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Original Article

Microbiology Section

Optimising Urinary Pathogen Detection: A Cross-sectional Comparative Study of CLED Agar, HiCrome UTI Agar and MacConkey Agar

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ABSTRACT

Introduction: Urinary Tract Infections (UTIs) are commonly caused by both Gram-negative and Gram-positive bacteria. In clinical microbiology, urine culture plays a crucial role in diagnosis, with traditional media like blood agar and MacConkey agar being widely used. However, Cystine Lactose Electrolyte Deficient (CLED) agar offers a more cost-effective alternative by inhibiting the swarming of *Proteus* species while supporting a wide range of uropathogens. Chromogenic media, such as HiCrome UTI agar, enable rapid identification but are limited by their high cost.

Aim: To compare the diagnostic efficiency of CLED agar, HiCrome UTI agar, and MacConkey agar for the isolation and identification of common urinary tract pathogens.

Materials and Methods: This cross-sectional comparative study was conducted in the microbiology department of Shri M.P. Shah Govt. Medical College, Jamnagar, Gujarat, India from October 2019 to December 2020. A total of 400 urine samples from patients with suspected UTIs, collected from both the outpatient and inpatient departments of a tertiary care hospital, were included for bacterial examination. Patients were instructed to collect clean-catch midstream or catheter-catch urine in sterile containers following aseptic protocols. Demographic data such as age, sex, and clinical symptoms were recorded. Statistical analysis was performed using the Chi-square test, with a p-value of <0.05 considered statistically significant.

Results: Out of the 400 urine samples processed, 189 (47.3%) showed significant growth, with 158 (83.6%) exhibiting pure bacterial growth and 31 (16.4%) showing mixed bacterial growth. A total of 220 bacterial isolates were identified, predominantly Gram-negative bacilli (85%), with *Escherichia coli* (44.1%) and *Klebsiella* spp. (30.5%) being the most common pathogens. Among Gram-positive cocci, *Staphylococcus aureus* (9.1%) and *Enterococcus* spp. (5.9%) were prevalent. CLED agar, MacConkey agar, and HiCrome UTI agar all isolated Gram-negative bacteria, but only CLED and HiCrome UTI agar supported the growth of Gram-positive cocci. Mixed bacterial growth was observed in 31 samples, with *E. coli* and *Klebsiella* spp. being the most frequent combination. A cost comparison revealed CLED agar as the most economical and effective choice for routine use in resource-limited settings.

Conclusion: This study emphasises the need for strategic selection of culture media to enhance the diagnostic efficiency of UTIs, particularly in resource-limited settings. The comparative analysis highlights CLED agar as a cost-effective and reliable option, balancing diagnostic accuracy with affordability. While HiCrome UTI agar offers the advantage of rapid presumptive identification, its higher cost may limit its routine use. The findings provide valuable insights for clinical microbiology laboratories aiming to optimise resource allocation without compromising diagnostic quality.

Keywords: Antibacterial agents, Bacteriuria, Culture media, Drug resistance, Urinary tract infections

INTRODUCTION

The UTIs are among the most common infections encountered globally, both in healthcare settings and in the community. As a result, urine is one of the most frequently cultured clinical specimens. UTIs are primarily caused by Gram-negative bacteria such as *Escherichia coli*, *Proteus* spp., *Pseudomonas aeruginosa, Acinetobacter* spp., *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. Among Gram-positive bacteria, *Staphylococcus saprophyticus, Enterococcus* spp., and coagulase-negative *Staphylococcus* are frequently involved [1,2]. Over 95% of UTIs are due to a single pathogenic species [3]. These organisms possess virulence factors such as adherence, colonisation, biofilm formation, urease production, and antimicrobial resistance. UTIs often result from the ascending spread of bacteria from the perineal area to the urethra, bladder, and kidneys. Females are more prone to UTIs due to anatomical factors. If left untreated, UTIs can lead to serious complications, such as renal scarring or failure [4].

Urine culture plays a key role in diagnosing UTIs, particularly in developing countries like India. Traditionally, a combination of blood agar and MacConkey agar has been used [5]. However, CLED agar is now widely adopted due to its cost-effectiveness, ability to inhibit *Proteus* swarming, and its support for a broad range of organisms. CLED agar also allows differentiation of lactose fermenters from non fermenters, especially when Andrade's indicator is added [6,7]. Yet, CLED agar lacks genus-specific indicators and may not reliably detect mixed infections, necessitating further subcultures and increasing turnaround time. To address these limitations, chromogenic media such as HiCrome UTI agar have been developed [5,8]. These media contain substrates that react with species-specific enzymes, producing distinct colony colors for presumptive identification. For example, *E. coli* forms pink colonies due to β-D-galactosidase activity, while *Enterococcus* spp. appear blue due to β-glucosidase [9,10]. Chromogenic media offer the added advantage of detecting tryptophan deaminase activity, aiding in the identification of organisms such as *Proteus*, Morganella, and *Providencia* species. Furthermore, simple biochemical tests—such as catalase, oxidase, and indole—can be conducted directly from the primary culture plate, allowing for prompt initiation of antimicrobial susceptibility testing without the need for subculturing. However,

the relatively high cost associated with these media presents a significant barrier to their widespread adoption, particularly in low-resource settings. Therefore, there is a continued need to refine urine culture protocols to balance diagnostic accuracy, operational efficiency, and economic feasibility.

This study seeks to critically evaluate and compare the diagnostic efficacy of CLED agar, HiCrome UTI agar, and MacConkey agar for the isolation and identification of common uropathogens. The rationale for this investigation stems from the need for more efficient, cost-effective, and reliable methods in routine urine culture, particularly in resource-constrained clinical settings. While MacConkey agar has traditionally been the standard medium, the introduction of CLED and HiCrome UTI agars offers potential advantages in enhancing both pathogen isolation and differentiation.

The novelty of this study lies in its comparative assessment of the three culture media, with particular emphasis on the capacity of HiCrome UTI agar to allow rapid presumptive identification of uropathogens through distinct colony colour differentiation. The study was undertaken with the hypothesis that HiCrome UTI agar would offer greater ease of identification and better overall isolation performance compared with the other two media.

The present study aimed to compare the diagnostic efficiency of three commonly used culture media—CLED agar, HiCrome UTI agar, and MacConkey agar—in the isolation and identification of common urinary tract pathogens. The primary objective of this study was to evaluate the comparative performance of CLED agar, HiCrome UTI agar, and MacConkey agar in supporting the growth and facilitating the identification of urinary tract pathogens in clinical urine specimens. The secondary objectives include analysing the distribution and prevalence of isolated uropathogens, assessing the media's ability to detect mixed infections, and determining their effectiveness in isolating Gram-positive organisms such as *Staphylococcus aureus* and *Enterococcus* spp. Additionally, a cost comparison will be conducted to evaluate the economic feasibility of each medium, particularly in resource-limited diagnostic settings.

MATERIALS AND METHODS

This was a cross-sectional comparative study conducted in the Department of Microbiology at Shri M.P. Shah Government Medical College, Jamnagar, a tertiary care teaching hospital in western India. The study was carried out over a 15-month period, from October 2019 to December 2020. It was a time-bound investigation, and all patients presenting with suspected UTIs during this interval, who fulfilled the inclusion criteria, were enrolled consecutively. The study protocol was approved by the Institutional Ethics Committee of Shri M.P. Shah Government Medical College, Jamnagar, under reference number IEC/CERTI/102/03/2019. Written informed consent was obtained from all participants or their legal guardians in accordance with the ethical standards outlined in the Declaration of Helsinki.

Sample size and sampling method: A total of 400 urine samples were processed during the study period. The sample size was not derived from formal statistical calculation but was determined based on a time-bound sampling frame. A non probability consecutive sampling method was used, including all eligible urine specimens received from clinically suspected UTI patients during the study period.

Inclusion criteria:

- Patients of all ages and both sexes presenting with clinical symptoms suggestive of urinary tract infection, such as dysuria, urinary frequency, urgency, suprapubic pain, or unexplained fever.
- Patients attending both inpatient and outpatient departments.
- Patients willing to provide informed written consent.

Exclusion criteria

- Patients who had received antimicrobial treatment within 48 hours prior to sample collection.
- Repeat or follow-up samples from the same individual.
- Improperly collected, contaminated, or leaking specimens.
- Patients not consenting to participate in the study.

Study Procedure

A total of 400 urine specimens were collected using standard aseptic methods. Clean-catch midstream urine samples were collected from ambulatory patients, while catheter-catch urine was collected from catheterised patients, using sterile, wide-mouthed containers. Patients were instructed on proper collection techniques to avoid contamination. All specimens were transported promptly to the microbiology laboratory and processed immediately upon receipt.

Urine microscopy was performed on centrifuged deposits. Samples demonstrating ≥5 pus cells per High-power Field (HPF) were selected for culture.

Urine culture was performed using a standard calibrated loop delivering 0.001 mL of urine, inoculated onto the following media:

- Blood agar
- MacConkey agar
- CLED
- HiCrome UTI agar

The inoculated media were incubated aerobically at 35°C-37°C for 18-24 hours. A growth of ≥100 colonies (equivalent to 10^5 colony-forming units per mL of urine) was considered significant for bacteriuria [3]. Colonial characteristics observed on the respective culture media were used for the initial presumptive identification of isolates. Further confirmation was carried out using a series of conventional biochemical tests appropriate to the morphological and Gram-staining characteristics of the organisms.

For Gram-negative bacilli, the tests performed included oxidase, catalase, indole production, citrate utilisation, urease activity, motility, Triple Sugar Iron (TSI) agar reactions, and methyl red-Voges-Proskauer (MR-VP) tests. Gram-positive cocci were identified using catalase and coagulase tests, along with bile esculin hydrolysis, salt tolerance, and Pyrrolidonyl Arylamidase (PYR) tests where applicable. These procedures were conducted in accordance with standard microbiological guidelines to ensure accurate and reliable identification of uropathogens.

Antimicrobial Susceptibility Testing (AST): AST was performed on all significant isolates using the Kirby-Bauer disk diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. Zones of inhibition were measured, and results were interpreted as susceptible, intermediate, or resistant based on CLSI standards [11].

Media Preparation and Quality Control

MacConkey agar [12] preparation: Suspend 49.53 g of the dehydrated medium in 1000 mL of distilled water. Heat to boiling until completely dissolved. Sterilise by autoclaving at 121°C (15 lbs pressure) for 15 minutes. Cool to 45-50°C, mix well, and pour into sterile petri plates.

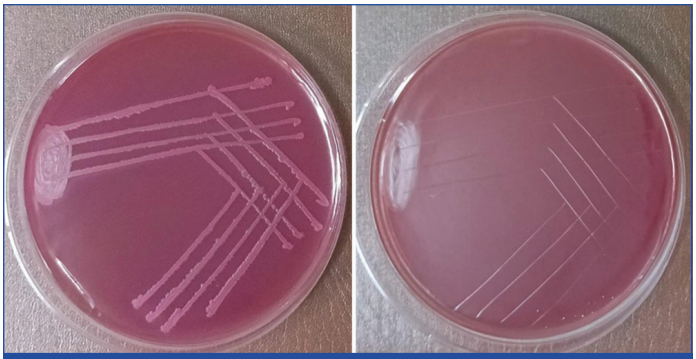
Quality control:

- *Escherichia coli* ATCC 25922 - pink colonies [Table/Fig-1]
- *Staphylococcus aureus* ATCC 25923 - no growth [Table/Fig-2]

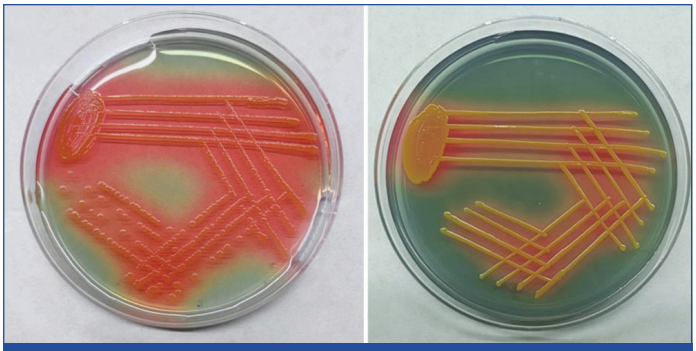
CLED Agar with andrade indicator [6] preparation: Suspend 36.25 g in 1000 mL of distilled water. Heat to dissolve completely. Sterilise at 121°C for 15 minutes. Cool to 45–50°C and incorporate Andrade indicator as modified by Bevis for differentiation between Lactose Fermenters (LF) and Non Lactose Fermenters (NLF).

Quality control:

- *E. coli* ATCC 25922: Bright pink colonies [Table/Fig-3].
- S. aureus ATCC 25923: Golden yellow colonies [Table/Fig-4].



[Table/Fig-1]: *E. coli* on MacConkey agar. Strain: ATCC 25922.
[Table/Fig-2]: *S. aureus* on MacConkey agar. Strain: ATCC 25923. (Images from left to right)

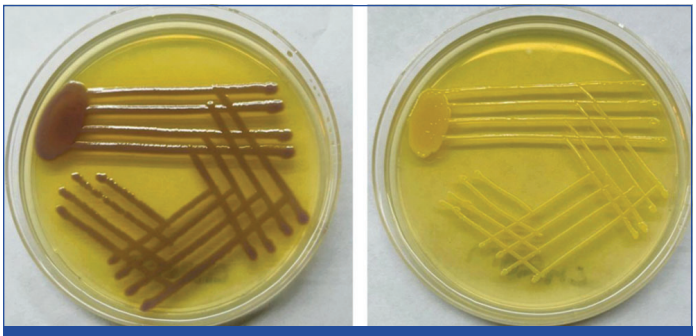


[Table/Fig-3]: *E. coli* on CLED agar. Strain: ATCC 25922.
[Table/Fig-4] *S. aureus* on CLED agar. Strain: ATCC 25923. (Images from left to right)

Preparation: Suspend 56.8 g in 1000 mL of distilled water. Heat to boiling to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C, mix well, and pour into sterile Petri plates.

Quality control:

- *E. coli* ATCC 25922: Pink-purple colonies [Table/Fig-5].
- *S. aureus* ATCC 25923: Golden yellow colonies [Table/Fig-6].



[Table/Fig-5]: *E. coli* on HiChrome UTI agar. Strain: ATCC 25922.
[Table/Fig-6] S. aureus on HiChrome UTI agar, Strain: ATCC 25923. (Images from left to right)

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 24.0. Descriptive statistics were presented as frequencies and percentages. The Chi-square (χ^2) test was used to analyse categorical variables, including the distribution of growth patterns (single, mixed, or sterile) and differences in organism isolation across various culture media. Fisher's exact Test was applied where expected cell frequencies were less than 5, particularly in the analysis of Gram-positive cocci growth across different media. A p-value of <0.05 was considered statistically significant for all comparisons.

RESULTS

Out of 400 urine samples processed, 189 (47.3%) showed significant growth, while 211 (52.7%) were sterile. Among the 189 culture-positive samples, 158 (83.6%) showed pure bacterial growth, and 31 (16.4%) showed mixed bacterial growth. A total of 220 bacterial isolates were identified, of which 187 (85%) were Gram-negative bacilli (GNB) and 33 (15%) were Gram-positive cocci (GPC). Among the GNB, 164 (88%) were lactose fermenters and 23 (12%) were non lactose fermenters.

The most frequently isolated pathogen was *Escherichia coli* (97/220, 44.1%), followed by *Klebsiella* spp. (67/220, 30.5%). Among the GPC, *Staphylococcus aureus* (20/220, 9.1%) was the most common, followed by *Enterococcus* spp. (13/220, 5.9%).

Growth patterns and isolation rates: Out of 400 urine samples, 158 (39.5%) exhibited single bacterial growth, 31 (7.8%) showed mixed bacterial growth, and 211 (52.7%) were sterile [Table/Fig-7]. A statistically significant difference was observed in the distribution of growth types (chi-square=83.33, p<0.001), indicating that the observed proportions were not due to chance.

Growth type	n (%)	Significance
Single bacterial growth	158 (39.5)	Chi-square value: 83.33 p-value: <0.001 (statistically significant)
Mixed bacterial growth	31 (7.8)	
Sterile	211 (52.7)	

[Table/Fig-7]: Different types of growth on urine culture (n=400).

Distribution of microorganisms isolated: Among the 220 isolates, the majority were GNB, the distribution of GNB and GPC is as shown in [Table/Fig-8].

Microorganism	n (%)
Escherichia coli	97 (44.1)
Klebsiella spp.	67 (30.5)
Pseudomonas aeruginosa (NFL)	5 (2.3)
Other non lactose fermenters	18 (8.2)
Staphylococcus aureus	20 (9.1)
Enterococcus spp.	13 (5.9)

[Table/Fig-8]: Distribution of microorganisms from urine culture (n=220).

Comparison of isolation media: When comparing media types (CLED agar, MacConkey agar, and HiCrome UTI agar), all 187 GNB were isolated on all three media. However, a significant difference was observed in the isolation of GPC. Both *Staphylococcus aureus* and *Enterococcus* spp. grew on CLED agar and HiCrome UTI agar, while MacConkey agar failed to support growth for either organism. The difference was statistically significant (Fisher's exact test, p-value <0.001), as shown in [Table/Fig-9].

Organism	CLED agar	MacConkey agar	HiCrome UTI agar	p-value
Staphylococcus aureus	20	0	20	<0.001
Enterococcus spp.	13	0	13	<0.001

[Table/Fig-9]: Gram-positive cocci growth on different media (n=33).
Statistical test applied: Fisher's exact test; p<0.001

Mixed growth analysis: A total of 31 samples exhibited mixed bacterial growth. The most common combination was *E. coli* with *Klebsiella* spp. (13/31, 41.9%), followed by E. coli with Enterococcus spp. (9/31, 29.0%), E. coli with Pseudomonas aeruginosa (5/31, 16.1%), and Klebsiella with Enterococcus spp. (4/31, 12.9%). The variation in combination frequency was not statistically significant (chi-square=3.21, p-value=0.36) [Table/Fig-10].

Combination	n (%)
E. coli+Klebsiella spp.	13 (41.9)
E. coli+Pseudomonas aeruginosa	5 (16.1)
E. coli+Enterococcus spp.	9 (29.0)
Klebsiella+Enterococcus spp.	4 (12.9)

[Table/Fig-10]: Distribution of mixed bacterial growth (n=31).

Cost comparison: A cost analysis was conducted to assess the economic feasibility of three commonly used culture media for UTI diagnostics. Pricing data were obtained from HiMedia Laboratories' official catalog [13]. The cost per 500 g pack was considered, which typically yields approximately 20-25 standard 90 mm Petri plates depending on laboratory practices. A comparative summary is presented in [Table/Fig-11].

Culture medium	Product code	Price (INR per 500 g)	Remarks
CLED agar	M031-500G	₹945.00	Most cost-effective; inhibits *Proteus* swarming; supports broad spectrum of uropathogens.
MacConkey agar	MH081-500G	₹3,570.00	Traditional medium for Gram-negative bacilli; lacks chromogenic identification.
HiCrome UTI Selective agar	M1505	₹3,493.00	Enables rapid presumptive identification; chromogenic medium; relatively high cost.

[Table/Fig-11]: Comparative pricing of culture media from HiMedia Laboratories (India) [13].

These data indicate that CLED agar is the most economical medium, priced substantially lower than both MacConkey agar and HiCrome UTI Selective agar. Although HiCrome UTI agar provides the advantage of rapid presumptive identification through chromogenic differentiation—potentially reducing downstream testing—its higher cost may be prohibitive in low-resource settings. MacConkey agar, though traditionally used for Gram-negative organisms, offers limited identification capability and is similarly expensive.

In resource-limited diagnostic setups, CLED agar represents a cost-effective yet diagnostically adequate choice, balancing affordability with microbiological utility.

DISCUSSION

The current investigation revealed that 47.3% (189/400) of urine samples exhibited significant bacterial growth, while 52.7% (211/400) were sterile. Among the culture-positive samples, 83.6% showed pure bacterial growth, while 16.4% presented with mixed infections. This finding aligns with that of Chaturvedi A et al., (2017), who reported that 42.2% (211/500) of urine samples yielded significant growth, with a predominance of GNB (63.3%) [5]. Mixed growth was less common, occurring in only 4.8% (24/500) of samples. In contrast, Khalid M, observed a slightly higher rate of mixed growth (7.2%) among 208 urine samples, with CLED agar yielding growth in 45.2% of samples, while 47.6% showed no bacterial growth [14].

Escherichia coli was the predominant uropathogen across all studies. In the present study, *E. coli* accounted for 44.1% of isolates, followed by *Klebsiella* spp. at 30.5%. This finding is consistent with Chaturvedi A et al., (2017), who observed *E. coli* in 40.5% of the isolates and *Klebsiella* spp. in 28.6% [5]. Notably, the study by Nahar SG et al., reported a higher prevalence of *E. coli* (62.75%) and a lower proportion of *Klebsiella* spp. (12.41%), with *Enterococcus* spp. accounting for 11.03% of isolates [15]. In contrast, *Pseudomonas* spp., though less frequently isolated, was notably present in both Chaturvedi A et al., (2017) and Biji S et al., (2017) [5,16]. These differences may be attributed to variations in patient demographics, geographical location, and the evolving antimicrobial resistance profiles of pathogens.

The effectiveness of various culture media for the isolation of uropathogens was assessed in all studies, revealing some notable trends. In the present study, all GNB were successfully cultured on CLED, MacConkey, and HiCrome UTI agar, although MacConkey agar failed to support the growth of GPC, unlike CLED and HiCrome UTI agar. This finding aligns with those of Biji S et al., who reported that HiCrome UTI agar exhibited superior performance, isolating 100% of the uropathogens, including yeast isolates, and identifying all mixed bacterial growths by the second day [16]. Sachu A et al., (2022) similarly demonstrated that HiCrome UTI agar was the most effective medium, achieving a 100% isolation rate and a higher presumptive identification rate compared to CLED and MacConkey agar [17]. Khalid, also noted that HiCrome UTI agar had a statistically significantly higher presumptive identification rate (94%) compared to CLED agar (84%) [14]. These results underscore the superiority of HiCrome UTI agar in terms of both isolation efficiency and the accuracy of presumptive identification, particularly for GPC and yeast isolates.

Polymicrobial growth was detected at varying frequencies across the studies. In the present study, mixed infections involving *E. coli* and *Klebsiella* spp. were the most common, constituting 41.9% of mixed infections, although the differences in growth patterns were not statistically significant. Chaturvedi A et al., also observed mixed growth in a small proportion of samples (2.4% of 500 samples) [5]. However, Biji S et al., found a higher incidence of mixed bacterial growth, with 16.99% of samples exhibiting such growth [16], and Sachu A and Samuel AK, observed polymicrobial growth in 18 samples, accounting for 36 bacterial growths [17]. This variability highlights the complexity of UTIs and the importance of employing comprehensive diagnostic methods to accurately identify polymicrobial infections.

Overall, *Escherichia coli* remained the most prevalent uropathogen across studies, with a substantial degree of variation in the relative abundance of other pathogens, such as *Klebsiella* spp., *Enterococcus* spp., and *Pseudomonas* spp., depending on local epidemiology. In terms of culture media, HiCrome UTI agar emerged as the most effective medium for isolating and identifying uropathogens, outperforming traditional media such as CLED and MacConkey agar in isolation rates, presumptive identification, and polymicrobial growth detection. These findings emphasise the utility of chromogenic media like HiCrome UTI agar for clinical microbiology, particularly in improving diagnostic accuracy and streamlining the identification process for uropathogens in UTIs.

The clinical implications of these findings are profound, particularly in enhancing diagnostic precision and optimising treatment strategies for UTIs. The superior efficacy of HiCrome UTI agar in isolating and identifying uropathogens, including polymicrobial infections, facilitates faster and more accurate detection, thereby enabling clinicians to initiate targeted antimicrobial therapy. This is of paramount importance in the context of escalating antimicrobial resistance, where precise pathogen identification is crucial for minimising the inappropriate use of antibiotics and curbing resistance development.

Additionally, the heightened sensitivity of HiCrome UTI agar in detecting mixed infections emphasises its potential for providing comprehensive insights into complex clinical scenarios that may be overlooked with traditional media. Looking ahead, future research should prioritise refining culture media to encompass a broader spectrum of uropathogens, including emerging antimicrobial-resistant strains, and assess their diagnostic performance across diverse patient populations. The integration of molecular diagnostic techniques alongside conventional culture methods could further enhance the accuracy of pathogen detection and resistance profiling, offering a more integrated approach to managing UTIs.

Moreover, the development of advanced diagnostic platforms, coupled with rapid antimicrobial susceptibility testing, will be instrumental in improving clinical outcomes, enhancing therapeutic decision-making, and addressing the growing global challenge of antimicrobial resistance.

Study strengths: This study's strengths include its comprehensive comparison of three widely used culture media-CLED, HiCrome UTI, and MacConkey agars-providing clear insights into their growth characteristics, isolation performance, and practical utility in routine urine culture workflows. The use of a substantial sample size

and strict adherence to standardised laboratory protocols enhance the robustness, reproducibility, and applicability of the findings, particularly for laboratories operating in resource-limited settings.

Limitation(s)

Nevertheless, the study was limited by its single-centre design, which may not fully capture the variability in microbiological practices across different healthcare settings. Additionally, the analysis was based on retail pricing from a single supplier, which may not reflect regional pricing discrepancies or the impact of bulk purchasing agreements. Furthermore, the study did not account for potential confounding factors such as variations in incubation conditions or other laboratory variables that could influence the performance of the media.

CONCLUSION(S)

In conclusion, this study highlights the importance of selecting the right culture media for diagnosing urinary tract infections, especially in settings where resources may be limited. CLED agar stands out as the most cost-effective option, offering reliable results for isolating a wide range of uropathogens, making it a practical choice for many laboratories. While HiCrome UTI Selective agar can speed up the identification process with its chromogenic properties, its higher cost may limit its use in routine practice. On the other hand, MacConkey agar, though effective for Gram-negative bacteria, falls short in isolating Gram-positive organisms. Overall, this study suggests that CLED agar provides a good balance between cost and diagnostic capability, making it an ideal option for most clinical settings, while HiCrome UTI agar could be considered in environments where faster identification is a priority.

Authors' contribution: All authors listed have made substantial, direct, and intellectual contributions to the work and approved it for publication.

Data availability: All data generated or analysed during this study are included in the manuscript.

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