

ISOLATION AND IDENTIFICATION OF ENTEROHEMOLYTIC ESCHERICHIA COLI FROM SLAUGHTERED COW'S LIVER AND LUNG

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ABSTRACT

Liver and lung samples (25 for each) were collected from slaughtered cows and examined bacteriologically for the presence of *Escherichia coli* Eosin-Methylene Blue (EMB) and MacConkey agar positive culture (15 from liver and 12 from lung) were produced by cultivation of liver and lung samples. *Escherichia coli* presence was confirmed biochemically in 53.3% of liver isolates and in 33.3% of lung isolates. Enterohemolytic activity was detected in 75% of both liver and lung isolates.

INTRODUCTION

Coliform constitute a group of bacteria that are aerobic and facultative anaerobes. The rational of coliforms test was required by many agencies due to *Escherichia coli* which is a member of the coliform group. It is common in the faces of man and animal. Other coliform such as *Aerobacter* and *Klebsiella* are found to be wide spread in soil, water and plants(1).

Escherichia coli, *Aerobacter* and *Klebsiella* are three indicators for the sanitary quality of foods and the presence of one or more of them could easily give rise to public health hazards. The isolation of these organisms may indicate a possible contamination with potential pathogen such as coliform(2).

Raw beef is a major source of pathogenic *Escherichia coli* and it has been assumed that such organisms in the faces and hid of cattle spread in meat during slaughtering and processing(3).

Midyley and Desmarchelier(4) found that cattle and their environment are among the most important sources of pathogenic *Escherichia coli* and they may be the origin of meat contamination.

The aims of this study were to investigate the possibility of the presence of human pathogen associated with food poisoning outbreaks such as *Escherichia coli* and to assess the

microbial quality of liver and lung in order to ensure that these material present no health hazard to the human consumers.

MATERIALS AND METHODS

Samples collection :

Liver samples (25) and lung sample (25) were collected from slaughtered cows seen in the Basrah slaughter house. The liver and lung area used for sampling were 25 cm². According to method of (Hall and Maurer)(5). Two test tubes were used for each sample . First one containing 10 ml of 0.1% peptone water and the second sterilized tube contain cotton swabs. At the time of samples collection, a sterile swab was removed from its tube and moistened with peptone water by dipping it into the first tube. The sterile metallic template was pressed against the surface to be sampled. The tip of moistened swab was rubbed over the area to be sampled. The swab was broken off into tube containing peptone water.

Bacteriological analysis:-

After the swab sample were shaken thoroughly, aliquots, 0.1ml was streaked on to surface of MacConkey agar and Eosin Methylene Blue agar (EMB) and incubated at 37°C for 24hr. Suspected coliform colonies were tested biochemically by hydrogen sulfide production on triple sugar iron agar(TSI), urea hydrolysis , citrate utilization and indol production. Isolation and identification of coliform isolates were performed according to method of (Quinn et al)(6)

Detection of Enterohemolysis activity :

Enterohemolytic activity of *Escherichia coli* isolates were detected on blood agar plates (blood agar base supplemented with 5% sheep blood as described previously)(7)

The inoculated plates were observed for hemolysis after 3h of incubation (for detection of alpha- hemolysis) and after overnight incubation at 37°C (for detection of enterohemolysis or non –hemolysis)(7).

RESULTS

EMB and MacConkey positive culture (15 from liver and 12 from lung) were produced from cultivation of liver and lung samples. Biochemical tests revealed that 8 (53.3%) of liver isolates and 4 (33.3%) of lung isolates showed positive results specific for *Escherichia coli* (Table-1).

Table-1-The distribution of Escherichia coli in liver and lung isolates:-

organ	Examened No.	E coli +Ve	
		No.	%
liver	15	8	53.3
lung	12	4	33.3
Total	27	12	44.4

* +Ve = positive

Identification of Escherichia coli isolates:-

Escherichia coli isolates were identified by its cultural characteristics on MacConkey agar plates and EMB agar plates. The identification of Escherichia coli isolates depend on the biochemical test as shown in table-2.

Table -2- the results of Escherichia coli identification tests:-

samples	+Ve samples	No %					
		EMB	MacC.	I	C	U	TSI
liver	8	metallic sheen	pink col.	+	-	-	Y/Y/-H2S
lung	4	metallic sheen	pink col.	+	-	-	Y/Y/-H2S

EMB= Eosin Methylene blue, MacC =. MacConkey, I= Indol production ,

c= citrate utilization, U= urea hydrolysis, TSI= Triple sugar Iron Agar., Y= Yellow, H2S= Hydrogen sulfide. Col= colour ,+ve = positive

Detection of EHEC

In liver isolates comparative study between biochemical tests and enterohemolytic activity, concordance positive results between both were 6(75%) concordance negative results were 6(58.7%). Negative results for biochemical tests and positive for enterohemolysis was only one case 14.3% (Table3).

In case of lung isolates concordance positive results between both were 3(75%) and concordance negative results were 7(87.5%). Negative results for biochemical tests and positive for enterohemolysis was only one case 12.5% (Table3). The positive reported enterohemolytic activity was for positive results after 24h.

Table -3- Comparative study between biochemical tests versus enterohemolysin production

Sample	Biochem. Test	Enterohemolysin Positive		Enterohemolysin Negative		Total	
		No.	%	No.	%	No.	%
Liver	Positive No.	6	75	2	25	8	100
	Negative No.	1	14.3	6	58.7	7	100
Total No.		7	46.7	8	53.3	15	100
lung	Positive No.	3	75	1	25	4	100
	Negative No.	1	12.5	7	87.5	8	100
Total No.		4	33.3	8	66.7	12	100

DISCUSSION

In general the presence of *Escherichia coli* in the food has been considered as a criterion for the existence of unsanitary conditions. However, presence of *Escherichia coli* in liver and lung of slaughtered cow (53.3% in liver and 33.3% in lung) may indicate contamination from either faecal or nonfaecal sources. These results were in agreement with results of Alaa(8) who report that 53.13% of beef carcasses show positive cultures of coliform.

Escherichia coli is an important member of the normal intestinal microflora. However, *Escherichia coli* is more than just intestinal inhabitant, it can also be a highly pathogenic. Several different *Escherichia coli* strains cause diverse intestinal and extra intestinal diseases by means of virulence factors that effect a wide range of cellular processes.(9).

Shiga toxin producing *Escherichia coli* (STEC) is an important emerging food born pathogen, it has been associated with bloody and non – bloody diarrhea, hemorrhagic colitis and, hemolytic uremia syndrome (HUS). The cattle have been shown to be the major reservoir of STEC(10). Rapid identification of STEC types performed by enterohemolysin production as a marker. In the present study enterohemolysin was detected in 75% of both liver and lung isolates. EHEC hemolysin, which causes an enterohemolytic phenotype on blood agar, was detected in many STEC strains of different origins in previous reports(11) and(12). That's to say enterohaemolysin production could be considered as good phenotypic marker for STEC

From this study we can conclude that EHEC isolated from liver and lung of slaughtered cows and this isolation represent health hazard to human consumers

عزل وتشخيص الشريكيا القولونية المحللة للدم من اكباد و رئات الأبقار المذبوحة

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الخلاصة

تم جمع عينات من اكباد ورئات (25 عينة لكل منها) الأبقار المذبوحة وفحصت جرثومياً. وظهرت نتائج موجبة عند زرع هذه العينات على وسطي EMB و MacConkey الزرعين (15 نتيجة موجبة للكبد و 12 عينة موجبة للرئة) وثبت وجود الشريكيا القولونية بواسطة الاختبارات الكيمياء حيوية ونسبية 53.3% من عزلات الاكباد و 33.3 % من عزلات الرئتين. وتم الكشف عن فعالية التحلل الدموي في 75% من عزلات كل من الاكباد والرئتين.

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