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BACTERIOLOGICAL AND SEROLOGICAL STUDIES ON *ESCHERICHIA 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. 027 (8 strais), 086, 0148, 0152, 0159, 0164, 0167 (2 streins each), 0128 (one strain and 3 strains untyped.

In vitro, antibiogram showed that the majority of isolated strains of *Escherichia coli* were sensitive to ampicillin, cefadroxil, cefataxime, chloramphenicol, gentamicin, neomycin and nitrofurantoin, but all strains were resistant to amoxicillin, duracef, lincomycin and oxytetracyclin.

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 (1969) and Abdel Karim and El-Ashmawy (1979) isolated bacteria causing bovine mastitis including *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. Simmon'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, cefataxime, chloramphenicol, duracef, 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 "8" 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 thouroughly 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 MacCarteny 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

tentatively identified as those of *Escherichia coli.*

Escherichia coli were confirmed by IMVC reaction, fermentation of glucose with gas production and absence of H_2S 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 antisera 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 37oC for 2 hours until turbidity was noticed.

2. Disc diffusion technique

The disc diffusion technique of antibiogram was adopted according to Cruickshank *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 1. Results of biochemical tests on the *Escherichia* coli isolates.

Biochemical test	Reaction
Glucose with gas production	+ ve
Sucrose	+ ve
Sorbitol	+ ve
Dulcitol	Variable
Raffinose	Variable
Lactose	+ ve
Indole	+ ve
Methyl red	+ ve
Voges-Proskauer	- ve
Citrate utilization	- ve
TSI	Acid/acid+gas
H_2s production	-ve

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), 027 (8 strains), followed by 020, 086, 0148, 0152, 0159, 0164, 0167 (2 strains each), 0128 (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 (1983), 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 (1979) 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 identfied *Escherichia coli* (06, 027, 020, 086, 0148, 0152, 0159, 0164, 0167, 0167, 0128 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 06, 025, 026, 055, 078, 086, 0111, 0119, 0124, 0125 and 0127. Farag (1987), El-Bagoury (1988), Hafez *et al.* (1988) Moawad (1988), Arocha *et al.* (1992) and Adesiyun (1995) isolated *Escherichia coli* 0157 from milk samples in a percentage of 75.6%.

Table 2. Serogroups of isolated *Escherichia coli* from mastitic milk.

Serogroup	Number	Percentage
O6	12	31.58
027	8	21.05
020	2	5.26
086	2	5.26
0148	2	5.26
0152	2	5.26
0199	2	5.26
0164	2	5.26
0167	2	5.26
0128	1	2.63
Untyped	3	7.90
Total	38	100

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

Chemotherapeuti agents	Concentration*	Reaction	Number	Percentage
Amoxicillin	AMX_{25}	R	10	100
Ampicillins	SAM_{30}	S	4	40
Cefadroxil	CFR_{30}	S	10	100
Cefataxime	CTX_{30}	S	10	100
Chloramphenicol	C_{30}	S	6	60
Duracef	DA_{30}	R	10	100
Gentamicin	GM_{10}	S	10	100
Linocmycin	L_2	R	10	100
Neomycin	NE30	S	6	60
Nitrofurantoin	FIM_{300}	S	10	100
Oxytetracycline	OT_{30}	R	10	100

* = 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, cefataxime, 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 amoxacillin, duracef, linocmycin and oxytetracycline. Similar results were obtained by Diaz-de Aguayo *et al.* (1992).

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دراسات بكتريولوجية وسيرولوجية عن الميكروب القولوني في البان الضرع المصاب في محافظة الغربية

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تم فحص عدد ١٠٠ عينة البان من ابقار مصابة بالتهاب الضرع. وقد تم عزل ٣٨ عترة من الميكروب القولوني بنسبة ٣٨٪. كما تم إجراء الأختبارات البيوكيميائية للعترات المعزولة. أثبتت الفحوص المصلية للعترات المعزولة وجود ١٠ أنواع من الأنتيجين البدني" O وهي كالتالي O6 (١٢ عترة)، O27 (٨ عترات)، 0167, 0164, 0159, 0152,0148, 086, 020 وكل منهم عدد (٢ عترة) و ٣ عترات لم تصنف.

كما تم إجراء اختبار الحساسية للعترات المعزولة للمضادات الحيوية المختلفة وكانت معظم العترات المعزولة من الميكروب القولوني حساسة الي الأمبسللين، سيفادروكسيل، سيفاتاكسيم، الكلورامفنيكول، الجينتاميسين، النيوميسين والنيتروفيورانتين ولكن كانت العترات المعزولة غير حساسة الي الأموكسيسيللين، ديوراسيف، لينكوميسين والأوكسي تتراكسيكلين.