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A Study on Prevalence and Evaluation of Clinical Isolates from Community Acquired Infections Using Different Media in Semiurban Areas

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Abstract: This study reports the prevalence of community acquired pathogens in clinical isolates from semi urban areas and evaluates the detection using conventional and chromogenic agar media. One hundred clinical samples which included midstream urine, stool, pus, high vaginal swab, sputum samples and blood samples were collected from outpatients attending Rajah Muthiah Medical College and Hospital, Annamalainagar, Tamilnadu, India. A total of 71 bacterial isolates were obtained, of which *Escherichia coli* [39 (54.9%)] was the highest, it was predominant in all samples, especially in urine samples. This was followed by *Klebsiella pneumoniae* [15 (21.1%)], isolated from urine, stool and pus samples. *Pseudomonas aeruginosa* [8 (11.2%)], *Enterococcus faecalis* [8 (11.2%)], had even numbers isolated, from urine samples only and *Proteus mirabilis* [1 (1.4%)] was least isolated from urine sample only. This study showed a high prevalence of bacterial pathogens in outpatient samples. All clinical samples and positive blood cultures were inoculated in parallel onto Hichrome UTI agar, Eosin Methylene Blue agar, Cystine-Lactose-Electrolyte-Deficient agar with Andrade indicator and MacConkey agar plates. From the growth characteristics, this study revealed the highest recovery of pathogens was seen in chromogenic medium than in routinely used media, proving to be an easy and attractive diagnostic tool for pathogen detection.

Key words: Community acquired infections · Chromogenic media

INTRODUCTION

Bacterial infections in developing countries still remain to be the major cause of morbidity and mortality [1]. The prevalence of community acquired infections, are higher in rural and semi urban areas than in metropolitans [2, 3].Community acquired infections can be defined as, onset of infections prior to hospital admission and not within 10 days of hospital discharge [4]. The problems associated with community acquired infections, extends from urinary tract infections to acute gastroenteritis and pneumonia to blood stream infections [3, 5].

Recently antibiotic resistant pathogens have emerged and possess a serious threat to existing antibiotic treatment [1, 6]. This necessitates the precise detection of pathogens from existing micro flora, in short time period using chromogenic media [7] which are highly efficient and are gradually replacing routinely used media in identification and isolation of clinical pathogens, reducing time consumption and facilitating early antibiotic therapy. The first selective chromogenic medium was used by Kilian and Bulow in 1976 [8] for the direct detection of *Escherichia coli* in primary culture of urine.

Chromogenic media have been introduced for easy detection of both, some gram negative and gram positive pathogens based on colony colors produced by interaction of specific enzymes with chromogenic substrates [7]. It has broader application as not only a nutritional medium, but can also be made selective by antibiotic supplementation for detecting, infection causing resistant pathogens [9]. This study evaluates the advantages of chromogenic media as a primary medium in comparison to other conventional media in detecting pathogens not only from urine samples but also from various other samples, especially its utility in semi urban areas.

MATERIALS AND METHODS

Clinical Samples: This study comprises of 100 clinical samples, which included urine, stool, pus, high vaginal swab (hvs), sputum samples and blood samples, which were collected in sterile containers and blood culture bottles respectively from outpatients attending Rajah Muthiah Medical College and Hospital, Annamalainagar,

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Tamilnadu, India. Samples were labeled and transported within 30 minutes of collection, to the Division of Microbiology, Bacteriology Laboratory, Rajah Muthiah Medical College and Hospital, Annamalainagar, Tamilnadu, India.

Culture Medium: Hichrome UTI agar, Eosin Methylene Blue agar (EMB agar), Cystine-Lactose-Electrolyte-Deficient agar with Andrade indicator (CLED agar), MacConkey agar plates (Himedia Laboratories, Mumbai, India), were prepared as per manufacturer's instructions. Freshly prepared media were tested with *Escherichia coli* ATCC (25922) to ensure the ability of the media to support growth.

Inoculation and Incubation: Each of the collected samples and positive blood cultures were simultaneously inoculated, in parallel, onto all four different media using calibrated loop and incubated at 37°C for 24 hours. The isolates on conventional media and on chromogenic medium were confirmed by colony morphology and standard biochemical tests [10].

RESULTS

The frequency of bacterial pathogenic isolates, in clinical samples is as shown in Table 1. Of the 100 clinical samples studied, 71% of isolates, showed significant growth, including both unimicrobial and polymicrobial growth. Detection of various microorganisms on different media was done. Detection of pathogens on chromogenic medium was based on colony color (Figure 1). Of the total 71 bacterial isolates, 63 gram negative and 8 gram positive bacteria were obtained. It was observed, a high incidence of isolates 60.5% were from urine samples, while 19.7, 8.4, 5.6, 4.2, 1.4% were from stool, pus, high vaginal swab, blood culture and sputum samples respectively (Table 1).

Fig. 1: Clinical isolates plated on chromogenic media (Hichrome UTI agar) 1. *Eschericha coli* 2. *Pseudomonas aeruginosa* 3. *Klebsiella pneumoniae* 4. *Proteus mirabilis* 5. *Enterococcus faecalis*

The prevalence of bacterial pathogens, obtained were *Escherichia coli* in various samples, was 39 (54.9%), followed by *Klebsiella pneumoniae* 15 (21.1%), *Pseudomonas aeruginosa* 8(11.2%), *Enterococcus faecalis* 8 (11.2%) and *Proteus mirabilis* 1 (1.4%). *Escherichia coli* was the most common bacterium from urine samples, followed by *Klebsiella pneumonia,* whereas, both *Pseudomonas aeruginosa* and *Enterococcus faecalis* had even number of positive urine isolates, while *Proteus mirabilis* was the least isolate from urine. In all urine samples more isolates were from females than in males were observed. Both *Escherichia coli* and *Klebsiella pneumonia* were recovered from stool and pus samples, while stool samples had even number of male and female isolates, *Escherichia coli* from pus samples had more isolates from female, while *Klebsiella pneumonia* from pus sample was recovered only from a male. *Escherichia coli* were the only isolates from blood

Table 1: Distribution of pathogens in various clinical samples

	Escherichia coli		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Enterococcus faecalis*		*Proteus mirabilis*		
Pathogen Sample Type	M	F	M	F	M	F	M	F	M	F	Total % in each sample
Urine	4	12	2	8	1	7	2	6	0	1	43(60.5%)
Stool	5	5	2	2	0	0	0	0	0	0	14(19.7%)
Pus	2	3	1	0	0	0	0	0	0	0	6(8.4%)
Hvs	0	4	0	0	0	0	0	0	0	0	4(5.6%)
Blood culture	0	3	0	0	0	0	0	0	0	0	3(4.2%)
Sputum	1	0	0	0	0	0	0	0	0	0	1(1.4%)
Total % of pathogen	39(54.9%)		15(21.1%)		8(11.2%)		8(11.2%)		1(1.4%)		Total 71

Total number of clinical samples = 100; Total number of positive clinical isolates = 71
Hvs -high vaginal swab. M- Male, F-Female

Table 2: Age and sex distribution of the out patients with positive clinical isolates

	Positive Clinical Isolates		
Age in Year	Male	Female	Total
0-1	3	2	5
1-4	9	3	12
5-10	4	4	8
11-19	0	1	1
20-29	0	10	10
30-39	1	7	8
40-49	2	4	6
50-59	1	12	13
>60	3	5	8
Total	23	48	71

Table 3: Isolation of pathogens in various media

Media	*Eschericha coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*	*Proteus mirabilis*	Total number of Isolates
Hichrome UTI agar	39	15	8	8	1	71(100%)
EMB agar	39	15	6	4	0	64(90.1%)
CLED agar	37	15	6	4	1	63(88.7%)
MacConkey agar	35	15	6	4	0	60(84.5%)

Colony count of all isolates were clinically significant (> 1000 cfu/ml)

cultures, high vaginal swab and sputum samples. Both high vaginal swabs and blood cultures had isolates recovered only from females, whereas only one isolate from male was recovered from sputum.

The male to female ratio was 1:2 and the age distribution of patients was as shown in Table 2. The majority of the patients with community acquired infections were above 50 years of age followed by 1-4 years of age group and 20-29 years of age group. The age group 20-59 years of age constituted more than 50% of all patients.

The ability of all the media to support growth was similar, except for few variations as shown in Table 3. Eosin Methylene Blue agar, Cystine-Lactose-Electrolyte-Deficient agar with Andrade indicator, MacConkey agar failed to recover seven, eight and eleven clinical isolates respectively, whereas the highest recovery was seen in Hichrome UTI agar with best sensitivity when compared to other 3 media. The specificity of chromogenic agar medium was increased, by inclusion of Indole spot test by 100% for *Escherichia coli*.

DISCUSSION

Community acquired infections, with potential acquired bacterial resistance, are global problem with geographical variations. The present study showed that *Escherichia coli* (54.9%) predominated in all outpatients samples [5] and most of the isolates were from urine [11, 12], similar to the findings of other workers [13, 14]. Other pathogens isolated in order of prevalence included *Klebsiella pneumoniae* (21.1%), *Pseudomonas aeruginosa* (11.2%), *Enterococcus faecalis* (11.2%) and *Proteus mirabilis* (1.4%) but 54.9% high prevalence of *Escherichia coli* showed, it to be still, the dominant aetiological agent causing community acquired infections in semi urban areas, especially urinary tract infections, as previously demonstrated by most workers [5, 6, 15, 16].

Acute gastroenteritis is the major source of morbidity in developing countries, especially in young children and immunocompromised persons [3, 17, 18]. In this study the highest incidence of 60.5% of isolates were from urine, followed by 19.7% stool isolates, both *Escherichia coli* and *Klebsiella pneumonia* were the gastroenteritis causing pathogens isolated from stool samples [2, 18, 19]. In the present investigation, blood stream infection was detected 4.2% and the causative pathogen was found to be *Escherichia coli* as in prior studies [20, 21, 22].

The frequency of community acquired infections was increased in elderly patients over 50 years of age, due to decrease in immunity with advanced age [5, 23]. The male to female ratio in this study was 1:2, this may be due to high number of urine and high vaginal swab samples from female patients, affected with urinary tract infections [14].

Community acquired pathogens may cause, difficult to treat infections, due to high incidence of antibiotic resistance strains, which require, effective and precise surveillance of various clinical samples from intestinal and extra intestinal origin, as they may be the reservoir for plasmid mediated resistance [3, 6].

Chromogenic agar medium have diagnostic importance in detecting pathogens. The principles involved in chromogenic medium [7] evaluated in this study, was the utilization of the substrate by the enzymatic action of bacteria, thereby imparting, a unique colony color that can be visually detected, they can be species specific or genus specific [24]. Hichrome UTI Agar consists of two chromogenic substrate. The enzyme ß-glucosidase produced by *Enterococcus* and *Klebsiella* species, cleaves the ß-glucosidase chromogenic substrate resulting in distinct blue colored colonies [25]. The enzyme ß-D-galactosidase produced by *Escherichia coli* [26] that cleaves the other chromogenic substrate forming the characteristic pink to red colored colonies which can be confirmed with indole test. *Proteus* species produces brown colored colonies due to tryptophan deaiminase activity [7]. Peptic digest of animal tissue, incorporated in this medium, provides the essential carbon and nitrogen compound which enhances the growth of most uropathogens [27].

The introduction of chromogenic agar into clinical labs had made easy and early recognition of frequently occurring microorganism in primary culture, thus reducing workload [28]. This study was conducted in semi urban areas using conventional media and chromogenic agar medium, for urine samples and various other clinical samples, such as stool, pus, high vaginal swab, sputum samples and positive blood cultures were also included for primary isolation of pathogens. Hichrome UTI Agar was formulated for detection of uropathogens, but surprisingly it was also able to support significant growth from other clinical samples, similar to the works of Filus *et al.* [29]. This chromogenic agar medium also prevented swarming of *Proteus mirabilis*, mucoid *Klebsiella pneumoniae* and *Escherichia coli* strains thereby increasing the better detection rate of pathogen from mixed flora [30]. Our result agrees with other studies, that chromogenic media also favors the detection of some Gram positive organisms by color differentiation from Gram negative organisms, facilitating easy detection [31]. Detection rate of Hichrome UTI agar medium, in comparison with other media were similar, with few exception, as observed in previous studies by Laskshmi *et al.* [24], but its best advantage over other medium was, easy recognition of mixed growth and preventing the need for sub culturing and performing multiple biochemical test [7]. In the conclusion of present study, Hichrome UTI agar was found to be an innovative medium, which has the potentiality to reduce time and labor and was cost effective especially in semi urban areas, where there is a constant inflow of various clinical sample and demand to produce reports in short time span and this can also be utilized, in prevalence study of pathogens.

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