

## A STUDY ON BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF INFECTIONS IN HOSPITALISED CHILDREN WITH NEPHROTIC SYNDROME

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### ABSTRACT

**Background:** Children with nephrotic syndrome are highly susceptible to infections, increasing morbidity and guiding treatment decisions. **Aims and Objectives:** This study aimed to determine the spectrum of bacterial infections in children with nephrotic syndrome, identify the bacteria, and determine their antibiotic susceptibility patterns of the isolated pathogens. **Materials and Methods:** This cross-sectional study included 100 children with nephrotic syndrome conducted at Inst of Microbiology, MMC & admitted to the Institute of Child Health, Madras Medical College, Chennai, between 2015 and 2016. Data were collected from children with nephrotic syndrome, including demographic details and clinical data. Samples collected were processed for microbiological analysis. **Results:** Among 100 children, 56 were males and 44 were females. Most children were aged 5–12 years (25 males, 27 females). Urinary tract infection (UTI) was the most common (35/100), followed by acute upper respiratory tract infection (9/100). *Escherichia coli* (54%) and MSSA (11%) predominated in urine; MSSA (78%) and *Streptococcus pyogenes* (22%) in throat swabs. Nephrotic syndrome types: first episode 14%, steroid-dependent 49%, steroid-resistant 37%. Most *E. coli* isolates were sensitive to AK (95%), COTRI (100%), PT (100%), and IMP (100%). ESBL isolates showed variable susceptibility; GPC isolates were largely sensitive to PEN, GM, and VAN. **Conclusion:** Infections are a significant complication of nephrotic syndrome, with urinary tract infections being the most prevalent. *E. coli* is the primary pathogen, sensitive to several antibiotics, and no AmpC  $\beta$ -lactamase or metallo- $\beta$ -lactamase producers were detected.

## INTRODUCTION

Children with nephrotic syndrome are at a heightened risk of infection due to multiple factors, including urinary protein loss leading to hypogammaglobulinemia, impaired complement activity, and frequent use of immunosuppressive therapy.<sup>[1]</sup> Infections in children with nephrotic syndrome contribute significantly to morbidity and may complicate clinical management, precipitate relapse, or prolong hospitalisation. Recognising the types of infections and causative organisms is critical to guiding empirical therapy and improving outcomes.<sup>[2]</sup>

Among reported infectious complications in nephrotic syndrome, spontaneous bacterial

peritonitis, pneumonia, urinary tract infections, and sepsis are frequently described.<sup>[3]</sup> In a cohort of hospitalised nephrotic children, major infections occurred in 43.8% of admissions, with peritonitis being the most common, followed by pneumonia.<sup>[4]</sup> Another study in India found pneumonia to be the most frequent infection in children with idiopathic nephrotic syndrome, with urinary tract infection being the next in line.<sup>[5]</sup> In a tertiary-centre Indian series, 73% of nephrotic children experienced relapse episodes, with infection.<sup>[6]</sup> Urinary tract infections (UTIs) deserve special attention in nephrotic syndrome because they are often asymptomatic, may remain undetected, and can predispose patients to relapse or renal damage if untreated.<sup>[7]</sup> The pooled global prevalence of UTI in

children with nephrotic syndrome has been estimated at 21.6% (95% CI: 17.1–26.5%).<sup>[8]</sup> In one Indian cross-sectional study, among nephrotic children, *Escherichia coli* (*E. coli*) accounted for 33.3% of UTIs, followed by *Klebsiella* spp and *Staphylococcus aureus*. Knowledge of the local bacterial profile and antibiotic sensitivity in children with nephrotic syndrome is essential for empirical therapy, especially in settings where culture results may be delayed or not always interpretable.<sup>[9]</sup>

Patterns of antibiotic resistance among pathogens isolated from children with nephrotic syndrome vary by region and over time. Some studies have reported emerging extended-spectrum  $\beta$ -lactamase (ESBL)-producing enterobacteria and multidrug-resistant strains, complicating empirical treatment choices. Regular surveillance of antimicrobial susceptibility in nephrotic populations ensures that empirical regimens remain effective and helps prevent the development of resistance.<sup>[10]</sup>

#### Aim

This study aimed to determine the spectrum of bacterial infections in children with nephrotic syndrome, identify the bacteria, and determine their antibiotic susceptibility patterns of the isolated pathogens.

## MATERIALS AND METHODS

This cross-sectional study included 100 children with nephrotic syndrome conducted at Inst of Microbiology, MMC & admitted in the paediatric nephrology ward of the Department of Nephrology, Institute of Child Health, Madras Medical College, Chennai, between September 2015 and August 2016. This study was approved by the Institutional Ethics Committee prior to initiation, and informed consent was obtained from all their parents or guardians.

#### Inclusion Criteria

Paediatric patients with nephrotic syndrome aged 1–12 years and hospitalised for reasons such as relapse, re-evaluation, non-response to therapy, cyclophosphamide therapy, and infectious complications were included.

#### Exclusion Criteria

Paediatric patients with urogenital anomalies and those diagnosed with acute or chronic renal failure were excluded.

**Methods:** Demographic details (name, age, sex, address, and date of admission) and clinical data (presenting complaints, history, treatment history, clinical diagnosis, and investigations) were collected. Urine, blood, and throat swab samples were collected. For urine collection, a sterile, leak-proof wide-mouth container was provided, and 10 ml of clean-catch midstream urine was collected. Blood was collected using sterile precautions by disinfecting the venipuncture site with 70% alcohol, followed by 1% povidone-iodine solution. Five millilitres of blood was transferred into 25 ml of Brain Heart Infusion broth in a blood culture bottle. A sterile swab was inserted through the mouth, and specimens were collected from the posterior pharyngeal wall.

**Direct Gram staining:** A drop of urine was transferred to a glass slide, air-dried, heat-fixed, gram-stained, and examined under oil immersion. Gram stain morphology and the presence of pus cells and bacteria were documented.

**Culture:** A calibrated Nichrome loop (0.01 ml) was used to streak urine onto MacConkey and blood agar plates. The plates were incubated at 37 °C overnight and observed after 24–48 h. A colony count >100,000/ml ( $10^5$ /ml) was considered to indicate significant bacteriuria. Blood culture bottles were incubated at 37 °C for 48 h and sub cultured on MacConkey and blood agar plates. Throat swabs were Gram-stained and cultured on MacConkey and blood agar plates, incubated in a candle jar at 37 °C for 24 h. Antibiotic susceptibility testing was done for the isolated pathogens from culture. Data were entered into Microsoft Excel, presented as frequencies and percentages, and analysed using MS Excel and SPSS.

## RESULTS

Among the children studied, 12 males and 12 females were aged 1 –  $\leq 3$  years, 19 males and 5 females were aged >3 –  $\leq 5$  years, and 25 males and 27 females were aged >5 –  $\leq 12$  years. Urinary tract infection was the most common infection, seen in 19 males and 16 females, followed by acute upper respiratory tract infection in 5 males and 4 females, while both infections occurred together in 1 male child. [Table 1]

**Table 1: Demographic details and spectrum of infections according to gender**

Parameter	Category	Gender	
		Male	Female
Age group (years)	1 to $\leq 3$	12	12
	>3 to $\leq 5$	19	5
	>5 to $\leq 12$	25	27
Sex	Child	56	44
Spectrum of infections	Urinary tract infection	19	16
	Acute upper respiratory tract infection	5	4
	Both (UTI and AURI)	1	-

In urine samples, *E. coli* was the most common isolate (54%), followed by MSSA (11%), *Klebsiella pneumoniae* (9%), ESBL-producing *E. coli* (6%),

ESBL-producing *K. pneumoniae* (6%), ESBL-producing *K. oxytoca* (6%), *Pseudomonas* spp. (6%), and *Acinetobacter baumannii* (3%). From throat

swabs, MSSA was isolated in 78% of cases and *Streptococcus pyogenes* in 22% of cases. Regarding the types of nephrotic syndrome, 14% were first

episodes, 49% were steroid-dependent, and 37% were steroid-resistant. [Table 2]

**Table 2: Pathogens isolated in the urine, throat swab samples and infections in types of nephrotic syndrome**

Parameter	Name of the organism	Number of isolates (%)
Urine sample	<i>Escherichia coli</i>	19 (54%)
	<i>Escherichia coli</i> (ESBL)	2 (6%)
	<i>Staphylococcus aureus</i> (MSSA)	4 (11%)
	<i>Klebsiella pneumoniae</i>	3 (9%)
	<i>Klebsiella pneumoniae</i> (ESBL)	2 (6%)
	<i>Klebsiella oxytoca</i> (ESBL)	2 (6%)
	<i>Pseudomonas spp</i>	2(6%)
Throat swab sample	<i>Acinetobacter baumannii</i>	1 (3%)
	<i>Staphylococcus aureus</i> (MSSA)	7 (78%)
	<i>Streptococcus pyogenes</i>	2 (22%)
Types of nephrotic syndrome	First episode	14%
	Steroid-dependent NS	49%
	Steroid-resistant NS	37%

Among 19 *E. coli* isolates, most were sensitive to amikacin (AK, 30 µg, 95%), cotrimoxazole (COTRI, 1.25/23.75 µg, 100%), piperacillin-tazobactam (PT, 100/10 µg, 100%), cefotaxime (CTX, 30 µg, 84%), tetracycline (TETRA, 10 µg, 100%), imipenem (IMP, 10 µg, 100%), norfloxacin (NOR, 10 µg, 74%), and nitrofurantoin (NITRO, 300 µg, 68%). The three *Klebsiella pneumoniae* isolates were completely

sensitive to PT, TETRA, and IMP and partially sensitive to AK (67%), COTRI (67%), and CTX (67%) but fully sensitive to NOR and NITRO. Both *Pseudomonas spp.* isolates were 100% sensitive to AK, PT, ceftazidime (CAZ, 30 µg), and IMP and 50% sensitive to NOR. The single *Acinetobacter baumannii* isolate was fully sensitive to AK, COTRI, PT, ciprofloxacin (CIP, 5 µg), and TETRA. [Table 3]

**Table 3: Antibiotic sensitivity of pathogens in urine in percentage (%) – GNB**

Parameter	Name of the organism			
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas spp</i>	<i>Acinetobacter baumannii</i>
Total no of isolates	19	3	2	1
AK 30µg	95%	67%	100%	100%
COTRI 1.25/23.75µg	100%	67%	NA	100%
PT 100/ 10µg	100%	100%	100%	100%
CIP 5µg	NA	NA	NA	100%
CAZ 30µg	NA	NA	100%	R
CTX 30µg	84%	67%	NA	NA
TET RA 10µg	100%	100%	NA	100%
IMP 10µg	100%	100%	100%	100%
NOR 10µg	74%	100%	50%	NA
NITRO 300µg	68%	100%	NA	NA

The antibiotic susceptibility of the six ESBL-producing isolates showed variable patterns of susceptibility. Among the two *E. coli* (ESBL) isolates, 50% were sensitive to AK (30 µg) and COTRI (1.25/23.75 µg), 100% to PT (100/10 µg), TETRA (10 µg), IMP (10 µg), and NITRO (300 µg), and resistant (R) to CTX (30 µg) and NOR (10 µg). Both *Klebsiella pneumoniae* (ESBL) isolates were fully sensitive to AK (30 µg), COTRI (1.25/23.75

µg), PT (100/10 µg), IMP (10 µg), and NOR (10 µg), partially sensitive to TETRA (10 µg, 50%), and resistant to CTX (30 µg), with 50% sensitivity to NITRO (300 µg). Both *Klebsiella oxytoca* (ESBL) isolates were completely sensitive to AK (30 µg), COTRI (1.25/23.75 µg), PT (100/10 µg), TETRA (10 µg), IMP (10 µg), and NITRO (300 µg) but resistant to CTX (30 µg) and NOR (10 µg). [Table 4]

**Table 4: Antibiotic sensitivity of ESBL pathogens in urine in percentage (%) – GNB**

Parameter	Name of the organism		
	<i>Escherichia coli</i> (ESBL)	<i>Klebsiella pneumoniae</i> (ESBL)	<i>Klebsiella oxytoca</i> (ESBL)
Total no of isolates	2	2	2
AK 30µg	50%	100%	100%
COTRI 1.25/23.75µg	50%	100%	100%
PT 100/10µg	100%	100%	100%
CTX 30µg	R	R	R
TETRA 10µg	100%	50%	100%
IMP 10µg	100%	100%	100%
NOR 10µg	R	100%	R
NITRO 300µg	100%	50%	100%

The antibiotic susceptibility of gram-positive isolates varied according to the sample type. Among 4 urine *Staphylococcus aureus* (MSSA) isolates, 100% were sensitive to penicillin (PEN, 10 µg), gentamicin (GM, 10 µg), COTRI (1.25/23.75 µg), TETRA (10 µg), NOR (10 µg), NITRO (300 µg), and vancomycin (VAN). The seven throat MSSA isolates were fully

sensitive to PEN, erythromycin (ERY, 15 µg), GM, CIP (5 µg), COTRI, TETRA, clindamycin (CK, 30 µg), and VAN. Both *Streptococcus pyogenes* isolates showed complete sensitivity to PEN, TETRA, CK, VAN, and CTX (30 µg) and 50% sensitivity to ERY and ofloxacin (OF, 5 µg). [Table 5]

**Table 5: Antibiotic sensitivity of pathogens in urine & throat swab in percentage (%) -GPC**

Name of the organism	Urine sample		Throat swab sample	
	<i>Staphylococcus aureus</i> (MSSA)		<i>Staphylococcus aureus</i> (MSSA)	<i>Streptococcus pyogenes</i>
Total no of isolates	4		7	2
PEN 10µg	100%		100%	100%
ERY 15µg	-		100%	50%
GM 10µg	100%		100%	NA
CIP 5µg	-		100%	NA
COTRI 1.25/23.75 µg	100%		100%	NA
TETRA 10µg	100%		100%	100%
CK 30µg	-		100%	100%
VAN	100%		100%	100%
CTX 30µg	-		NA	100%
OF 5µg	-		NA	50%
NOR 10µg	100%		-	-
NITRO 300µg	100%		-	-

## DISCUSSION

In our study, 52% of paediatric patients with nephrotic syndrome were > 5 to ≤ 12 years of age, followed by 24% in the 1 to ≤ 3 year (24%) in the >3 to ≤ 5 years (24%). Senguttuvan et al. stated that the mean age at onset of nephrotic syndrome was 5.95 years.<sup>[11]</sup> Alfakeekh et al. reported 111 children with primary childhood nephrotic syndrome (PCNS), of whom 78 (70%) were boys and 33 (30%) were girls, with a mean age at onset of 4.17 ± 2.1 years. The overall incidence of infection was similar between genders (boys 76%, girls 76%), and children <10 years had a slightly higher infection rate (76%) compared to older children (71%).<sup>[12]</sup> In our study, the prevalence of nephrotic syndrome was higher (56%) in male than in female children. Adedoyin et al. study showed a male-to-female ratio of 2:6:1.<sup>[13]</sup> In our study, the percentage of infections varied among the different types of nephrotic syndromes. The percentage of infections was higher in patients with steroid-dependent nephrotic syndrome (49%) than in those with steroid-resistant nephrotic syndrome (37%). The first episode of nephrotic syndrome (14%) showed that infections are more common in children on immunosuppressants for treatment, indicating that steroid intake causes more immunosuppression than other drugs. Alfakeekh et al., reported that children receiving secondary immunosuppressive agents had a 100% risk of infection. Higher annual cumulative doses of corticosteroids (median 45.8 mg/kg) and secondary immunosuppressants were significantly associated with increased infection risk (p<0.01 and p<0.001, respectively).<sup>[12]</sup> According to Senguttuvan et al., there was no difference in the incidence of infections between children who received steroid therapy alone and those who received both steroids and cyclophosphamide.<sup>[11]</sup>

In our study, the percentage of infection in children with nephrotic syndrome was higher in male children (24%) than in female children (22%). Moorani et al. showed that 72.58% were male children and 27.42% were female children in a ratio of 2:5:1.<sup>[14]</sup> In our study, the spectrum of bacterial infections showed that urinary tract infection was the most common (35%), followed by acute upper respiratory tract infection (9%). Gulati et al. showed urinary tract infections being the commonest at 13.7% and upper respiratory infections at 5.2%.<sup>[15]</sup>

In our study, male children with UTI were more likely to be infected (19%) than female children (16%), and acute upper respiratory tract infections were more common in males (5%) than in female children (4%). Among the Gram-negative bacilli, the most common pathogen was *E. coli* (60%) followed by *Klebsiella pneumoniae* (15%), *Klebsiella oxytoca* (6%), *Pseudomonas* spp (6%) and *Acinetobacter baumannii* (3%). Adedoyin et al. showed that coliforms commonly cause UTI in children with NS, including *Klebsiella* (8.6%) and *Pseudomonas* (5.7%). *Staphylococcus aureus* (11%) is a gram-positive pathogen isolated from urine.<sup>13</sup> The study done by Ibadin showed 54.3% growth of *Staphylococcus aureus*.<sup>[16]</sup>

In our study, the predominant pathogen isolated from the throat swabs was gram-positive cocci, *Staphylococcus aureus* (MSSA) (78%), followed by *Streptococcus pyogenes*. Among the antibiotic sensitivity patterns of pathogens in urine, the most common pathogen was *E. coli*, which was 100% sensitive to trimethoprim-sulfamethoxazole, piperacillin-tazobactam, tetracycline, and imipenem. 95% sensitivity to amikacin and 84% sensitivity to cefotaxime. Sensitivity to norfloxacin was 74%. The sensitivity to nitrofurantoin was 68%. The study was done by Ibadin which showed 100% sensitivity to cefotaxime and amikacin.<sup>[16]</sup>

In our study, *K. pneumoniae* showed 100% sensitivity to piperacillin-tazobactam, tetracycline, imipenem, norfloxacin, and nitrofurantoin. 67% sensitive to amikacin, trimethoprim-sulfamethoxazole, and cefotaxime, and *Pseudomonas* spp were 100% sensitive to amikacin, piperacillin-tazobactam, ceftazidime, and imipenem, 50% sensitive to norfloxacin. The study done by Ibadin showed a 100% sensitivity to amikacin and ceftazidime.<sup>[16]</sup>

Nephrotic syndrome was more common in male children, and infections were more common in those receiving steroids or other immunosuppressants, consistent with trends reported in previous studies. Urinary tract infections, mainly caused by *E. coli*, were the most frequent, aligning with the findings of the study by Gulati and Ibadin,<sup>[12,16]</sup> and most pathogens showed good antibiotic sensitivity.

## CONCLUSION

Nephrotic syndrome predominantly affects children aged 5–12 years and is associated with a higher incidence of infections, particularly in those with steroid-dependent nephrotic syndrome, than in steroid-resistant cases and first episodes. Urinary tract infections are the most common, followed by acute upper respiratory tract infections, with *E. coli* identified as the primary pathogen in UTIs, which is sensitive to trimethoprim-sulfamethoxazole, piperacillin-tazobactam, tetracycline, and imipenem. No AmpC  $\beta$ -lactamase or metallo- $\beta$ -lactamase producers were detected among the isolated pathogens, indicating a lower risk of antimicrobial resistance.

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