Original Article

Use of chromogenic Agar in detection of urinary tract pathogens and antimicrobial Susceptibility

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Summary:

Background: HROM agar Orientation is a chromogenic medium used for the detection and differentiation of Gram's negative and Gram's positive pathogenic microorganisms in urine samples. Evaluation of CHROM agar Orientation for identification of urinary pathogens and susceptibility determinations in comparison to the ordinary media used.

Patients and Methods: A total of 375 midstream urine sample collected from patients with urinary tract infection (UTIs). CHROM agar Orientation, blood agar and macConkey agar media were used for direct inoculation.

Results: CHROM agar Orientation succeeded in detecting all the urine pathogens that were detected by the reference media, and antimicrobial Susceptibility tests were performed directly from primary isolates in all cases without the need for subcultures.

Conclusion: HROM agar Orientation medium excellent detection of urinary pathogens and antimicrobial Susceptibility tests without the need for subcultures. Therefore, can replace the standard primary plating media used in routine diagnosis of urinary tract infection.

Fac Med Baghdad 2009; Vol. 51, No. 1
Received May 2008
Accepted Oct.2008

Key words: UTI, CHROM agar Orientation, urine, antimicrobial Susceptibility

Introduction:

Urinary tract infections (UTI) are among the most common conditions causing individuals to seek Medicare. They are also among the most common bacterial infections in humans, both in the community and hospital settings (1, 2). Urinary tract infections (UTIs) are important because they cause acute morbidity and may result in long-term medical problems, including hypertension and reduced renal function (3). In almost all cases there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTI s and their resistance patterns may help the clinician to choose the right empirical treatment(4). For many years blood and macConkey agars have been used for the detection of Urinary tract pathogens as well as for the differentiation of a few of them (5). In the last few years several chromogenic media have been developed and commercialized, allowing for more specific direct differentiation of Microorganisms on primary plates. A new one, CHROM agar Orientation, offers simultaneous presumptive identification of Gram's positive and Gram's negative bacteria and yeasts on a single medium by means of distinct colony colors produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrate (6, 7). The aims of this study were to evaluate the sensitivity of this medium and its ability to differentiate urinary pathogens. The accuracy of antimicrobic susceptibility testing by standard methods was also tested by picking isolates directly from CHROM agar Orientation.

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Materials and Methods

Study population and Specimens: A total of (375) midstream urine sample collected from outpatients and hospitalized patients (from Al-Khadymia Teaching Hospital).Media Preparation: CHROM agar Orientation was prepared according to the manufacturers instructions (CHROM agar company, France), where (32.4) gm of CHROM agar Orientation powder were dissolved in one liter distilled water then the medium was boiled under continuous stirring then autoclaved for 15 min. The medium then cooled to (45) C° and then dispensed in sterile Petri dishes and allowed to solidify at room temperature. The plates then were stored at (4) C° in a dark container.

Other media used blood agar, macConkey agar and Muller Hinton agar. All were prepared according to the manufacturers directions.

Methods: - After inoculation of the specimens on blood, macConkey and CHROM agar media the plates were incubated at (37) C˚ for (16-24) hours in the dark. - Biochemical test used for identification of Gram's negative bacteria including: catalase, oxidase, indole, MR, VP, Simmons citrate utilization and API reference profile system (Biomerieux, France).
- Biochemical test used for identification of Gram's positive bacteria: catalase and Mannitol Salt agar (8, 9). - Antimicrobial susceptibility of the isolates were tested by the disk diffusion technique according to National Committee for Clinical Laboratory Standards (NCCLs) recommendations (10, 11). The accuracy of antimicrobic susceptibility testing was evaluated by picking isolates directly from CHROM agar Orientation to Muller Hinton agar and comparing the results with those parallel tests of isolates picked from reference media. The gram–positive bacteria were picked from blood agar and the gram – negative were picked from MacConkey agar (6).

Results:
Out of (375) urine samples tested, only (150) specimens revealed growth cultures. The results have also shown that (75.33) % of the UTI were caused by Gram's negative bacteria and (24.66) % Gram's positive bacteria. E. coli (n=50) was found to be the predominant isolates from positive urine sample of the isolates tested , all produced red to pink colonies on CHROM agar Orientation. Enterococcus spp. (n=25) were obtained diffuse blue. Proteus spp. (n=19) resulted in colorless with brown halo. Enterobacter spp. (n=2) and Klebsilla pneumoniae (n=15) demonstrated metallic blue colonies. Pseudomonas aeruginosa (n=21) produced translucent creamy colonies. Acinetobacter spp. (n=4) shown nontransparent entire edges white. Only two isolate of Serratia spp. Were obtained light blue colonies. S. aureus isolate (n=9) gave golden, opaque small white. Finally Streptococcus spp. (n=3) gave turquoise, small. Table (1) Shown the distribution of the different urine pathogen among the (150) positive urine samples and the ability of the media to detect species is given in Table (2). Fig (1). Susceptibilities were obtained in all cases and very few differences between zone diameters (1) to (2) mm were detected. Note that none of these differences were out of the range specified by (NCCLs) for the disk diffusion method of Susceptibility testing. The numbers of the Susceptibilities isolates picked from CHROM agar Orientation were exactly the same as the numbers of those picked from the reference media Table (3) shown Susceptibilities result for Gram's negative isolates. Table (4) shown Susceptibilities result for Gram's positive isolates.

Table (1) the color of colonies on CHROM agar Orientation.

Microorganism	No. of isolates (%)	Description of color and colony morphology
E. coli	50(33.33)	Pink red
Enterococcus spp.	25(16.66)	diffuse blue
Proteus spp	19(12.66)	colorless with brown halo
Klebsilla pneumoniae	15(10)	metallic blue
Enterobacter spp.	2(1.33)	metallic blue
Pseudomonas aeruginosa	21(14)	translucent creamy
Acinetobacter spp.	4(2.66)	nontransparent entire edges white
Serratia spp.	2(1.33)	light blue
S. aureus	9(6)	gave golden, opaque small white
Streptococcus spp.	3(2)	turquoise , small
Total	150(100)	

Table (2) distribution of the isolates among positive urine specimens.

Microorganism	No. of isolates on:		
	CHROM agar Orientation	blood agar	macConkey agar
E. coli	50	48	50
Enterococcus spp.	25	25	0
Proteus spp	19	19	19
Klebsilla pneumoniae	15	14	14
Enterobacter spp.	2	2	1
Pseudomonas aeruginosa	21	21	21
Acinetobacter spp.	4	3	4
Serratia spp.	2	2	2
S. aureus	9	9	0
Streptococcus spp.	3	3	0
Total	150	146	111

Table (3) Susceptibilities result for (113) Gram's negative isolates according to the NCCLs criteria

Antimicrobic agent disk content (µg)	No. of isolates					
	CHROM agar Orientation			macConkey agar		
	S	I	R	S	I	R
Ampicillin (10)	10	3	100	10	1	102
Amoxicillin(20) / clavulanic acid(10)	75	1	37	75	2	36
Cefotaxime(30)	80	1	32	80	1	32
Ceftazidime(30)	85	1	27	85	2	26
Gentamicin(10)	89	1	23	89	2	22
Amikacin (30)	92	1	20	92	2	19
Ofloxacin (10)	99	0	14	99	0	14
Ciprofloxacin(5)	96	0	17	96	0	17
Imipenem (10)	105	0	8	105	1	7
Aztreonam (30)	106	1	6	106	0	7
Piperacillin (100)	65	2	46	65	2	46
Nitrofurantion(30)	70	3	40	70	1	42

* S: Sensitive I: Intermediate R: Resistance

Table (4) Susceptibilities result for (37) Gram's positive isolates according to the NCCLs criteria

Antimicrobic agent disk content (µg)	No. of isolates					
	CHROM agar Orientation			blood agar		
	S	I	R	S	I	R
Ampicillin (10)	28	2	7	28	1	8
Amoxicillin(20) / clavulanic acid(10)	30	0	7	30	1	6
Cefotaxime(30)	15	1	21	15	1	21
Cephalothin(30)	13	0	24	13	0	24
Amikacin (30)	27	1	9	27	2	8
Gentamicin(10)	20	0	17	20	0	17
Erythromycin (15)	17	0	20	17	0	20
Clindamycin(2)	10	0	27	10	0	27
Ofloxacin (10)	30	0	7	30	1	6
Nitrofurantion(30)	30	1	6	30	1	6
Chloramphicol (30)	25	2	10	25	1	11

* S: Sensitive I: Intermediate R: Resistance

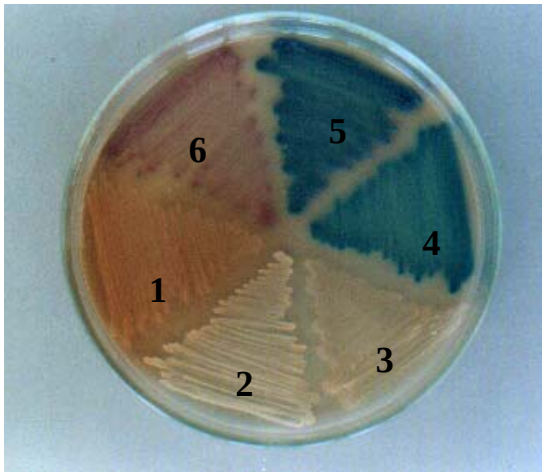

Fig (1): The color reaction of microorganism on CHROM agar Orientation**.**
1. E. coli
2. Proteus spp
3. S. aureus
4. Pseudomonas aeruginosa
5. Klebsilla pneumoniae
6. Enterococcus spp

Discussion:

CHROM agar Orientation media modification of a chromogenic agar developed for urine culture (12). A total of 375 urine samples were tested by parallel inoculation on CHROM agar Orientation and on two reference media (blood and macConkey agar). CHROM agar Orientation showed the same ability to detect urine pathogens as the combination of the two reference media. Color and Morphology characteristics on CHROM agar Orientation allowed for easy differentiation of the bacteria colonies, several studies have reported similar conclusion (6, 7, 14) This study shown that (113) of UTI was caused by Gram's negative bacteria considering that the majority were E. coli. All these isolates grew on CHROM agar Orientation in pink red colonies and were very easy to distinguish (15). The medium failed to differential Klebsiella spp. and Enterobacter spp. Owing to similarity of color produced and final identification among them require other biochemical tests. While Proteus spp. was easily distinguished on the primary plates because of their characteristic brown halo colonies (16). Acinetobacter spp. grew in nontransparent white, entire edges colonies. There strains were very distinct from Pseudomonas aeruginosa isolates which grew in translucent creamy. Serratia spp. grew in light blue. CHROM agar Orientation succeeded in detecting all Grams' positive microorganism that grew on blood agar, including S. aureus appear golden, opaque small white colonies and Streptococcus spp. that grew small turquoise colonies (17). Enterococcus spp. one of the most commonly encountered Gram's positive pathogens in UTI were distinguished on CHROM agar Orientation by diffuse blue colonies on agar. Antimicrobial Susceptibility tests of microorganisms pinked from CHROM agar Orientation showed an excellent correlation with test results of microorganisms pinked from reference media (5). In conclusion CHROM agar Orientation medium is recommended as a single medium for direct isolation and presumptive identification of UTI pathogens and antimicrobial Susceptibility tests without the need for subcultures. The use of this medium for other clinical specimens requires further evaluation.

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