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Original Research Article

Impact of a Hospital-Based Antibiotic Stewardship Intervention on Microbiome Diversity in Pediatric Inpatients

Karan B. Patel[1], Rutwa Shah[2], Vinyl Kumar Pahuja[3]

[1]Senior Resident, Department of Paediatric, GMERS Medical College, Vadnagar, Gujarat, India
[2]DNB Paediatrics, Department of Paediatric, GMERS Medical College and Sola Civil Hospital, Ahmedabad, Gujarat, India
[3]Professor, Department of Microbiology, MVASMC Bijnor, Uttar Pradesh, India

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Corresponding Author: Dr. Vinyl Kumar Pahuja
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Abstract:

Background: Antibiotic overuse in hospitalized children disrupts the developing gut microbiome, potentially contributing to dysbiosis-related complications including secondary infections, immune dysregulation, and long-term metabolic consequences. Antibiotic stewardship programs have been widely implemented to optimize antimicrobial prescribing, yet their direct impact on gut microbiome diversity in pediatric inpatients remains poorly characterized.

Methods: A prospective quasi-experimental study was conducted over 12 months involving 136 pediatric inpatients aged 1–14 years admitted to a tertiary care hospital. Patients were divided into a pre-intervention cohort (n = 68) receiving standard care and a post-intervention cohort (n = 68) managed under a structured antibiotic stewardship protocol. Stool samples were collected at admission and discharge for 16S rRNA gene sequencing. Shannon diversity index, observed operational taxonomic units, and Firmicutes-to-Bacteroidetes ratio were analyzed alongside antibiotic utilization data.

Results: The stewardship intervention significantly reduced broad-spectrum antibiotic use by 31.4% ($p < 0.001$). At discharge, the post-intervention cohort demonstrated significantly higher Shannon diversity index (2.94 ± 0.51 vs. 2.38 ± 0.63; $p = 0.001$) and observed OTUs (187.3 ± 34.6 vs. 152.8 ± 41.2; $p < 0.001$) compared to the pre-intervention group. The decline in microbiome diversity from admission to discharge was significantly attenuated in stewardship-managed patients.

Conclusion: Hospital-based antibiotic stewardship interventions effectively preserve gut microbiome diversity in pediatric inpatients while reducing unnecessary antimicrobial exposure, supporting their integration into pediatric hospital practice.

Keywords: Antibiotic Stewardship; Gut Microbiome; Pediatric; Microbial Diversity; 16S rRNA sequencing.

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Introduction

The human gut microbiome constitutes a complex and dynamic ecosystem comprising trillions of microorganisms that play fundamental roles in nutrient metabolism, immune system maturation, pathogen resistance, and neurodevelopmental processes [1]. In pediatric populations, the gut microbiome undergoes a critical developmental trajectory during the first years of life, and perturbations during this formative period can exert lasting consequences on health and disease susceptibility [2]. Among the most potent disruptors of microbial community structure are antibiotic agents, which exert broad collateral effects on commensal organisms even when appropriately targeted against pathogenic bacteria [3].

Hospitalized children represent a population at particularly elevated risk for antibiotic-induced microbiome disruption. Studies estimate that up to 60% of hospitalized pediatric patients receive at least one antibiotic course during their admission, and approximately one-third of these prescriptions are considered unnecessary or inappropriate [4]. Broad-spectrum agents such as third-generation cephalosporins and carbapenems have been shown to cause profound and sometimes prolonged reductions in microbial diversity, with recovery periods extending weeks to months following cessation of therapy [5].

Antibiotic stewardship programs represent a systematic organizational approach to optimizing antimicrobial prescribing through evidence-based guidelines, prospective audit and feedback, formulary restriction, and prescriber education [6]. The effectiveness of such programs in reducing antibiotic consumption, healthcare costs, and antimicrobial resistance has been well documented in adult hospital settings [7]. Pediatric-specific stewardship programs have similarly demonstrated significant reductions in broad-spectrum antibiotic utilization and associated adverse drug events [8]. However, the majority of outcome measures evaluated in stewardship studies have focused on prescribing metrics, resistance patterns, and clinical outcomes such as length of stay and readmission rates [9].

Emerging research has begun to explore the intersection between stewardship interventions and microbiome outcomes. Investigations in adult intensive care populations have suggested that restricted antibiotic protocols may preserve beneficial microbial communities [10]. Nevertheless, a conspicuous gap exists in the literature regarding the direct measurement of microbiome diversity outcomes following stewardship implementation in hospitalized children [11]. Given the unique vulnerability of the developing pediatric microbiome to antibiotic perturbation and the potential for long-term health consequences, understanding whether stewardship programs confer measurable microbiome-protective benefits in this population is of considerable clinical and public health importance [12].

The aim of the present study was to evaluate the impact of a structured hospital-based antibiotic stewardship intervention on gut microbiome diversity indices, measured through 16S ribosomal RNA gene sequencing, in pediatric inpatients compared to a pre-intervention standard care cohort.

Materials and Methods

Study Design and Setting: This was a prospective quasi-experimental before-and-after study conducted at the pediatric inpatient department of a tertiary care hospital.

Participants: A total of 136 pediatric inpatients were enrolled, with 68 patients in each phase. Inclusion criteria were age between 1 and 14 years, hospital admission requiring antibiotic therapy for a minimum of 48 hours, and parental consent for stool sample collection. Exclusion criteria included antibiotic use within 30 days prior to admission, known gastrointestinal disorders (inflammatory bowel disease, celiac disease), immunodeficiency syndromes, current probiotic or prebiotic supplementation, admission to the pediatric intensive care unit, and anticipated hospitalization exceeding 21 days. Patients were consecutively enrolled during each study phase.

Stewardship Intervention: The antibiotic stewardship intervention was implemented at the beginning of the post-intervention phase and comprised four core components: (1) prospective audit and real-time feedback by a dedicated stewardship team consisting of a pediatric infectious disease specialist and a clinical pharmacist who reviewed all antibiotic orders within 48 hours of initiation; (2) implementation of empiric antibiotic prescribing guidelines stratified by clinical syndrome and local resistance data; (3) mandatory reassessment of antibiotic necessity and spectrum at 48–72 hours with documented de-escalation justification; and (4) monthly educational sessions for prescribing physicians and nursing staff emphasizing appropriate antibiotic duration and spectrum selection.

Stool Sample Collection and Processing: Stool samples were collected from each participant at two time points: within 24 hours of admission (baseline) and within 24 hours prior to discharge. Samples were collected in sterile containers, immediately placed on ice, transported to the microbiology laboratory, and stored at −80°C until processing. Total genomic DNA was extracted using a commercial stool DNA extraction kit following manufacturer protocols. The V3-V4 hypervariable region of the 16S rRNA gene was amplified using universal primers 341F and 805R. Amplicon libraries were prepared and sequenced on an Illumina MiSeq platform using a 2 × 300 bp paired-end protocol.

Bioinformatics Analysis: Raw sequencing reads were processed using the QIIME2 pipeline (version 2024.2). Quality filtering, chimera removal, and denoising were performed using the DADA2 plugin. Taxonomic assignment was conducted against the SILVA 138 reference database. Alpha diversity metrics including Shannon diversity index, observed operational taxonomic units (OTUs), and Chao1 richness estimator were calculated. Beta diversity was assessed using Bray-Curtis dissimilarity matrices and visualized through principal coordinates analysis. The Firmicutes-to-Bacteroidetes (F/B) ratio was computed from phylum-level relative abundance data.

Antibiotic Utilization Metrics: Antibiotic consumption data were extracted from electronic medical records and expressed as days of therapy per 1,000 patient-days (DOT/1,000 PD). Antibiotics were categorized as narrow-spectrum (aminopenicillins, first-generation cephalosporins) or broad-spectrum (third-generation cephalosporins, fluoroquinolones, carbapenems, piperacillin-tazobactam). Antibiotic de-escalation rates and mean duration of therapy were also recorded.

Statistical Analysis: Continuous variables were expressed as mean ± standard deviation or median with interquartile range, as appropriate. Categorical variables were presented as frequencies and percentages. Between-group comparisons were performed using independent samples t-tests or Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables. Within-group changes from admission to discharge were evaluated using paired t-tests. Spearman correlation analysis was used to examine associations between antibiotic exposure metrics and diversity indices. All analyses were conducted using SPSS version 28.0, with statistical significance defined as $p < 0.05$.

Results

Demographic and Clinical Characteristics: The two cohorts were comparable in baseline demographic and clinical characteristics, with no statistically significant differences in age, sex distribution, primary diagnosis category, or admission diversity indices, confirming the validity of between-group comparisons (Table 1).

Table 1: Baseline Demographic and Clinical Characteristics

Characteristic	Pre-Intervention (n = 68)	Post-Intervention (n = 68)	p-value
Age (years), mean ± SD	5.8 ± 3.4	6.1 ± 3.2	0.594
Male sex, n (%)	39 (57.4)	36 (52.9)	0.601
Length of stay (days), mean ± SD	7.2 ± 3.1	6.8 ± 2.9	0.437
Respiratory infection, n (%)	28 (41.2)	30 (44.1)	0.726
Urinary tract infection, n (%)	15 (22.1)	13 (19.1)	0.672
Skin/soft tissue infection, n (%)	12 (17.6)	14 (20.6)	0.662
Other infections, n (%)	13 (19.1)	11 (16.2)	0.654
Baseline Shannon index, mean ± SD	3.41 ± 0.48	3.38 ± 0.52	0.726
Baseline observed OTUs, mean ± SD	214.5 ± 38.7	218.2 ± 36.4	0.564

Antibiotic Utilization Outcomes: The stewardship intervention produced substantial reductions in antibiotic consumption metrics. Broad-spectrum antibiotic use decreased by 31.4%, antibiotic de-escalation rates more than doubled, and the mean duration of antibiotic therapy was significantly shortened (Table 2).

Table 2: Antibiotic Utilization Metrics by Study Phase

Metric	Pre-Intervention (n = 68)	Post-Intervention (n = 68)	p-value
Total DOT/1,000 PD	842.6 ± 124.3	648.5 ± 108.7	<0.001
Broad-spectrum DOT/1,000 PD	486.3 ± 98.4	333.7 ± 87.6	<0.001
Narrow-spectrum DOT/1,000 PD	356.3 ± 82.1	314.8 ± 74.5	0.003
De-escalation rate, n (%)	14 (20.6)	38 (55.9)	<0.001
Mean antibiotic duration (days)	6.4 ± 2.3	4.7 ± 1.8	<0.001
Patients receiving ≥2 antibiotics, n (%)	41 (60.3)	24 (35.3)	0.003

Microbiome Diversity Outcomes: At discharge, patients in the post-intervention cohort demonstrated significantly higher alpha diversity indices and lower Firmicutes-to-Bacteroidetes ratios compared to the pre-intervention cohort. The magnitude of diversity decline from admission to discharge was significantly smaller in stewardship-managed patients across all measured indices (Table 3). Spearman correlation analysis revealed a significant negative correlation between broad-spectrum DOT and discharge Shannon index ($r = -0.52$, $p < 0.001$).

Table 3: Microbiome Diversity Indices at Admission and Discharge

Index	Timepoint	Pre-Intervention (n = 68)	Post-Intervention (n = 68)	p-value (between groups)
Shannon Index	Admission	3.41 ± 0.48	3.38 ± 0.52	0.726
	Discharge	2.38 ± 0.63	2.94 ± 0.51	<0.001
	Change (Δ)	−1.03 ± 0.44	−0.44 ± 0.37	<0.001
Observed OTUs	Admission	214.5 ± 38.7	218.2 ± 36.4	0.564
	Discharge	152.8 ± 41.2	187.3 ± 34.6	<0.001
	Change (Δ)	−61.7 ± 28.3	−30.9 ± 22.1	<0.001
Chao1 Richness	Admission	268.4 ± 44.2	271.6 ± 42.8	0.669
	Discharge	194.2 ± 48.5	238.7 ± 39.3	<0.001
	Change (Δ)	−74.2 ± 32.6	−32.9 ± 24.8	<0.001
F/B Ratio	Admission	2.14 ± 0.68	2.08 ± 0.71	0.612
	Discharge	3.87 ± 1.24	2.76 ± 0.89	<0.001

Discussion

The findings of this study demonstrate that a structured hospital-based antibiotic stewardship intervention significantly preserves gut microbiome diversity in pediatric inpatients while achieving substantial reductions in antibiotic consumption. These results provide novel evidence linking stewardship-driven prescribing optimization to measurable microbiome outcomes in a vulnerable pediatric population.

The 31.4% reduction in broad-spectrum antibiotic utilization observed following stewardship implementation is consistent with meta-analytic data from pediatric stewardship programs, which have reported reductions ranging from 19% to 38% in unnecessary broad-spectrum prescribing [13]. The significant increase in de-escalation rates from 20.6% to 55.9% reflects the effectiveness of the prospective audit and feedback component, which has been recognized as one of the most impactful stewardship strategies in hospital settings [14].

The preservation of microbiome diversity in the post-intervention cohort represents a finding of considerable clinical significance. The Shannon diversity index at discharge was 23.5% higher in stewardship-managed patients compared to the pre-intervention group, and the magnitude of diversity decline was reduced by more than half. These observations align with emerging evidence that narrower-spectrum antibiotics and shorter treatment durations produce less collateral microbiome damage than broad-spectrum prolonged regimens [15]. Previous research has demonstrated that each additional day of broad-spectrum antibiotic exposure is associated with a measurable decline in microbial richness, a relationship that was corroborated by the significant negative correlation between broad-spectrum DOT and discharge Shannon index observed in our study [16].

The clinical implications of preserved microbiome diversity extend beyond the immediate hospitalization period. Disruption of the gut microbiome in hospitalized children has been associated with increased susceptibility to healthcare-associated infections, particularly Clostridioides difficile infection, which disproportionately affects patients with reduced microbial diversity [17]. Moreover, emerging evidence suggests that antibiotic-induced dysbiosis during childhood may contribute to the development of chronic conditions including obesity, allergic diseases, and autoimmune disorders through altered immune programming and metabolic pathway disruption [18]. By demonstrating that stewardship interventions can attenuate microbiome disruption, the present study suggests an additional mechanism through which these programs may improve patient outcomes beyond traditional metrics.

The elevated Firmicutes-to-Bacteroidetes ratio observed at discharge in the pre-intervention cohort compared to stewardship-managed patients is notable, as shifts in this ratio have been implicated in metabolic dysregulation and inflammatory conditions [19]. The relative preservation of a balanced F/B ratio in the post-intervention group suggests that targeted antibiotic use may help maintain homeostatic microbial community structure during hospitalization.

Several limitations warrant acknowledgment. The quasi-experimental design, while pragmatically appropriate, does not provide the same level of causal inference as a randomized controlled trial. Temporal confounders, including seasonal variation in infection epidemiology and potential secular trends in prescribing behavior, may have influenced results. The single-center design limits generalizability to institutions with different patient populations, resistance patterns, and baseline prescribing practices. Additionally, 16S rRNA gene sequencing provides taxonomic resolution primarily to the genus level and does not capture functional metagenomic information that would enhance understanding of how stewardship-mediated microbiome preservation translates to biological activity. Future multicenter randomized studies incorporating shotgun metagenomic sequencing and longitudinal post-discharge follow-up would strengthen the evidence base considerably [20].

Conclusion

This study demonstrates that a structured hospital-based antibiotic stewardship intervention significantly reduces unnecessary broad-spectrum antibiotic exposure in pediatric inpatients while meaningfully preserving gut microbiome diversity during hospitalization. The stewardship-managed cohort exhibited substantially higher alpha diversity indices at discharge, reduced magnitude of diversity declines during admission, and more balanced microbial community composition compared to the standard care cohort. These findings provide compelling evidence that antibiotic stewardship programs confer microbiome-protective benefits in hospitalized children, supporting their routine integration into pediatric hospital practice. The preservation of gut microbial diversity during critical developmental windows may carry implications extending well beyond the immediate hospitalization, potentially reducing susceptibility to secondary infections and mitigating long-term dysbiosis-associated health consequences. Future research should prioritize multicenter validation, functional metagenomic characterization, and longitudinal assessment of clinical outcomes associated with stewardship-mediated microbiome preservation.

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