Original Article

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Microbiology Section

Early Detection of Drug Resistant Enterobacteriaceae in Urinary Tract Infections using Chromogenic Agar Medium in a Tertiary Care Hospital, Kakinada, Andhra Pradesh, India

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ABSTRACT

Introduction: The irrational and inappropriate use of beta lactam antimicrobial drugs has led to the advent of Extended Spectrum Beta-Lactamase (ESBL) resistant strains. ESBL producing Enterobacteriaceae strains are frequent causative agents both in community and in acquired nosocomial infections and Urinary Tract Infections (UTI). The phenotypic confirmatory tests rarely identify all ESBLs. Chrom ID (Chromogenic identification Media) ESBL – Bx (bioMerieux) is a completely new and innovative chromogenic medium designed specifically for the screening of ESBL producing Enterobacteria directly from urine samples. It is a ready to use selective media which is sensitive and specific for rapid and presumptive identification of ESBL producing Enterobacteriaceae.

Aim: Early detection of ESBL producing Enterobacteriaceae directly from urine samples on chromogenic medium (Chrom ID-ESBL- Bx) and confirmation of ESBL producing Enterobacteria using Disc Potentiation Test (DPT).

Materials and Methods: The present cross-sectional study was conducted in the Department of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India from November 2019 to March 2020 (five months duration). The study was done on 70 urine samples from patients with UTI. All samples were subjected to wet mount, inoculated directly for culture on Chrom ID ESBL- Bx agar and MacConkey agar. Antibiotic Susceptibility testing of ceftazidime and cefotaxime was done by Kirby-Bauer disc diffusion method and conformation of ESBL production by DPT using Clinical and Laboratory Standards Institute (CLSI) method. The Statistical Package for the Social Sciences (SPSS) Statistical package version (18.0) was used.

Results: A total of 56 (80%) isolates were obtained from 70 urine samples, out of them 28 (50%) were *Escherichia coli*, 21 (37.5%) were *Klebsiella* spp., 7 (12.5%) were *Proteus* spp., 23 (82.14%) isolates of *Escherichia coli*, 15 (71.43%) of *Klebsiella* spp., 6 (85.71%) of *Proteus* spp., isolated were screened positive using Chrom ID ESBL-Bx agar. About 44 (78.57%) of total Enterobacteria (56) were screened for ESBL production. 20 (86.96%) of *Escherichia coli*, 11 (73.33%) of *Klebsiella* spp., and 5 (83.33%) of *Proteus* spp., that were screened positive using Chrom ID ESBL agar were confirmed (by DPT) as ESBL producers and 2 (16.6%) of total (12) isolates that were screened negative by Chrom ID ESBL agar were confirmed as ESBL producers when screened and confirmed by DPT. So sensitivity and specificity CHRO Magar was 94.73% and 55.5%.

Conclusion: ESBL continues to become a serious public health threat. Results from present study showed that CHROMagar ESBL has a high sensitivity and a convenient method for making provisional diagnosis of drug resistant Enterobacterial infections in 24 hours. Chrom ID ESBL- Bx agar medium allows easy differentiation of different bacteria based on colony colouration.

Keywords: Chromogenic identification media, Disc potentiation test, Extended spectrum beta- lactamase

INTRODUCTION

The UTI are a serious threat to human health resulting in high morbidity and mortality. UTI is common in males and females but females are more susceptible than males. Enterobacteriaceae species like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Enterobacter aerogenes*, *Citrobacter freundii* are the commonest uropathogens resulting in bacteraemia and hospital acquired infections and are the common pathogens producing ESBL [1]. The injudicious and misuse of beta lactam antimicrobial drugs has led to the emergence of ESBL resistant strains worldwide. ESBL producing Enterobacteriaceae strains are frequent causative agents both in community and in acquired nosocomial infections. The phenotypic confirmatory tests rarely identify all ESBLs. Chrom ID ESBL-Bx (bioMerieux) is a completely new and innovative chromogenic medium designed specifically for the screening of ESBL producing enterbacteria directly from urine samples. It is a ready to use selective media which is sensitive and specific for rapid and presumptive identification of ESBL producing Enterobacteriaceae [2].

Chrom ID ESBL agar is a rich nutrient medium with a mixture of antibiotics, including cefpodoxime which is recognised as being the marker of choice for this resistance mechanism. There is incorporation of chromogenic enzyme substrate as a detection system. Chromogenic substrates consist of chromophor linked to an enzyme recognising part such as carbohydrate, aminoacids or phosphate. Specific enzymes produced by the target microorganisms will cleave to the chromogenic substrate liberating the chromophor which highlight the microorganism by coloration of the grown colony. The addition of antibiotics to the chromogenic media has been a revolution for the explicit detection of ESBL from clinical specimen. This media selectively inhibits gram positive bacteria and yeasts. It allows early identification bacteria depending on colony colour [3]. This media are increasingly being used as versatile tools in early differentiation and identification of gram positive and gram negative isolates from clinical specimens [4].

Chromogenic culture media have wide range of uses in diagnostic clinical microbiology and it is also being used to found carbapenamases and have a role in molecular diagnostics [5].

The very good sensitivity (97%) of chrom ID ESBL agar in ESBL detection represents a convenient method for the identification and recovery of ESBL producing Enterobacteriaceae [6]. The sensitivity of chrom ID ESBL agar can be increased at 48 hours of incubation and its selectivity are particularly useful in specimens containing resident associated flora [7].

The aim of the present study was to detect ESBL producing enterobacteria directly from the urine samples inoculated on chromogenic agar medium and confirmation of ESBL producing enterobacteria using DPT among multidrug resistant pathogens causing UTI.

MATERIALS AND METHODS

The present study was a cross-sectional study carried out within a period of five months duration from November 2019 to March 2020 in the Department of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India. Informed consent was obtained from all the patients before collecting the sample. Ethics clearance was not required as only urine samples were taken as routine testing which was a non inversion procedure.

Inclusion criteria: Urine samples from patients with UTI and clinical symptoms and cell count of more than 8 pus cells per high power field by wet mount.

Exclusion criteria: Urine samples of patients who were already on antibiotic treatment.

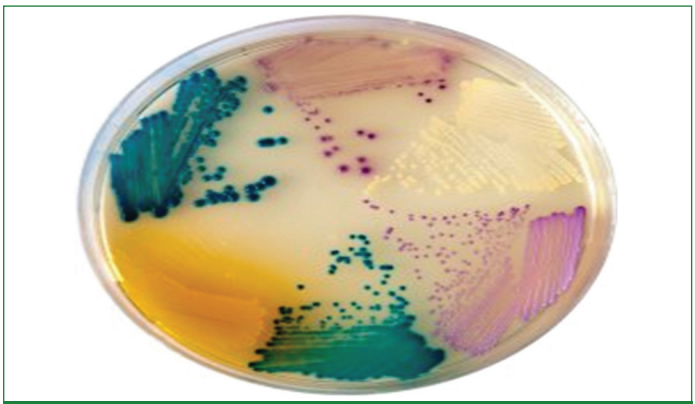
Sample size: A total of 126 samples were collected from patients admitted in hospital and subjected for wet mount, 56 samples did not showed any pus cells and so were excluded for culture and total 70 samples were included in the study.

Sample collection: Midstream clean catched urine samples were collected and samples were processed without delay. Direct plating on ChromID ESBL agar was performed in parallel on to MacConkey agar and nutrient agar. The isolates from these media were identified using various biochemical reactions [8] and then were subjected to screening test for ESBL production and positive screened isolates were subjected to confirmatory test for routine confirmation of ESBL production.

Identification of ESBL- producing isolates: Chrom ID ESBL agar is a rich nutrient medium with a mixture of antibiotics, including cefpodoxime which is recognised as being the marker of choice for this resistance mechanism. After inoculation on both media all culture plates were interpreted. Any colored colonies on ESBL – Bx (bioMerieux) were considered as presumptive ESBL producers. For Chrom ID ESBL, the colour and intensity of the colonies was recorded according to the colour chart provided by the manufacturer. All media were incubated at 37°C for 18-24 hours [Table/Fig-1].

Escherichia coli- pink /burgundy

Klebsiella/Enterobacter/Serratia- blue /green.



[Table/Fig-1]: Showed ChromID – ESBL agar showing ESBL producing colonies of *Escherichia coli* (2 samples) (pink to burgundy coloration), *Klebsiella* spp (2 samples) (green to blue coloration) and *Proteus* spp (1 sample) (light brown coloration) and *Acinetobacter* spp (1 sample) (cream color).
Total six samples inoculated on one plate sir out of which 2 were *E.coli*, 2 were *Klebsiella* spp, 1 was *Proteus* spp, and 1 was *Acinetobacter* spp

Proteus spp.- light to dark brown.

Acinetobacter spp.- cream colour

Then the colonies was subjected (those screened positive) for antibiotic susceptibility testing of ceftazidime and cefotaxime and confirmation of ESBL production by DPT using CLSI method [9,10]. The isolates from MacConkey agar and nutrient agar were identified using various biochemical reactions and then were subjected to screening test (Kirby-Bauer disc diffusion test showed evidence of resistance that zone of inhibition of <22 mm with ceftazidime and cefotaxime) for ESBL production and the screened positive isolates were subjected to confirmatory test.

Screening test for screened negative isolates on CHROMagar by Kirby Bauer disc diffusion test: Isolates from MacConkey agar and CHROMagar seperately prepared in suspension equivalent to 0.5 MacFarland standards were used for antibiotic susceptibility testing with following antibiotic discs. The test was conducted in accordance with Kirby-Bauer disc diffusion method. Zones of inhibition were interpreted according to CLSI guidelines [9]. Controls were utilised as endorsed by CLSI.

Isolates which had diameter of zone of inhibition of <17 mm with cefpodoxime and <22 mm with ceftazidime and cefotaxime were suspicious for producing ESBL and thus subjected to confirmatory test by DPT [Table/Fig-2].

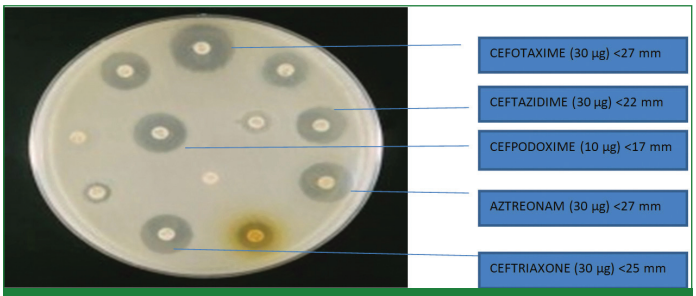
Cefpodoxime [10 µg) <17 mm

Ceftazidime [30 µg) <22 mm

Aztreonam [30 µg) <27 mm

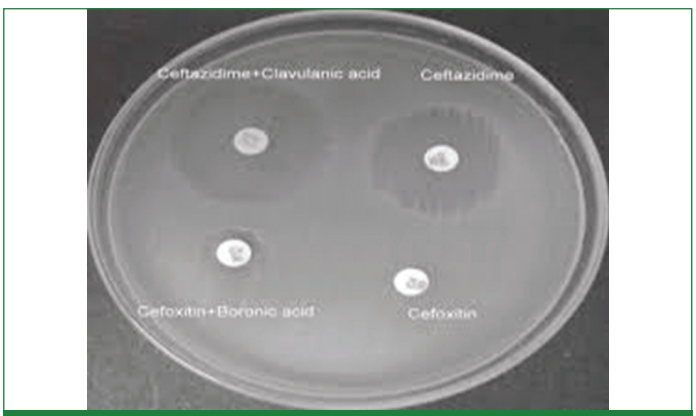
Cefotaxime [30 µg) <27 mm

Ceftriaxone [30 µg) <25 mm



[Table/Fig-2]: Showed Enterobacteriaceae suspicious for producing ESBL.

Confirmation test [10] by DPT: Muller Hinton agar was inoculated with an overnight culture of test strain, previously adjusted to 0.5 McFarland standard turbidity using broth or saline according to CLSI recommendations [9], ceftazidime and ceftazidime-clavulanic acid discs were placed at a distance of 10 mm from one another in the centre of the plate. The plates were examined after 18-24 hours of incubation at 37°C. The organism is said to be ESBL producer when the zone of inhibition around the ceftazidime- clavulanic acid disc is more than 5 mm compared to zone of inhibition around ceftazidime disc alone [Table/Fig-3].



[Table/Fig-3]: Showing disc potentiation test: ESBL positive Enterobacteriaceae.

Escherichia coli ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as the control strains obtained from the department.

STATISTICAL ANALYSIS

The SPSS Statistical package version (18.0) was used. The data was entered in Microsoft excel and results were expressed in terms of frequency and percentage.

RESULTS

Total 56 (80%) isolates were obtained from 70 urine samples. Out of them 28 (50%) were *Escherichia coli*, 21 (37.5%) were *Klebsiellla* spp., 7 (12.5%) were *Proteus* spp. 23 (82.14%) of *E. coli*, 71.43% of *Klebsiella*, 85.71% of *Proteus* spp. isolated were screened positive using chrom ID ESBL agar and 44 (78.57%) of total Enterobacteria isolated were screened positive for ESBL production (by chrom ID ESBL agar) [Table/Fig-4].

Organism	Total isolates	Positive	Negative
E. coli	28	23 (82.14%)	5 (17.86%)
Klebsiella spp.	21	15 (71.43%)	6 (28.57%)
Proteus spp.	7	6 (85.71%)	1 (14.23%)
Total	56	44 (78.57%)	12 (21.43%)

[Table/Fig-4]: Screening for ESBL production using chrom ID ESBL agar.

86.96% of *E.coli*, 73.33% of *Klebsiella* and 83.33% of *Proteous* spp. that were screened positive using chrom ID ESBL agar were confirmed as ESBL producers [Table/Fig-5].

Organism	ESBL positive	ESBL negative	Total
E. coli	20 (86.96%)	3 (13.4%)	23
Klebsiella spp.	11 (73.33%)	4 (26.67%)	15
Proteus spp.	5 (83.33%)	1 (16.67%)	6
Total	36 (81.82%)	8 (18.18%)	44

[Table/Fig-5]: Confirmation of presumptive ESBL positives [on chrom ID ESBL medium) by disc potentiation test.

2 (16.6%) of total isolates that were screened negative by chromID ESBL were confirmed as ESBL producers when screened and confirmed using CLSI guidelines. 36 (81.8%) of total isolates that were screened positive by chrom ID ESBL were confirmed as ESBL producers when screened and confirmed using CLSI guidelines [Table/Fig-6]. Sensitivity and specificity of CHROMagar was 94.73% and 55.5% repectively.

Organism	ESBL positive	ESBL negative	Total
E. coli	2	3	5
Klebsiella spp.	0	6	6
Proteus spp.	0	1	1
Total	2	10	12

[Table/Fig-6]: Confirmation of presumptive ESBL negatives [on chromID ESBL medium) by disc potentiation test.

DISCUSSION

UTI are most common hospital acquired infections and are life threatening with drug resistant microorganisms due to ESBL production. Enterobacteriaceae group of organisms are most important causative agents of drug resistant UTIs. UTIs are a serious threat to human health and affecting millions of people every year results in high morbidity and mortality [1]. So the present study highlights that early detection of ESBL producing enterobacters from urine samples by directly inoculating on new Chrom ID agar can give the quick report to the patient. Methods to detect ESBL producing organisms from clinical samples should have high sensitivity and specificity [2].

The most common isolates in the present study were *Escherichia coli* (50%), followed by *Klebsiella* (37.5%) and *Proteus* (12.5%) which was similar to study done by Ahmed NF et al., [3] (*E.coli*-48.3%, *Klebsiella* 10%, *Proteus*-11.7%) and also with the study of Sharmin S et al., [4]. In the present study, 78.57% of Enterobacteria were screened positive for ESBL production using Chrom ID agar when compared to study of Prabha R et al., [1] where 35% of ESBL producers, study of Glupczynski Y et al., [2] (29.7% of ESBL producers), study of Blane B et al., [11] (39% of ESBL producers) and 35% of ESBL producers were seen in the study of Uyanga FZ et al., [12] so more percent (78.5%) of Enterobacteria were screened positive for ESBL production when compared to the other studies.

In the present study 86.96% of *E.coli*, 73.33% of *Klebsiella* and 83.33% of *Proteus* spp. that were screened positive using chrom ID ESBL agar were confirmed as ESBL producers. So 81.82% of ESBL producers were confirmed positive with DPT correlating with the study of Teklu DS et al., [10] (84.5% of screened ESBL positive Enterobacteria were confirmed as ESBL producers) and two of total isolates that were screened negative by CHROMagar were confirmed as ESBL producers when screened and confirmed by CLSI guidelines. So sensitivity is 94.73% and specificity is 55.5%.

Sensitivity (94.73%) in the present study coincide with the study of Ahmed NF et al., (91.7%) [3], Glupczynski Y et al., (97.7%) [2], Tuchilus C et al., (97%) [6], and Uyanga FZ et al., (98%) [12].

Specificity (55.5%) in the present study correlating to study of Tuchilus C et al., [6] (66%) and lesser than (may be due to other mode of resistance mechanism in enterobacteria, regional variation and concerning the low specificity, all suspected ESBL producing strains should be verified with additional tests.) the study of Glupczynski Y et al., (89%) [2], Ahmed NF et al., (100%) [3].

In current study, ESBL producers were isolated more from inpatients were 86% (samples collected before the use of antibiotics) whereas, in the study of Davoodabadi A et al., [13], 68.16% of urinary isolates of *Escherichia coli* were from communities.

Chrom ID ESBL medium permitted the differential growth of many with ESBL and carbapenemases and is a potential medium to detect many other resistant bacteria [14]. In recent studies, HiCromUTI agar is also using as a primary urine culture media [15].

CHROMagar TM Serratia Chromogenic medium is also available detection of carbapenemases producing *Serratia marcescens* and give an advantage of its implementation in nosocomial outbreaks [16]. The highest sensitivity showed that chrom ID ESBL agar is a suitable method for making provisional diagnosis of drug resistant enterobacterial infections in 24 hours. Concerning the low specificity, all suspected ESBL producing strains should be verified with additional tests.

Limitation(s)

One of the limitation of CHROM agar included lack of difficulty in distinguishing *Candida* species and other enteric microbes like *Shigella* spp. and small sample size of the study.

CONCLUSION(S)

ESBL continues to become a serious public health threat and accounts for high morbidity and mortality. Results from this study showed that CHROMagar ESBL has a high sensitivity and a convenient method for making provisional diagnosis of drug resistant Enterobacterial infections in 24 hours and so appropriate treatment can be given to the patients by which we can reduce the drug resistant strains.

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REFERENCES

[1] Prabha R, Easow JM, Swapna M. Phenotypic detection of Extended Spectrum Beta- Lactamase producing uropathogens using DDST, PCT, Chrom agar and E-test- A comparative study. Int J Curr Microbiol App Sci. 2016;5 (4):565-77.

[2] Glupczynski Y, Berhin C, Bauraing C. Evaluation of a new selective chromogenic agar medium for detection of Extended Spectrum Beta – Lactamase producing Enterobacteriaceae. J Clin Microbiol. 2007;45(2):501-05.

[3] Ahmed NF, Ziada KW, Mohammad TAH, Abdelkader WF. Evaluation of ChromID CPS for isolation & identification of urinary tract pathogens.QJM Int J Med. 2018;3(1):119.

[4] Sharmin S, Alamgir F, Begum F, Jaigiridar QH. Use of chromogenic agar media for identification of uropathogens, Bangladesh. J Med Microbiol. 2010;04 (01):18-23.

[5] 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):449-79.

[6] Tuchilus C, Poiata A, Buiuc D. Chromogenic medium for detection of Enterobacteria producing beta-lactamases in North-Eastern Romania. Rev Med Chris Soc Med Nat Iasi. 2010;114 (2):522-25.

[7] Réglier-Poupet H, Naas T, Carrer A, Cady A, Adam JM, Fortineau N, et al. Performance of chrom ID ESBL, a chromogenic medium for detection of Enterobacteriaceae producing Extended Spectrum Beta – Lactamases. J Med Microbio. 2008;57 (3):310-15.

[8] Mackie & McCartney, Practical Medical Microbiology. Churchill Livingstone, 1996. 14th edition, page-152.

[9] Performance Standards for Antimicrobial Susceptibility testing, volume, 26th edition, CLSI supplement M100s. Wayne, PA: Clinical and Laboratory Standard Institute (2016).

[10] Teklu DS, Legese MH, Eshetu K, Negeri AA, Fentaw S, Addis T, et al. Comparision of double disc synergy test and combination disc test, methods for detection of ESBL production among Enterobacteriaceae E. C Microbiology. 2019;15 (6):411-20.

[11] Blane B, Brodrick HJ, Gouliouris T, Ambridge KE, Kidney AD, Ludden CM, et al. Comparision of 2 chromogenic media for the detection of ESBL producing Enterobacteriaceae stool carriage in nursing home residents. Diagn Microbiol Infect Dis. 2016;84 (3):181-83.

[12] Uyanga FZ, Ekudayo EO, Nawnkwo EO, Inimfon AI. Evaluation of CHROM agar ESBL and double disc synergy test for screening of ESBL producing uropathogens in South – South Nigeria, JAMB. 2019;17(4):01-11.

[13] Davoodabadi A, Farahani A,Ranjbaran M, Antibiotic resistance in *E.coli* strains isolated from urine of inpatients and outpatients. Zahedan. J Res Med Sci. 2012;14(8):95.

[14] Romo-Ibáñez Á, Calatrava-Hernández E, Gutiérrez-Soto B, Pérez-Ruiz M, Navarro-Marí JM, Gutiérrez-Fernández J, et al. High clinical impact of rapid susceptibility testing on CHROM ID ESBLR medium directly from swabs. Ann Transl Med. 2020;8 (9):604.

[15] Khalid M. Comparision of Chromogenic (HiChrom Urinary Tract infection agar) medium with cysteine lactose electrolyte deficient agar in a resource-limited setting. Int J Appl Basic Med Res. 2021;11(1):09-13.

[16] Perez-Viso B, Aracil-Gisbert S, Coque TM, Campo RD, Ruiz–Garbajosa P, Canton R, et al. Evaluation of CHROMagar™-Serratia agar, a new chromogenic medium for the detection and isolation of Serratia marcescens. Eur J Clin Microbiol Infect Dis. 2021;40 (12):2593-96.

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