Vol 7 (2), July 2016; 6-9

Screening of *Escherichia coli* O157 Strain from Stool Samples in Karachi, Pakistan

Arshia Sohail[1], Erum Mazhar[1]

[1]Department of Microbiology, Jinnah University for Women, Karachi.

ABSTRACT

***Escherichia coli* are the leading non-pathogenic flora of the human intestine. However, some *E. coli* strains have developed the capability to cause infection in gastrointestinal, urinary, or central nervous system in human hosts. O157 strain is an in frequent source of infection, although it can be severe and may lead to a serious intestinal infection along with bloody diarrhea. Most of the people fully convalesce from a O157 infection. However, in only some people, it can be lethal. In this study, the rapid detection method of *Escherichia coli* O157 in feces by using the latex agglutination test kit (Remel-wellcox) and latex agglutination reagents (Remel-wellcolex,) was studied. The latex test was found to be a straightforward, highly competent and reliable test for detecting *E. coli* O157. Out of 52 samples, 20 samples were tested positive tested. The existence of O157 positive clinical sample appeared to be an indicator of presence of this hemorrhagic strain. For the strains of *E. coli* O157 latex reagents used with high (100%) sensitivity and specificity. Our results revealed that the commercial latex reagents are fine substitutes to typical serologic methods for categorizing the O157 antigens of *E. coli*.**

Keywords: *E. coli O157, Remel latex agglutination test, wellcolex latex agglutination test.*

INTRODUCTION

Escherichia coli is a widespread inhabitant of the gastrointestinal tract of humans and animals. *Escherichia coli* are effortlessly grown in the clinical laboratory, although the characterization of the different pathogens involves detection of virulence factors that are not normally available in all clinical laboratories. *E. coli* is one of the best understood and exemplified living organisms along with laboratory studies from biochemical, physiological and genetic view point (Trabulsi *et al.*, 2002).

Some types of *E. coli* are relatively harmless and they live in the intestines as a normal flora without causing any problems. However, other types of *E. coli* may cause intestinal infections. The pathogenic strains of *E. coli* release a poisonous substance known as toxins. The toxins released by *E. coli* damage the intestines and cause inflammation. Among the intestinal pathogens there are six well-described categories: entero pathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), entero toxigenic *E. coli* (ETEC), entero aggregative *E. coli* (EAEC), entero invasive *E. coli*(EIEC) and diffusely adherent *E. coli* (DAEC)(Stephen, 2009). The *E. coli* pathotypes can also cause infections in animals employing many of the same or unique virulence attributes that are present or absent in human strains. (Hartland and Leong, 2013; Jelacic *et al.*, 2008).

E. coli O157 is the most widespread part of pathogenic *E coli* strains known as Entero-haemorrhagic, Verocytotoxin-producing, or Shiga-toxin-producing strain. The infection caused by EHEC O157:H7 includes; asymptomatic mild infection with uncomplicated diarrhea. Screening of EHEC O157:H7 is usually made by biochemical characterization of *E. coli* isolates, serological tests for O157, and H7 flagellar antigen detection by agglutination with the respective anti-serum. Presumptively positive strains are further confirmed by molecular techniques,

such as polymerase chain reaction& pulsed-field gel electrophoresis.

Recently, Latex agglutination tests have become commercially available for rapid presumptive detection of *E. coli* belonging to the serogroup 0157. Latex Agglutination is a complex mechanism that is not yet fully understood. Usually antigen (Ag) Exists in the latex-Ag reagent in two physical states: free in solution (FAg) and bound to latex (BAg). Bound Ag Particles aggregate with the corresponding antibody both in the presence and in the absence Of FAg. When Ag is prevented from adsorbing to the latex, it reacts with antibody in solution, resulting in agglutination of uncoated particles. Standard test procedures For the Identification of *Escherichia coli* serotypes involve agglutination or immune fluorescence whole cells or gel precipitation with soluble antigens. The relative insensitivity or complexity of these tests is a major disadvantage when the quantity of antigen or the time available is limited (Komatsu *et al.*, 1997). The Remel-line of wellcolex rapid latex agglutination test kits is made for fast plus precise characterization of enteric pathogens such as *Salmonella*, *Shigella* and *E. coli*. Wellcolex tests can save testing time over the conventional screening methods and it's comprehensible (Chapman, 1989).

The main aim of this research was to detect *Escherichia coli* O157 strain from stool samples using latex agglutination test.

MATERIALS AND METHODS

Samples Collection: *Escherichia coli* strains were obtained from culture collection of IMAM CLINIC in Karachi, Pakistan. Total fifty two (52), strains of *E. coli* are used for the detection of *Escherichia coli* 0157 strain.

Identification of E. coli: Isolated cultures of *E. coli* from stool samples streaked on Nutrient agar slant. Cultures of *E. coli* streak onto MacConkey agar and Eosin Metallic Green agar for the fermentation of lactose. Biochemical identification was also performed.

Detection of E. coli O157: The reliability of Sorbitol MacConkey agar aid in the identification of *E. coli*0157:H7 in stool cultures. Cultures of *E. coli* inoculated on this agar was incubated at 37°C for 24 hrs.

Latex Agglutination Test: T test was performed according to the manufacturer's instruction.

For every test sample, place 1 drop of test latex in 1 circle and 1 drop of control in the other circle with the emulsified culture. Mix the contents of the circles cautiously dispersing the latex over the whole circle.Circulate the card gradually for about 30 sec and then observe for the agglutination.

RESULTS

The isolation of *E. coli*, stool sample were streaked onto EMB agar plates and incubated at 35°C for 24hours. The colonies which were able to grow on eosin methylene blue (EMB), confirmed the presence of *E. coli* strain on the basis of green metallic sheen and shiny appearance then the colonies of pure cultures of *E. coli* streaked on MacConkey agar for the confirmation of lactose fermentation in the result of pink colonies appear shown in Figure 1 & 2.Colonies of *E. Coli* streaked on TSI it gives characteristics acidic, acidic butt it indicates the fermentation of dextrose, lactose and/or sucrose, absence of black color of the medium occurs shows the non-production of H 2 S. Bubbles or cracks in the agar indicate the production of gas shown in Figure 3. In the field trial of SMAC medium, *E. coli* 0157 was isolated from stools obtained from patients with diarrhea and not from patients without diarrhea. This difference in the connection of *E. coli* 0157 with non diarrheal illness was noteworthy. In all positive stool samples, the growth of *E. coli* 0157 on Sorbitol MacConkey agar was heavy and obtained as colorless non

sorbitol fermenting colonies.

A total of 52 strains of *E. coli* were collected for the detection of Escherichia. coli 0157 strain from stool samples by the Remel kit method. Out of 52 samples, agglutination was observed in twenty samples. While in another samples, agglutination was not observed. Agglutination indicated the presence of *E. coli* 0157(as shown in Figure 4).

DISCUSSION

E. coli is a frequent in habitant of the gastro intestinal tract of human and animal. It is easily grown in laboratory however the characterization of different pathogenic strains necessitates virulence factors detection systems which are not usually available in majority of local clinical laboratories. In the present study, *E. coli* and their 0157 strain has been isolated from stool samples. 32 fecal samples obtained from 20 healthy persons and 12 patients who had diarrhea by *E. coli* O157 were examined. While 20 samples from healthy persons were all negative in the direct inoculation, but 12 samples from diarrheal patients were all positive. In Our study, the rate of *E. coli*O157 infection was found to be 35%. According to the previous research, some of our findings were found to be consistent with other findings (Wetzel and LeJeune, 2006).

Agglutination was observed in twelve e samples of *E. coli* from stool it indicate the presence of *E. coli* 0157 and they are not fermenting sorbitol. Agglutination was not observed in other samples of *E. coli* from stool it means the strains of *E. coli* is not 0157 because, they are fermenting sorbitol. Agglutination of the test latex within one (1) minute is a positive result. This indicates the presence of *E. coli* sero group O157. No agglutination occurring within one minute is a negative result. This indicates the absence of *E. coli* sero group O157; these results were compatible with A. M. Hamza (2013). Some strains of *E. coli* are difficult to emulsify in saline and may give a stringy type reaction with the test reagents. The prevalence of *E. coli*O157 from human feces were compatible with previous study conducted by Omisakin (2003), they reported that the occurrence of *E. coli*O157 in feces was found to be 7.5% and with study of Alam, *et al* (2006).

Detection of *E. coli* 0157:H7 on Sorbitol MacConkey agar had a high sensitivity (100%), specificity (85%), and accuracy (86%). Routine use of SMAC medium is recommended particularly for culturing stools with blood. All our isolates of *E. coli*0157:H7 were verotoxin positive and failed to ferment sorbitol. Therefore, our findings specify that, since *E. coli*0157:H7 does not ferment sorbitol, its colonies on SMAC medium were colorless, and hencet hey are readily recognizable. They are impossible to be differentiated from fecal flora in cultures obtained on MacConkey agar. Our data also indicate that the peculiarity of nonsorbitol fermenting colonies of *E. coli* 0157:H7 on SMAC Medium makes confirmation of presumptive colonies by additional tests straightforward and easy.

Figure 1. Remel Latex Agglutination Test

CONCLUSION

We have evaluated the performance of common laboratory test for the identification of *Escherichia coli* serotype O157. In our experiment we observed that O157 strain of *Escherichia coli* showed agglutination on Remel latex agglutination kit. This test is a straightforward, highly resourceful and reliable test in the screening of *E. coli* O157 with high sensitivity and specificity. Remel Latex agglutination kit test with wellcolex reagents was a speedy, easy-to-perform procedure to provide preliminary results within a short time.

REFERENCES

Alam MJ, Zurek L. (2006). Seasonal prevalence of *Escherichia coli* O157:H7 in beef cattle feces. *J. Food Prot.*, 69(12): 3018- 3020.

Chapman PA. (1989). Evaluation Of commercial latex slide test for identifying Escherichia Coli 0157 Public Health Laboratory, Northern General Hospital, Sheffield. *J. Clin. Pathol.*,42:1109-110 .

Hamzah AM. (2013). Isolation of *Escherichia coli* 0157:H7 Strain from Fecal Samples of Zoo Animal. *Scientific World J.*, 843968. Doi: 10.1155/2013/843968.

Hartland EL and Leong JM. (2013). Enteropathogenic and enterohemorrhagic *E. coli*: ecology, pathogenesis, and evolution. *Front Cell Infect Microbiol.*, 3: 15. doi: 10.3389/fcimb. 2013.00015, PMCID: PMC3639409. .

Jelacic JK *et al.* (2008). Shiga Toxin–Producing *Escherichia coli* in Montana: Bacterial Genotypes and Clinical Profiles. *Oxford J.Med. & Health J. Infect. Dis.,*188(5): 719-772.

Komatsu M, Aihara M, Nagasaka Y, Nakajima H, Iwasaki M, Takahashi M, Shimakawa K, Yamanaka T, Matsuo S. (1997). Rapid detection of *Escherichia coli*0157:H7 in feces by latex agglutination and immunochromatographic assay. *Kansenshogaku Zasshi*, 71(11):1124-30.

Omisakin F, MacRae M, Ogden ID, Strachan NJ.(2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl. Environ. Microbiol*. 69: 2444–2447.

Stephen A. (2009). Enterohaemorrhagic *Escherichia coli* 0157:H7 Prevalence in meat and vegetables sold in Benin City, Nigeria. *Afr. J. Microbiol*. Res., 3(5): 276-279.

Trabulsi LR, Keller R, Gomes TA. (2002). Typical and Atypical Enteropathogenic *Escherichia coli .Emerg. Infect. Dis.*, 8(5): 508 – 513.

Wetzel AN and LeJeune JT. (2006) Clonal dissemination of *Escherichia coli* O157:H7 subtypes among dairy farms in northeast Ohio. *Appl. Environ. Microbiol*., 72: 2621–2626.

Vol 7 (2), July 2016; 10-12

Moraxella Catarrhalis: A Threat For Hospitalized Patients

Maha Jamil[1] & Naheed Afshan[1]

[1]Department of Microbiology, Jinnah University for Women, Karachi.

ABSTRACT

Over the past two decades Branhamella catarrhalis (now known as *Moraxella catarrhalis*) has come out as a major pathogenic bacteria in humans. Different researches have uncovered its association in respiratory tract infections (e.g., otitis media, sinusitis, pneumonia and bronchitis). In children eye infections while in adult laryngitis, bronchitis, and pneumonia commonly occurred. There is rising number of beta lactamase positive strains in this genus, we designed a study project in which we evaluated the sensitivity pattern of *Moraxella catarrhalis* isolated from sputum. Total 124 sputum samples were analysed. Confirmation was done by using chocolate agar a typical golden yellow which can be distinguished by hockey puck like colony. Further identifications were done by gram staining, oxidase test &other biochemical tests. Antibiogram was done by CLSI method. Sensitivity pattern showed Amoxicillinclavunate v 99%, Teicoplanin 49%, Ceftrioxone 01%, Erythromycin 30%, Trimethoprim -sulphamethoxazole 04%, Ciproflaxacin 50%,Cefexatin 84%. To control the resistivity of this organism some preventive measurements should be taken.

Keywords: *Antibiogram, beta lactamase, Moraxella catarrhalis, resistance.*

INTRODUCTION

Neisseria catarrhalis or *Micrococcus catarrhalis* was the formal name of *M.catarrhalis* is a gram negative and aerobic diplococcus organism. *Moraxella catarrhalis* is recognized as a commensal of the upper respiratory tract (Helminen*et al.*, 1994; Winstanley and Spencer, 1986).It is named after the Swiss ophthalmologist Victor Morax. *Moraxella catarrhalis* is an infectious exclusively human. Recent reports suggest this as a pathogenic microbe. Transmission occurs by to direct contact by saliva, air, coughing, & fecal-oral route, etc. Isolation can be done from sputum, urine, blood, naso-pharynx, middle-ear-effusion, trans-tracheal or trans-bronchial aspirates, sinus aspirates, peritoneal fluid, and wounds. Isolation of *Moraxella catarrhalis* may be done by the help of different diagnostic techniques, depending on the infected site and severity. Colonial appearance of *Moraxella catarrhalis* may have an irregular or rough surface and powdery in texture, golden-yellow in colour and opaque in opacity, whereas *Neisseria spp*. have an optimal growth temperature of 35°C-37°C. *Moraxella catarrhalis*is not routinely isolated from oropharynx of fit and healthy adults; however, it is carried more commonly in children and older adults. The bacterium is a frequent source of otitis media as well as sinusitis and an in frequent source of laryngitis, bronchitis and pneumonia in children. It leads to infection of the host cell by sticking to the cell using a Trimeric Auto transporter Adhesin (TAA). In adults with Chronic Obstructive Pulmonary Disease (COPD). It is infrequently a cause of bacteremia and meningitis, particularly in imunocompromised patients can be intricated as osteomyelitis or septic arthritis. It has discovered that an increasing number of beta lactamase positive (Mcleod *et al.*, 1983; Doern and Tuber, 1987).

Incidence rate of lower respiratory tract infection caused by *Moraxella catarrhalis* is has become higher mostly in elderly patients

and its antimicrobial susceptibility pattern in Karachi has modified.

MATERIALS AND METHODS

A total 250 sputum samples were analyzed collected from different Hospitals & pathological labs. Cultured on chocolate & blood agar, a typical goldenish yellow hockey puck like colony indicates the presence of *M.catarrhalis*. Further identifications were done by gram-staining, oxidase -test, Dnase test ,Nitrate reduction & catalase test.

Antibiogram was done by CLSI method in which: Amoxicillinclavulanate (AMC), Tetracycline (T), Moxifloxacin (MXF), erythromycin (E), Trimethoprim -sulphamethoxazole (SXT), Cefoxitin (C), Ceftriaxone (CRO) were used for susceptibility test.

RESULTS & DISCUSSION

After the complete review of the conducted study and observation chart the sensitivity pattern of M. catarrhalis isolated from sputum specimen of hospitalized patients showed that antibiotic discs Ampicillin clavunate was 99% sensitive while only 1% resistant, Tetracycline 49% sensitive while ceftriaxone 51% resistant , 01% sensitive while 99% resistant, Erythromycine 30% sensitive while 70% resistant, Trimethoprim -sulphamethoxazole04 % sensitive while 96% resistant,Moxifloxacin01% sensitive while 99% resistant, Cefoxitin 84% sensitive while 16% resistant. So the result showed the sensitivity pattern of M. catarrhalis isolated from hospitalized patients among 250 samples the M. catarrhalis were isolated from 124 (49.6%) samples. In which 40% female &60% male were infected. Sensitivity pattern showed Ampicillin clavunate 99%, Tetracycline 49%, Moxifloxacin 01%, Erythromycin 30%, Trimethoprim -sulphamethoxazole 04%, ceftriaxone 50%, and Cefoxitin 84%.As we know that M. catarrhalis is consider as normal flora but they become resistant to several antibiotics mentioned in this research indicates that researchers should pay the serious attention towards the organisms. This research also indicates the Nosocomial transmission of M. catarrhalis is has increased.

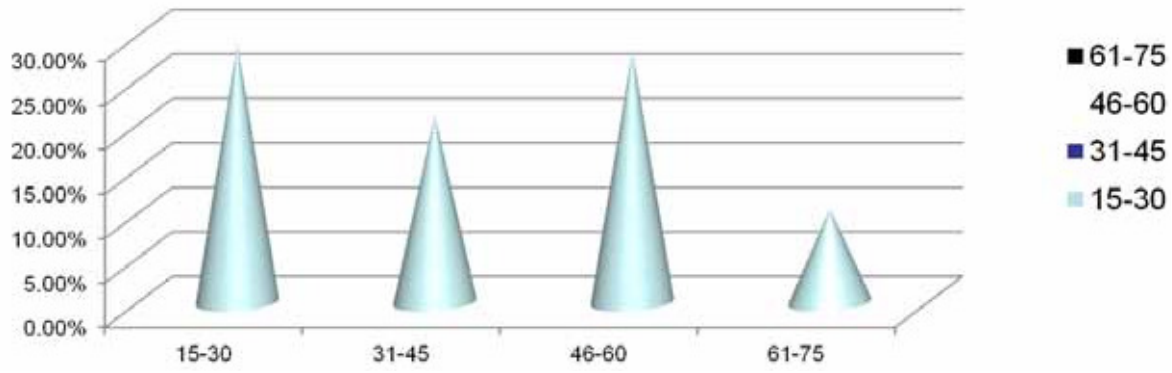

Figure 1. Age groups distribution

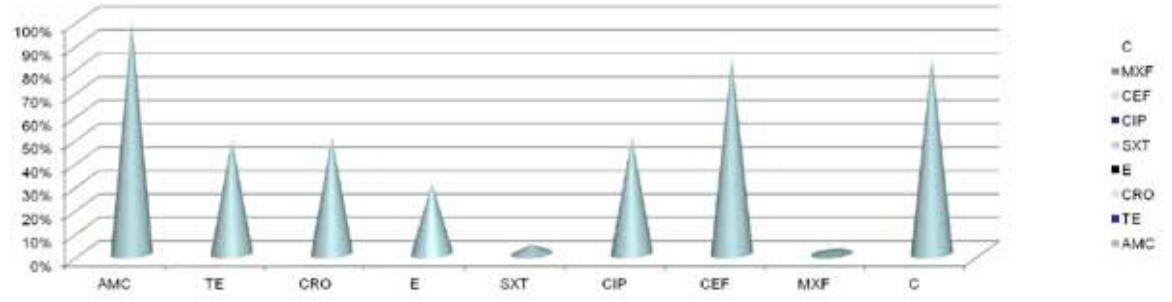

Figure 2. Antibiogram sensitivity pattern of *M. catarrhalis.*

Figure 3. Antibiotic susceptibility testing.

We here worked with minimum amount of samples but to obtain the clear scenario of this organisms we should gathered the large data.

CONCLUSION

Morexallacatarhalis infection may spread from person to person via droplet and expectorated sputum; general hygiene measures should be followed to prevent spread in the community cases. As far as resistivity of these organisms is concerned some preventive measurements like Proper identification and selective medication regimens should also be emphasized.

REFERENCES

Doern GV, Tuber RA. 1987. Detection of beta lactamase activity among clinical isolates of Branhamellacatarrhalis with six different beta lactamase assays. J.Clin.Microbiol., 25:1380-3.

Helminen, Merja E., Isobel Maciver, Jo L. Latimer, Julia Klesney-Tait, Leslie D. Cope, Maria Paris, George H. McCracken, Jr., and Eric J. Hansen. 1994. A Large, Antigenically Conserved Protein on the Surface of *Moraxella catarrhalis*Is a Target for Protective Antibodies. J. Infect. Dis., 170(4): 867-872.

Mcleod DT, Ahmed F, Margaret JT. 1983. Bronchopulmonary infection due to Br catarrhalis. BMJ, 87:144-7.

Winstanley TG, Spencer RC. 1986. Moraxeacatarrkar: antibiotic susceptibility with special reference to trimethoprim. J. AntimicrobialChemother., 18(3): 425-426.