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Original Article

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The association between vitamin D levels and the incidence of urinary tract infections in children under seven years old in Larkana

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Abstract

Background: Children are particularly vulnerable to urinary tract infections (UTIs), which are bacterial diseases. *Escherichia coli* (E. coli) is the most often implicated etiological agent, accounting for 80% to 90% of UTIs in children. **Objectives:** The main goal was to find out how often vitamin D insufficiency is in kids who have diagnosed UTI. **Methods:** In the Larkana district of Pakistan, 144 children less than 7 years old who were diagnosed with acute bacterial meningitis were included in this prospective research. In accordance with established laboratory procedures, we obtained blood and urine samples to analyze vitamin D levels, isolate bacteria, and test for antibiotic susceptibility. Inclusion required residence in Larkana and no antibiotic use within 3 days; children ≥7 years, outside Larkana, or recently on antibiotics were excluded. **Results:** The mean vitamin D level was significantly lower in UTI-positive children (18.08 mg/dl) compared with controls (35.48 mg/dl, $p = 0.05$). Vitamin D deficiency (< 12 mg/dl) was observed in 84% of UTI patients versus 23% of controls. Chi-square analysis confirmed a significant association between vitamin D deficiency and culture-positive UTIs. **Conclusion:** This study concluded that vitamin D deficiency was significantly associated with culture-positive UTI cases, suggesting a potential role of hypovitaminosis D as a risk factor for pediatric UTIs.

Keywords:
Vitamin D, UTI, Children, *E. coli*, ABM

1. Introduction

Studies show that UTIs are rather frequent in youngsters and rank high among bacterial infections.[1] *Escherichia coli* (*E. coli*) is the most often implicated etiological agent in children's UTIs, accounting for 80 to 90% of cases.[2] Some congenital genitourinary tract abnormalities may increase the risk of UTI progression to more severe consequences such as sepsis and renal scarring, which can cause irreversible kidney damage.[3]

UTIs are caused by a combination of bacteria and host factors.[4] In recent years, vitamin D's role in host innate immunity has come into sharp focus.[5] Vitamin D, long regarded as an antibacterial agent, also protects the urothelium by promoting the local synthesis of AMP.[6] Cathelicidin prevents infections in the urinary tract by stimulating the synthesis of cytokines, an effect that is amplified by 1,25-dihydroxy vitamin D.[7] Additionally, 1,25-dihydroxy vitamin D may affect another naturally occurring AMP, increasing β-defensin levels, which can be seen in UTIs.[8] Vitamin D also helps keep the urothelium in good repair because the vitamin D receptor (VDR) regulates the activity of epithelial cell junctions.[9] The epithelial cells directly interact with uropathogenic *E. coli*, which disrupts the epithelial barrier by downregulating claudin and occludin.[10]

Taking vitamin D supplements before and during an episode of UTI helps improve the patient's condition.[11]

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There are vitamin D supplements on the market, and they do not cost a fortune. Taking vitamin D pills in addition to medicines can help control UTIs more effectively. By decreasing the overuse of antibiotics, this technique has the potential to lower the cost of management. The initial line of defense against the bacteria that cause UTIs is epithelium-derived cathelicidin. When the urinary tract becomes infected, the epithelium secretes cathelicidin.[12] Vitamin D is essential for the cathelicidin pathway to work. When bacteria attach to the mucosa lining the urinary tract, they release cathelicidin.[13] For macrophage cathelicidin synthesis to be at its best, serum vitamin D levels must be within normal ranges.[14]

Vitamin D levels and UTIs in children have not been the subject of any local investigations as of yet. Because it allows comparison with results published in the worldwide literature and provides region-specific data on the frequency of vitamin D insufficiency among pediatric patients with UTI, the current research is particularly relevant. The main goal was to find out how often vitamin D insufficiency occurs in children who have a diagnosed UTI.

2. Materials and Methods

2.1. Study Design and Setting

This prospective, laboratory-based study was conducted to investigate vitamin D levels and identify bacterial uropathogens in children clinically diagnosed with acute bacterial meningitis (ABM) and suspected urinary tract infections (UTIs). The study population consisted of children under seven years of age residing in the Larkana district of Pakistan.

Blood and urine samples were collected from the Children's Medical Center (CMC) Children's Hospital, Larkana, and several private clinics. Vitamin D estimation, primary urine culture, and preservation of pure bacterial isolates were performed at Bhittai Pathology Laboratory, Kambar. Biochemical identification, recovery of isolates, and antimicrobial susceptibility testing (AST) were conducted at the Institute of Microbiology, Shah Abdul Latif University (SALU), Khairpur. Ethical approval was granted by the Research and Ethics Review Board (RSB), Shah Abdul Latif University Khairpur Mir's.

2.2. Participant Selection

A total of 144 children diagnosed with ABM were enrolled after obtaining written informed consent from parents or guardians. Of these, 94 were male and 50 were female. Age-wise distribution included:

- 60 children: <2.5 years
- 52 children: 2.5–5 years
- 32 children: 5–7 years

All enrolled children were diagnosed and treated at private hospitals in Larkana or the SMBB University Hospital.

2.3. Inclusion Criteria

- Children under 7 years of age.
- Residents of Larkana district.
- No antibiotic use within the preceding 3 days.

2.4. Exclusion Criteria

- Children ≥ 7 years of age.
- Non-residents of Larkana.
- Antibiotic use within the last 3 days.
- Refusal to participate.

2.5. Sample Size Calculation

Sample size was calculated based on the expected prevalence of *Proteus* spp. in UTIs (11.33%), reported in previous studies. The sample size formula used was:

$$n = \frac{Z_{1-\alpha/2}^2 \cdot p(1-p)}{d^2}$$

Where:

$$p = 0.1133 \quad \text{(prevalence)},$$
$$Z_{1-\alpha/2} = 1.96 \quad \text{(95\% confidence interval)},$$
$$d = 0.05 \quad \text{(margin of error)}.$$

The estimated sample size was approximately 144, which was increased to 148 (blood + urine samples) to compensate for potential attrition.

2.6. Sample Collection and Laboratory Processing

2.6.1. Vitamin D Estimation

Blood samples were analyzed at Bhittai Pathology Laboratory using standard clinical chemistry methods for serum vitamin D quantification. Results were documented for correlation with UTI status.

2.6.2. Urine Culture

All 144 urine samples were cultured on Cystine–Lactose–Electrolyte–Deficient (CLED) agar using a calibrated-loop technique. Plates were incubated aerobically at 37°C

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for 18–24 hours. Colony counts of $\geq 10^5$ CFU/mL were considered significant.

Culture results were categorized as:

- No growth
- Positive growth
- Mixed growth
- Yeast growth

2.6.3. Gram Staining

All culture-positive isolates were subjected to Gram staining. Microscopy revealed:

- 84% Gram-negative bacteria
- 16% Gram-positive bacteria

2.6.4. Biochemical Identification

Standard biochemical tests routinely used in Pakistani microbiology laboratories were performed.

For Gram-negative bacilli:

- Indole test
- Urease test
- Citrate utilization
- Motility test
- Oxidase test
- Triple Sugar Iron (TSI) reactions

For Gram-positive cocci:

- Catalase test
- Coagulase test (where applicable)

Based on biochemical profiling, the isolates were identified as:

- *Escherichia coli* — 52%
- *Proteus* spp. — 16%
- *Klebsiella* spp. — 11%
- *Enterococci* spp. — 11%
- *Streptococci* spp. — 5%

2.6.5. Antimicrobial Susceptibility Testing (AST)

AST was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following CLSI guidelines.

Gram-negative antibiotic panel:

The antibiotics tested included Cefoperazone/Sulbactam, Nitrofurantoin, Meropenem, Amoxicillin–Clavulanic Acid, Trimethoprim–Sulfamethoxazole, Amikacin, and Tazobactam.

E. coli isolates were multidrug resistant (MDR), remaining susceptible only to Amikacin and Piperacillin/Tazobactam.

Gram-positive antibiotic panel:

- Carbapenem
- Amoxicillin–Clavulanic Acid
- Vancomycin
- Erythromycin
- Ampicillin
- Ciprofloxacin
- Clindamycin

Several *Streptococcus* spp. showed resistance to carbapenem, ciprofloxacin, and clindamycin.

2.7. Quality Control

Quality control procedures were maintained using standard ATCC strains:

- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 25923

All media, biochemical reagents, and antibiotic disks were evaluated for performance prior to use.

3. Results

3.1. Simple Frequency Distribution

Our analysis of the ages and genders of the 144 participants revealed that there were 94 males and 50 females. The age distribution of the participants showed that 60 children were in the 0–2.5 years age group, 52 were in the 2.5–5 years age group, and the remaining 32 were in the 5–7 years age group.

This comprehensive study emphasizes the higher prevalence of UTIs and antibiotic sensitivity patterns in children under seven years in the Larkana district. Further investigation into these associations is warranted.

3.2. Pattern of Growth Distribution in Urine Samples

Figure 1 shows the results of inoculating 144 urine samples onto Cystine-Lactose-Electrolyte-Deficient (CLED) agar to identify bacteria responsible for UTIs. Out of 117 samples, no bacterial growth was observed (negative results). Nineteen samples exhibited positive bacterial growth, while seven samples showed mixed growth. Additionally, as shown in Figure 2, two samples demonstrated yeast growth. These findings highlight the microbial composition of the study's urine samples.

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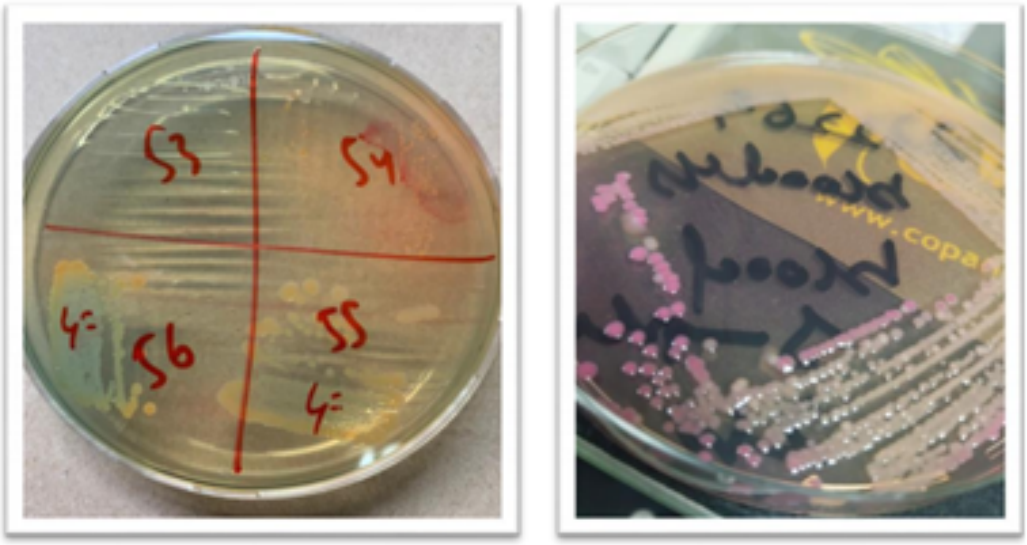

Figure 1: Isolates on culture media after inoculation and incubation

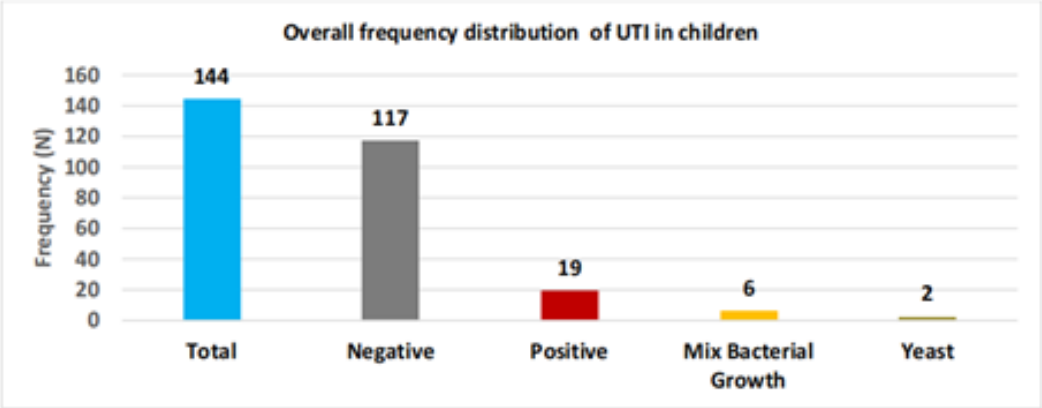

Figure 2: Overall frequency distribution including positive, negative and mixed growth

3.3. Gram Staining Results

Seventeen urine samples showed evidence of bacterial growth. Gram staining revealed that 16% of the isolates were Gram-positive bacteria, whereas 84% were Gram-negative bacteria. The Gram-staining microscopy images are presented in Figure 3.

3.4. Bacterial Biochemical Detection

Catalase and coagulase tests were used to confirm the presence of *Streptococci* spp. in Gram-positive isolates. Figures 4 shows the distribution of bacterial species: *Escherichia coli* 52%, *Proteus* 16%, *Klebsiella* 11%, *Enterococci* 11%, and *Streptococci* 5%.

3.5. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed on Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus*) and Gram-positive *Streptococcus* spp. using standard antibiotic panels.

Gram-negative panel: Cefoperazone/Sulbactam, Nitrofurantoin, Meropenem, Amoxicillin-Clavulanic Acid, Trimethoprim/Sulfamethoxazole, Amikacin, Tazobactam. Two *E. coli* isolates were multidrug-resistant, remaining susceptible only to Amikacin and Piperacillin/Tazobactam.

Gram-positive panel: Carbapenem, Amoxicillin-Clavulanic Acid, Vancomycin, Erythromycin, Ampicillin, Ciprofloxacin, Clindamycin. Some *Streptococcus* isolates exhibited resistance to Carbapenem, Ciprofloxacin, and Clindamycin, while others were susceptible.

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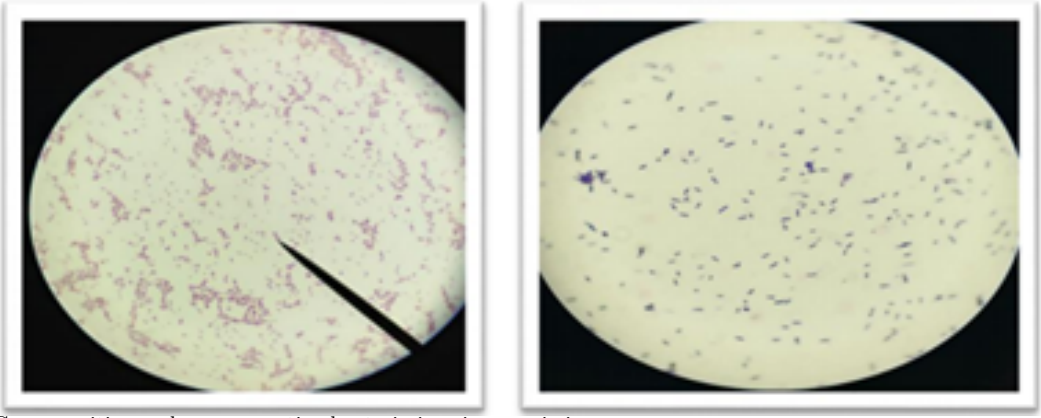

Figure 3: Gram-positive and gram-negative bacteria in microscopic images

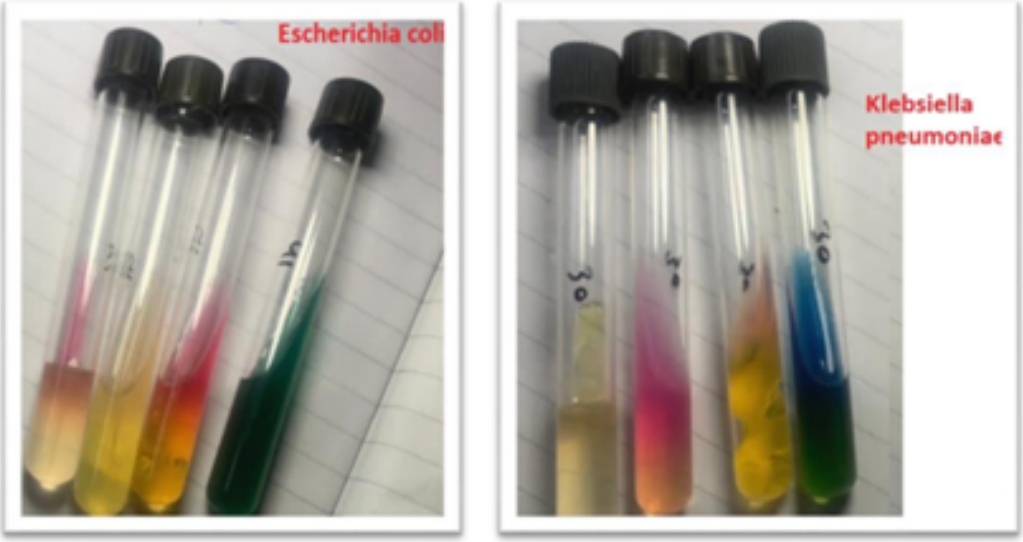

Figure 4: Biochemical identification tests

Representative AST images are shown in Figures 6 and 5.

3.6. Prevalence of Urinary Tract Infection

The 13% of the Larkana population had a urinary tract infection. Among them, 84% exhibited vitamin D insufficiency, whereas 16% were UTI-negative but had other contributing factors such as limited sun exposure, darker skin tone, malnutrition, kidney or liver disease, certain medications, hereditary predisposition, or unexplained rickets in infancy.

3.7. Association between Vitamin D and Urinary Tract Infections

Vitamin D levels were compared between infected and non-infected participants. The UTI-positive (case) group had a mean vitamin D level of 18.08 mg/dL, whereas the UTI-negative (control) group had a mean level of 35.48 mg/dL. Independent t-test analysis showed a significant difference ($p = 0.05$).

Vitamin D status was further classified into normal (25–50 mg/dL) and deficient (<12 mg/dL). In the case group, 11 children had insufficient vitamin D, whereas 101 children in the control group were sufficient. Two patients (16%) in the case group and 31 patients (23%) in the control group were vitamin D deficient. The culture-positive samples had lower vitamin D levels compared to culture-negative samples.

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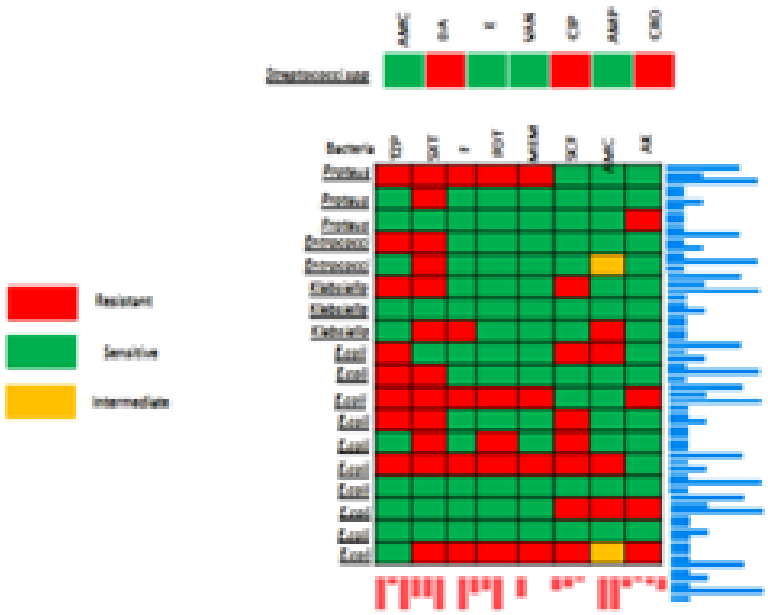

Figure 5: Graph of antimicrobial susceptibility testing pattern of bacterial isolates

4. Discussion

The current research found that younger age may increase the risk of vitamin D insufficiency and deficiency. This observation is consistent with earlier investigations reporting significant age-related differences in vitamin D status, with deficiency increasing as age advances [15,16]. Prior studies primarily focused on school-age children, attributing the higher risk to reduced outdoor activity [17] or to the inclusion of wide age ranges (0–16 years) in participant cohorts [18]. In contrast, our study concentrated on preschool-aged children (25–72 months), stratified into 12-month intervals. Consistent with previous findings, we observed a progressive decline in mean 25(OH)D concentrations and sufficiency rates with increasing age. Spearman correlation further demonstrated an inverse association between vitamin D levels and age, corroborating reports that age-related gastrointestinal changes may influence vitamin D absorption efficiency [19]. Nevertheless, the biological mechanism underlying this association remains incompletely understood [20], warranting further investigation.

A significant relationship between vitamin D status and urinary tract infection was also observed. The mean vitamin D concentration was markedly lower in the UTI group (18.08 mg/dL) compared with controls (35.48 mg/dL), and Chi-square analysis confirmed that vitamin D deficiency was more common among culture-positive cases. These findings parallel those of a previous case-control study [21]. Together, these results support a potential role of vitamin D deficiency as a predisposing factor for UTIs in children.

Sex distribution in our cohort revealed a higher prevalence of UTIs in females (54.9%) compared with males (45.1%), consistent with established anatomical and physiological risk factors. Shorter urethral length in females and phimosis in male infants increase susceptibility to infection, while toilet training in toddlers may further promote bladder stasis and bacterial colonization [22,23]. Shalaby et al. similarly reported female predominance, with 60% of cases occurring in girls [24], corroborating our observations.

Microbiological profiling demonstrated that *Escherichia coli* was the predominant uropathogen (52%), followed by *Proteus* spp. (16%), *Klebsiella* spp. (16%), *Enterococci* spp. (11%), and *Streptococci* spp. (5%). This distribution aligns with previous literature, where *E. coli* consistently emerges as the leading etiologic agent in pediatric UTIs [25–27]. Other studies also identify *Klebsiella*, *Proteus*, *Pseudomonas aeruginosa*, *Enterobacter*, and *Enterococcus* species as common isolates, albeit with variability in their relative frequencies [28,29].

The overall prevalence of pediatric UTIs in our setting was 24.1%, comparable to reports from Uganda (26.8%) [31], Tanzania (20.65%) [32], and Hawassa, Ethiopia (27.5%) [30]. However, our prevalence exceeded that observed in Dar es Salaam, Tanzania (16.7%) [36], Abakaliki, Nigeria (3%) [34], and Bahirdar, Ethiopia (16.7%) [33]. These discrepancies may reflect differences in nutritional status,

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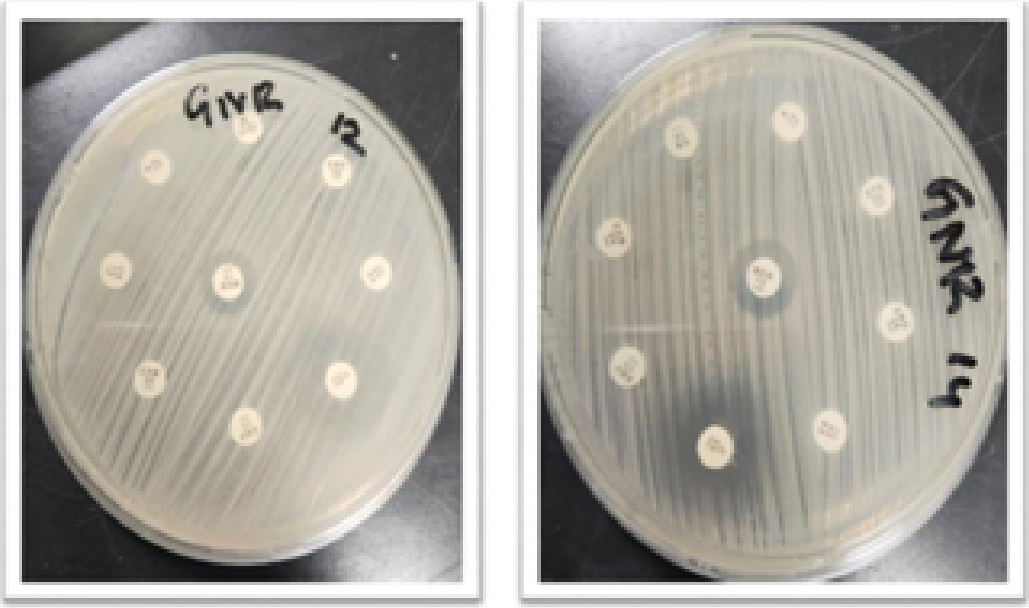

Figure 6: Antimicrobial susceptibility testing pattern of Bacterial Isolates

host immunity, diagnostic criteria, study design, or bacteriuria definitions[37]. In the Larkana region specifically, the prevalence was 13%, of which 84% of children had concomitant vitamin D deficiency, reinforcing the association between poor vitamin D status and increased susceptibility to UTIs.

Antimicrobial susceptibility testing revealed variable resistance patterns among Gram-negative isolates. While some *E. coli* strains remained susceptible to all tested agents, others exhibited multidrug resistance, with amikacin and piperacillin-tazobactam demonstrating the highest efficacy. *Streptococcus* spp., among Gram-positive isolates, showed resistance to clindamycin, ciprofloxacin, and carbapenems, while retaining sensitivity to amoxicillin-clavulanic acid, vancomycin, and erythromycin. These findings are consistent with earlier reports documenting high resistance rates to ampicillin, tetracycline, cefazolin, and trimethoprim-sulfamethoxazole, with better activity of meropenem (92.7%), ciprofloxacin (83.1%), and amoxicillin-clavulanic acid (77.9%) against Gram-negative isolates[?]. Our data similarly demonstrated marked resistance to ampicillin and tetracycline (>80%) in *E. coli* strains, while maintaining substantial susceptibility to meropenem and amoxicillin-clavulanic acid.

5. Conclusions

This study demonstrated that the overall prevalence of urinary tract infection in children under seven years in the Larkana region was 13%, with *Escherichia coli* as the predominant pathogen, followed by *Proteus* and *Klebsiella* species. Antimicrobial susceptibility patterns revealed variable resistance, highlighting the need for careful antibiotic stewardship. Importantly, vitamin D deficiency was significantly associated with culture-positive UTI cases, suggesting a potential role of hypovitaminosis D as a risk factor for pediatric UTIs. These findings emphasize the necessity of integrating vitamin D assessment into clinical management and encourage further multicenter studies to validate the observed association.

6. List of abbreviations

UTI	Urinary tract imfection
AST	Antibiotic susceptibility testing
ABM	acute bacterial meningitis
E. coli	Escherichia coli

7. Acknowledgment

We sincerely thank the participants and their families for their cooperation throughout this study. We are grateful to the staff of the microbiology laboratory in Larkana for their support in sample processing and analysis. We also acknowledge the institutional assistance that facilitated the successful completion of this research.

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8. Authorship

All authors are accountable for this work, meet ICMJE authorship criteria, and have approved the final version for publication.

9. Authors' Contributions:

M.A.M Collected and analyzed the data, Writing – Review & Editing, Formal analysis, Data curation. M.M.A.T Supervision, conceptualized and designed the study, Writing – Original Draft.
All authors reviewed and approved the final manuscript.

10. Conflicts of interest

None

11. Funding

None

12. Consent for publication

Not Applicable

13. AI Use Disclosure

N/A

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