

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 الحيوية لها

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الخلاصة

أجريت هذه الدراسة لفحص (100) عينة حليب مأخوذة من (25) جاموسة (مصابة بالتهاب الضرع سريريا و غير مصابه سريريا) في مناطق مختلفة من محافظة النجف. أظهرت النتائج انه 36 (36%) عينة مصابة بالتهاب الضرع تتكون من 12 (33.3%) عينة كانت سريريا و 24 (66.6%) عينة كانت من التهاب الضرع تحت السريري وفقا لنتيجة CMT. ستة وثلاثين عزلة بكتيرية كانت موجبة النمو على وسط اكار الدم، تم عزل وتشخيص العزل البكتيري بواسطة الطرق الاعتيادية والكلاسيكية واختبارات الكيمياء الحيوية (عدة جاهزة) ، 10 من أصل 36 عزلة بكتيرية كانت هي الإشريكية القولونية. وظهرت نتيجة اختبار انتشار القرص لعزلات الإشريكية القولونية والتي كانت حساسة للتتراسيكلين بنسبة (70%)، أميكاسين و ميثوبريم / سلفاميثوكسازول (60%)، جنتاميسين وسيفروفلوكساسين (50%) ، في حين كانت عزل الإشريكية القولونية مقاومة للسيفوتاكسيم والأمبيسلين.

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 recorded 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 ; ciprofloxacin 30µg ,Ampicillin 10 µg, Tetracycline

30µg,Amikacin 30 µg, Gentamycin 10 µg,Cefotaxime 30 µg and Trimethoprim/Sulfamethaxol 5/5 µg (Cypriss 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.

No.Milk samples	Mastitic milk sample (%)	Clinical mastitis (%)	Subclinical mastitis (%)
100	36(36%)	12 (33.3%)	24 (66.6%)



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 don 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.

NO. samples	Blood agar	Macconky agar	EMB agar
36	36(100%)	15 (41.6%)	10 (66.6%)

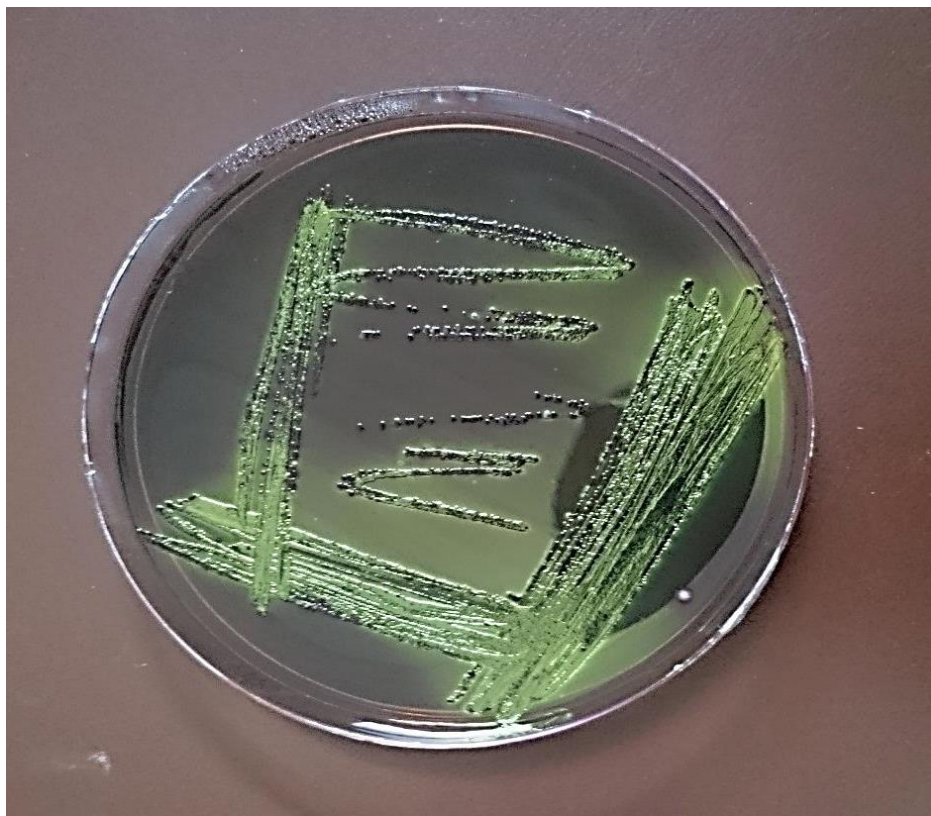


Figure (2): positive growth *E.coli* on EMB agar



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 Ampicillin .

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

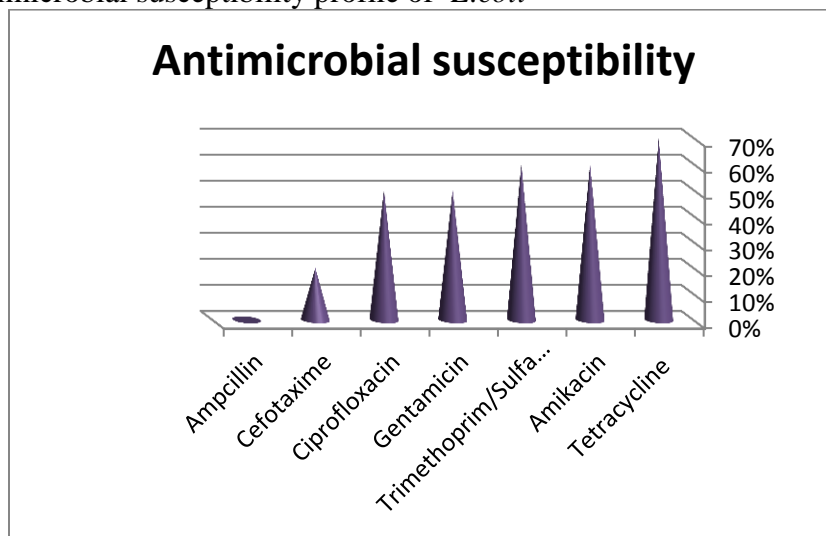


Figure (7): show disk diffusion test of *E.coli* isolates

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 sample 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 Ampicillin. This pattern of Susceptibility was agree with previous report by (31), who found the resistance of *E.coli* to Ampicillin(100); Cefotaxime(100).

But (22), who found *E.coli* isolates had sensitivity to Penicillin, Enrofloxacin, Cefotaxime, Amoxicillin/clavulanic 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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