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Comparison of CHROMagar Orientation versus CLED (cystine-lactose-electrolyte-deficient) Agar, VITEK-XL and MALDI-TOF in a Tertiary Laboratory Setting Processing Urine Culture Samples at Dr. *Lal Path Labs*, Delhi

By Puneeta Singh, Shalabh Malik & Vandana Lal

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Strictly as per the compliance and regulations of:

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Conclusion: CHROMagar orientation was found useful as a primary urine culture medium in both higher rate of isolation and presumptive identification of uropathogens and use as a replacement of conventional CLED agar. It would improve the detection rate of contaminated urine samples to enhanced identification that helps to distinguish species, facilitating the monitoring of bacterial resistance in support of the national antibiotic strategy.

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I. Introduction

Urinary tract infections (UTIs) are the second most common infections, only after respiratory tract infections. Conventionally, Blood agar (BA), Mac Conkey agar (MAC), and Cysteine Lactose Electrolyte Deficient (CLED) medium used routinely for processing of urine samples[1]. Several chromogenic media are now available, which are used to allow more specific and direct differentiation of bacterial colonies on the primary plate itself [1-9]. The following study conducted to evaluate the advantages of CHROMagar orientation over isolation of most common urine isolates (*E.coli, Enterococcus faecalis, E. faecium, Staphylococcus aureus, streptococcus sps Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Proteus mirabilis* and *Enterobacter species& Candida species*) represent a global threat to human health[2-6]. Urine samples contribute greatly to the daily workload of a microbiology laboratory, CHROMagar orientation has the advantage of being technically simple, rapid and cost-effective method for the diagnosis of urinary tract infections as compared to the conventional methods [6,9].

In our lab continually, we strive to streamline and improve their urine culture algorithms because we received high volumes of urine specimens and the modest numbers of different species of bacteria that are ultimately considered clinically significant. In the current study, we quantitatively measured the impact of CHROMagar orientation media used as tools in the early differentiation and identifying of bacterial isolates from urine specimens. We have evaluated the CHROMagar orientation, a newly introduced chromogenic medium, for its utility as primary isolation and identification medium for correctly identify more-frequently occurring bacteria and yeasts organism groups on primary culture with no further testing or a minimum number of confirmatory tests. Substrates present in chromogenic

media target specific classes of enzymes produced by certain bacteria and yeasts [6].

CHROMagar orientation media may facilitate improved sensitivity of identifying of some Gram-positive cocci (e.g., *Enterococci*) in mixed cultures with *Enterobacteriaceae*. They may promote the uniform interpretation of urine culture plates by less experienced bench technologists [4]. The purpose of the current study for implementing CHROMagar orientation could be realized by use as the primary medium for urine culture and reduce workload of test, turnaround time, and labor costs.

II. Material and Methods

An evaluation of two commercial media undertaken using isolates of known identity to assess the level of accuracy of presumptive identification. Subsequently, an assessment of the two best-performing media in our laboratory adopting a standardized protocol to determine isolation rates and detect mixed cultures. The study was conducted at *Dr. Lal Path Labs* largest clinical microbiology laboratory in North India, which collectively processes approximately 500,000 urine specimens per year.

a) Media preparation, inoculation, and incubation

CHROMagar orientation (CO) (CHROMagar company, Paris, France) and CLED agar (Hi-Media Laboratories Pvt. Ltd. Mumbai-400086, India)were obtained as a dehydrated powder form. All culture petri plates were prepared in house by following manufacturer's instructions and recommendations. Every fresh batch of media was tested for its ability to support the growth of *Escherechia coli* ATCC (25922) to ensure the quality of the media. Urine samples were inoculated onto CLED agar and CO medium plates using a calibrated 0.001-ml loop and streaked manually. The inoculated plates (CLED agar or CO medium) were incubated at 37°Cover night (18-24 hrs) and examined at the intervals of 6hrs 12hrs, 18hrs 24hrs, and 48 hrs. Samples showing significant bacterial growth were further recorded. This study was carried out in the Department of Microbiology, at Dr. Lal path Labs, Delhi from 1st November 2020 to 31st January 2021. In total, 2,550 routine urine samples (predominantly in boric acid) received in our laboratories during a three months in 2020-2021, from both hospital and general practice, were included in the study.

b) Plate reading

CHROMagar media utilize synthetic chromogenic enzyme substrates to specifically target pathogenic species (or groups of species) based on their enzyme activity. Such enzyme activity is never completely species-specific, necessitating complementary enzyme substrates and selective agents.

For the purpose of our study, plates were recorded according to colonial morphology. The numbers of each colony type were also recorded to support the evaluating of the contributing organism counts of mixtures. The organism obtained from the CHROMagar orientation agar media was of different colors. *E.coli* gives dark pink to reddish color colony, *Klebsiella, Enterobacter, Citrobacter* → metallic blue*Proteus* → brown halo, *Pseudomonas* → greenish translucent, *Acinetobacter baumanii* →cream, round translucent, bacterial isolates *S. aureus* → golden, opaque, small, *S. saprophyticus* → pink, opaque, small However, MALDI-TOF techniques were used to confirm the identification of organism at species level of yeast and bacterial isolates.

c) Presumptive identification

Presumptive identification of bacterial growth was done on CHROMagar orientation agar according to colony morphology and colour as depicted by the manufacturer (Figure1, 2) whereas when using CLED agar plates other tests and procedures were often required to differentiate between organisms. The final identification of the isolates was done using standard identification protocol such as VITEK -2XL (Biomerieux, France) and MALDI-TOF (Bruker Daltonics) as appropriate for the isolates.

d) Statistical methods

For the study, data were collected and entered into an Excel spreadsheet.

III. Results

The present study undertaken to validate the usefulness of CHROMagar orientation UTI agar as a primary urine culture medium for its rate of isolation and presumptive identification of uropathogens in comparison to CLED in a *Dr. Lal Path Labs.* Out of the 2550 urine samples processed, 587samples were positive (23.1%) and 1963 samples (76.9%) were negative.

Among the 587 positive samples Escherichia coli was the predominant Gram-negative isolate and *Enterococcus faecalis* was the predominant Gram-positive isolate. This study included (587) positive isolates consecutively collected from both male and female population aged 0-100 midstream and/or catheter catch urine samples obtained from patients having bacteriuria in urinary tract infection. Based on data extracted from our Laboratory Information System from 2019- 2020, the use of CHROMagar orientation medium resulted in a 28% reduction in workload for additional procedures such as Gram stains, subcultures, identification panels, agglutination tests, and biochemical tests and MALDI-TOF.

In the present study, CHROMagar Orientation was evaluated as a direct isolation medium for clinical

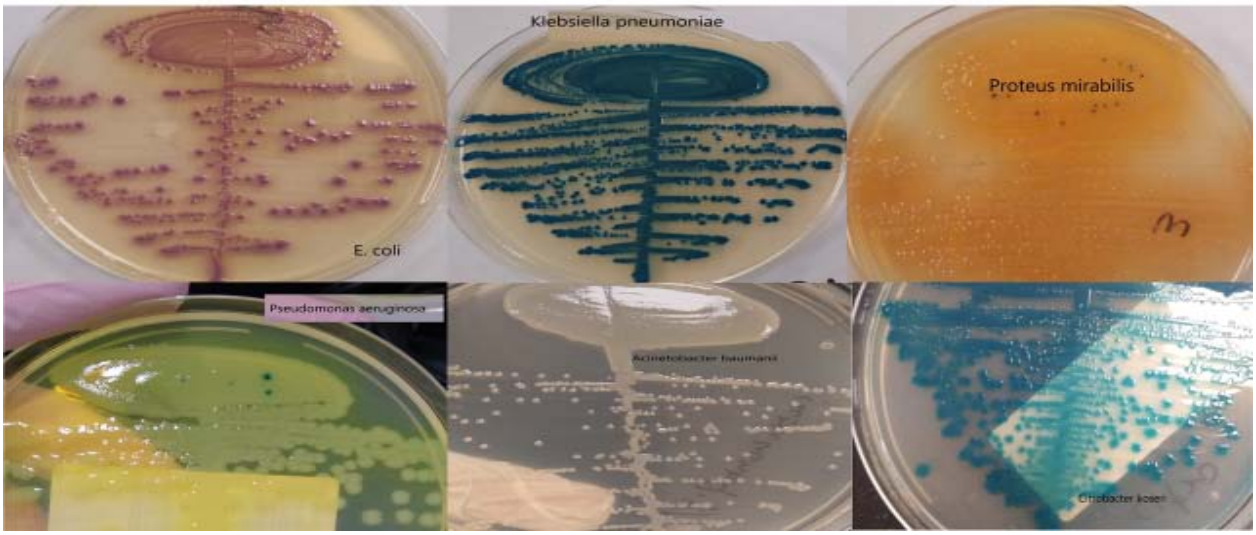
specimens. 587 positive urine samples were tested by parallel inoculation on CHROMagar Orientation and on other reference media, CLED agar.

The analysis of the data obtained from CLED, CHROMagar Orientation agar for the detection of different bacteria, result indicated that the growth pattern of the uropathogens were different. It could be due to the different constituents and properties of the media. From the study, it observed that the growth of organism over the media was according to the characteristics of the media. Mixed cultures were differentiate easily on CHROMagar orientation. On CLED agar lactose fermenting organism grows which gives yellow color colonies. However, The overall impression of the color changes produced on CHROMagar orientation media by *E. coli* (pink-red) which was the predominant species (32.5%). All these isolates grew on CHROMagar Orientation in reddish colonies and were very easy to distinguish. Since E. coli is responsible for many of the UTI in nosocomial patients *Klebsiella* spp., (blue) and the *Acinetobacter* spp. should be added to the list of gram-negative microorganisms that can be presumptively differentiated directly on CHROMagar Orientation. They grew in nontransparent, white, entire-edge colonies. These strains were very distinct from *Pseudomonas* isolates, which grew in diffuse, yellow-to-green colonies with serrated edges that they were distinct and easy to perceive. Similarly, tryptophan is also present in the medium to detect members of the Proteus group, which generates a diffuse brown coloration background because of tryptophan deaminase production.

In the study gram positive bacteria were also isolated as one chromogenic substrate cleaved by ß-glucosidase possessed by *Enterococci* resulting in formation of turquoise colonies and *S.aureus* gives golden yellow color colonies.

The results of the study to CHROMagar Orientation differentiate the most commonly encountered gram-negative pathogens gram-positive and fungal uropathogens because of color and morphology alone compared to CLED agar. CHROM agar supported the growth of all common routine urinary isolates can be recommended as a primary plating medium for recovery of uropathogens and the ease of distinguishing when multiple probable pathogens were present (Figure1).

Different Gram-negative bacterial isolates on CHROMagar orientation isolated from urine culture



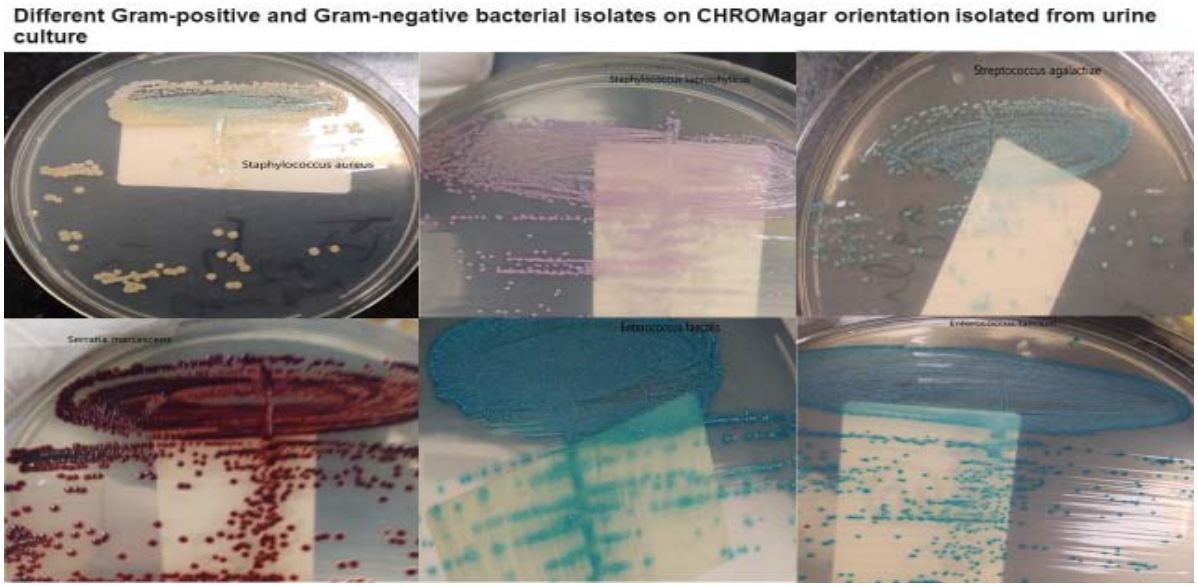


Figure 1: Growth of Uropathogens isolated from urine culture on CHROMagar orientation media

Comparison between CHROMagar Orientation media, CLED agar VITEK 2-XL and MALDI-TOF used for the identification of uropathogens: Patterns of 587 culture-positive samples yielded different bacterial isolates including 491 single and 96 (two bacteria in each plate account for polymicrobial growths from urine culture shown in Table1 and Table 2 respectively.

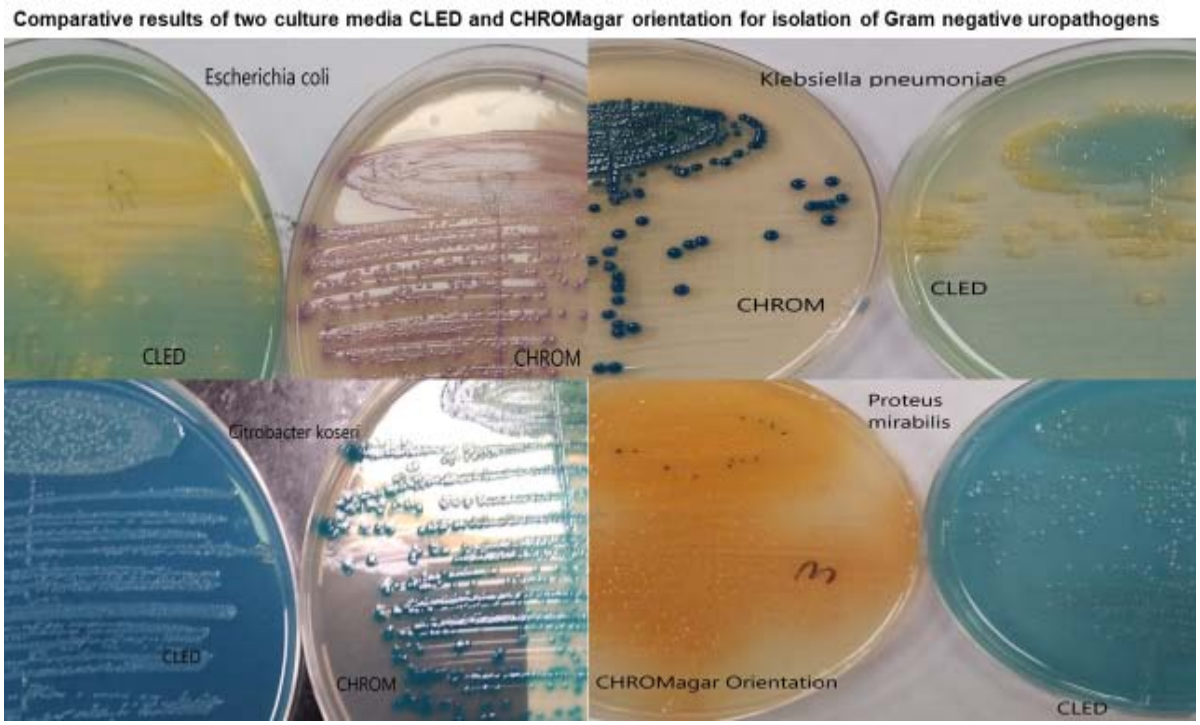
For presumptive identification of bacterial species by colony characteristics on primary culture plate, of 491 bacterial and yeast isolates, 491(100%), 488(99.4%), 484 (98.5%) and 388(79%) could be differentially identified on MALDI-TOF, Vitek2-XL,CHROMagar Orientation and CLED agar respectively. The rate of presumptive identification of the isolates was found significantly higher on CHROMagar Orientation agar than CLED agar as primary urine culture medium (Table 1; Figure 2). E. coli was the leading bacteria isolated from 171 (34.8%) samples followed by *Klebseilla pneumoniae* 89 (18.1%), *Enterococcus* spp. 73 (14.8%), *Pseudomonas aeruginosa* 54 (10.9%), *Acinetobacter spp.* 21 (4.3%), *Staph.aureus* 16 (3.3%), *Proteus mirabilis* 13 (2.6%), *Candida spp.* 13 (2.6%), *Enterobacter spp.* 9 (1.8%), *Staph. saprophyticus* 11 (2.2%), and *Streptococcus agalactiae* 6 (1.2%) respectively.

Presumptive identification of mostly gram-negative and gram-positive common uropathogens such as *E.coli, K.pneumoniae, Proteus, Pseudomonas, Morganella morganii,* and *Enterococci* spp. was correct on the CHROMagar media. *E. coli* was correctly identify in 99 to 100% of the cases. 4-5 of total 54 isolates of *Pseudomonas aeruginosa* were not correctly presumptively identify on the CLED media. Six of *Citrobacter* spp., 9 of *Enterobacter* spp. isolates presumptively misidentified as *E. coli* on the CLED agars. The colony appearance of *Serratia* on the chromogenic media was either Red in 4 of the 9 isolates and 5 strains from the typical colony appearance of the *Klebsiella- Enterobacter-Serratia* group (i.e., blue, mucoid) as described by the manufacturers. The overall impression of the color changes produced on chromogenic media by *E. coli, Enterococci, Klebsiella* spp., *Serratia* spp., and the *Proteus-Morganella-Providencia* group that are distinct and easy to perceive. All the isolates of *Enterococcus faecalis and E. faecium* correctly identified at genus level and were easily distinguished from *Streptococcus agalactiae* isolates. *Staphylococcus saprophyticus* isolates were easy to identify only on the CHROMagar orientation medium whereas in CLED agar *S. saprophyticus* and *E. faecalis* have shown same colony characteristics (Figure 2).All of the gram-positive isolates were misidentified on CLED agar.

In this study, a total 21isolates of *Acinetobacter* spp. we presumptively identified 18 isolates of *Acinetobacter baumanii* on CHROM agar whereas species level differentiation of *Acinetobacter spp* were showed difficulty in CHROMagar. Similarly remaining 3 isolates of *Acinetobacter* spp. were identify as *A. junii* (2), and *A. iwofii* (1) by MALDITOF however, in CLED agar *Acinetobacter spp* were poorly identified. The identification results obtained from the Vitek-2XL system were not consistent with those from the MALDI-TOF for few Candida spp. Furthermore, identification results of 10 Candida spp. isolates from the MALDI-TOF system were the same as those from the Vitek 2 system (data not listed). In this study, we evaluate the identification performance of MALDI-TOF MS for identification of enteropathogens and yeast isolates with a lower identification error rate, MALDI-TOF MS has better performance than VITEK 2 in identifying yeast found routinely in the clinical laboratory.

Table 1: Comparison of two culture media, VITEK 2-XLand MALTI-TOF for the Rate of identification of Uropathogens

Uropathogens N=491	CHROMagar orientation N=484 (98.6%)	MALDI-TOF Identification N=491(100%)	VITEK-XL identification N=488 (99.4%)	CLEDagar N=388 (79%)
Escherichia coli (171)	171 (100%)	171 (100%)	171 (100%)	169 (98.8%)
Klebsiella pneumonia (89)	89 (100%)	89 (100%)	89 (100%)	87 (97.8%)
Proteus mirabilis (13)	13 (100%)	13 (100%)	13 (100%)	13 (100%)
Enterobacter spp. (9)	9 (100%)	9 (100%)	9 (100%)	0 (0%)
Citrobacter koseri (6)	6 (100%)	6 (100%)	6 (100%)	0 (0%)
Pseudomonas aeruginosa (54)	54 (100%)	54 (100%)	54 (100%)	49 (90.7%)
Acinetobacter spp. (21)	18 (85.7%)	21 (100%)	21 (100%)	9 (42.8%)
Serratia marcescens (9)	9 (100%)	9 (100%)	9 (100%)	4 (44.4%)
Enterococcus faecalis (52)	52 (100%)	52 (100%)	9 (100%)	43 (58.9%)
Enterococcus faecium (21)	21 (100%)	21 (100%)	21 (100%)	9 (42.8%)
Staphylococcus aureus (16)	16 (100%)	16 (100%)	16 (100%)	0 (0%)
Staphylococcus saprophyticus (11)	11(100%)	11 (100%)	11 (100%)	0 (0%)
Streptococcus agalactiae (6)	6 (100%)	6 (100%)	11 (100%)	0 (0%)
Candida spp.(13)	9 (69.2%)	13 (100%)	10 (76.9%)	5 (38.4%)



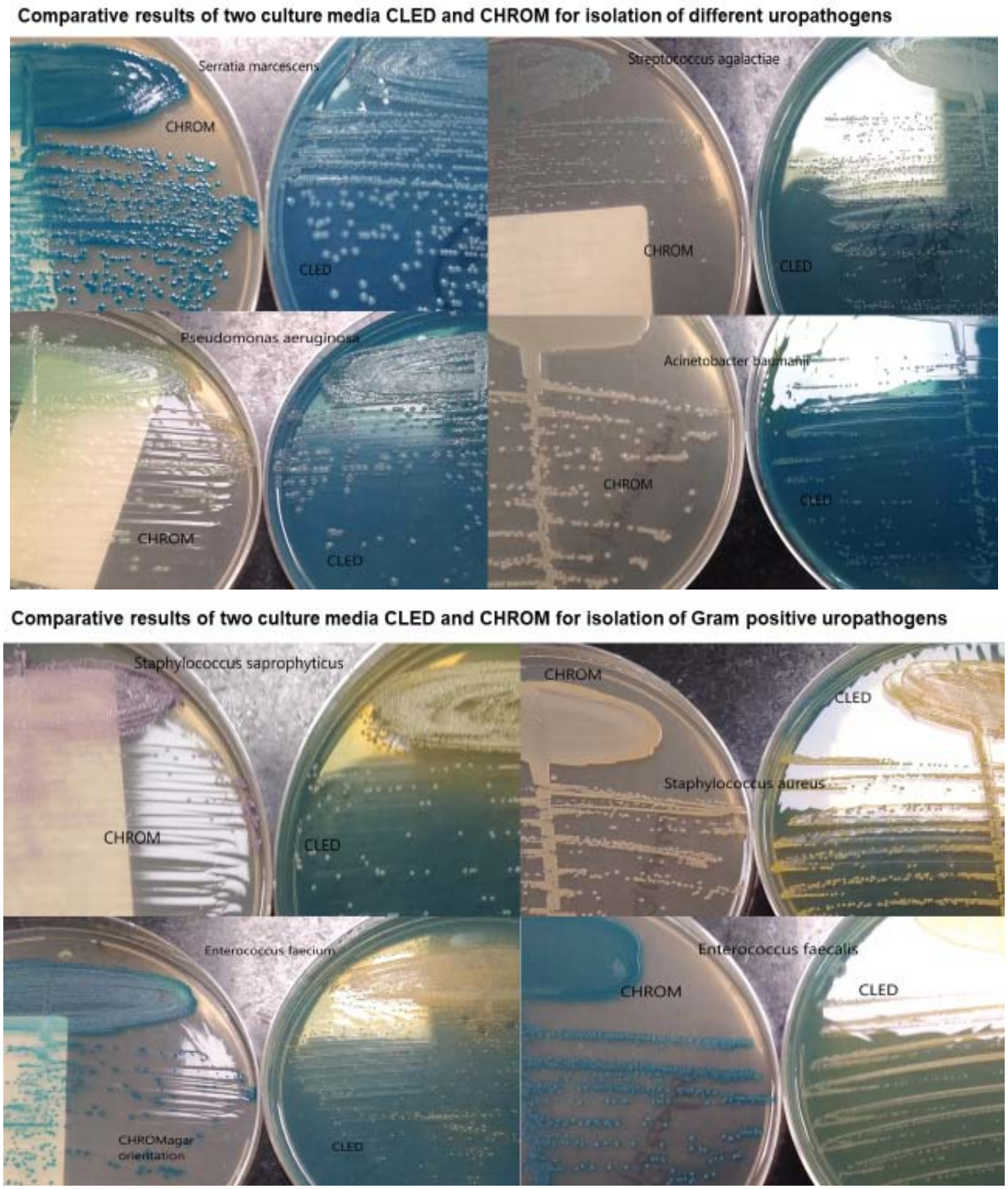


Figure 2: Comparison between CLED and CHROMagar Orientation media used for the isolation of uropathogens.

Table 2 Shows the rate of presumptive identification of polymicrobial growth in different culture media. All 139 (100%) polymicrobial growths distinctly identified only on Chromagar Orientation agar medium, in contrast except in a single case consisting of *E. coli* and *Proteus mirabilis,* no mixed bacterial growths could be identified on CLED agar media. The detection of Gram positives and yeasts organisms diminishes in the presence of increasing numbers of Gram-negative organisms, because of the white or colorless appearance of the colonies on the CHROMagar orientation media for Gram-positive organisms and yeasts, CHROMagar performed better than other UTI medium such as CLED (Table.2).

In our study, CHROMagar showed a superior differentiation of mixed cultures because different species may generate colonies with different colors and may not easily differentiate on conventional agars. *Enterococci* spp. and *S.aureus* presumptively identified (Figure 3) on the CHROMagar and were not in CLED agar.

Table 2: Comparison of Rate of Isolation of polymicrobial Uropathogens on CHROMagar Orientation and CLED culture media

Uropathogens N=96 (16.4%)	CHROMagar orientation N=96 (100%)	CLED agar N =74 (77%)
E. coli and *K. pneumoniae* (23)	23(100%)	22(95.7%)
E coli and *Enterococcus spp*. (29)	29 (100%)	17 (58.6%)
K. pneumoniae and *Pseudomonas aeruginosa* (12)	12 (100%)	11 (91.6%)
E.coli and *Pseudomonas aeruginosa* (19)	19 (100%)	18 (94.7%)
Proteus mirabilis and E.faecalis (3)	3 (100%)	0 (0)
Proteus mirabilis and E.coli (6)	6 (100%)	6 (100%)
Staphylococcus aureus and E. coli (4)	4 (100%)	0 (0)

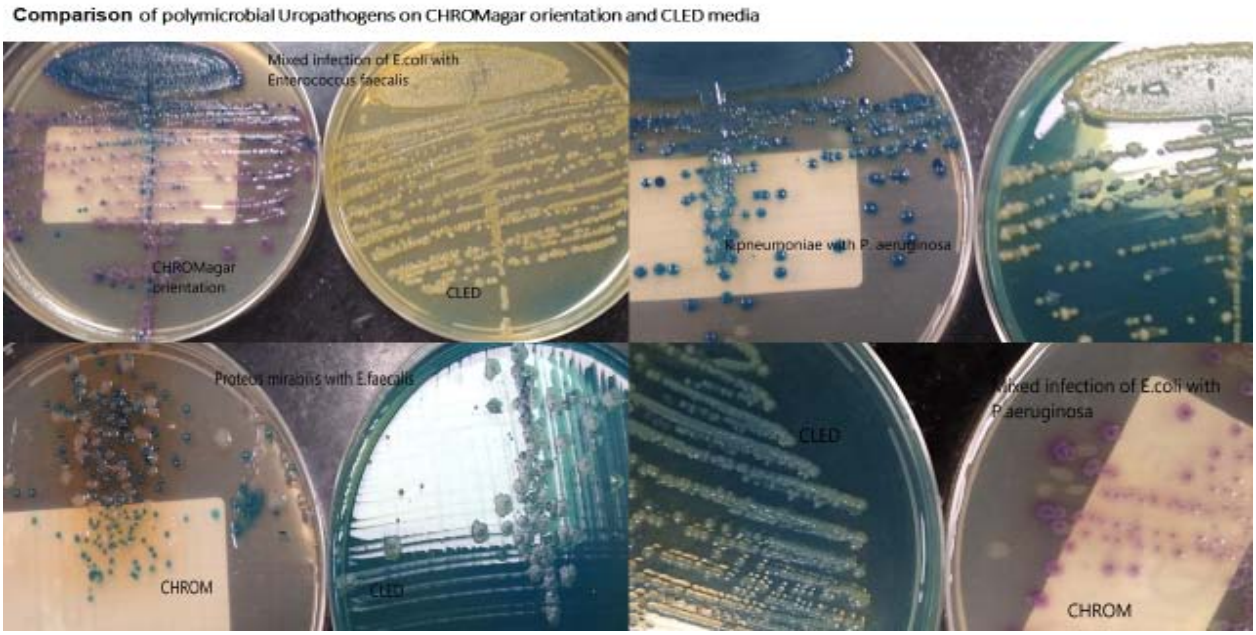


Figure 3: Comparison between CLED and CHROMagar Orientation media used for the isolation of polymicrobial uropathogens

In this study, we evaluated CHROMagar Orientation from The CHROMagar Company [Paris, France] for routine diagnosis of bacteriuria at our laboratory concerning isolation frequency and presumptive identification of urine isolates. CLED (cysteine-, lactose-, and electrolyte-deficient) agar, were used as the reference media. We also compared the interval of 6hrs incubation to 48hrs of incubation; to our knowledge, this has not done previously. The media evaluation were listed in (Table 3).

Table 3: Comparison of culture media for the Rate of presumptive isolation as Primary culture plate of Uropathogens

Incubation period	0-6 hrs		7-12 hrs		13-18 hrs		19-24 hrs	
Bacterial isolates	CLED	CHROMagar orientation	CLED	CHROMagar Orientation	CLED	CHROMagar Orientation	CLED	CHROMagar Orientation
E. coli (n=145)	No growth	No growth	10^2cfu/ml	10^4cfu/ml	10^5cfu/ml	>=10^5cfu/ml	>=10^6cfu/ml	>=10^6cfu/ml
K.pneumoniae (n=85)	No growth	No growth	10^2cfu/ml	10^3cfu/ml	10^5cfu/ml	>=10^5cfu/ml	>=10^6cfu/ml	>=10^6cfu/ml
P. mirabilis(n=13)	No growth	No growth	10^2cfu/ml	10^3cfu/ml	10^5cfu/ml	>=10^5cfu/ml	>=10^6cfu/ml	>=10^6cfu/ml
P.aeruginosa(n=54)	No growth	No growth	10^2cfu/ml	10^3cfu/ml	10^5cfu/ml	>=10^5cfu/ml	>=10^5cfu/ml	>=10^5cfu/ml
Enterococcus spp. (n=73)	No growth	No growth	10^1cfu/ml	10^4cfu/ml	10^4cfu/ml	>=10^5cfu/ml	>=10^4cfu/ml	>=10^5cfu/ml
Acinetobacter baumanii (n=21)	No growth	No growth	10^2cfu/ml	10^3cfu/ml	10^4cfu/ml	>=10^4cfu/ml	>=10^5cfu/ml	>=10^5cfu/ml
Enterobacter spp.(n=9)	No growth	No growth	10^2cfu/ml	10^3cfu/ml	10^3cfu/ml	>=10^4cfu/ml	10^5cfu/ml	>=10^5cfu/ml
Streptococcus agalactiae(n=6)	No growth	No growth	10^1cfu/ml	10^2cfu/ml	10^3cfu/ml	>=10^4cfu/ml	10^5cfu/ml	>=10^5cfu/ml
S.saprophyticus (n=11)	No growth	No growth	10^1cfu/ml	10^2cfu/ml	10^3cfu/ml	>=10^4cfu/ml	10^4cfu/ml	>=10^4cfu/m
S. aureus(n=16)	No growth	No growth	10^1cfu/ml	10^2cfu/ml	10^3cfu/ml	>=10^4cfu/ml	10^4cfu/ml	>=10^4cfu/ml
Candida spp. (n=13)	No growth	No growth	$10^{1/2}$cfu/ml	10^2cfu/ml	10^1cfu/ml	10^3cfu/ml	10^2cfu/ml	>=10^3cfu/ml

According to the technical data, when the total number of isolates recovered from both of the media was compared to the number of isolates growing on the individual media types after an interval of6-48 hours incubation period. The percentage for CHROMagar Orientation media shows approximately 20% high in colony count in 13-18 hours incubation that was evident in the present study. Although incubation longer than overnight (up to 24-48 hours) does not significantly increase the yield of common, urine isolates on CHROMagar orientation or traditional media CLED. In this study, we found that most common gram- negative isolates such as *E.coli, K.pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Acinetobacter spp.* in 0-6 hrs incubation period no growth were seen in both media (Table 3, Figure 4). However, 7-18 hrs incubation period showed that CHROMagar Orientation performing better growth than CLED whereas after 18hrs incubation, there growth pattern were similar in both media. CHROMagar Orientation, performed better growth of Gram-positive isolates in a short incubation period and easily identified after 18 hrs incubation (Table 3). Similarly, CHROMagar Orientation given the best result for isolation of yeast species in 18-24hrs incubation period (Table-3).

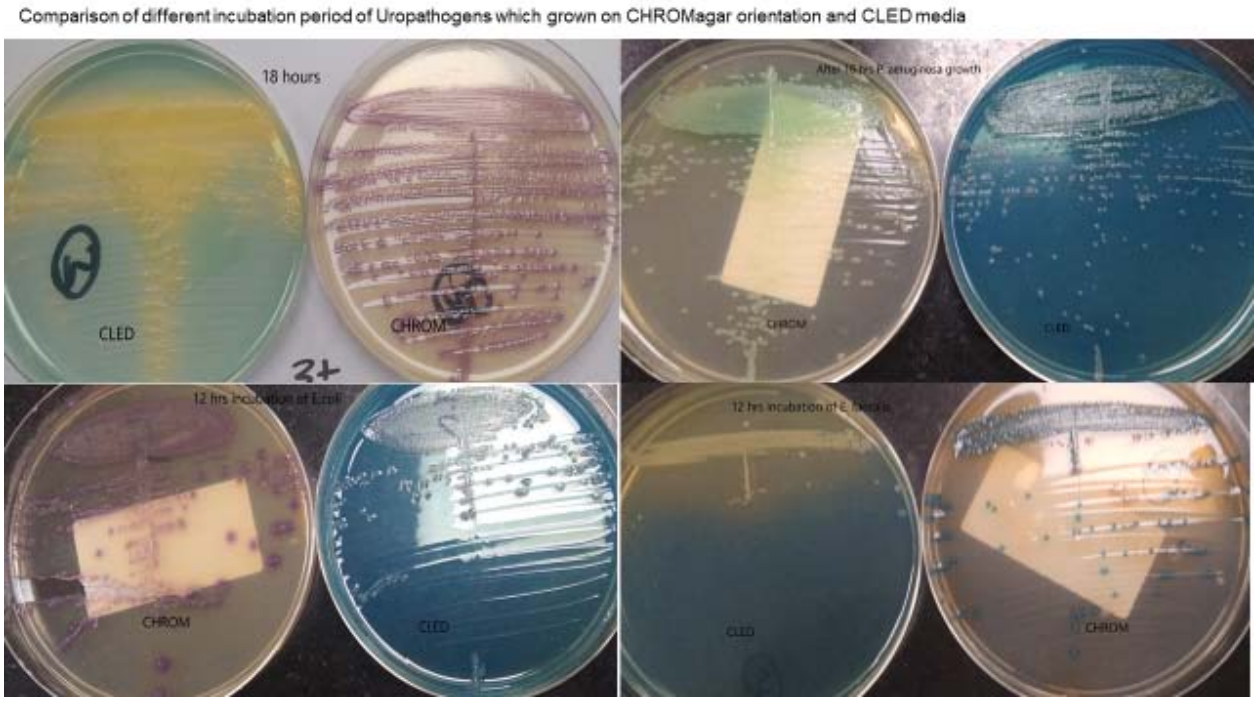


Figure 4: Comparison of incubation period culture media for the Rate of presumptive isolation as Primary culture plate of uropathogens

IV. Discussion

Every clinical microbiology laboratory's daily workload of urine cultures account for a diagnosis of urinary tract infection because only 20 to 30% of urine samples result in significant growth[1,3]. Therefore, any new medium or method with the ability to streamline urine culture processing in a meaningful way, such as reducing technologist workload, improving result turnaround times (TATs), or reducing laboratory costs, would be welcomed and has the potential to have considerable laboratory impact. Our study confirmed the superiority of CHROMagar orientation over CLED agar in detecting mixed cultures, Gram-positive organisms, and yeasts; these results corroborate earlier studies [2-6].

Traditionally conventional media like Blood agar (BA) the majority of urine isolates as an enriched medium but its performance in the identification of bacteria is very deficient. Similarly, differentiation of lactose fermenter and non-fermenter is possible on MAC and CLED agar. Moreover, none of these media singly or in combination can support the growth and identification of possible urine isolates [1,7]. As a result, further species identification necessitates subculture or divergent tests with longer reporting time and cost. The present findings were in concordance with the findings of (Aspevall *et al.,* 2002) observed that the CHROMagar Orientation media tested in this study was better than CLED agar. A similar observation was also reported by (Fallon et al., 2003) using BBL CHROMagar, UTI medium, or CPS ID2 chromogenic agar, as a replacement for Cystine Lactose Electrolyte Deficient agar (CLED) would improve the detection rate of contaminated urine samples. *"A cost comparison of the agars suggests that as the use of chromogenic agar in laboratories increases, the purchase cost is decreasing"* (Fallon et al., 2003)[6].

In the present study, the time interval between plating and final organism identification was decreased on CHROMagar orientation and it was seen that were evident within 18-hours versus CLED using the entire required standard microbiological tests; it was an average of 38 hours. Using CHROMagar orientation, clinically significant cultures required less hands-on time. Similarly in a study by Bajoria et al., concluded that conventional media requires 24-48 hours to give positive results [3]. Articles reported the effect of incubation time on results of urine culture on traditional media [2]. All agree that common urine isolates detected after overnight incubation and that a longer incubation time is required for the detection of yeasts.

Hence, it concluded that the cost comparison of the agars suggests that the use of CHROMagar orientation in laboratories increases, the purchase cost is decreasing due to the needs for repeat samples, and avoided antimicrobial therapy because of improved mixture detection [1,2]. In a few studies comparing CHROMagar Orientation media with traditional ones, its advantages including a 20% reduction in time for identification, reduction in workload [5, 6, 8]. When using traditional media requires a great deal of experience for presumptive identification of isolates, whereas CHROMagar media, is easier, requiring less training and interpreted by personnel with less

experience in microbiology. Thus, the use of CHROMagar Orientation media may improve the quality of urine culture by contributing to a uniform interpretation of urine culture plates by the different personnel engaged in this task at the laboratory. All these factors have a direct impact on ultimate cost reduction. Our data support the findings of these investigators [2-8]. Also, MALDI-TOF MS showed to be simple, rapid, and accurate tool for the identification of enteropathogens and rare yeast species, At the same time the Vitek 2 XL system is a popular commercial method commonly used in clinical microbiology laboratories for bacterial identification.

Most of the isolates analyzed in our study largely commonly found pathogens, and the construction of the MALDI-TOF MS database may offer higher identification accuracies for these pathogens. Additionally, MALDI-TOF MS dramatically shortened identification time from 6-8 hours to just a few minutes. However, MALDI-TOF MS made no errors at the genus and species level while VITEK -2XL made 0.6% errors at the species level of rare yeast species[10, 11].

V. Conclusion

CHROMagar Orientation provided the highest overall organism recovery rates, convenient for rapid identification, and the greatest ability to detect mixed cultures. The use of CHROMagar orientation medium as a replacement for Cystine Lactose Electrolyte Deficient (CLED) agar would improve the detection rate of contaminated urine samples and has the potential to streamline urine culture processing in a meaningful way, such as reducing technologist workload, improving result of turnaround times and reducing costs. It would improve identification that helps to distinguish species, facilitating the monitoring of bacterial resistance in support of the national antibiotic strategy.

Ethical Approval: It is not applicable.

Conflicts of interest: There are no conflicts of interest.

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