DIAGNOSTIC ACCURACY OF CHROMAGAR™ ECC FOR DETECTION AND ENUMERATION OF *ESCHERICHIA COLI* AND COLIFORMS IN WATER SAMPLES

Sundas Shabir[1], Umar Khursheed[1], Mariam Sarwar[1], Farooq Ahmad[1], Rabia Shabir[2], Saira Saleem[1]

[1]Armed Forces Institute of Pathology Rawalpindi (National Institute of Medical Sciences) Pakistan
[2]Combined Military Hospital, Muzaffarabad Pakistan

ABSTRACT

Objective: To determine the diagnostic accuracy of CHROMagar™ ECC for detection and enumeration of Coliforms and *E. coli* in water samples keeping the membrane filtration method as gold standard.

Material & Methods: This comparative cross-sectional was conducted from June to December 2019 in Department of Microbiology, AFIP, Rawalpindi, Pakistan with a sample size of 336. Both the membrane Filtration technique and CHROMagar™ ECC media were employed for enumerating E. coli and Coliforms in water samples. Data was analyzed and processed on SPSS version 22.

Results: The water samples tested had *E. coli* average count of 38 CFU/100 ml on CHROMagar™ ECC as compared to 43 CFU/100 ml on MacConkey agar by Membrane filteration Method. For Coliforms, average colony count on CHROMagar™ ECC was 46 CFU/100 ml as compared to 49 CFU/100 ml on MacConkey agarby Membrane filteration method. For detection and enumeration of Coliforms on CHROMagar™ ECC against Membrane filteration method, the Sensitivity was found 56.8 %, specificity 43.1%, positive predictive value 95.3%, negative predictive value 96.5% and diagnostic accuracy 0.95 (95%).For detection and enumeration of *E.coli* on CHROMagar™ ECC against Membrane filteration method, the Sensitivity was 44.3%, specificity 55.6%, positive predictive value is 92.7%, negative predictive value is 95.1% and diagnostic accuracy is 0.94 (94%).

Conclusion: This study suggested that the CHROMagar™ ECC is a unique method that can be incorporated in the routine methods of laboratory for bacteriological examination of water to detect bacterial contamination

Key Words: CHROMagar™ ECC, Coliforms, *E. coli*, Fecal contamination, Membrane Filtration Technique, Water contamination.

This article can be cited as: Shabir S, Khursheed U, Sarwar M, Ahmad F, Shabir R, Saleem S. Diagnostic accuracy of CHROMagar™ ECC for detection and enumeration of *Escherichia coli* and coliforms in water samples. Pak J Pathol. 2021; 32(2): 56-60.

INTRODUCTION

One of the major millennia developing goal adopted by world community in 2015 is sustainable access to clean drinking water and basic sanitation [1]. In drinking water major health risks are established by microorganisms, coming from the water source, entering storage or distribution systems or growing in the water [2]. At start of 21st century there was no access to safe drinking water for 2.4 billion (40%) persons of world population [3]. In one study it is found that in Rawalpindi, 70.06% of water samples were contaminated [4]. In another study approx 67%–93% of water samples in Sukkur, Hyderabad and Karachi were contaminated. In Abbottabad, Mardan, Peshawar and Mingora approx 55% and in other cities like Quetta 60% drinking water samples were contaminated [5].

One of the main routes of water contamination is fecal or sewage for transmission of human pathogens like viruses, parasites and bacteria in the developing world. Intestinal helminths infections because of utilizing contaminated water are very common, affecting approximately 133 million people worldwide [6]. According to WHO, between 11 and 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually worldwide [7]. Pakistan is already facing ongoing outbreak of XDR Typhoid [8]. For controlling or minimizing the incidence and outbreaks of water borne diseases, rapid detection of fecal or sewage contamination detecting the indicator organisms (total coliforms and *Escherichia coli*) is required [9]. Membrane filtration method is not cost effective, this technique takes 48-72 hours in interpreting results and it require biochemical testing API 20E for identification of *Escherichia* coli/ Coliforms. Use of CHROMagar™ ECC is convenient, rapid, does not require sophisticated technical expertise and can be performed easily like in the small laboratories.

This study is planned to found the diagnostic accuracy of CHROMagar™ ECC so that this medium can be incorporated in our laboratory procedures as an alternative to membrane filtration method.

Correspondence: Dr Sundas Shabbir, Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

Email: **sundasshabir741@gmail.com**

Received: ***22 Feb 2021****; Revised:* ***16 May 2021****; Accepted:* ***24 Jun 2021***

MATERIAL AND METHODS

This comparative cross-sectional study was conducted in Department of Microbiology, AFIP Rawalpindi, Pakistan and was completed within 6 months (June to December 2019) after the approval by Institutional Ethical Committee. Sample size was calculated using WHO calculator. Non-probability convenience sampling technique was used. All water samples received during six months period of data collection at Microbiology department AFIP were included. Samples were excluded if it was not collected in sterilized bottle, not properly sealed, time of collection not known or >4 hours and sample quantity <200 ml.

Membrane Filtration Method for Determining *Escherichia coli* and Coliforms in Water Samples: Sterile grid membrane filters were used with a pore size of 0.2 µm and 47 mm diameter; it was used to filter a 100ml volume of water under vacuum. The membrane was then placed on MacConkey agar medium which was then incubated at 35ºC +/- 2 temperature for 24 hours. If the water samples contain any type of coliforms then characteristic colonies were identified by lactose fermentation, colony morphology, gram stain, motility, catalase and oxidase and indole test. Indole positive colonies were tested by API-20E to confirm the presence of *E.coli*.

As per colony count and membrane filtration techniques, a cluster of bacteria or a particle with bacteria attached or even a single bacterium will lead to a single visible colony. Thereby, each of these particles or clumps is a colony forming unit (CFU). The results are then presented as colony forming units per unit volume (cfu/ml) [9].

CHROMagar™ Ecc Method for Determining and Enumeration of *Escherichia coli* and Coliforms in Water Samples: For preparation of media 1 liter of purified water was slowly dispersed in 32.8 g of powder. Stirred continuously till the agar became thick, while stirring regularly, heat it till it boiled. The boiled media was placed in a water bath and cool down up to 45-50 °C. Stirred softly to homogenize and then poured into Petri dishes. Finally, it was dried and solidified. After preparing media in aseptic conditions 47 mm diameter sterile membrane filter with pore size of 0.2 µm was placed in sterile filtration unit (Millipore). 100ml sample of water was filtered through filter membrane. Membrane filter was then be on pre-warmed culture plates containing CHROMagar™ ECC. In aerobic condition plates were turned upside down and were incubated at 36 ± 2 °C for 18-24 hrs.

For quality control *E. coli* ATCC 25922 and *Klebsiella pneumoniae ATCC 13833* strains were used as controls. Results were interpreted by color changes as under according to manufacturer's instructions.

Escherichia coli give metallic blue to violet color colonies. Other coliforms give pink to red colonies. Other bacteria appeared colorless or were inhibited. Results were interpreted according to WHO guidelines [10].

RESULTS

In this study total water samples analyzed were 336. Out of these samples, 153 (46%)/144 (43 %) were found satisfactory and 183 (54 %)/192 (57 %) unsatisfactory for drinking purpose according to WHO criteria by Membrane filtration method (MFT) (Gold standard) /CHROMagar™ ECC method respectively as shown in Figure-II & III Figure-I: Frequency distributions of satisfactory and unsatisfactory drinking water samples in membrane filtration test (MFT) (Gold standard).

Among all drinking water samples (336), 140/151 was bacteriologically contaminated with *E. coli* and 183/192 with coliforms by Membrane filtration method/CHROMagar™ ECC respectively. Further enumeration of Coliform /*E. coli* in total water sample was performed. The total water samples tested had *E. coli* colony count of 6463 in Gold standard method, i.e. average count was 43 CFU/100 ml; while the colony count of *E. coli* on CHROMagar™ ECC was 5780 i.e. average 38 CFU/100 ml. For Coliforms, the colony count on CHROMagar™ ECC was 8860 i.e. average was 46 CFU/100 ml and 9350 colony count of Coliform on Gold standard method i.e. Average is 49 CFU/100 ml as shown in Figure-I.

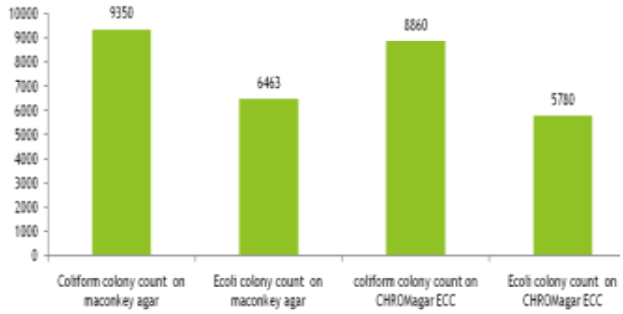

Figure-I: Comparison of Colony Count of *E. coli* and Coliforms on Membrane filtration method using (MacConkey agar and API 20E) and CHROMagar™ ECC.

Out of total 336 drinking water samples, 175 were tap water, 64 well water, 87 bore water and 10 bottled water samples respectively. Among all these samples, 195 (58%) were treated (filtered / chlorinated) and 141 were untreated. Out of 195

treated samples, 120 (61%) and among 141 untreated samples, (39%) were found satisfactory. Distribution of satisfactory and unsatisfactory treated and untreated water samples by gold standard method from different water sources are shown in Figure-II and III.

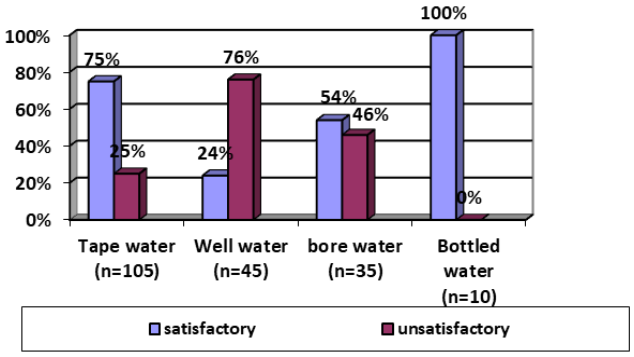

Figure-II: Distribution of satisfactory and unsatisfactory treated water samples by membrane filtration method from different water sources.

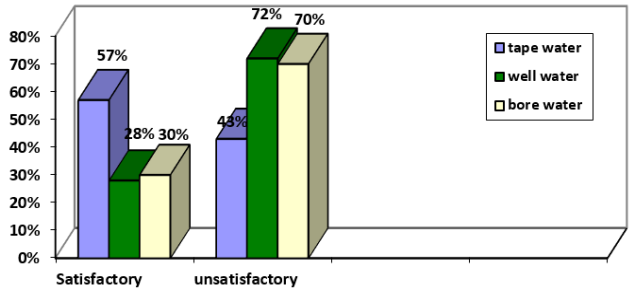

Figure-III: Distribution of satisfactory and unsatisfactory untreated water samples by Membrane filtration method from different water sources.

For detection and enumeration of *E.coli* on CHROMagar™ ECC against MFT, the Sensitivity (true positive) was found 44.3%, specificity (true negative) 55.6%, positive predictive value 92.7%, negative predictive value 95.1% and diagnostic accuracy is 0.94 (94%) with *p*-value of 0.01as shown in table 1.For detection and enumeration of Coliforms on CHROMagar™ ECC against MFT, the Sensitivity (true positive) was 56.8%, specificity (true negative)43.1%, positive predictive value95.3%, negative predictive value96.5% and diagnostic accuracy is 0.95 (95%) with *p* value of 0.01 as shown in Table-I.

Table-I: Diagnostic accuracy comparison of CHROMagar™ ECC against Membrane Filtration Method (Gold standard) for detection and enumeration of *E. coli* & Coliforms in water sample.

	Sensitivity	Specificity	Positive productive values	Negative productive values	Diagnostic accuracy	P value
Coliforms	56.8%	43.1%	95.3%	96.5%	0.95	0.01[1]
E. coli	44.3%	55.6%	92.7%	95.1%	0.94	0.01

DISCUSSION

This study figured out that for detection and enumeration of *E.coli* on CHROMagar™ ECC in comparison of MFT, the diagnostic accuracy is 0.94; and for detection and enumeration of Coliforms on CHROMagar™ ECC against MFT, the diagnostic accuracy is 0.95. Its results show easy differentiation between *E. coli* and Coliform colonies i.e. Coliforms colonies appeared mauve in color and typical *E. coli* colonies appeared blue in color.

In this study total water samples analyzed were 336, out of which 153 (46%)/144 (43 %) were found satisfactory for drinking purpose according to WHO criteria and 183 (54 %)/192 (57%) were found unsatisfactory in Gold Standard Membrane filtration method /CHROMagar™ ECC respectively. Hussain and colleagues also used the CHROMagar and under aseptic condition, sterile bottles, as per the criteria of drinking water by WHO, the results were interpreted. 298 (64%) were found fit for drinking purpose while, 164 samples (35.5%) out of total 462 water samples were found to be polluted with fecal contamination and were declared unsatisfactory for use; rest of the samples were safe to use. Bacteriological examination of water samples depicted that among the tested samples, a high frequency of unsatisfactory drinking water samples was found [11].

A study conducted by Hanan *et al*, also used Membrane Filtration Technique (MFT) with CHROMagar for enumeration of *E. coli* and coliforms in 100 samples of drinking water. The study declared that only 40-60% of population in Pakistan gets safe and clean water for drinking purpose. Hanan, *et al*., thus, employed the Membrane Filtration Technique (MFT) using CHROMagar which is much more effective than the MPN method. 42% samples were *E. coil* positive; 54% samples were coliforms positive [12].

Similar to this, a study carried out by Khan, Ali, and Hassan, found out the identification and frequency of different bacterial isolates in water samples. The study was conducted at National Institute of Health, Islamabad and 521 samples of

water were total sample size of study. Out of 521 total samples, only 168 (32.2%) samples of water were satisfactory and fit for drinking; while, 353 (67.8%) samples of water were unsatisfactory. In addition to this, Sarwar, *et al* studied bacteriological contamination of drinking water in Peshawar. Total 224 samples were tested for contamination. Forty-two (19%) treated water samples and ninety-two (81%) untreated water samples were positive for coliforms. 43.28% samples were *E. coli* positive which showed that the water has fecal contamination [13].

Similarly, Chowdhury, esteemed to detect the absence or presence of *E. coli* in water samples; as it is the indicator of fecal contamination. Positive results for *E. coli* were shown by around 10% of samples in entire study [14].

Li, *et al*, described in a study that Membrane filtration method is commonly used for bacteriological examination of water but it is not cost effective, and this technique takes 48-72 hrs in interpreting results and it require biochemical testing API 10S/20E for identification of *Escherichia coli*/ Coliforms. Another shortcoming of membrane filtration method is that it is not suitable for turbid water. On the contrary, use of CHROMagar™ ECC for identification and enumeration of coliforms and *Escherichia coli* in water samples is convenient, rapid and does not require sophisticated technical expertise and can be performed easily like in the small laboratories. CHROMagar™ ECC is a selective medium for the immediate enumeration and detection of *E. coli* and other coliforms in water and food samples [15].

Manafi, demonstrated in his study that quick identification and detection of microorganisms. Generally, chromogenic and fluorogenic substrates are significant resources in determining microbial presence in water through using the ability of certain organisms to produce certain enzymatic activities. These chromogenic and fluorogenic substrates sometimes work in collaboration with traditional methods or work independently. Detection and enumeration of bacteria can be easily done by incorporating primary selective media and synthetic chromogenic and fluorogenic substrates, directly on the isolation plate. Faster detection and improved accuracy of target organisms is now easy and rapid by the incorporation of many media and identification tests. This incorporation has also reduced the requirement of confirmatory tests and isolation of pure cultures [16].

Amirat, Wildeboer, Abuknesha, and Price, (2012) conducted a research which used membrane filtration technique along with culturing on selective chromogenic media for assessing the water quality of river Thames. Successful identification of *E. coli*, *Klebsiella pneumoniae*, *Enterococci* and Salmonella was carried out. 60% of the samples showed fecal contamination of river Thames. This high-level contamination was due to heavy rainfall [17].

Nabeela *et al*, highlighted the issue of bacterial contamination of water in Pakistan, the contributing factors for this contamination and the resultant risks to health of public. In Pakistan, water supplied to the public is polluted and contaminated at all points like consumer tap, in distribution network and at water source with fecal coliforms and total coliforms. More than 7000 samples of water were tested and it was found out that fecal coliforms were 58% and total coliforms were 71%. An average of 20-40% diseases in the country is due to the consumption of contaminated drinking water.

This study recommended that new policies should be developed for the safety of quality of water and measures should be taken to protect the water resources from bacterial pathogens [18].

In Pakistan, approximately 84% of its rural population and 62% of its urban population do not undergo water treatment or filtration for drinking purpose and as a consequence 40% w2of deaths are caused due to polluted water consumption. Water borne diseases like diarrhea cases are reported regularly and accounts for a number of deaths in children [19]. On the other side, another research study reviewed various conventional, emerging and biosensor-based methods for enumerating and detecting *E. coli*. Generally, a long duration of time is required for enumeration and detection of *E. coli* bacteria in laboratory settings. The culturing process of samples requires 24 hours to 72 hours before getting the results. Though, new and advanced techniques like Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) have been developed for the detection of E. coli n samples of water. Currently, biosensor device has been developed which is selective, sensitive, portable and easy to perform. In water samples, this device has remarkably detected enormously lower consolidation of *E. coli* bacteria [20].

Findings of the study have confirmed that CHROMagar™ ECC can be used as alternative method as compared to traditional membrane filtration method. The diagnostic accuracy of CHROMagar™ ECC media is high as compared to MFT, and has proved to be more suitable and rapid method for enumeration of target organisms in water samples.

This study has certain limitations like, sensitivity of CECC for E. col i is 97%, Rare ß-glucuronidase negative E. coli strains are false negative on this medium (typically 0157 E. coli), Few Hafnia are false negative in this medium and have a colorless appearance.

CONCLUSION AND RECOMMONDATION

In our study it is concluded that CHROMagar™ ECC is reliable test for detection of Coliform and *E.coli* in water samples and this procedure can be adopted instead of conventional MFT (Gold standard) for bacteriological examination of drinking water samples because It has good specificity, sensitivity, positive predictive value, negative predictive value and overall diagnostic accuracy, this method does not require sophisticated technical expertise in the laboratory settings, timely identification of pathogens or microbial in water can lead to rapid process of cleaning the water resource and making it consumable, It is cost effective as compared to Gold standard method MFT that further requires API 20 E for confirmation of bacteriological contamination of water with *E.coli* .It provided the rapid identification of organisms based on their colour morphology even in a mixed bacterial culture growth within 24 hours and differentiating Coliform and *E.coli* easily.

AUTHOR CONTRIBUTIONS

Sundas Shabir: Data collection, drafting and data analysis.

Umer Khursheed: Revision and final approval.

Mariam Sarwar: Data interpretation and study design.

Rabia Shabir: Data collection

Farooq Ahmad and Saira Saleem: Discussion and literature review.

REFERENCES

1. World Health Organization, WHO/UNICEF Joint Water Supply and Sanitation Monitoring Programme, 2015. Progress on sanitation and drinking water: 2015 update and MDG assessment. World Health Organization.
2. Højris B, Christensen SCB, Albrechtsen HJ, Smith C. Dahlqvist M. A novel, optical, on-line bacteria sensor for monitoring drinking water quality. Sci Rep. 2016; 6: 23935.
3. www.who/unicef. 2015. Available from. https://www.un. org/ sustainable devepopment/water-and-sanitation/.
4. Khan MJ, Ali MF, Hassan M. Microbial evaluation of drinking water and frequency of bacterial isolates from Rawalpindi Pakistan. Pak J Pathol. 2017; 28(1): 28-32.
5. Daud MK, Nafees M, Ali S, Rizwan M, Bajwa RA, Shakoor MB, *et al* 2017. Drinking water quality status and contamination in Pakistan. BioMed Res Int. 2017: 7908183. https://doi.org/10.1155/2017/7908183.
6. Globalsanitation. 2013.Availablefrom.https://www.Psai. org /page/GlobalSanitation.
7. World Health Organization. Typhoid vaccines: WHO position paper–March 2018. Weekly Epidemiol Rec. 2018; 93(13): 153-172.
8. Qamar FN, Yousafzai MT, Khalid M, Kazi AM., Lohana H, Karim S, *et al*. Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. Lancet Infect Dis. 2018; 18(12): 1368-1376.
9. Chesbrough M. District laboratory practice in tropical countries. Cambridge university press; 2006. http://fac.ksu.edu.sa/sites/default/files/BookDistrict_Laboratory_Practice_in_Tropical_Countries_Part2_Monica_Cheesbrough.pdf.
10. WHO (Geneva)? Microbiological criteria. Guide lines for drinking water quality. ed 4th. Austria 2011.
11. Hussain W, Ahmed U, Zaman G, Jamil B, Khursheed U, Mirza IA. Bacteriological examination of drinking water in Rawalpindi and Islamabad area. Pak J Pathol. 2019; 30(4): 101-104.
12. Hannan AB, Shan S, Arshad UM. Bacteriological analysis of drinking water from 100 families of Lahore by membrane filtration technique and chromagar. Biomedica. 2010; 26:152-156.
13. Sarwar G, Khan J, Iqbal R, Afridi AK, Khan A, Sarwar R.. Bacteriological analysis of drinking water from urban and peri-urban areas of Peshawar. J Postgraduate Med Inst. 2004; 18(1): 64-69.
14. Chowdhury JA. Detection of *Escherichia coli* in drinking water sources of filter units and supply water. Bangladesh Pharmaceutical J. 2016; 19(2), 206-210.
15. Li J, Wang H, Liu Y, Lin M, Liu X, Hu X. Distribution and diversity of coliform bacteria in estuary of Jiahe River, China. Int J Environ Res. 2014; 8(2): 501-508.
16. Manafi M. Fluorogenic and chromogenic enzyme substrates in culture media and identification tests. Int J Food Microbiol. 1996; 31(1-3): 45-58.
17. Amirat L, Wildeboer D, Abuknesha, RA, Price RG. E scherichia coli contamination of the river T hames in different seasons and weather conditions. Water and Environ J. 2012; 26(4): 482-489.
18. Nabeela F, Azizullah A, Bibi R, Uzma S, Murad W, Shakir SK, et al. Microbial contamination of drinking water in Pakistan—a review. Environ Sci Pollution Res. 2014; 21(24): 13929-13942.
19. Jabeen A, Huang X, Aamir M. The challenges of water pollution, threat to public health, flaws of water laws and policies in Pakistan. J Water Resource and Protection. 2015; 7(17): 1516.
20. Nurliyana MR, Sahdan MZ, Wibowo KM, Muslihati, A, Saim H, Ahmad SA, et al. The detection method of *Escherichia coli* in water resources: A review. J Phys. 2018; 995: 012065.