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ISOLATION AND IDENTIFICATION OF ESCHERICHIA COLI O157:H7, O26:H8 AND O111:H8 USING CULTURE AND SEROLOGICAL TECHNIQUE IN KHARTOUM STATE SUDAN – 2019

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ABSTRACT

Most *E.coli* are considered as commensal of the human and animals intestine and are harmless. Some serotypes are pathogenic. One of the most important pathogenic serotype is *E. coli* O157:H7 which cause Enterohaemorrhagic diarrhea. The non-O157 strains were found to cause similar illnesses, mostly by Enterohaemorrhagic *Escherichia coli* (EHEC) O26:H11 and O111:H8 This study was conducted to look for the presence of *E. coli* O157:H7 and related serotypes *E. coli* O26:H11 and *E. coli* O111:H8 in Sudanese patients having diarrhea using MacConkey agar (MAC) and Sorbitol-MacConkey agar (S-MAC) and serology at hospitals in Khartoum State, Sudan. A total number of 100 patients having diarrhea were included in this study. Identification of the organism was done using culture of the stool specimens on (MAC) and (S-MAC) followed by biochemical tests and serological identification. The result of culture showed that 89 isolates were identified as *E.coli* on MAC. 29 were identified as non sorbitol fermenter *E.coli* and 60 were identified as sorbitol fermenter *E.coli* on S-MAC. The serological tests showed that 25 out of 29 non sorbitol fermenter were *E.coli* O157 H7 while 53 out of the 60 sorbitol fermenter were identified as *E.coli* O26 H11 and the remaining 7 were *E.coli* O111 H8. As conclusion the commonest serotype identified in Sudan was *E.coliO26:H11* (53 isolates) followed by *E.coli* O157 H7(25 isolates).

KEYWORDS: Enterohaemorrhagic *Escherichia coli* (EHEC), sorbitol, serotypes.

INTRODUCTION

E. coli; is a common commensal in the lower intestine of human and animals. More than 700 serotypes of *E.coli* have been identified. Most of *E.coli* strains are harmless, but some serotypes can cause serious diseases in humans.[1] Fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause the disease.[2]

Virulent strains of *E. coli* can cause gastroenteritis that may end up with hemolytic uremic syndrome(HUS).[3] Certain strains of *E. coli*, refered to Enterohaemorrhagic *Escherichia coli* (EHEC) such as O157:H7, O111:H8, O26:H11, O104:H4 and O104:H21 produce potentially lethal toxins which causes diarrhea or bloody diarrhea. *E.coli* O157:H7 is the most notorious strains.

A stool culture can detect the bacterium, although it is not a routine test and must be specifically requested. Routinely the stool specimens are cultured on MacConkey agar (MAC) producing pink colonies as *E.coli* is lactose-fermenter.[4] On Sorbitol-MacConkey (S-MAC) agar, *E.coli* O157:H7 produces pale colonies due to their inability (unlike other *E. coli* serotypes) to ferment sorbitol.[5] The biochemical reactions of *E. coli* O157 are similar to other strains of *E. coli*.[5]

***Escherichia coli* O157:H7** produces shiga-like toxins(*stx)* which has multiple variants (e.g., *Stx1*, *Stx2*, *Stx2c*) and cause severe illness like hemorrhagic diarrhea, and occasionally may lead to kidney failure, especially in young children, elderly and of patients whose immune systems are otherwise compromised.[6,7]

E. coli O157:H7 was first recognized as a pathogen as a result of an outbreak of unusual gastrointestinal illness in 1982. The outbreak was traced to contaminated hamburgers, and the illness was similar to other incidents in the United States and Japan.[8]

***Escherichia coli* O26:H11 and *Escherichia coli* O111:H8** were estimated to cause 36,000 or more illnesses, 1,000 hospitalizations and 30 deaths in the United States yearly Food safety specialists recognize toxin producing strains; O26, O45, O103, O111, O121, and O145[9] Enterohaemorrhagic *Escherichia coli* (EHEC) O26:H11 emerged as the most important non-O157:H7 EHEC, with respect to their ability to cause diarrhoea and haemolytic uraemic syndrome (HUS). Since 1996, EHEC O26, was found to produce Stx2 only and appear to have enhanced virulence.[10]

However, non-O157 have been isolated with a frequency similar to that of *E. coli* O157:H7 from diarrheal stool specimens.[11]

This study is meant to look for the identification of *E. coli* O157:H7, O26:H11 and O111:H8 by culturing and serological tests from the stool of Sudanese patients presenting with diarrhea.

MATERIAL AND METHODS

This is a cross sectional laboratory based study .One hundred stool specimens were collected from patients suffering from diarrhea and or bloody diarrhea, both gender adult and children from different hospitals in Khartoum State were included (table 1) The Specimen were received into a sterile plastic containers and were processed immediately for the detection of pathogenic *E. coli*. The Isolation of *E. coli* from the stool specimens was done by culturing directly onto MAC agar plates (Oxoid Basingstoke England), and S-MAC (Oxoid Basingstoke England). S-MAC can assist in the isolation of *E. coli* O157:H7 since it is known to be non sorbitol fermenter colonies producing pale yellow colonies.

All plates were incubated aerobically for 24 hours at 37°C and examined for significant growth of *E. coli*. *E. coli* isolates were identified on the basis of cultural characteristics, gram stains, biochemical tests, and serological test where the detection of O antigen and H antigen was done according to the British Cteq Company.

RESULTS

Out of the 100 specimens 96 reveled lactose fermenting colonies. Out of 96 lactose fermenting colonies 89 were identified to be *E.coli* by the biochemical tests. The 89 isolates were subcultured on S- MAC agar. twenty nine of them were non sorbitol fermenter *E.coli* and 60 were sorbitol fermenter *E.coli (*figure 1*).*

By using the antisera for the three targeted serotypes twenty five out of the twenty nine non sorbitol fermenter were positive for *E.coli O157 H7*. Out of the sixty sorbitol fermenter *E.coli*. 53 were found to be reactive with *E.coli O26 H11* and seven with *E.coli O111 H8.* Four out of the 29 non sorbitol fermenter were nontypeable (figure.2).

Table 1: Details of the participants' gender and ages.

Participants	Frequency	Percentage	Mean age
Females	26	26%	45
Males	34	34%	40
Children	40	40%	6
Total	100	100%	

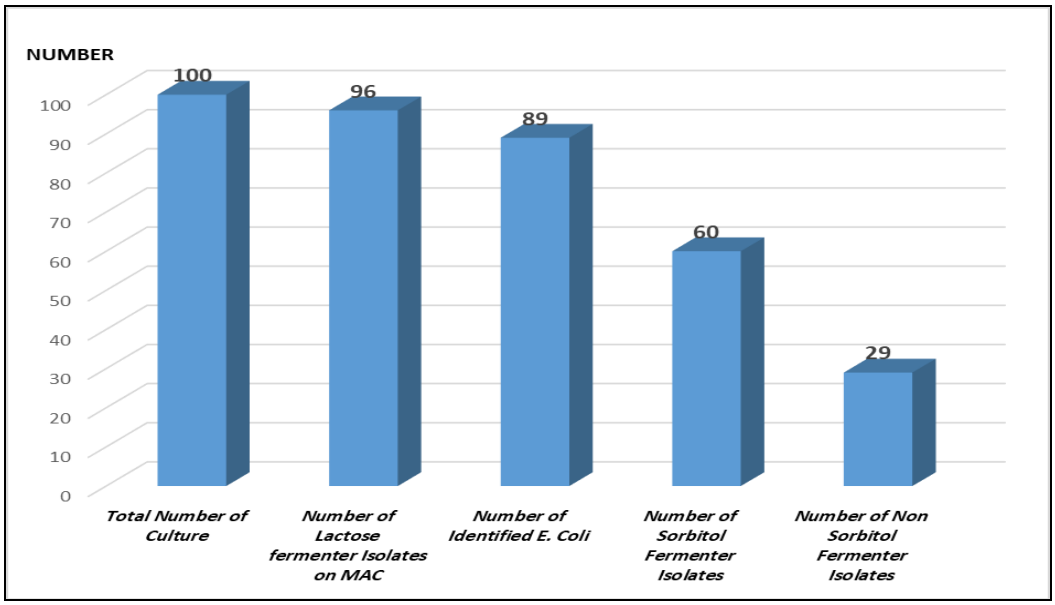

Figure 1: The Outcome of growth on MAC Agar and S-MAC Agar.

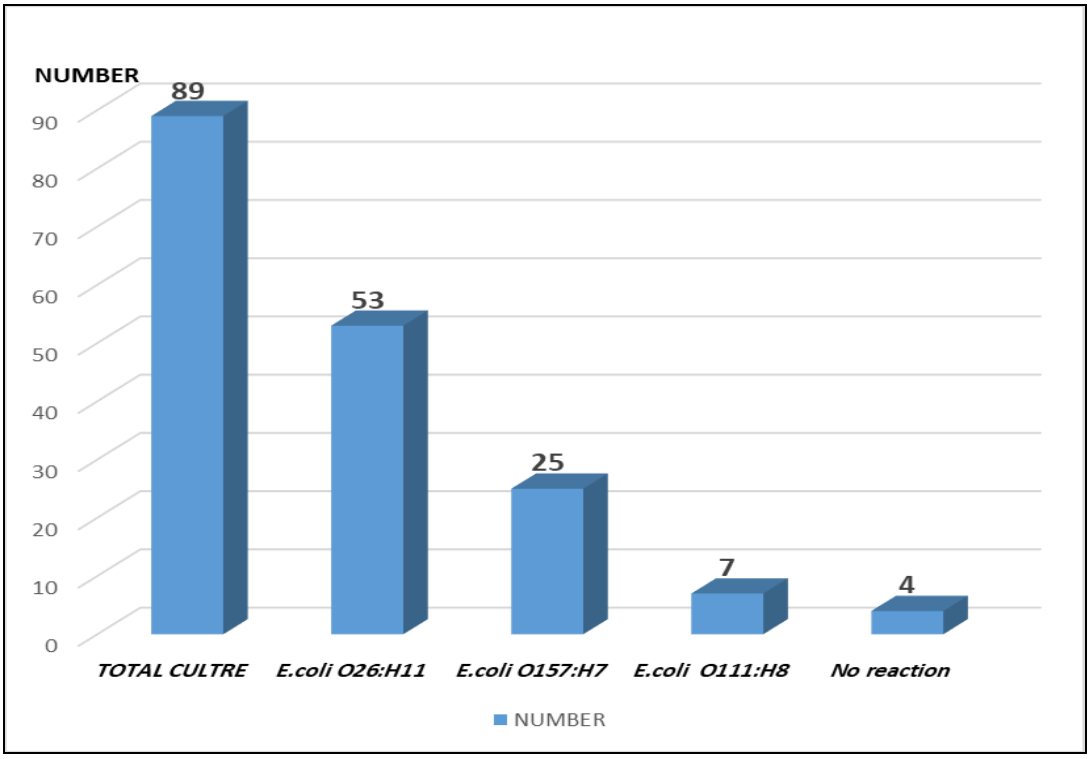

Figure 2: Serological Identification of the Isolate *E. Coli.*

TOTAL CULTURE MUST REPLACED WITH TOTAL NUMBER OF ISOLATES E.COLI NO REACTION WITH NO REACTIV

DISCUSSION

In this study the number of the non sorbitol fermenter *E.coli* was 29. Out of them 25 were reactive with the antisera of *E.coli* O157 H7. Four of them were non reactive which means that not all the non sorbitol fermenter should be considered as *E.coli* O157 H7. Hence confirmatory serology is necessary to conclude the proper diagnosis of *E.coli* O157 H7.

The sorbitol fermenter *E.coli* were sixty isolates. Out of them 53 were proved to be *E. coli* O26 H11. *E.coli* 026 H11 emerged as a highly virulent strain in Europe.[12] This mean that not all sorbitol fermenter are harmless and underline the importance of microbiological diagnostic approach to detect sorbitol fermenter non O157 H7 EHEC.

Seven of the sorbitol fermenter were confirmed serologically to be *E.coli* O111 H8. Wenlan Z *et al* (2007 isolate 72 isolates of Enterohaemorrhagic *E.coli* O111 from patient with diarrhea or HUS as an emerging EHEC.[13] Consequently *E.coli O26 H111* should be monitored carefully to detect shiga like toxin production in cases of diarrhea or HUS.

CONCLUSION

The commonest (EHEC) in Sudanese patient with diarrhea is *E.coli* O26:H11 according to serology.

Serological confirmation of the virulent serotypes of (EHEC should be routinely adopted.

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