BACTERIOLOGICAL AND SEROLOGICAL STUDIES ON ESCHERICHI A COLI IN MASTITIC MILK IN GHARBIA PROVINCE
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Abstract
The present study included the examination of 100 quarter milk samples from mastitic udder. Thirty-eight (38%) Escherichia coli isolates were recovered. Most isolated strains showed typical morphological and biochemical reaction of Escherichia coli.
Antigenic studies of Escherichia coli revealed 10 "O" different serogroup of Escherichia coli. The serogroups were O6 (12 strains), untyped, O27 (8 strains), O98, O148, O152, O153, O154, O167 (2 strains each), O128 (one strain and 3 strains untyped).
In vitro, antibiogram showed that the majority of isolated strains of Escherichia coli were sensitive to ampicillin, cefadroxil, cefotaxime, chloramphenicol, gentamicin, neomycin and nitrofurazone, but all strains were resistant to amoxicillin, duracet, lincomycin and oxytetracycline.

INTRODUCTION
Mastitis is the most important disease affecting udder; it is a problem of considerable economic importance in dairy industry. Mastitis has a quite vital importance due to its association with many zoonotic diseases in which milk acts as a vehicle of infection. Zakarya (1963) and Abdel Karim and El-Ashmary (1979) isolated bacteria causing bovine mastitis includig Escherichia coli.

The present work was conducted to enumerate Escherichia coli associated with mastitis and its different "O" serogroup. Also, a trial was made for antibiograms to ensure rapid and prompt recovery of affected udder.

MATERIALS AND METHODS

Materials
1. Samples
A total of 100 quarter milk samples were collected aseptically from cows with mastitic udder. The samples were taken during the different seasons of year from private and governmental dairy farms.
2. Media used for isolation
   1. MacConkey's agar medium (Difco, 1977).
   2. Eosin methylene blue.

3. Media for biochemical identification
   1. Triple sugar iron agar media.
   2. Pepton water 1% for indole test.
   3. Glucose phosphate broth for methyl red and Voges proskauer test.
   4. Simon's citrate media.
   5. Media for sugar fermentation, 1% of the following sugars: sucrose, sorbitol, dulcitol, raffinose, and lactose.
   6. Antibiotic sensitivity test (Antibiogram). Antibiotic sensitivity discs obtained from Oxoid Laboratories were used. Such discs contained the following antibiotics:
      1. Amoxicillin, ampicillin, cefadroxil, cefetaxime, chloramphenicol, duracuf, gentamicin, lincomycin, neomycin, nitrofurantoin and oxytetracycline.
      2. Muller Hinton broth.
      3. Muller Hinton agar for sensitivity test.

4. For serological test

   Forty-three *Escherichia coli* "O" antisera used for typing were divided into "B" polyvalent groups, each one contained numbers of monovalent antisera.

Methods

Collection of milk samples

The udder of each cow was palpated before sampling for detection of any abnormalities such as swelling or any other changes.

Each udder was thoroughly washed with soap and water, carefully dried with clean toilet-paper, then, the teats were swabbed with 70% alcohol. The first few streams of milk were rejected, then, about 15-20 ml of milk were drawn from 4 quarters of the udder into sterile screw-capped MacCartyen bottles. All samples were transported immediately to the laboratory, where they were kept for bacteriological examination.

Initial isolation of *Escherichia coli* from milk samples was made on MacConkey's agar medium; lactose fermenting colonies were plated on eosin methylene blue (EMB) agar. Colonies showing characteristic metallic sheen were
tentaive identified as those of *Escherichia coli*.

*Escherichia coli* were confirmed by IMVC reaction, fermentation of glucose with gas production and absence of H₂S production on (TSI). Fermentation of other sugar, viz, sucrose, sorbitol, dulcitol, dulcitol, raffinose and lactose were applied.

For serotyping, the procedure outlined by Sojka (1965) by using slide agglutination test was used. One drop of saline was placed on a clean slide and a loopful of *Escherichia coli* culture was emulsified on saline, one drop of antiserum was placed on antigen and recorded within 2 minutes.

**Antibiogram**

1. Preparation of medium

1) Five colonies of similar morphology were transferred using sterile loop to a tube containing 5 ml Muller Hinton broth.

2) The broth was incubated at 37°C for 2 hours until turbidity was noticed.

2. Disc diffusion technique

The disc diffusion technique of antibiogram was adopted according to Cruikshank et al. (1975).

**RESULTS**

From the 100 milk samples studied, 38 *Escherichia coli* cultures were isolated. The biochemical characterization of *Escherichia coli* isolates are presented in Table 1. Approximately, all the isolates fermented lactose, sucrose, sorbitol, and glucose, but variable results were recorded with other sugars (dulcitol, raffinose).

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose with gas production</td>
<td>+ ve</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+ ve</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>+ ve</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>Variable</td>
</tr>
<tr>
<td>Raffinose</td>
<td>Variable</td>
</tr>
<tr>
<td>Lactose</td>
<td>+ ve</td>
</tr>
<tr>
<td>Indole</td>
<td>+ ve</td>
</tr>
<tr>
<td>Methyl red</td>
<td>+ ve</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>- ve</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>- ve</td>
</tr>
<tr>
<td>TSI</td>
<td>Acid/acid+gas</td>
</tr>
<tr>
<td>H₂S production</td>
<td>- ve</td>
</tr>
</tbody>
</table>
Other biochemical tests were encountered in all isolates; most strains gave positive results with indole test and methyl red test, but negative results occurred with Voges-Proskauer and citrate utilization tests.

The results of serogrouping of 38 isolates of *Escherichia coli* isolated from mastitic milk were serogrouped into 10 “O” serogroup, namely O6 (12 strains), O27 (8 strains), followed by O20, O86, O148, O152, O159, O164, O167 (2 strains each), O128 (one strain) and 3 strains untyped.

In vitro, antibiogram of these *Escherichia coli* isolates showed different results as shown in Table 3.

**DISCUSSION**

Mastitis constitutes a great problem to milking herds. Several methods have been reported for the detection of mastitis, of which the isolation of the causative micro-organism is the most accurate.

The results of bacteriological examination of the 100 samples of mastitic milk showed 38 isolates of *Escherichia coli* in an incidence of 38%. This finding nearly coincides with the observation obtained by Verma et al. (1978) who isolated *Escherichia coli* in a percentage of 27% from mastitic milk. Kinabo and Assey (1989), El-Sagheer et al. (1989), Arocha et al. (1992), Mohamed (1993), Adesiyun (1994), Adesiyun et al. (1995), Yu and Bruno (1996) and Todd (1996) reported that *Escherichia coli* was the most frequent isolate from mastitic cows milk. On the contrary, Abd El-Karim and El-Ashmawy (1975) reported lower percentage of isolated *Escherichia coli* from mastitic milk.

The morphological, cultural and biochemical characteristics observed in different serogroups corresponded with typical description by Subba Rao et al. (1975) and Koneman et al. (1988).

The results of serogrouping revealed 10 identified *Escherichia coli* (O6, O27, O20, O86, O148, O152, O159, O164, O167, O128 and 3 strains untyped). Many authors recorded many serogroups of *Escherichia coli* from mastitic and raw milk. Gogov and Kaloyanov (1978) isolated *Escherichia coli* from milk which were serogrouped into O6, O25, O26, O55, O78, O86, O111, O119, O124, O125 and O127. Farag (1987), El-Bagoury (1988), Hafez et al. (1988) Moawad (1988), Arocha et al. (1992) and Adesiyun (1995) isolated *Escherichia coli* O157 from milk samples in a percentage of 75.6%.
Table 2. Serogroups of isolated *Escherichia coli* from mastitic milk.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>O8</td>
<td>12</td>
<td>31.58</td>
</tr>
<tr>
<td>027</td>
<td>6</td>
<td>21.05</td>
</tr>
<tr>
<td>029</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>086</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>0148</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>0152</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>0199</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>0184</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>0167</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>0128</td>
<td>1</td>
<td>2.03</td>
</tr>
<tr>
<td>Untyped</td>
<td>3</td>
<td>7.90</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. In vitro sensitivity test of 10 "O" Serogroups of *Escherichia coli* recovered from mastitic milk to different chemotherapeutic agents.

<table>
<thead>
<tr>
<th>Chemo therapeutic agents</th>
<th>Concentration</th>
<th>Reaction</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>AMX_{10}</td>
<td>R</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>AMX_{50}</td>
<td>S</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>CFX_{50}</td>
<td>S</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>CFX_{50}</td>
<td>S</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>C_{500}</td>
<td>S</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Darcafe</td>
<td>DA_{50}</td>
<td>R</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GM_{500}</td>
<td>S</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>L_{2}</td>
<td>R</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Neomycin</td>
<td>NE_{300}</td>
<td>S</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>FM_{100}</td>
<td>S</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>OT_{30}</td>
<td>R</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

* = Concentration in micrograms.
S = Sensitive.
R = Resistant.
Treatment after sensitivity test will also help to overcome drug fastness due to the occurrence of resistant bacteria, specially, *Escherichia coli* which carry plasmid of R-factor (drug resistant factor), which emerges as a mutant due to the repeated use of drug, or the incorporation of antibiotics and chemotherapeutics in the ration as feed additives.

The antibiogram presented in Table 3 shows that, all isolated strains of *Escherichia coli* from mastitic milk were highly sensitive to cefadroxil, cefaxime, gentamicin, nitrofurantoin. Intermediate sensitivity was shown with chloramphenicol, ampicillin, and neomycin. These results were nearly in agreement with those of Baladssi et al. (1988) and Bassiony (1992), but, all strains were resistant to amoxicillin, duracef, lincomycin and oxytetracycline. Similar results were obtained by Diaz-de Aguayo et al. (1992).
REFERENCES


دراسات بكتيرиولوجية وسريرولوجية عن الميكروبات القولونى في النبات
القشر المصابة في محافظة الغربية

ماشية رجب علي
معهد بحوث سمية الحيوان - مركز البحوث الزراعية - الدقي - مصر

تم فحص عدد 100 عينة النبات من أبقار مصابة بالتشبع القشر. وقد تم عزل 28 مثرة
من الميكروبات القولونى بنسبة 28%. كما تم إجراء الاختبارات البيوكيميائية للمثرات
المعزولة. أثبتت الفحوصات المخبرية للمثرات المعزولة وجود 10 أنواع من الأنترجينات المبكرة
O وهي كالتالي: 06 (21 مثرة)، 027 (8 مثرات), 029 (6 مثرات), 0164, 0159, 0152, 0148, 0200, 0203
وكل منهم عدد (1 مثرة) و (2 مثارات لم تصنف.

كما تم إجراء اختبار الحساسية للمثلثات المعزولة للمضادات الحيوية المختلفة وكانت
معظم المثلثات المعزولة من الميكروبات القولونى حساسة إلى الأسيتاميسلين، سيفرادوكسيل،
سيفاداكسيم، كلاوناسفاز، أزميناميسين، لينكوسين، والدينورفسفين ولكن
كانت المثلثات المعزولة غير حساسة إلى الأموكسيسيلين، نوراسيف، لينكوسين،
والأوكسي نتركسكسيم.