Original Article

Phenotypic identification on the virulence status of Escherichia coli strains isolated from the clinical specimens of tertiary care teaching hospital, Tamil Nadu

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Abstract

Introduction: Escherichia coli is belonging to the family Enterobacteriaceae and it mostly isolated in Urinary tract Infection, diarrheal, Respiratory infections as well as normal commensal in the intestine of humans.
Aim: To determine the virulence status of Escherichia coli isolated from clinical samples using hemolytic activity, sorbitol fermentation and invasiveness.
Materials and Methods: This Cross-sectional study was carried out for a period of six months from (June 2020 – December 2020),in the department of microbiology, Superspeciality Hospital, Chennai with a total 250 specimens of urine as well as stool samples received from different clinical departments.
Result: Among 88 E. coli strains, 32 (36.3%) were collected from faeces and 56 (63.6%) from Urine samples for this research study. Out of 88 patients, 47(53.4%) were female and 41 (46.5%) male patients. In the present study, only 51 isolates (57.95%) showed hemolytic and remaining 37 (42.04%) were non hemolytic activity. In sorbitol MacConkey (SMAC) agar tested, 54 (61.36%) ferment sorbitol and 34 (38.63%) non sorbitol fermenters. Out of 88 isolates, 33 (37.5%) showed virulent and remaining 55 (62.5%) were non-virulent.
Conclusion: This preliminary study insists that to include at least one biochemical marker in the routine diagnostic assay for the detection of virulence status of pathogenicE. coli strains from faeces and urine samples.

Keywords: Escherichia coli, Congo Red Agar, Hemolysin, Virulence, Urinary tract infection

Introduction:

Escherichia coli is a rod-shaped Gram-negative bacterium which is commonly found in the human intestine and other vertebrates. E. coli belongs to the family Enterobacteriaceae and is mostly a pathogenic species in humans that is isolated from clinical specimens, such as urine, pus, and sputum [1-10]. This bacterium causes various diseases directly or indirectly involving tissues and their visceral parts. E. coli predominantly causes urinary tract infections followed by wound infections, pneumonia, and meningitis in neonates or other common infections [11]. E. coli is considered a normal commensal of the human intestine.The most common organisms, such as E. coli and Klebsiella pneumoniae, are involved in sepsis and endotoxin-induced shock. Nonvirulent strains are commonly found in the intestine, and they benefit the host by producing vitamin K2 and preventing the entry of virulent bacteria in the intestine [7]. Urinary tract infection (UTI) is a predominant disease caused by the entry of microorganisms into the genitourinary tract that further extends to the kidney, followed by most respiratory infections [10]. Compared with other organisms, E. coli is the most common causative agent of urinary tract infection and is isolated from approximately 80%–90% of patients. Other bacteria that cause UTI are K. pneumoniae, Pseudomonas aeruginosa, Citrobacter freundii, Enterococcus species, Enterobacter species, Proteus species, Providencia species, and Morganella species [1]. Women are more prone to UTI because of their shorter ureter that allows bacteria to be easily introduced into the bladder during sexual intercourse and the proximity of the urethra to the anus, which facilitates the entry of pathogen into the urinary tract. E. coli is differentiated into different groups: uropathogenic E. coli (UPEC), which cause infection in the urogenital tract, and extraintestinal pathogenic E. coli (EPEC), which are the causative agent of 70%–95% of community-acquired UTIs and nosocomial UTIs. Diarrhoeagenic infection is caused by enterotoxigenic E. coli (ETEC), enteroinvasive E. coli, EPEC, enterohaemorrhagic E. coli, and enteroaggregative E. coli (EAEC), and diffuse adhering E. coli. Among these types, ETEC is the most common, especially in developing countries [1-2]. Virulence of E. coli is multifactorial and primarily associated with other virulent strains. Emergence of multidrug resistant strains of E. coli is considerably progressing among hospitalized patients because of their ability to mutate and transmit plasmids and other mobile genetic elements from resistant to sensitive strains [9]. E. coli is an intestinal pathogen mostly associated with diarrhoeal diseases and UTI; however, its diagnostic testing is lacking in developing countries [14-16]. The objective of this study is to explore the virulence status of pathogenicE. coli strains by screening the haemolytic activity, sorbitol fermentation, and invasiveness by Congo Red (CR) Agar in a clinical isolate.

Materials & Methods:

This prospective study was conducted for the period of six months (June – December 2020), in the Microbiology department, tertiary teaching hospital, Tamil Nadu. Totally, 250 urine and stool samples were collected from the various clinical departments to the microbiological laboratory for routine culture and sensitivity testing were included in the study. Routine stool examination like Rice watery stool, Stool for Ova/cyst and hemorrhagic stools were excluded. The collected sample was processed for aerobic culture followed by Biochemical test to identify the pathogenic bacteria like E. coli, Klebsiella, and other Enterobacteriaceae family. The above samples were inoculated on to Blood agar, MacConkey agar for stool samples and Cystine Lysine Deoxycholate (CLED) agar for Urine samples and incubated at 37°c for 24 hours and observed for the growth. Suspected colonies were identified from the culture plate and to carried out the preliminary test like gram stain, catalase and oxidase to confirm the genus of the organism. In Blood agar-colonies were characterized as circular, flat, large, thick, regular, mucoid, grey moist non-hemolytic. Due to lactose fermentation, a pink color colony was observed in MacConkey agar. Few colonies were taken and inoculated into peptone water by comparing with 0.5 McFarland standard and incubated at 37ºC for two hours to perform biochemical as well as antibiotic sensitivity testing for the isolates. The following routine biochemical tests like IMViC (Indole, Methyl Red, Voges Proskauer, Citrate Utilization, Triple Sugar Iron Agar and Urease test), were performed in bacteriology for the identification of bacteria. Virulence tests like Hemolysin, Sorbitol fermentation and Invasiveness tests using Congo Red Agar (CR) were performed for the isolates and the results have been noted based on the CLSI guidelines 2019 [13].

Results:

Out of 250 samples, 88 E. coli strains have retrieved, 32 (36.3%) from faeces and 56 (63.6%) from urine for this research study. Mean Age of the patients were 31±15.5 and the patients were grouped under three categories viz., 29 patients are under 18 to 30 years followed by 22 from 31 to 50 years and remaining 37 were above 51 years.

Out of 88 patients, 47(53.4%) were female and 41 (46.5%) male patients. Table– 1 have listed the study samples were received from the different clinical departments. E. coli strains have identified from the clinical samples based on the preliminary and biochemical findings. Antibiotic sensitivity testing was recorded as per the CLSI guidelines [13]. In the present study, only 51 E. coli isolates (57.95%) were found to be hemolytic and remaining 37 (42.04%) were non hemolytic activity. In sorbitol MacConkey (SMAC) agar tested, 54 (61.36%) was observed to ferment sorbitol and 34 isolates (38.63%) were non sorbitol fermenters showed in [Figure – 2].

Congo Red Agar:

Out of 88 isolates, 33 (37.5%) were showed orange/pink color which implies to be virulent strains and it tends to be producing a biofilm formation followed by remaining 55 (62.5%) with white color colonies which indicates non-virulent.

Discussion:

Pathogenic E. coli is mostly isolated from the samples of children and adults with acute watery diarrheal diseases in the developing countries, and it causes approximately more than 400 million diarrheal cases and 3,80,000 deaths annually in children from <5 years of age [11]. Because diarrhoea is a nonspecific disease, it occurs with a common medical problem in developed tropical and subtropical regions. Many DEC strains are prevalent in developing nations because of inadequate sanitary conditions [10]. Statistical surveillance has been conducted for prevalent strains, and the results may vary from region to region. The virulent nature of E. coli was determined based on Congo Red (CR) binding on agar plate, where 33 isolates (37.5%) exhibited that virulent in nature, whereas the remaining 55 (62.5%) isolates are tend to be nonvirulent. CR is one of the most vital assays for the detection of invasive and non-invasive E. coli among the clinical samples [12]. However, researchers from different regions have demonstrated the ability of E. coli strains to produce with the increased levels of curli fibres on the bacterial cell surface, and these fibres are mostly present in the fresh urine sample of infected patients. In the present study, only 54 isolates (61.36%) were able to ferment sorbitol, and the remaining 34 (38.63%) were designated as non-sorbitol. SMAC medium can be used to determine the virulence of E. coli, from faecal specimens that does not ferment sorbitol and coloured colonies; they are indistinguishable colonies grown on MacConkey agar. Virulence of UPEC strains which contributes to cause UTI in humans which is derived from the colonic flora of the affected individuals. Kahalii et al. reported that 75.2%, 64.5%, 45.5%, and 43% of patients with EAEC infection had watery diarrhoea, vomiting, abdominal pain, and severe dehydration [18]. In this study, 51 isolates (57.95%) were haemolytic and the remaining 37 (42.04%) were non haemolytic. E. coli strains produce haemolysin, which can cause an intestinal and extraintestinal diseases in humans.

Conclusion:

To conclude, the preliminary study insists that to include at least one biochemical marker in the routine diagnostic assay for the detection of virulence status of E. coli strains from faeces and urine. Genotypic methods will be performed for these isolates to identify the resistant genes of E. coli strains in future.

CONFLICT OF INTEREST: The authors declares that there is no conflict of Interest

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AUTHOR'S CONTRIBUTION:

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication

- Conception and design, acquisition of data, or analysis and interpretation of data has been done by the Nandha Gopal D, Sunil Indernath & Vinod babu
- Either drafting the article or revising it critically for important intellectual content has been done by the Nandha Gopal, Prem Kumar & Usha
- The final approval of the version to be published has been given by the Sudha, Vinod babu & Shanmugavadivoo

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DATA AVAILABILITY:

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT:

Not Applicable

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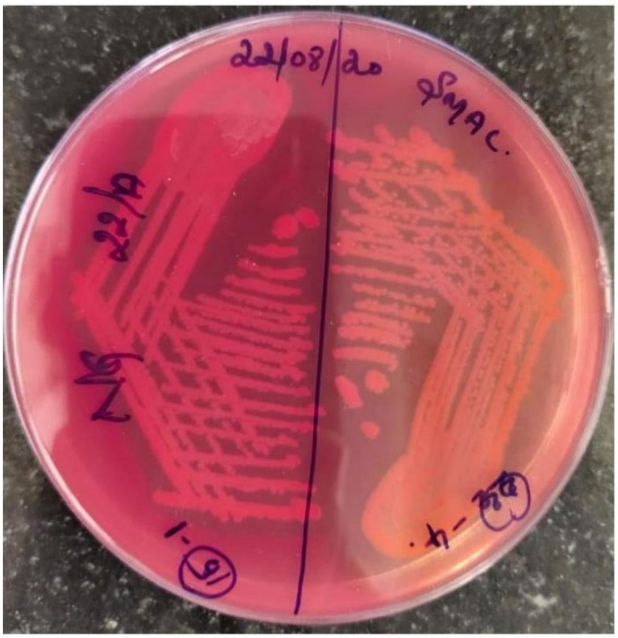
Table - 1: List of specimens received from different clinical departments

CLINICAL DEPARTMENT	NO OF SPECIMENS (n=88)
GENERAL MEDICINE	27
EMERGENCY MEDICINE	8
OBG	14
UROLOGY	8

SURGERY	9
GASTROENTEROLOGY	12
ICU	3
COVID WARD	7



/Fig 2: SORBITOL MACCONKEY AGAR MEDIUM