Int. J. Mendel, Vol. 35(1-2), 2018 & 36(1-2), 2019 75-78 ISSN0970-9649

A COMPARATIVE STUDY OF GRAM NEGATIVE UROPATHOGENS

Murli Dhar Mishra*, Gunjan Sharan Sinha, Priyanka Sinha, M.K. Mishra and A.K. Sinha

Key words : Uropathogens, *E.coli, K.pneumoniae , P. aeruginosa,* IMViC test.

Association of Gram negative bacteria with urinary tract infections in human beings has been the most common infectious presentation in medical practice. Common treatment for Urinary Tract infection against Gram negative uropathogens is antibiotic therapy. Antibiotic therapy against uropathogens requires urine culture and sensitivity test as a diagnostic tool to detect the causative organisms and to determine the type of antibiotic therapy method. In the present investigation, comparative growth of bacterial colonies on different culture media has been observed. After isolation the Gram negative uropathoens were characterized on the basis of morphological, cultural and biochemical characteristics. Three different culture media namely Nutrient agar, MacConkey agar and Hi-Chrome UTI agar media have been taken for observing the growth rate of different pathogens. It was observed that Escherichia coli grew on all the media but the maxmum percenage of colonies was found on MacConkey agar media. On the other hand the two bacteria namely *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* did not grow on nutrient agar media but their growth on the other two media was clearly visible. It was interesting to find that Hi-Chrome UTI agar medium was better for the growth of *K.pneumoniae* and *P. aeruginosa*. Finally biochemical characterization of the isolates were performed with lactose fermentation test and IMViC test.

INTRODUCTION

Urinary tract infections have been described since ancient times and the first documented description was recorded in the Ebers Papyrus in 1550 BC (Al-Achi Antone, 2008). They (UTIs) are the most frequent clinical problem affecting millions of people each year. It is estimated that about 35% of healthy women suffer symptoms of urinary tract infection at some stage in their life. About 5% of women each year suffer with the problem of painful urination and frequency (Hootan, 2003).

UTI has a number of causes. Most are caused by bacteria normally present on the skin or in the intestinal tract that invade the urinary tract. Leading biological agents of UTIs include *Escherichia coli, Enterococcus faecalis*, *Pseudomonas aeruginosa*,*Klebsiella pneumoniae* and *Proteous mirabili*s (Abiramasundar *et. at.,* 2011). The distribution pattern of UTI causing microorganisms changesfrom time to time and from place to place (Okonko *et. at.,* 2009).

The single medium cannot support the growth of all uropathogens .The commonly used media for urine culture in different hospitals of Gaya town are Blood agar, MacConkey agar and Eosin methylene blue agar. The aim of this study was to evaluate which medium is the best for the routine culture of uropathogens. In the present investigation microbiological performance of nutrients agar medium and MacConkey agar medium were compared with Hi-Chrome UTI agar medium for isolation and rapid identification of uropathogens. Data of present investigation indicate that Hi-Chrome UTI agar medium is better medium.

Hi-Chrome UTI agar medium contains chromogenic substances and is used for rapid and fast identification of most uropathogens (Ciragil *et. at.,* 2006). There was no earlier study and published information on UTI in the study area. This study was conducted in order to assess the prevalence of bacterial uropathogens in Gaya town.

MATERIALS & METHODS

1. **Collection of urine sample:** A total of 200 urine samples have been collected from clinically diagnosed patients. Urine sample have been collected from different hospitals likes Piligrim (JPN) Hospital, A.N.M.C.H., Gaya and from other laboratories located in Gaya town. Before collecting samples the women were instructed to swab the vulva and men to retract the foreskin and cleanse the glance penis. Urine samples were collected in wide mouthed containers. After collection of samples, the containers were closed tightly to avoid any leakage during transportation. Containers were finally transported to laboratory in an ice cold condition by adding boric acid at a final bacteriostatic concentration of 1.8% without delay (Porter et al., 1969).
2. **Isolation of Bacteria causing UTI from urine sample:** The media used in the present investigation include Nutrient agar medium, MacConkey agar and Hi-Chrome UTI agar medium. For the isolation of UTI causing organism loopful of urine sample was streaked on the above said three different media and incubated at 37°C for 24 hours (Inabo *et. at.,* 2006).

***Department of Biotechnology, Gaya College, Gaya**
Email ID : murlidharmishra25@gmail.com

3. **Identification of Bacteria:** After incubation, colonies were selected and characterized on the basis of morphological, cultural and biochemical characteristics.

   a. **Cultural observations:** colour, size and colony morphology are observed from the incubated plates.

   b. **Morphological analysis of urine specimen:** Slides were prepared from different colonies observed on the plates and gram staining was performed and the morphological studies were made.

   c. **Biochemical Examinations:** Six biochemical tests were performed for each organism; they are lactose fermentation test, IMViC test and catalase activity test (Salle,1961 and Cruickshank *et. at.,* 1975).

OBSERVATIONS

First of all, a total of 200 urine samples of clinically diagnosed patients were collected from government hospitals and other laboratories of Gaya town. It was observed that 123 persons (61.50%) out of 200 cases studied had UTIs. The cultural characteristics of UTI isolates collected from 123 samples was performed on different culture medias via Nutrient agar medium, MacConkey agar medium and Hi-Chrome UTI agar medium. On NAM large white opaque, mist colonies were observed which indicated that these colonies may be of *E. coli* while on MacConkey agar three different types of colonies like red colonies, circular mucoid colonies and colourless colonies were observed which may be of *E. coli, Klebsiella pneumoniae* and *P. aeruginosa* respectively. Lastly on Hichrome UTI medium violet, green and creamy yellow colonies indicated that *E. coli, K. pneumoniae* and *P. aeruginosa* might have been present (Table=1).

Table-01

Cultural Characteristics of UTI isolates on different media.

Media	Uropathogens	Color and Shape
1. Nutrient agar medium	*Escherichia coli*	Large, White, Smooth opaque colonies
	Klebsiella pneumoniae	No Growth
	Pseudomonas aeruginosa	No Growth
2. MacConkey agar medium	*Escherichia coli*	Red / Pink colonies
	Klebsiella pneumoniae	Circular mucoid small Colonies
	Pseudomonas aeruginosa	Colourless colonies
3. Hi- Chrome UTI agar medium	*Escherichia coli*	Violet
	Klebsiella pneumoniae	Green
	Pseudomonas aeruginosa	Cream Yellow

Comparative growth analysis of uropathogen was performed on three different media namely Nutrient agar medium, MacConkey agar and Hi-Chrome UTI Agar. It was observed that out of 123 samples 86 were containing *E. coli*, 24 were *K. pneumoniae* and 13 samples were *P. aeruginosa* (Table-3). Growth of *E. coli* was maximum on MacConkey agar, while K. pneumoniae showed maximum growth on Hi-Chrome UTI agar whereas growth of *P. aeruginosa* was more or less equal on MacConkey and HiChrome UTI agar media. There was complete absence of growth of *K. pneumoniae* and *P. aeruginosa* on NAM.

By performing Gram's staining, it was identified that three different uropathogens namely E. coli, K. pneumoniae and *P. aeruginosa* were present (Table-2). Biochemical tests were performed to identify uropathogens. The biochemical test included lactose utilization, catalase and IMViC test. These were performed for each of the different UTIs isolate colonies as shown in Table-2.

Table-02

Identification of Isolates microbes using biochemical test

Biochemical Tests	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Gram's Staining	Rods	Rods	Rods
Lactose utilization	+ (AG)	+ (AG)	- (No AG)
Catalase test	+	+	+
Indole production test	+	-	-
Methyl – Red test	+	-	-
Voges – Proskauer test	-	+	-
Citrate utilization	-	+	+

(+ = positive) and, (– = negative)

Table-03

Comparative Growth of Bacterial Colonies in Different Culture media

Name of the organism	Total Number	On Nutrient Agar Medium (NAM)	Percentage	On MacConkey Agar Medium	Percentage	On Hi-chrome UTI Agar	Percentage
Escherichia coli	86	24	27.09%	36	41.86%	26	30.23%
Klebsiella pneumoniae	24	–	–	9	37.51%	15	62.50%
Pseudomonas aeruginosa	13	–	–	6	46.15%	7	53.84%

DISCUSSION:

In the present study out of 86 *E. coli,* 27.09% (24) were identified in Nutrient agar medium, whereas 41.86% (36) were in MacConkey agar medium and 30.23% in Hi-Chrome UTI agar media. Current investigation showed difference in detection rate of *E. Coli* on different growth medium. The different types of bacteria found in urine sample cannot be covered by a single growth medium (Ramzan *et. at.,* 2004). The present investigation also evaluated the ability of MacConkey agar as medium for detection and identification of routine urinary pathogens. Out of 123 positive cases, 51 samples showed significant bacterial growth on this media. Table-1 shows the rate of isolation of UTI causing bacteria in different media. The major isolates in MacConkey agar was E. coli (41.86%), and P. aeruginosa (46.15%). The rate of isolation of UTI causing pathogens was highest on MacConkey agar medium however studies carried by Praveen *et. at.,* (2011), and Rani *et at.* (2012) are not in accordance with our observation.

These workers found high isolation rate on Hi-Chrome UTI agar. In this study, Hi-chrome. UTI agar was evaluated as a medium for isolation of UTI causing bacteria. The ability of Hi-Chrome UTI agar as medium for detection of uropathogen was almost similar to MacConkey agar. *K. pneumoniae* showed highest percentage (62.5%) followed by *P. aeruginosa* (58.34%). Other studies also showed HiChrome UTI agar as an excellent medium for isolation of uropathogens (Lakshmi *et. at.,* 2004). The cultural characterization of all isolated bacteria as reported in the present case has also been recorded by other workers such as Cheesbrough (1985). The result of present investigation showed that among the causative organisms of UTI. The members of the family Enterobacteriaceae are the predominant pathogens. These findings are consistent with reports published from other countries (Hootan *et. at.*,1997, Zhanel *et. at.,* 2000). In this study colony morphology and gram staining characteristics were recorded. These finding were more or less similar to the finding of Buxton and Fraser (1997). Biochemical characterization of the isolates was performed. The biochemical properties of all isolated bacteria were in agreement with Cheesbrough (1985). From this study it was concluded that *E. coli*, *K. pneumoniae* and *P. aeruginosa* were the predominant bacterial flora of human urine sample.

REFERENCES

Al-Achi, Antoine. 2008. An introduction to botanical medicines, history, Science, uses and dangers. Westport, conn: Pralger Publishers. P. 126.

Abiramasundari, P., Priya, V., Jeyanthi, G.P., ayathri, Devi. S. 2011. Evaluation of the antibacterial activity of Cocculushirsutus. Hygeia. Journal for drugs and Medicines Vol. 3,2:26-31

Buxton, A., s Fraser, G. 1997. Animal Microbiology Vol 1. Blackwell Scientific Publication Oxford, London, Edinburg, Melbourne. 85-86, 99.

Cruickshank, R., Dugid, JP., Marmion, BP., and Sawin, RH. 1975. Medical Microbiology 12thEdn. Churchill Livingstone (Pub). Edinburg, London and New York. Vol II.

Cheesbrough, M. 1985. Medical Laboratory Manual for tropical countries vol 2. Microbiology. 400-480.

Ciragil, P., Gill, M., Aral, M. &Ekerbicer, H. 2006. Evaluation of a new chromogenic medium for isolation and identification of common urinary tract pathogens. Eur J clin Microbial infect Dis 25:108-111.

Hootan, TM., &Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North AM. 1997; 11:551-81.

Hootan, T.M. 2003. Urinary tract infection in adults, In: Johnson R.J., Feechally J., (Eds). Comprehensive clinical nephrology, 2nd ed, London: Mosby, 731-744.

Inabo, H.I, and H.B.J. obanibi, 2006. Antimicrobial susceptibility of some urinary tract clinical isolates to commonly used antibiotics. African J. Biotechnal., 5(5) :487-489.

Lakshmi, V., Satheeskumar, T., & Kulkarni G. 2004. Utility of Urochrome II-A chromogenic medium for uropathogens. Indian J of Med. Microbial. 22 (3): 153-158.

Okonko, I.O., L.A. Ijandipe, O.A. Ilusanya *et. at.*, 2009. Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. Afr. J. Biotechnology. 23(8) : 6649-6657.

Praveen, R., Saha, S.K., Shamshu, Zaman., Rashid, S.M., chowdhary, A. L., &Muazzam, N. 2011. Detection of uropathogen by using chromogenic media (HiChrome UTI agar), CLED agar and other conventional media. Faridpur Med. Coll. J., 6(1): 46-50.

Porter, A., and Brodie, J. 1969. Boric acid preservation of urine samples. British Medical Journal. 2: 353-355.

Ramazan, M., Bakhah, S., Salman, A., Khan, GM., & Junaid, M. 2004. Comparative study of various growth media in isolation of urinary tract pathogens. Gomal J. Med. Sci. 2 (1): 16-19.

Rani, L., pinnelli, V.B.K., Hemavathi, Belwadi, S., Rajendran, R. 2012. Utility of HiChrome urinary tract infection (UTI) agar medium for identification of uropathogens. A comparative study with other conventional media. J. Chem. Pharm. Res.

Salle, A.J., 1961. Fundamental principle of Bacteriology. 5thEdn. McGraw Hill Book Co., London, UK.

Zhanel, G G., karlowsky, JA., Harding GK., Carrie, A., Mazzullis T., & Low, DE. 2000. A Canadian national surveillance study of urinary tract isolates from outpatients: Comparison of the activities of trimethoprim-Sulfamethoxazole, ampicillin, mecillinam-nitrofurantoin and ciprofloxacin. Antimicrobe Agent che-mother 44: 1089-92.