-70 (%)	71-80 (%)	81-90 (%)	91-100 (%)	
Escherichia coli	(15)	11 (5.50)	(74)	(66)	(24)	(24)	(20)	(8)	17 (8.50)	5 (2.50)	1 (0.5)	200 (100)
Klebsiellapneumoniae	1 (4.76)	8 (38.10)	(4.76)	(9.52)	3 (14.29)	(14329)	(9.52)	(4.76)	(0)	(0)	(0)	21 (100)
Klebsiellaoxytoca	(0)	(0)	(100)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	1 (100)
Proteus mirabilis	(50)	(0)	1 (16.67)	(0)	1 (16.67)	(0)	1 (16.67)	(0)	(0)	(0)	(0)	6 (100)
Proteus vulgaris	(0)	(0)	(0)	(0)	(0)	(40)	(20)	(0)	(20)	(20)	(0)	5 (100)
Citrobacterkoseri	(0)	(0)	(0)	1 (33.33)	1 (33.33)	(0)	(0)	(0)	1 (33.33)	(0)	(0)	3 (100)
Citrobacterfreundii	(0)	(0)	(0)	(0)	(0)	(0)	1 (33.33)	2 (66.67)	(0)	(0)	(0)	3 (100)
Enterobacter species	(0)	(0)	(0)	(0)	(100)	(0)	(0)	(0)	(0)	(0)	(0)	1 (100)
Pseudomonas aeruginosa	(0)	(0)	1 (16.67)	2 (33.33)	(0)	(0)	(0)	(0)	2 (33.33)	(0)	1 (16.67)	6 (100)
Acinetobacter species	(0)	2 (13.33)	2 (13.33)	5 (33.33)	2 (13.33)	1 (6.67)	(0)	(6.67)	(0)	2 (13.33)	(0)	15 (100)
Staphylococusaureus	(0)	(0)	4 (18.18)	8 (36.36)	3 (13.63)	1 (4.55)	(455)	(9209)	(9209)	(455)	(0)	22 (100)
CoNS	(0)	(0)	(30)	(40)	20)	(0)	(0)	(0)	(10)	(0)	0 (0)	10 (100)
Streptococcus species	(0)	(0)	(0)	1 (14.29)	2 (28.57)	(0)	2 (28.57)	(0)	2 (28.57)	(0)	(0)	7 (100)
Enterococcus species	(0)	(0)	(0)	(0)	(20)	(0)	(10)	(40)	(10)	(20)	(0)	10 (100)
Candida albicans	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(120)	(0)	2 (100)
Non-albicans Candida species	(0)	(0)	(0)	0 (0)	1 (33.33)	(0)	(0)	(0)	2 (66.67)	(0)	0 (0)	3 (100)
Total	14 (4.44)	21 (6.67)	27 (8.57)	89 (28.25)	42 (13.33)	31 (9.84)	29 (9.21)	18 (5.71)	29 (9.21)	13 (4.13)	2 (0.64)	315 (100)

Table 3. Distributionof isolates in male and female patient

Isolates	Patients sex		
	Male (%)	**Female (%)**	**Total (%)**
Escherichia coli	57 (28.50)	143 (71.50)	200 (100)
Klebsiella pneumonia	5 (23.81)	16 (76.19)	21 (100)
Klebsiellaoxytoca	1 (100)	0 (0)	1 (100)
Proteus mirabilis	1 (16.67)	5 (83.33)	6 (100)
Proteus Vulgaris	3 (60)	2 (40)	5 (100)
Citrobacterkoseri	2 (66.67)	1 (33.33)	3 (100)
Citrobacterfreundii	2 (66.67)	1 (33.33)	3 (100)
Enterobacter species	1 (100)	0 (0)	1 (100)
Pseudomonas aeruginosa	4 (66.67)	2 (33.33)	6 (100)
Acinetobacter species	5 (33.33)	10 (66.67)	15 (100)
Staphylococcus aureus	8 (36.36)	14 (63.64)	22 (100)
CoNS	2 (20)	8 (80)	10 (100)
Streptococcus species	0 (0)	7 (100)	7 (100)
Enterococcus species	3 (30)	7 (70)	10 (100)
Candida albicans	1 (50)	1 (50)	2 (100)
Non-albicans*Candida* species	1 (33.33)	2 (66.67)	3 (100)
Total	96 (30.48)	219 (69.52)	315 (100)

years. However, Dhungana and Shakya (2015) reported a higher infection rate in the age groups 11-20, 51-60, 61-70 years than this study. The sex-wise distribution of isolates is listed in Table 3.

The rate of infection in females was found higher compared to that in males. More females (69.52%) were in fected than males (30.48%), which correlates with the study of Raja et al., (2011), Dhungana, and Shakya (2015); Khadka et al., (2012). The majority of the isolates; Klebsiella oxytoca, Proteus Vulgaris, Citrobacter koseri, Citrobacter freundii, Enterobacter species, and Pseudomonas aeroginosa was found causing UTI in male than in the female. The ward-wise distribution of the isolates is listed in table 4.

The majority of infections was observed in the emergency ward (24.44%), followed by obs/gyne (20.32%), while 17.14% in the nephrology ward. The resistance pattern of bacterial isolates deduced from the antibiotic susceptibility test is shown in Table 5.

Penicillin and cefoxitin were tested only for Gram-positive bacteria, while nalidixic acid was tested only for Gram-negative bacteria. Most of the Gram-positive isolates were resistant to penicillin and cefoxitin. The majority of the isolates of Gram-positive, as well as Gram-negative bacteria, were resistant to ampicillin. E. coli isolates were resistant to most of the antibiotics tested except for nitrofurantoin and Gentamycin. Sah et al., (2016); Dhungana and Shakya (2015) reported above 72% ampicillin resistance in E. coli isolates, which is in concordance with this study. A total of 49.5% isolates were resistant to ceftriaxone, which was much higher in comparison with the study of Sah et al., (2016); Raja et al., (2011); Dhungana and Shakya (2015). Cotrimoxazole resistance pattern was higher than the study of Sah et al., (2016); Dhungana and Shakya (2015), but lower than that of Raja et al., (2011). Nalidixic acid resistance was much higher than that found in the study of Dhungana and Shakya (2015). Higher resistance was observed in ciprofloxacin than the study of Raja et al., (2011) and Dhungana and Shakya (2015). Twenty-four percent of E. coli isolates were resistant to Gentamycin, which was similar in the study of Raja et al., (2011), while Dhungana and Shakya (2015) reported 11.12% resistance.

Klebsiella pneumoniae isolates were

Table 4. Distribution of the isolates in different wards

Isolates	Wards									Total (%)
	Emergency (%)	OPD (%)	Nephrology (%)	Surgical (%)	Paediatric (%)	Orthopedic (%)	Obs/Gyne (%)	Medicine (%)	ICU (%)	
Escherichia coli	(60)	9 (4.50)	29 (14.50)	(98)	19 (9.50)	(0.50)	41 (20.50)	(22)	1 (0.50)	200 (100)
Klebsiellapneumoniae	(9.52)	(0)	(14329)	(0)	8 (38.09)	0 (0)	(23581)	3 (14.29)	(0)	21 (100)
Klebsiellaoxytoca	(0)	(0)	1 (100)	(0)	(0)	(0)	(0)	(0)	(0)	1 (100)
Proteus mirabilis	(0)	(0)	(16167)	1 (16.67)	2 (33.33)	(0)	(16167)	1 (16.67)	(0)	6 (100)
Proteus vulgaris	(60)	(0)	(20)	(0)	(0)	(0)	(20)	(0)	(0)	5 (100)
Citrobacterkoseri	(0)	(0)	(0)	2 (66.67)	(0)	(0)	(33133)	(0)	(0)	3 (100)
Citrobacterfreundii	(0)	(0)	(0)	(0)	(0)	(100)	(0)	(0)	(0)	3 (100)
Enterobacter species	(100)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	1 (100)
Pseudomonas aeruginosa	(16167)	(0)	(16167)	(0)	(0)	(16167)	(33233)	1 (16.67)	(0)	6 (100)
Acinetobacter species	(33533)	(0)	(20)	2 (13.33)	(6.67)	(6.67)	(6.67)	1 (16.67)	1 (6.67)	15 (100)
Staphylococusaureus	(4.55)	1 (4.55)	(36836)	(4.55)	(4.55)	(4.55)	(18418)	5 (22.72)	(0)	22 (100)
CoNS	(30)	(10)	(20)	(10)	(0)	(0)	(30)	(0)	(0)	10 (100)
Streptococcus species	(0)	(0)	(14129)	1 (14.29)	1 (14.29)	(0)	(28257)	(0)	2 (28.57)	7 (100)
*Enterococcus*species	(10)	(0)	(30)	(0)	(10)	(0)	(30)	(20)	(0)	10 (100)
Candida albicans	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100)	(0)	2 (100)
Non-albicans*Candida* species	(0)	(0)	(33133)	(0)	(0)	(0)	(0)	(0)	2 (66.67)	3 (100)
Total	77 (24.44)	11 (3.49)	54 (17.14)	26 (8.25)	33 (10.48)	(2.22)	64 (20.32)	37 (11.75)	(1.91)	315 (100)

Table5. Antibiotic susceptibility pattern of the isolates

Isolates	Resistance pattern of the antibiotics tested											
	Ampicillin (%)	Cefazolin (%)	Ceftriaxone (%)	Cotrimoxazol (%)	Nalidixic acid (%)	Ciprofloxacin (%)	Norfloxacin (%)	Nitrofurantoin (%)	Gentamycin (%)	Penicillin (%)	Cefoxitin (%)	Total
Escherichia coli	155 (77.5)	-	99 (49.5)	115 (57.5)	159 (79.5)	115 (57.5)	124 (62)	62 (31)	48 (24)	-	-	200 (100)
Klebsiellapneumoniae	18 (85.71)	9 (42.86)	10 (47.62)	12 (57.14)	11 (52.38)	9 (42.86)	8 (38.10)	16 (76.19)	8 (38.10)	-	-	21 (100)
Klebsiellaoxytoca	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	-	1 (100)
Proteus mirabilis	6 (100)	3 (50)	2 (33.33)	4 (66.67)	4 (66.67)	4 (66.67)	3 (50)	6 (100)	1 (16.67)	-	-	6 (100)
Proteus vulgaris	5 (100)	5 (100)	4 (80)	4 (80)	5 (100)	3 (60)	3 (60)	4 (80)	2 (40)	-	-	5 (100)
Citrobacterkoseri	3 (100)	1 (33.33)	1 (33.33)	3 (100)	3 (100)	1 (33.33)	1 (33.33)	2 (66.67)	1 (33.33)	-	-	3 (100)
Citrobacterfreundii	3 (100)	3 (100)	0 (0)	0 (0)	2 (66.67)	0 (0)	0 (0)	0 (0)	0 (0)	-	-	3 (100)
Enterobacter species	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	-	1 (100)
Pseudomonas aeruginosa	6 (100)	6 (100)	2 (33.33)	6 (100)	6 (100)	2 (33.33)	1 (16.67)	6 (100)	2 (33.33)	-	-	6 (100)
Acinetobacter species	12 (80)	12 (80)	11 (73.33)	5 (33.33)	8 (53.33)	2 (13.33)	6 (40)	13 (86.67)	4 (26.67)	-	-	15 (100)
Staphylococusaureus	11 (50)	6 (27.27)	11 (50)	6 (27.27)	-	4 (18.18)	7 (31.82)	0 (0)	5 (22.73)	22 (100)	10 (45.46)	22 (100)
CoNS	7 (70)	5 (50)	8 (80)	6 (60)	-	3 (30)	3 (30)	1 (10)	1 (10)	9 (90)	9 (90)	10 (100)
Streptococcus species	2 (28.57)	7 (100)	3 (42.86)	3 (42.86)	-	4 (57.14)	3 (42.86)	2 (28.57)	4 (57.14)	7 (100)	5 (71.43)	7 (100)
Enterococcus species	4 (40)	10(100)	10 (100)	9 (90)	-	10 (100)	8 (80)	4 (40)	8 (80)	10(100)	8 (80)	10 (100)
Total	234	69	163	175	200	159	169	118	86	48	32	2 (100)

highly resistant to Gentamycin, nitrofurantoin, ciprofloxacin, cotrimoxazole, ceftriaxone, and norfloxacin while comparing the study of Raja et al., (2011). All the isolates of P. mirabilis and P. Vulgaris isolates were resistant to ampicillin, whereas Dhungana and Shakya (2015) reported that 60% Proteus species resistant to ampicillin. Similarly, the resistance pattern of norfloxacin, cotrimoxazole, ceftriaxone, and Gentamycin for Proteus mirabilis and Proteus Vulgaris was much higher than described for Proteus species in the study of Dhungana and Shakya (2015); Raja et al., (2011). Proteus species in this study were much more resistant to ciprofloxac in and nalidixic acid than the study of Dhungana and Shakya (2015). Higher drug resistance to nitrofurantoin was observed compared to that reported by Raja et al., (2011).

Raja et al., (2011) found high resistance of Citrobacter species against Gentamycin, norfloxacin, and ceftriaxone than that of Citrobacterkoseri and Citrobacter freundii in this study. However, resistance to cotrimoxazole and nitrofurantoin by Citrobacter koseriwas higher in this study while lower by Citrobacter freundii compared to resistance pattern of Citrobacter species in a study by Raja et al., (2011). A single Enterobacter species was isolated, which was resistant to all the antibiotics tested, whereas Raja et al., (2011) reported much lower antibiotic resistance than this study.

All the isolates of P. aeruginosa were resistant to nitrofurantoin and cotrimoxazole, which was higher than that found in the study of Raja et al., (2011). However, the resistance rate to Gentamycin, norfloxacin, ciprofloxacin, and ceftriaxone was found much lower than the study of Rajaet al (2011). Acinetobacter species were highly resistant to nitrofurantoin, ceftriaxone, and cotrimoxazole, while Gentamycin, norfloxacin, and ciprofloxacin resistance were comparatively low than that recorded by Raja et al., (2011).

S. aureus isolates were completely resistant to penicillin. Resistance pattern of S. aureus towards ciprofloxacin and norfloxacin was recorded much lower than that in Sah et al., (2016); Dhungana and Shakya (2015), whereas S.aureus isolates were highly resistant to Gentamycin and ceftriaxone in this study while comparing with the study of Sah et al., (2016); Raja et al., (2011); Dhungana and Shakya (2015). All the S. aureus isolates were sensitive to nitrofurantoin, whereas 27.27% S. aureus isolates were resistant to cotrimoxazole, which was higher than the study of Raja et al., (2011); Dhungana and Shakya (2015) but lower than Sah et al., (2016).

Conclusion

Urine specimens should be tested for culture and antimicrobial susceptibility for proper treatment and to get control over the antimicrobial resistance. Gentamycin and nitrofurantoin were found most effective antibiotics. Gentamycin is not recommended in pregnancy and children as it causes some adverse effects. Gentamycin is not available in oral forms, whereas, nitrofurantoin is available in oral form. Nitrofurantoin does not possess any adverse effects like Gentamycin does during pregnancy and in children. It makes nitrofurantoin the drug of choice for the treatment of urinary tract infections.

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