Isolation and identification study of *Escherichia coli* from buffalo mastitis and Antimicrobial susceptibility pattern of its.

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Abstract

This study was conducted to exam (100) milk samples taken from (25) buffalo within 2-6 years old (clinically mastitic and other non mastitic apparently) in different areas of Al-Najaf province. The results were showed 36(36%) mastitic milk sample as 12 (33.3%) clinical and 24(66.6%) subclinical mastitis according to CMT test. Thirty six bacterial isolates were positive growth on blood agar, the isolation and identification were diagnosed by classical microbiological and biochemical tests, 10 out of 36 bacterial isolates were *E.coli*. The result of disk diffusion test appeared *E.coli* isolates were sensitive to tetracycline (70%), Amikacin and Trimethoprim/Sulfamethaxol (60%), Gentamicin and Ciprofloxacin (50%), while the *E.coli* isolates were resistance to Cefotaxime and Ampicillin.

Kew word: Buffalo , Mastitis , Antimicrobial, *E.coli*

عزل وتحديد الإشريكية القولونية من التهاب الضرع الجاموس ونمط حساسية المضادات الحياتية لها

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الخلاصة

أجريت هذه الدراسة لفحص (100) عينة حليب مأخوذة من (25) جاموس (مصابة بالتهاب الضرع سريعا و غيرمصابة سريريا) في مناطق مختلفة من محافظة النجف. أظهرت النتائج أنه 36 (36%) عينة مصابة بالتهاب الضرع و تكون من (33.3%) عينة كانت سريريا و 24 (66.6%) عينة كانت من التهاب الضرع تحت السريري وفقاً نتيجة CMT. ستة وثلاثين عزلة بكتيريا كانت موجبة النمو على وسط أكار الدم، تم عزل وتشخيص العزل البكتيري بواسطة الطريق الاعتيادية والكلاسيكية واختبارات الكيمياء الحيوية (عدد جاهزة)، 10 من أصل 36 عزلة بكتيريا كانت هي الإشريكية القولونية. أظهرت نتيجة اختبارات القياس لمزيد من العزلات الإشريكية القولونية (التي كانت حساسة للتراسيلكين بنسبة (97%)، أميكاسين و ميتاميزول / سلاميتوكسوزول (60%)، جنتاميسين و سيبروفلوكساسين (50%), في حين كانت عزل الإشريكية القولونية مقاومة للسيفوكتاكسين والأمبيسيلين.

الكلمات المفتاحية: الجاموس،التهاب الضرع، الإشريكية القولونية، مضادات الميكروبات
Introduction:
Mastitis define as changes in physical, chemical and bacteriological of the milk and changes in glandular tissue of udder (1). Mastitis is an important economic diseases which cause high loss in dairy cattle (2). Bacteria are the most common pathogens caused mastitis in buffalo (4). Coliforms mastitis is common environmental mastitis caused by E.coli, Klebsiella pneumoniae and other environmental causes are Streptococcus uberis, dysagalactiae and Pseudomonas spp (5). Pathological disorder in udder as mastitis were related with parturient period, buffalo mastitis has been record in all worldwide with incidence rate 2%-60% (6). Escherichia coli is one of the major pathogens responsible for coliform mastitis and antimicrobial resistance of its considered the major problem in public health.(7,8,9, 10). Recent studies indicate that increase in antimicrobial resistance of E. coli, because of different antimicrobial drugs were used in practice, also some reasons of resistance yet not clear (11,12,13,14). In Iraq few data available about E.coli mastitis in buffalo ,therefore this study was aimed to isolation and identification E.coli from clinical and subclinical buffalo mastitis and antimicrobial susceptibility of its.

Materials and methods
In present work, several types of media and chemicals used in this study for the isolation and identification E.coli. The media were Blood agar , EMB agar,Macconky agar , and Mueller Hinton agar (Himedia ,India), macconky broth ,Nutrient broth (Tulip Diagnostic,Belgium) and chemicals were ;CMT kit( Immucell,U. S.A) , gram stain (Himedia ,India), Api 20 E test Kit (bioMérieux sa ,France).

Also , some types of antibiotics disks were used as ; ciprofloxacain 30µg, Ampicillin 10 µg, Tetracycline 30µg,Amikacin 30 µg, Gentamycin 10 µg, Cefotaxime 30 µg and Trimethoprim/Sulfamethaxol 5/5 µg (Cypress Diagnostics ,Belgium ).

Sample collection.
A total 100 milk samples were taken from 25 buffalo (12 clinically diseased animals and 13 apparently healthy).These samples were collected from some villages and townships of Al-Najaf province. All milk samples collected in sterile tube under a septic technique according to (15). The apparently healthy milk samples were examined by CMT. The mastitic samples immediately transported by cooling box to laboratory.

Bacterial Culture:
The samples obtained from mastitis buffalo were centrifuged (1500 rpm/ 15 minutes) and cultured on blood agar(7% ) defibrinated sheep blood, the positive growth streaking on Macconky and EMB as a selective media and the diagnosis confirmed by biochemical tests (API 20 E) test commercial kit ( 16).

Antimicrobial susceptibility tests:
Antimicrobial susceptibility pattern were performed via disk diffusion test on Mueller-Hinton agar , accordance with National Committee for Clinical Laboratory Standards ( NCCLS) (17).

Statistical analysis:
The data of the study were analysis by using SPSS software (18).

Results
One hundred milk sample which collected from buffalo (diseased and apparently healthy animals) ,the results of present work showed the percent of buffalo mastitis were 36 (36%). The prevalence of clinical was 12 (33.3%) while subclinical mastitis was 24(66.6%) as presented in table (1),figure (1).

Table (1) Numbers and percentage of clinical and subclinical mastitis of buffalo.
<table>
<thead>
<tr>
<th>No. Milk samples</th>
<th>Mastitic milk sample (%)</th>
<th>Clinical mastitis (%)</th>
<th>Subclinical mastitis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>36 (36%)</td>
<td>12 (33.3%)</td>
<td>24 (66.6%)</td>
</tr>
</tbody>
</table>

**Figure (1):** showed buffalo clinical mastitis

**Bacterial isolation**

The obtained results recorded (36) (100%), 15 (41.6%) and 10 (66.6%) bacterial isolates were recovered from blood agar, macconky agar and EMB agar respectively as showed in Figure (2,3), table (2). The isolation and identification of *E. coli* were done according to color and morphology of colony in addition to gram stain with biochemical tests by API 20 E test commercial Kit.

**Table 2: Number and percentage of bacterial isolation.**

<table>
<thead>
<tr>
<th>NO. samples</th>
<th>Blood agar</th>
<th>Macconky agar</th>
<th>EMB agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>36 (100%)</td>
<td>15 (41.6%)</td>
<td>10 (66.6%)</td>
</tr>
</tbody>
</table>
Figure (2): positive growth *E.coli* on EMB agar

Figure (3): positive growth *E.coli* on Macconky agar.
Antimicrobial susceptibility profile of *E. coli* isolates.
The results of Susceptibility tests of *E. coli* isolates against many antibiotics is presented in Chart (1). The *E. coli* isolates were observed sensitive for tetracycline (70%), Amikacin and Trimethoprim/Sulfamethaxol (60%), Gentamicin and Ciprofloxacin (50%), Figure (7). Least sensitive were observed against Cefotaxime (20%), while all isolates were sensitive for Ampcillin.

**Chart (1):** Antimicrobial susceptibility profile of *E. coli*

![Antimicrobial susceptibility chart](chart1)

**Figure (7):** show disk diffusion test of *E. coli* isolates
Discussion

The Prevalence of buffalo mastitis in the current research was (33.3%) which closely
with early study by (19) who had found percentage of buffalo mastitis (31.75%),
while some studies recorded low percent of prevalence of buffalo mastitis such as
20.4% in Brazile (20) and (9.64%) in Egyptian by (21) . The interpretation of
variation in prevalence of mastitis may be attributed to public management, breed and
age of animals(15).

Our results recorded high percent of subclinical mastitis were (66.6%), which
higher than previous reports obtained by (19) ,(21) and (22) ,who found the
prevalence of sub-clinical mastitis (59.64%), (37.75%) and (18.5%) respectively, while
another study observed high percent of subclinical mastitis in buffalo as (77.98%)
by(23).

The rate of clinical mastitis in the present work was (33.3 %).This result higher than
some studies ,which found low percentage of clinical mastitis such as (21.85% ) and
(9.64%) by (22,21) , while high prevalence of clinical mastitis was noticed in Pakistan
(59.64%) by (19).This distinction in mastitis rate may be due to age
,environment factor such as housing, health
management and season.(15).

The results of bacterial examination recorded the isolation of E.coli mastitis in
buffalo by conventional methods was (27.7 %) which match with (24) have been
isolated E coli from subclinical buffaloes mastitis in percent (25%) , While some
studies were reported low percent of E coli mastitis in buffalo as (20.1%) (18%),(17% ) , (16% ),(11%) and (4.55%) by (25) , (26) , (22) (27) (28) and (29)
respectively

We observed wide discrepancy in percentages of E.coli isolation in our study
when compare with another studies ,may be more than factors lead to negative bacterial
growth of milk simple such as presence of antibacterial agent cause suppression of
bacteria activity in milk. (30).

The Susceptibility test of E.coli isolates against many antibiotics showed (70%) of
isolates were sensitive to tetracycline, Amikacin and Trimethoprim/Sulfamethaxol
(60%),Gentamicin and Ciprofloxacin (50%),while we founded the E.coli isolates
resistance to Cefotaxime (80%) and (100%) to Ampcillin . This pattern of Susceptibility
was agree with previous report by (31),who found the resistance of E.coli to
Ampcillin(100); Cefotaxime(100).

But (22), who found E.coli isolates had sensitivity to Penicillin ,Enrofloxacin
Cefotaxime ,Amoxicillin/clauvilinic acid, Tetracycline and gentamycin , The Study of
(25) Observed the sensitivity of E. coli isolates to chloramphenicol and least to
cloxacillin .

The difference in antimicrobial resistance pattern of E.coli when compare with other
research may be attribute to miss choice and uses of antimicrobial agents and mastitis
treated without used sensitive test or bacterial isolation (32),(33).

Conclusions the present study reported high percentage of E.coli bacteria isolated
from buffalo mastitis and had resistance to Amikacin and Trimethoprim/Sulfamethaxol.

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