Open Access

Original Article

Diagnostic Accuracy of CHROMagar Orientation for Identification of Uropathogens

Nayab Ali, Wajid Hussain*, Irfan Ali Mirza*, Asima Niazi, Fatima Rubab*, Saira Salim*****

Frontier Medical & Dental College, Abbottabad Pakistan, *Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS), Rawalpindi Pakistan **Quetta Institute of Medical Science, Quetta Pakistan, ***Primary and Secondary Health Care Department, Lahore Pakistan

ABSTRACT

Objective: To determine the diagnostic accuracy of CHROMagar Orientation for its ability to support the growth and identification of uropathogens by keeping API 20E/20NE as a gold standard.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jun to Nov 2019.

Methodology: A total of 470 midstream specimen of urine from patients suspected of UTIs were analyzed. Urine specimens were inoculated by 1µL calibrated sterilized loop each on CLED Agar, Blood agar and CHROMagar Orientation media plates. Culture plates were incubated at 35.00±2.00ºC for 18 to 24 hours. As per standard protocols, isolates were identified by colony morphology, Gram stain, catalase, oxidase, DNAase, coagulase, and API 20E/20NE. In addition, the appearance of microorganism on CHROMagar Orientation media were identified according to the manufacturer's instructions.

Results: Out of 470 samples, 211 showed no growth, 90 were classified as mix growth (polymicrobial), and 169 samples were positive (unimicrobial) for the growth of uropathogens. All the samples positive for bacterial growth on conventional media were also positive on CHROMagar orientation and vice versa. The sensitivity and specificity of CHROMagar Orientation for differentiation of uropathogens were 99% and 100%, respectively.

Conclusion: The overall findings of our study suggested that CHROMagar supports the growth of all uropathogens. It provided the rapid identification of organisms based on their colour morphology, even in a mixed bacterial culture, growth within 24 hours easily.

Keywords: CHRO-Magar orientation, Polymicrobial growth, Uropathogens.

How to Cite This Article: *Ali N, Hussain W, Mirza IA, Niazi A, Rubab F, Salim S. Diagnostic Accuracy of CHROMagar Orientation for Identification of Uropathogens. Pak Armed Forces Med J 2023; 73(1): 17-20. DOI: https://doi.org/10.51253/pafmj.v73i1.7453*

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting all age groups in community and hospital settings.[1,2] It is estimated that almost 35 to 40% of the hospitalized population sometimes acquires UTI. In the day-to-day workload of a microbiology laboratory, most work attributes towards urine cultures. Blood agar (BA) and Cystine-Lactose-Electrolyte Deficient (CLED) are the most commonly used media for urine cultures.[3,4] Moreover, these routine cultures require considerable time of at least 48 to 72 hours, trained laboratory personnel, and further biochemical testing such as API 20E/20NE to confirm urinary pathogens. All these procedures are not only labour-intensive but also expensive and time-consuming.[5]

Originally discovered in 1979, chromogenic agar is a culture-based rapid method that requires less labour and fewer materials. It is cost-effective compared to routine conventional media and API 20E/20NE used to identify uropathogens. The limitation of CHROMagar medium is that it does not differentiate between Klebsiella, Enterobacter, and Citrobacter as they produce the same colour colonies, i.e., metallic blue.[6]

Considering the advantages of CHROMagar over conventional methods, the present study was proposed to evaluate the diagnostic accuracy of CHRO Magar orientation by keeping API 20E/20NE as the gold standard for identifying uropathogens and for incorporating it in routine laboratory practice.[7,8] If it turns out to be diagnostically accurate, the method under study will reduce laborious laboratory work and turn-around time and be a cost-effective method for reliable identification of uropathogens without any need for further biochemical testing panels as isolates are easily recognized based on their specific colours.[9,10] Furthermore, since the most common UTI pathogen is E. coli and CHROMagar reportedly claims a specificity of 99.3% for this bacterium, it is expected that isolates will be easy to recognize based on specific colours without needing highly trained staff. Moreover, it is also expected that isolate identification results will also

Correspondence: Dr Nayab Ali, Ass Prof of Microbiology, Frontier Medical & Dental College, Abbottabad, Pakistan

Received: 30 Sep 2021; revision received: 12 Mar 2022; accepted: 15 Mar 2022

be available within 24 hours, thereby supporting targeted or directed treatment for UTIs.

METHODOLOGY

The cross-sectional study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from June to November 2019. The Institutional Review Board approved the study, Armed Forces Institute of Pathology, Rawalpindi Pakistan, IRB number MP-CHP18-6/READ-IRB/19649A. Written informed consent was taken from patients. The sample size was calculated by using a WHO calculator. Based on the confidence level set to 95%, the anticipated population proportion having symptomatic UTI at 11.6%,[11] and precision set to 5%, the estimated sample size was 158. However, 470 patients were enrolled in the study owing to a greater influx of patients. Therefore, a non-probability consecutive sampling technique was used.

Inclusion Criteria: All urine samples collected at the AFIP laboratory from indoor and outdoor patients were included in the study.

Exclusion Criteria: Repeat samples of the same patient and samples received after two hours of the collection were excluded.

All urine specimens were processed by a conventional method per standard protocols and on the CHROMagar orientation media plate simultaneously. The urine specimen was inoculated by a 1µL calibrated sterilized loop each on CLED Agar media plate, Blood agar media plate and CHROMagar Orientation media plate. Culture plates were incubated at 35.00±2.00°C for 24 to 48 hours. The bacterial growth was defined as "positive (monomicrobial)", "negative (No growth)", and "mix growth (polymicrobial) for both conventional and CHROMagar media. As per standard protocols, isolates were further identified by colony morphology, Gram stain, catalase, oxidase, DNAase, coagulase, and API 20E/20NE. Isolates of enterococci were identified by growth in 6.5% NaCl, fermentation of 1% arabinose and bile esculin hydrolysis per standard protocols.[12,13] The growth appearance of microorganisms on CHROMagar Orientation media was identified according to the manufacturer's instructions. ATCC *Escherichia coli* 25922, *Staphylococcus aureus* 25923 and P*seudomonas aeroginosa* 27853 were quality control strains.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Descriptive statistics were calculated for age and gender variables in terms of frequencies. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were calculated.

RESULTS

Out of 470 samples, 211(44.89%) showed no growth on both conventional media and CHROMagar orientation, 90(19.14%) were classified as mixed growth (polymicrobial) on both media and 169(37.95%) samples were positive (unimicrobial) on both media for the growth of uropathogens as shown in Figure.

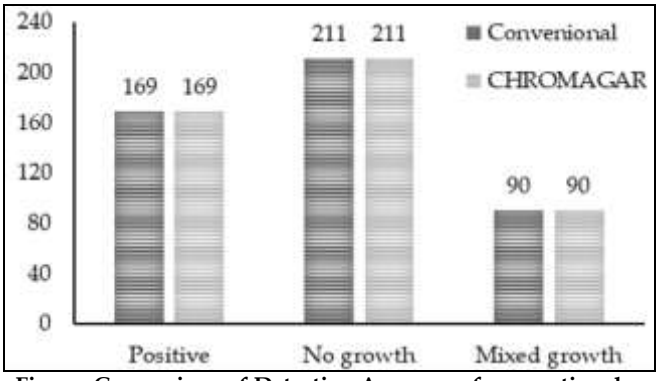

Figure: Comparison of Detection Accuracy of conventional and CHRO Magar (n=470)

No major differences in growth analysis were noted between conventional media and CHRO Magar as all those samples which yielded growth on conventional media also yielded growth on CHROMagar and vice versa.

Out of 259(55.10%) isolates yielded from monomicrobial and polymicrobial cultures, CHROMagar orientation identified 257(54.68%) correctly as compared to the conventional method using API 20E/20NE, 65g/1000ml of NaCl (6.5%), fermentation of 1g/9ml arabinose (1%) bile esculin hydrolysis for identification of different uropathogens. Two isolates identified as *E.coli* on conventional procedure using API 20E showed colourless colonies instead of dark pink/red colonies on CHROMagar orientation (false negative) after 48 hours of incubation. Comparison of detection accuracy of conventional and CHROMagar was shown in the Table-I.

Diagnostic accuracy comparison of conventional media versus CHROMagar Orientation for its ability to identify uropathogens from Unimicrobial cultures was shown in the Table-II. Diagnostic accuracy comparison of conventional media versus CHROMagar Orientation for its ability to identify uropathogens from Polymicrobial cultures was shown in the Table-III.

Table-I: Comparison of Detection Accuracy of Conventional and CHRO Magar (n=470)

CHRO Magar	Conventional Media		Total
	Positive	Negative	
Positive (unimicrobial+polymicrobial)	257(True Positive)	0(False Positive)	257
Negative	2(False Negative)	211(True Negative)	213
Total	259	211	470
Sensitivity	99%		
Specificity	100%		
Positive Predictive Value (PPV)	100%		
Negative Predictive Value (NPV)	99%		

Table-II : Diagnostic Accuracy Comparison of Conventional Media versus CHROMagar Orientation for its ability to identify Uropathogens from Unimicrobial Cultures (n=470)

CHRO Magar	Conventional Media		Total
	Positive	Negative	
Positive (unimicrobial)	167(True Positive)	0(False Positive)	167
Negative	2(False Negative)	211(True Negative)	213
Total	169	211	380
Sensitivity	99%		
Specificity	100%		
Positive Predictive Value (PPV)	100%		
Negative Predictive Value (NPV)	99%		

For polymicrobial samples, 100% accuracy was achieved.

Table-III: Diagnostic Accuracy Comparison of Conventional Media versus CHROMagar Orientation for its ability to identify Uropathogens from Polymicrobial Cultures (n=470)

CHROMagar	Conventional Media		Total
	Positive	Negative	
Positive (polymicrobials)	90 (True Positive)	0 (False Positive)	90
Negative	0(False Negative)	211(True Negative)	211
Total	90	211	301
Sensitivity	100%		
Specificity	100%		
Positive Predictive Value (PPV)	100%		

DISCUSSION

In the present study, we observed very good specific colour production by uropathogens on CHRO Magar orientation, enabling our laboratory technicians and us easy and correct the identification of different uropathogens not only in unimicrobial cultures but also in polymicrobial cultures.

In the present study, out of 470 urine samples, 169(36%) samples were classified as positive as they showed significant bacterial growth of single isolate and were called unimicrobial, whereas 90(19.1%) urine samples were termed as polymicrobial as they showed mixed bacterial growth isolates and 211(44.9%) showed no growth on both conventional media and CHROMagar orientation media indicating that CHROMagar supports the growth of uropathogens as equally as conventional blood agar and CLED media. The finding of our study was similar to the study conducted by Biji *et al.* which showed that 34.84% of urinary cultures were unimicrobial, 16.99% were polymicrobial, and 42.49% exhibited no growth.[14] The other studies done by Sharmin *et al.* also obtained significant growth between 40-50% and were in accordance with our results.[15] In contrast to the present study other studies by Lakshmi *et al.* reported a higher rate of microbial isolation.[16]

In the existing study, 169 strains were isolated in monomicrobial cultures. The major isolate was *Escherichia coli* (66.27%), followed by *Klebsiella* species (18.34%). Other isolates were *Enterococci spp.* (4%), *Candida species* (3%), *S. aureus*(3%), *Citrobacter* (1.18%), *p. aeruginosa* (1.8%), *Proteus species* (<1%) and *enter-obacter cloacae* (<1%). In various studies on both conventional media and CHROMagar *E.coli* was found to be the predominant pathogen isolated from urine samples which are in agreement with the present study.[17] Similar results were found in the study by Parveen *et al.* who reported *E.coli* (64.49%) followed by *Klebsiella spp* (11.21%), the most prominent bacteria in urinary samples.[18] The isolation rate of major uropathogens in the present study is in accordance with the studies carried out on both chromogenic and conventional media CHROMagar, BA and CLED supported the growth of all 269(100%) isolates of bacteria.[19,20] In our study, we observed that blood agar, CLED, and Chromogenic medium contain all the vital nutrients for the growth of all possible uropathogens in urine samples. Likewise, proteus species secrete tryptophan deaminase, which reacts with tryptophan in chromogenic media and gives brown colour to colony. In our study, two isolates identified as *E. coli* on API/20E did not produce proper colour (trans-lucent colonies) & were classified as a false negatives.

ACKNOWLEDGEMENT

We are extremely thankful to "Allah Almighty", the real source of knowledge and wisdom endowed to humankind, for providing us with the acumen and vision to complete this endeavour. We want to express our profound

gratitude to the supervisor, Dr.Irfan Ali Mirza and co-supervisor, Dr.Wajid Hussain, and we would like to extend our deepest gratitude to our family members.

LIMITATIONS

Limitation of the CHROMagar medium is that it does not differentiate between *Klebsiella, Enterobacter, and Citrobacter* as they produce the same colour colonies, i.e., metallic blue.

CONCLUSION

The overall findings of our study suggested that CHROMagar supports the growth of all uropathogens. Furthermore, it provided the correct and rapid identification of organisms based on their colour morphology, even in a mixed bacterial culture growth within 24 hours. Therefore, it should be incorporated into routine laboratory work to process urinary cultures.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

NA & WH: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

IAM & AN: Data acquisition, concept, critical review, approval of the final version to be published.

FR & SS: Critical review, data analysis, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Seifu WD, Gebissa AD. Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. BMC Infect Dis 2018; 18(1): 30. doi: 10.1186/s12879-017-2911-x.
2. Ullah A, Shah SRH, Almugadam BS. Prevalence of symptomatic urinary tract infections and antimicrobial susceptibility patterns of isolated uropathogens in kohat region of Pakistan. MOJ Biol Med 2018; 3(3): 85–89. doi: 10.15406/mojbm.2018.03.00082.
3. Leski TA, Taitt CR, Bangura U, Stockelman, MG, Ansumana R, Cooper WH, et al. 'High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone'. BMC Infect Dis 2016; 16(1): 167-170. doi: 10.1186/s12879-016-1495-1.
4. Ibrahimagić A, Bedenić B, Kamberović F, Uzunović S. High prevalence of CTX-M-15 and first report of CTX-M-3, CTX-M-22, CTX-M-28 and plasmid-mediated AmpC beta-lactamase producing Enterobacteriaceae causing urinary tract infections in Bosnia and Herzegovina in hospital and community settings. J Infect Chemother 2015; 21(5): 363-369.
5. Gharbi M, Drysdale JH, Lishman H, Goudie R, Molokhia M, Johnson AP, et al. Antibiotic management of urinary tract infection in elderly patients in primary care and its association with bloodstream infections and all cause mortality: population based cohort study. BMJ 2019; 364(1): l525. doi: 10.1136/bmj.l525.
6. Yarbrough ML, Wallace MA, Marshall C, Mathias E, Burnham CA. Culture of Urine Specimens by Use of chromID CPS Elite Medium Can Expedite Escherichia coli Identification and Reduce Hands-On Time in the Clinical Laboratory. J Clin Microbiol 2016; 54(11): 2767-2773. doi: 10.1128/JCM.01376-16.
7. Bischoff S, Walter T, Gerigk M, Ebert M, Vogelmann R. Empiric antibiotic therapy in urinary tract infection in patients with risk factors for antibiotic resistance in a German emergency department. BMC Infect Dis 2018; 18(1): 56. doi: 10.1186/s12879-2960-9.
8. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015; 13(5): 269-284. doi: 10.1038/nrmicro3432.
9. Davenport M, Mach KE, Shortliffe LMD, Banaei N, Wang TH, Liao JC. New and developing diagnostic technologies for urinary tract infections. Nat Rev Urol 2017; 14(5): 296-310. doi: 10.1038/nrurol.2017.20.
10. van der Zee A, Roorda L, Bosman G, Ossewaarde JM. Molecular Diagnosis of Urinary Tract Infections by Semi-Quantitative Detection of Uropathogens in a Routine Clinical Hospital Setting. PLoS One 2016; 11(3): e0150755. doi: 10.13711020/journal. pone. 01507552143614.
11. Akter L, Haque R, Salam MA. Comparative evaluation of chromogenic agar medium and conventional culture system for isolation and presumptive identification of uropathogens. Pak J Med Sci 2014; 30(5): 1033-1038. doi: 10.12669/pjms.305.5243.
12. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev 2017; 30(2): 449-479. doi: 10.1128/CMR.00097-16. Erratum in: Clin Microbiol Rev 2017; 30(4): vii.
13. Manickam K, Karlowsky JA, Adam H, Lagacé-Wiens PR, Rendina A, Pang P, et al. CHROMagar Orientation medium reduces urine culture workload. J Clin Microbiol 2013; 51(4): 1179-1183. doi: 10.1128/JCM.02877-12.
14. Biji SM, Sasikumari O, Bai JR. A Study on Evaluation of Different Culture Media for the Isolation of Routine Urinary Pathogens. Int J Curr Microbiol App Sci 2017; 6(2): 195-201. doi: 10.20546/ijcmas.2017.602.026.
15. Sharmin S, Alamgir F, Begum F, Jaigirdar MQH. Use of Chromogenic Agar Media for Identification of Uropathogen. Bangladesh J Med Microbiol 2010; 4(1): 18-23. doi:10.3329/bjmm.v4i1.8464.
16. Lakshmi V, Satheeshkumar T, Kulkarni G. Utility of Urichrom II - a chromogenic medium for uropathogens. Indian J Med Microbiol 2004; 22(3): 153-158.
17. Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P, et al. Urinary tract infections in women. Eur J Obstet Gynecol Reprod Biol 2011; 156(2): 131-136. doi: 10.10161020/j.ejogrb.2011.01.028.
18. Parveen R, Saha SK, Shamshuzzaman SM, Rashid AL, Chowdhury A, Muazzam N. Detection of uropathogens by using chromogenic media (Hicrome UTI agar), CLED agar and other conventional media. Faridpur Med Coll J 2011; 6(1): 46-50. doi:10.3329/fmcj.v6i1.7411.
19. Gaillot O, Wetsch M, Fortineau N, Berche P. Evaluation of CHROMagar Staph. aureus, a new chromogenic medium, for isolation and presumptive identification of Staphylococcus aureus from human clinical specimens. J Clin Microbiol 2000; 38(4): 1587-1591. doi: 10.1128/JCM.38.4.1587-1591.2000.
20. Mambatta AK, Jayarajan J, Rashme VL, Harini S, Menon S, Kuppusamy J. Reliability of dipstick assay in predicting urinary tract infection. J Family Med Prim Care 2015; 4(2): 265-268. doi: 10.4103/2249-4863.154672.