



Isolation Enterohemorrhagic *Escherichia coli* from gall bladder of buffaloes and determine its sensitivity for antibiotics

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Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, This study was preformed to find EHEC in gall bladder of buffaloes , (150 gall bladder) samples were collected from slaughter house and cultured on MacConky agar then identified by culture on Cefixim Tellurite Sorbitol-MacConkey, agar then tested with several types of antibiotics and serotyping of isolates to determine EHEC, in addition to conjugation methods were preformed .Results showed that 13 isolates of E.coli were obtained and 6 strainS were grown on Cefixim Tellurite Sorbitol-MacConkey agar and positive for specific antisera of (EHEC O157:H7), and all strains were only sensitive for nitrofurane, When conjugation done all recipient cell were resistant for antibiotics:

(ampicillin,streptomycin,sulfonamide,trimthoprine,tetracycline,chloramphenicol,genta mycin and amikacin).

Key words: *Escherichia coli*, Enterohemorrhagic , gall bladder, buffaloes.

عزل جرثومة الاشريشيا القولونية النزفية من مرارة الجاموس وتحديد حساسيتها للمضادات
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الخلاصة:

ترتبط جرثومة الاشريشيا القولونية النزفية المعوية بالتهاب القولون النزفي و هو شكل من اشكال الاسهال الشديد وايضا مع متلازمة البيلة الدموية. هذه الدراسة صممت لايجاد جرثومة الاشريشيا القولونية النزفية في مرارة الجاموس, 150 عينة مرارة جمعت من المجازروزرعت

على اكار الماكونكي وشخصت ايضا بواسطة الزرع على وسط سيفوكسم تليوريت سوربيتول ماكونكي اكار واختبرت العزلات لعدة انواع من المضادات وتم اجراء التتميط المصلي لتحديد النوع اضافة الى طرق الاقتران التي صممت ,اظهرت النتائج الحصول على 13 عزله ستة منها نمت على وسط سيفوكسم تليوريت سوربيتول ماكونكي اكاروكانت موجبة للمصل المضاد كل العزلات كانت حساسة للنايتروفيوران وعند الاقتران كانت الخلايا المستلمة جميعها مقاومة للمضادات.

Introduction:

Enterohemorrhagic *Escherichia coli* (EHEC) is a subset of pathogenic *E. coli* that can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS) (1), an important cause of acute renal failure in children and morbidity and mortality in adults. In the elderly, the case fatality rate for HUS can be as high as 50%. *E. coli* O157:H7 (EHEC O157:H7) has been recognized as a cause of this syndrome since the 1980s (2)

The reservoirs for EHEC O157:H7 are ruminants, particularly cattle and sheep, which are infected asymptotically and shed the organism in feces (3). Other animals such as rabbits and pigs can also carry this organism. Humans acquire EHEC O157:H7 by direct contact with animal carriers, their feces, and contaminated soil or water, or via the ingestion of underdone ground beef, other animal products, and contaminated vegetables and fruit. The infectious dose is very low, which increases the risk of disease (4).

E.coli is usually considered as an indicator organism for faecal

contamination and is important parameter in food and water

hygiene. These organisms transmitted by direct contact or through contaminated food and water. While generic *E.coli* is considered as an

intestinal pathogen, many strains of these species can be pathogenic leading to diarrhoeal diseases(5).

Materials And Methods**Collection of Samples:**

Hundred and fifty gall bladder samples were collected from buffalos at slaughter house.

Culture:

Bile were withdrew by sterile syringe and each of the bile samples in dilution of 1 ml in 9 ml buffered peptone water then 1 ml of diluted sample was plated on Eosin methylene blue (EMB) agar. They were then incubated at 37°C for 24 hours. Pure cultures of all colonies exhibiting colonies with typical metallic sheen which is characteristic of *E. coli* on EMB were then made ready for biochemical tests. Pure cultures of all positive *E. coli* were cultured on Cefixim Tellurite Sorbitol-MacConkey

agar and incubated at 37°C for 18 - 24 h, then confirmation by API kit (bioMerieux, France) . Suspected colonies of *E. coli* O157:H7 were confirmed using slide agglutination test with *E. coli* O157: H7 antiserum (Remel/USA) , Then determine serotypes with monovalent antisera for *E. coli* O157: H7(murex/ UK).

Conjugation method:-

Conjugation was performed according Oconnell(6) by using *E. coli* MM294 as a recipient cells and isolates.

Results:-

Thirteen samples(8.66%) were positive for *E. coli*, when cultured on MacConky agar where lactose ferment on macConky agar, with smooth,pink round colonies, *E. coli* were negative for VP, citrate utilization and oxidase, gave acid results on TSI with CO2 production and H2S, positive for indol, catalase and MR, all strain were motile as well as 6 strains were hemolysed blood agar.

Six strains were positive for growth on Cefixim Tellurite Sorbitol-MacConkey agar and latex agglutination with *E. coli* O157: H7 antiserum, serotyping results of isolates showed six strain were have O157: H7 (46%).

Results of conjugation :

All strain of *E. coli* O157: H7 were resistant (100%) for ampicillin and streptomycin, (83.33%) for sulfanamide , trimethoprine , tetracycline chloramphenicol ,gentamycin and (16.66%) for amikacin. While all isolates were sensitive to nitrofurane .

When conjugation done all recipient cell were resistant for ampicillin streptomycin, sulfanamide , trimethoprine , tetracycline chloramphenicol and gentamycin, except one isolates was resistant for amikacin.

Discussion

From results proved that *E.coli* found in gull bladder of buffalo, including *E. coli* O157: H7 this same that reported by Bach et al., (7)they said that Cattle are the main reservoir for *E. coli* O157:H7, which colonizes the gastrointestinal tract and is shed in the feces. Growth colonies on Cefixim Tellurite Sorbitol-MacConkey and biochemical results like that reported by(8) . According to the sensitivity test and cojugation results , most isolates were resistant to sulfa trimethoprin , streptomycin chloramphenicol and tetracycline due to multiple using of these antibiotics(9), in addition to the increase use of these antibiotics the plasmid content may be the cause behind the highly resistant and the ability of these plasmid to transfer among the bacterial strains and transfer the resistant character with them(10).

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