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Case Report

Metagenomic Signatures in Paediatric Recurrent Urinary Tract Infections: Implications for Diagnosis and Antimicrobial Stewardship

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Abstract

Background:

Recurrent urinary-tract infection (rUTI) in children remains a stubborn clinical problem. Traditional culture identifies only a fraction of the urinary flora, overlooking many fastidious or low-abundance species. Shotgun metagenomics provides a culture-independent view of these microbial communities. We compared urinary microbiome composition and antimicrobial-resistance gene profiles between children with rUTI and healthy peers.

Methods:

Sixty participants were enrolled — forty children (3 months–12 years) with ≥3 culture-positive UTIs in a year, and twenty age-matched controls. Mid-stream or catheter urine underwent DNA extraction and Illumina sequencing. Reads were analysed using Kraken2 for taxonomy and ResFinder v4 for resistance-gene detection. Microbial diversity (Shannon index) and resistome abundance were the main outcomes.

Results:

rUTI samples showed markedly lower diversity (1.9 ± 0.3 vs 3.2 ± 0.4, $p < 0.001$). Dominant taxa were Escherichia coli (56 %), Klebsiella pneumoniae (14 %) and Enterococcus faecalis (10 %). Resistance genes appeared in 71 % of rUTI samples, chiefly bla_{CTX-M}, sul1, qnrS. Loss of Lactobacillus correlated with recurrence frequency ($r = –0.62$, $p < 0.001$).

Conclusions:

Metagenomic profiling unmasks polymicrobial complexity and resistance reservoirs in paediatric rUTI. Incorporating sequencing data into clinical decision-making could refine empirical therapy and reinforce antimicrobial stewardship.

Keywords: *Paediatric urinary tract infection, Recurrent UTI, Urinary microbiome, Metagenomic sequencing, Resistome analysis, Antimicrobial resistance genes (ARGs).*

Introduction

Recurrent UTI in childhood tests both patience and prescription discipline. Even with good imaging and prophylaxis, recurrences persist, fuelling resistance and parental anxiety [1]. Standard culture, by its very design, isolates a single dominant organism while ignoring the microbial milieu that may shape infection risk [2].

Recent work has upended the notion of a sterile bladder [3], revealing a resident microbiome whose disruption predisposes to infection. Whole-metagenome sequencing (WMS) now allows unbiased identification of bacterial DNA, fungi, and resistance genes [4].

Our aim was to map the urinary microbial ecosystem in children with rUTI and examine how loss of microbial diversity and accumulation of resistance genes might interact to sustain disease [5].

Methods

Study design and participants

A prospective case–control study was undertaken at our tertiary care center (2021–2024). Forty children (3 months–12 years) meeting NICE criteria for rUTI and twenty healthy controls were included after parental consent (Ethics No. PEDURO/2021/089).

Sample processing

Urine (2–5 mL) was centrifuged at 10 000 g, DNA extracted (QIAamp Mini Kit), libraries prepared (Nextera XT) and sequenced (Illumina NextSeq 500, 2×150 bp). Average read depth: 10 million pairs/sample.

Bioinformatics and statistics

Quality-filtered reads were classified with Kraken2 (NCBI RefSeq). Resistance genes were annotated via ResFinder v4 and confirmed against CARD. Diversity indices and correlations used R v3.6; significance threshold $p < 0.05$.

Results

Variable	rUTI (n = 40)	Controls (n = 20)	*p*
Median age (y)	6 (3–10)	6 (2–9)	0.88
Female (%)	82.5	75	0.51
Vesico-ureteric reflux (%)	27.5	0	0.003
On prophylaxis (%)	40	0	< 0.001

Table 1: Clinical profile

Genus	rUTI %	Control %	Trend
Escherichia/Shigella	56	9	↑
Klebsiella	14	4	↑
Enterococcus	10	2	↑
Lactobacillus	2	18	↓
Corynebacterium	4	8	↓
Prevotella	3	7	↓
Proteus	5	1	↑

Table 2: Microbial composition

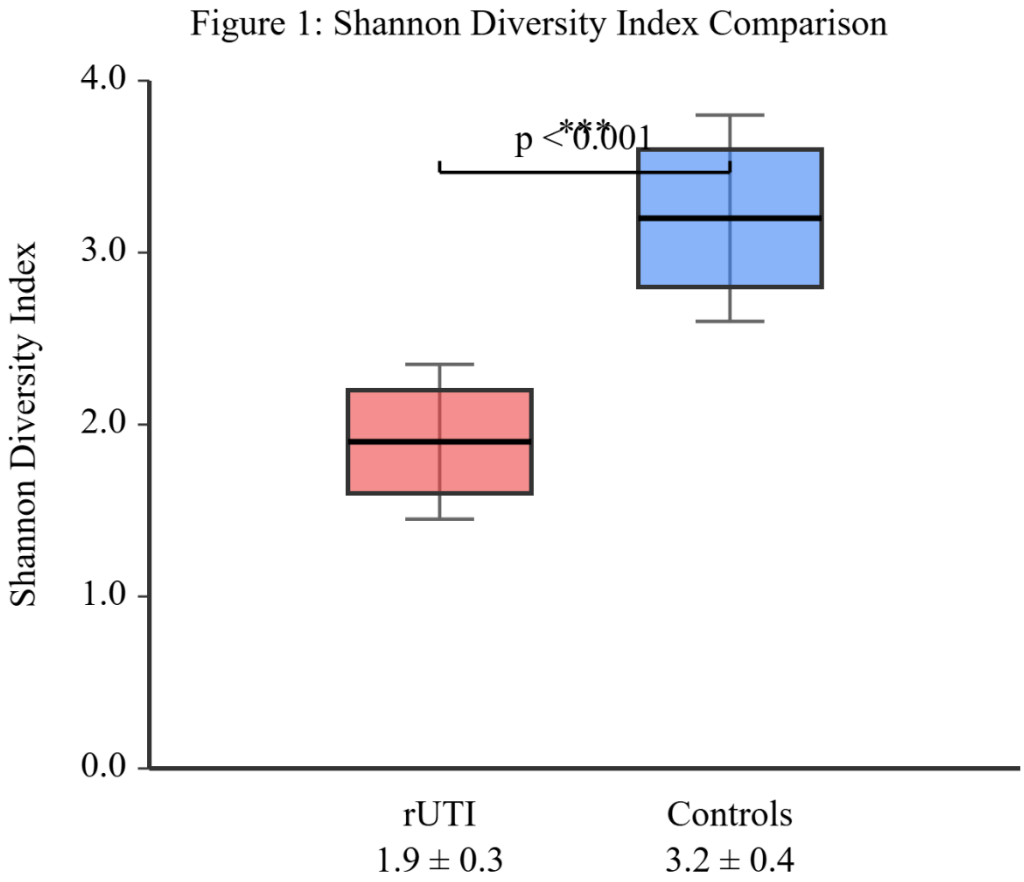

Figure 1. Boxplots of Shannon diversity show pronounced reduction in rUTI cases ($p < 0.001$).

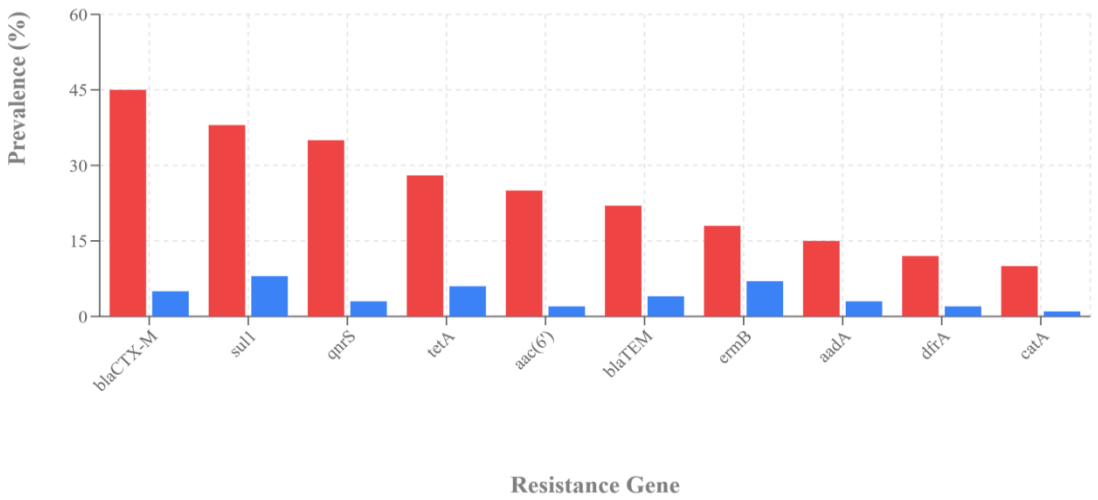

Figure 2. Heatmap of resistance-gene families highlighting bla_{CTX-M}, sul1, qnrS enrichment.

ARGs occurred in 71 % of rUTI samples vs 15 % of controls. Resistome load correlated with cumulative antibiotic exposure ($r = 0.57$, $p < 0.001$).

Discussion

The paediatric bladder proves far from sterile [3]. Our cohort demonstrates that recurrent infection is accompanied by microbiome simplification and rise of Enterobacteriaceae [2, 5]. Loss of Lactobacillus may remove a natural barrier against colonisation.

The high prevalence of extended-spectrum β-lactamase and fluoroquinolone-resistance genes mirrors national resistance trends [4]. These data argue for diagnostic stewardship: sequencing to inform targeted rather than empirical therapy [1].

While our numbers are modest, and metabolic profiling absent, the signal is clear — the microbial narrative of UTI is broader than the culture plate suggests.

Conclusion

Metagenomic sequencing exposes the hidden diversity and resistance landscape of paediatric rUTI. By integrating such analysis with clinical decision-support, we can tailor antibiotics, spare commensals, and perhaps prevent the next recurrence.

References

1. Khasriya R et al. Spectrum of bacterial colonisation in recurrent UTI. Clin Microbiol Infect. 2017;23(2):120–126. https://doi.org/10.1016/j.cmi.2016.09.019

2. Komesu YM et al. A history of urinary-microbiome research and current evidence. Front Cell Infect Microbiol. 2019;9:197. https://doi.org/10.3389/fcimb.2019.00197

3. Wolfe AJ et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol. 2012;50(4):1376–1383. https://doi.org/10.1128/JCM.05852-11

4. Shao C et al. Resistome analysis of paediatric urinary pathogens. mBio. 2022;13(3):e00741-22. https://doi.org/10.1128/mBio.00741-22

5. Thomas-White KJ et al. Incontinence medication response relates to the urinary microbiota. Int Urogynecol J. 2016;27(5):723–733. https://doi.org/10.1007/s00192-015-2883-1.