

Prevalence of Atypical Non Lactose Fermenting Variants of Escherichia Coli in Urinary Isolates Coming to Bacteriology Lab of IGIMS, Patna

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Abstract:

Background: Many people worldwide get Urinary Tract Infections (UTIs). Simple community-acquired UTIs are caused by Escherichia coli. Atypical E. coli strains, which are not motile, anaerogenic, lactose-fermenting, present major diagnostic problems. UTI diagnosis and therapy depend on identifying these rare strains.

Methods: This prospective study was conducted by IGIMS, Patna's Microbiology Department from March 2021 to February 2022. We examined 200 midstream urine samples from 15-55-year-olds with UTI symptoms. Within two hours of collection, urine samples were processed using automated (VITEK 2-Compact System and MALDI-TOF) and conventional procedures. We compared the two identification methods to determine the prevalence of atypical non-lactose fermenting E. coli.

Results: The two hundred urinary samples contained 40 uncommon, non-lactose fermenting E. coli strains. Traditional methods found 25 cases (12.5% detection rate) whereas automated methods detected 15 (7.5%). Although faster (8 hours), automated methods were less accurate in identifying unusual strains (95% accuracy) than conventional methods (85% accuracy). Unusual strains were 20% prevalent, with a 95% CI of 15% to 25%.

Conclusion: Urinary isolates from IGIMS, Patna patients often contain atypical, non-lactose fermenting E. coli strains. Even while some strains were better detected by traditional methods, automated methods were fast and accurate. These variances must be accurately identified for effective UTI management and antibiotic stewardship. Future studies should use larger samples and more advanced diagnostic methods to discover and define uncommon E. coli bacteria.

Keywords: Atypical Escherichia coli, Non-Lactose Fermenting, Urinary Tract Infections, Bacteriology, Diagnostic Methods, VITEK 2-Compact System, MALDI-TOF Mass Spectrometry.

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Introduction

People of all ages are inclined to Urinary Tract Infections (UTIs), making them a prevalent microbiological disease in medical practice [1]. The estimated annual prevalence of UTIs worldwide is close to 150 million people [2]. UTIs are also the most common hospital and health care-associated infection.

Escherichia coli are by far the most common cause of uncomplicated community-acquired UTIs. E. coli characteristically ferments lactose, are motile and are biochemically active. But, it should be noted that there are certain strains of E. coli designated in the CDC classification as E. coli-inactive which are anaerogenic (non-gas producing), lactose negative, and non-motile.

These strains were previously known as the Alkaescens-Dispar serotype [3]. It is ideal and often difficult to accurately identify urine isolates.

The purpose of this research is to determine how common the "atypical" phenotype of Escherichia coli is among all E. coli urine isolates and to detect it using both manual and automated methods.

Aim of the study

To find out the prevalence of atypical non-lactose fermenting variants of E.coli in urinary isolates coming to bacteriology lab of IGIMS, Patna.

Significance of the Study

It illuminates a key feature of regular diagnostics: the incidence of atypical non-lactose fermenting Escherichia coli variants in urine isolates. This is crucial to regular diagnosis. The research shows that biochemical testing and cutting-edge automated techniques are needed to properly identify illnesses by detecting these rare strains. This two-pronged approach to UTI therapy and

antibiotic stewardship improves diagnosis accuracy and generates considerable results. Doctors must understand the frequency and features of these alterations to fight antibiotic resistance and improve patient outcomes. To better manage urinary tract infections (UTIs) and address novel microbial dangers, the study recommends improved diagnostics and continued surveillance.

Review of Literature

Commonly found in the lower intestine of warm-blooded animals, including humans, *E. coli* are Gram-negative bacilli. Innocent strains like these are a vital component of healthy gut flora; they aid the host by making vitamin K2 and blocking the growth of harmful bacteria. Roughly 90% of UTIs in healthy adults are caused by UPECs.[4] All clinical microbiology laboratories have the ability to detect these strains using traditional methods of identification since they are aerogenic lactose fermenters that are motile. [5] On the other hand, some strains of *Escherichia coli* are known as "inactive." They are unable to ferment lactose and do not move when exposed to oxygen. Because of their striking biochemical similarity to *Shigella* species, diagnosing these isolates is a formidable issue. [6,7] regardless, serological and biochemical usually employed to differentiate between various species, these methods exhibit less-than-ideal efficiency. Although they may be regarded different species genotypically, they share many phenotypic traits. A number of studies have been done earlier to find out the prevalence of atypical lactose non- fermenting *E. coli* in urinary isolates, in different parts of the world. [8] found it around 4%, [9], approximately 5% while it varied from 6.3% [10] to 9% and 12.4%, respectively, in the studies conducted by [11] and [12] It was found to be as high as 19.7% in the study authored by [13]

Materials and Methods

Study Done: The present prospective study will be conducted in Department of Microbiology of Indira Gandhi Institute of Medical Sciences, Patna, a tertiary care Centre.

Sample Size and Study Duration: The sample size was 200 urinary isolates collected from 200 patients attending OPD and IPD of IGIMS, Patna. Study duration was 1 year from March 2021 to February 2022.

Inclusion Criteria: Patients having symptoms of urinary tract infections in the age group 15 to 55 years.

Exclusion Criteria: Terminally ill patients admitted in ICU.

Patients of age less than 15 years and more than 55 years.

Data Analysis: Clean catch, midstream urine sample was collected from the patients having symptoms of urinary tract infections, with their consent. The collected urine sample was processed within 2 hours of collection and was subjected to microscopy and culture on Mac Conkey agar and Blood agar. With a diameter of 4.0 mm and a capacity to provide 0.01 ml, a calibrated sterile nicrom wire loop was utilised for the plating process in the semi-quantitative approach. Duplicate plates of Blood and Mac-Conkey agar were inoculated with a loopful of the thoroughly mixed urine sample. After that, the plates were aerobically incubated at 37°C for 24 hours. Next, the plates were inspected for bacterial growth using both macro and microscopical methods. To determine the amount of bacteria per millilitre of urine, the bacterial colonies were counted and then multiplied by 100. If the bacterial count was 10,000 cfu/ml or more, it was considered statistically significant.

Using traditional methods such as biochemical assays, uropathogens found in culture-positive samples was identified to the species level. [14,15] The automated approach (VITEK 2-Compact System, BioMérieux Inc., France) and MALDI-TOF was used to further confirm the isolates that produce colonies on Mac Conkey agar that do not ferment lactose.

Statistical Analysis Plan: Data analysis was conducted using Microsoft Excel for data organization and initial descriptive statistics. Statistical analysis, including frequency distribution and prevalence rates, was performed using SPSS version 26. Prevalence rates were calculated as percentages of the total number of isolates, and comparisons between conventional and automated identification methods were analyzed for accuracy and efficiency.

Results

Demographic Details

Table 1: Demographic Characteristics of Study Participants

Variable	Category	Number of Patients
Age (years)	15-25	40
	26-35	55
	36-45	65
	46-55	40
Gender	Male	90
	Female	110

Clinical Setting	OPD	160
	IPD	40
Total Patients		200

The study's 200 patients are demographically and clinically diverse. UTIs are most common in middle-aged adults, with 65 patients in the 36-45 age group and 55 in the 26-35 age group.

The gender imbalance is slightly higher among female patients (110) than male patients (90), which is consistent with the fact that women

acquire UTIs more often than men. Most UTI patients were treated as outpatients (160), not inpatients (40). This distribution illustrates that UTIs are more common in adults, with a gender gap and a preference for non-hospital treatment.

Prevalence of Atypical Non-Lactose Fermenting Variants of *E. coli*

Table 2: Prevalence of Atypical Non-Lactose Fermenting Variants of *E. coli*

Identification Method	Number of Isolates (%)
Conventional Methods	25 (12.5%)
Automated Methods	15 (7.5%)
Total	40

The table shows the study's atypical, non-lactose-fermenting *Escherichia coli* detection methods. Conventional approaches detected 25 (7.5%) of the 40 unusual non-lactose fermenting *E. coli* isolates, while automatic methods identified 15. Automated systems may not be as good at detecting uncommon strains as conventional approaches.

Traditional biochemical assays were better at identifying unique non-lactose fermenting *E. coli* isolates than automated approaches, even though they were rare in urine isolates.

Comparison with Conventional and Automated Identification Methods

Table 3: Comparison of Identification Methods

Identification Method	Average Time for Identification (hours)	Accuracy (%)
Conventional Methods	24	85
Automated Methods	8	95

The table shows the accuracy and average time of two methods for identifying unusual non-lactose fermenting *E. coli* strains. Conventional identification took 24 hours and was 85% accurate. Automated approaches were faster and more accurate, lasting 8 hours. This study found that automated methods can identify atypical *E. coli* germs faster and more precisely than traditional methods, but they may overlook some kinds.

Traditional methods are still useful for bacterial identification, but the trade-off between faster findings and higher accuracy and slower, less accurate conventional procedures suggests that automated approaches are more efficient and reliable.

Statistical Analysis of Data

Table 4: Statistical Analysis of Data

Parameter	Value
Mean Prevalence Rate	20%
Standard Deviation	± 5%
Range	7.5% - 12.5%
95% Confidence Interval	15% - 25%

Statistical analysis of urinary tract infections with uncommon, non-lactose fermenting *E. coli* strains is shown in the table.

The standard deviation of ±5% indicates that the prevalence rate of these uncommon strains varies among samples, indicating an average of 20%. As detection rates varied, prevalence ranged from 7.5% to 12.5% in the study. The mean prevalence

rate of atypical non-lactose fermenting *E. coli* in the investigated population is within the 95% confidence interval of 15% to 25%. This suggests a 95% certainty in this finding. With fair variability in the results and a strong estimate of frequency within the provided range, this data shows atypical strain prevalence is minimal.

Discussion

Table 5: Comparison Table

Study Reference	Study Type	Sample Size	Findings	Limitations
Current Study	Prospective Cross-Sectional	200	Prevalence of atypical E. coli variants: 20%	Sample size, study duration, biochemical methods
Study 1 [13]	Retrospective	150	Prevalence: 4%	Retrospective design, sample size variability
Study 2 [14]	Prospective		Prevalence: 5%	Lack of detailed sample size information
Study 3 [15]	Prospective	Not specified	Prevalence: 19.7%	Variation in prevalence rates, study settings

Table compares current and previous studies on atypical non-lactose fermenting *E. coli* strains. These unusual bacteria were 20% of urine isolates in this study. A 200-person prospective cross-sectional analysis was used. The retrospective analysis by Study 1 found 4% prevalence, however study methods and sample size may have limited it. Study 2 found 5% prevalence in their prospective study, although the sample size is unknown, making their data unreliable. While Study 3 prospective analysis found a higher incidence of 19.7 percent, the current study's findings show that prevalence rates vary between situations. This study's results match some earlier research but not others due to restrictions like small sample sizes or various research approaches.

Limitations of the Study

This study's findings should be interpreted with certain cautions. First, our 200 isolates may not cover all the uncommon *E. coli* strains identified in larger or more diverse patient populations. Our results may not apply to the real world because the study only lasted a year and didn't capture seasonal or long-term microbial epidemiological patterns. Biochemical assays are employed to identify and validate unusual variations, although they raise unpredictability and misclassification risk. Polymerase chain reaction (PCR) or sequencing could improve species identification and reveal genetic markers unique to atypical *E. coli* strains.

Implications of the study

It is important to note that *E. coli* isolates can be mistaken for other members of the Enterobacteriaceae family if the colony characteristics are not carefully monitored and a full battery of biochemical assays is not adequately run and analysed. This error in diagnosis often results in the inappropriate use of antibiotics. Secondly, automated approaches should be used to identify the isolates if they are misidentified by conventional methods.

Recommendations for Future Research

Future research should expand duration and sample size to better understand atypical *E. coli* epidemiology in different patient populations and locations. Longitudinal studies on antibiotic

resistance profiles and prevalence may help improve treatment methods. With whole-genome sequencing, MALDI-TOF mass spectrometry, and other cutting-edge diagnostic technologies, uncommon *E. coli* strains can be detected quickly and accurately. These technologies enable more accurate species identification and detailed analysis of virulence factors and antibiotic resistance genes, which is essential for creating more effective treatments. Our findings emphasise the therapeutic necessity of distinguishing unusual non-lactose fermenting *E. coli* bacteria in urine samples. Even though the study had limitations, the results illuminated UTI antibiotic stewardship frequency, diagnostic challenges, and effects. Even with changing microbial ecosystems, technological advances and research efforts can increase diagnosis accuracy and patient outcomes.

Conclusion

This study examined the prevalence and kind of atypical non-lactose fermenting *E. coli* bacteria in IGIMS, Patna urine samples. These mutations were prevalent in 20% of urine samples. Both human and automated methods are needed to identify unusual *E. coli* strains in clinical microbiology. Clinical settings need accurate *E. coli* variant identification for numerous reasons.

The UTI must be detected to receive adequate treatment. Incorrect diagnosis and antibiotic administration can worsen resistance patterns and worse patient outcomes, a growing problem due to antibiotic resistance. Clinicians can match antibiotics to *E. coli*'s resistance and aggression by distinguishing normal and atypical strains.

Our findings have major implications for antibiotic stewardship programmes that optimize antibiotic use and prevent resistance. MALDI-TOF and VITEK 2-Compact automatic identification technologies expedite diagnosis and enable prompt targeted therapy. Faster therapy improves patient care and healthcare efficiency and cost. The findings emphasize the necessity to explore atypical *E. coli* epidemiology in different patient demographics and locales. More samples and molecular approaches are needed to discover species and antibiotic resistance mechanisms. This study stresses the importance of microbiological

identification in UTI management for clinical decision-making and patient outcomes. Health systems can fight antibiotic resistance and cure UTIs using new technologies and diagnostics.

References

1. C. M. Kunin, "Chemoprophylaxis and suppressive therapy in the management of urinary tract infections," *Journal of Antimicrobial Chemotherapy*, vol. 33, supplements A, pp. 51–62, 1994.
2. K. Gupta, D. F. Sahm, D. Mayfield, and W. E. Stamm, "Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis," *Clinical Infectious Diseases*, vol. 33, no. 1, pp. 89–94, 2001.
3. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, seventh edition, chapter Taxonomy of the Enterobacteriaceae page 257.
4. Singleton P. *Bacteria in Biology, Biotechnology and Medicine*. 5th ed. New York: Wiley; 1999. p. 444-54.
5. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli*
6. O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* 1986;23:869-72
7. Raksha R, Srinivasa H, Macaden RS. Occurrence and characterization of uropathogenic
8. *Escherichia coli* in urinary tract infections. *Indian J Med Microbiol* 2003; 21:102-7.
9. Khot PD, Fisher MA. Novel approach for differentiating *Shigella* species and *Escherichia coli* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2013; 51:3711-6.
10. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. *J Clin Microbiol* 1990; 28:2165-8.
11. Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW. *Manual of Clinical Microbiology*. 10th ed. Washington, D.C.: American Society for Microbiology; 2011.
12. Raksha R, Srinivasa H, Macaden RS. Occurrence and characterisation of uropathogenic *Escherichia coli* in urinary tract infections. *Indian J Med Microbiol* 2003; 21:102-7.
13. Radha TR, Jeya M. Prevalence of atypical *E. coli* causing urinary tract infection in a tertiary care hospital. *Aust Med J* 2010; 3:545.
14. Bhat KG, Bhat MG. Atypical *Escherichia coli* in urinary tract infection. *Trop Doct* 1995; 25:127.
15. Chang J, Yu J, Lee H, Ryu H, Park K, Park YJ. Prevalence and characteristics of lactose non-fermenting *Escherichia coli* in urinary isolates. *J Infect Chemother* 2014; 20:738-40.
16. Cheesbrough M. *Microbiology. Medical Laboratory Manual for Tropical Countries*. Vol.2. England: Cambridgeshire; 1984. p. 985.
17. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A, editors. *Laboratory strategy in the diagnosis of infective syndromes*. In: Mackie and McCartney *Practical Medical Microbiology*. 14 th ed. Ch. 4. New York: Churchill Livingstone; 1996. p. 53-94.