Bacteriological Affections Of Livers And The Associating Serum Biochemical Changes In Buffaloes In Menoufiea Governorate

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ABSTRACT

Lesion specimens from 75 condemned buffaloes livers were collected in abattoirs at El-Menoufiea Governorate, from slaughtered buffaloes of different age for bacteriological examination, in Animal Health Research Institute – Shebin El-Kom, in trials to detect the possible bacterial agents. Liver appeared congestion, dark brown in colour, hard, firm, tough in consistency condemned and some samples affected with abscesses.

Out of 75 samples of examined liver of buffaloes, 63 samples (84%) were positive for bacterial isolation. *Staph. aureus* was the most predominant isolales. *Staph. aureus, E. coli* (O157), *Streptococus pyogenes, C. perfringens* type "A", *Corynebacterium pyogenes, Klebsiella pneumoniae, Citrobacter freundi* and *Proteus vulgaris* were isolated with incidence of (30.6%, 12%, 10.7%, 8%, 8%, 6.7%, 5.3% and 2.7%, respectively).

The serum biochemical analysis of *Staph. aureus* infected buffaloes revealed a significant increase in Malondialdehyde (MDA) concentration, on contrary the total protein, and albumin were significantly decreased with significant increase in total globulins. Moreover, the serum levels of AST, ALT, urea, uric and creatinine were significantly increased.

In vitro antimicrobial sensitivity test, of the predominant isolates indicated that *Staph. aureus* were highly sensitive to cephalocin, erythromycin, kanamycin, doxycycline, enrofloxacin, and trimethoprim & sulphamethoxazole. There is marked difference between the sensitivity to antibiotics between different bacterial isolates. Kanamycin, doxycycline, ciprofloxacin and trimethoprim & sulphamethoxazole were considered the antimicrobial agents of choice for treatment of bacterial liver affections. On the other hand penicillin G and streptomycin were an ineffective chemotherapy for treatment of any isolates.

INTRODUCTION

The Egyptian buffaloes, is named river or water buffaloes, are mainly distributed in the Nile valley and Delta in about 2.8 million animals, 90 % of which are located in private small herds *(1)*. Buffaloes have been used in Egypt as agricultural work animals in the farms as well as the main source of both milk and meat necessary to fulfill the gap between the increased population and their demands from animal protein.

The liver is considered to be as one of the most important organs of mammalian metabolism and food converstion, which regulates, stabilizes, and protect the internal environment and the whole body. Any disturbance in this organ would have its reflection on the general health and causes a great economic losses in animal production represented by liver condemenation at slaughter houses. Liver infection may lead to serious morbidity with damage of liver and pathological affections of liver may be attributed to a variety of causes including viruses, mycoses, parasites and bacteria *(2)*. Many studies deal with the effect of bacterial pathogens on animal tissues particulary those used for human cosumption was performed *(3 - 7)*.

Bacteria were considered to be one of the most serious infectious agents which causes liver affections in buffaloes. Several Gram positive and Gram negative bacteria were associated with liver affections and abscesses,

these organisms orginated from the gastointestinal tract and arrived to liver by translocation via the blood stream *(7)*.

Liver abscesses are commonly observed in heavily fattened cattle, and causes condemenation of approximately 10% of the liver *(8)*. Numerous investigators evaluated the bacterial flora, both aerobic and anaerobic of bovine liver abscesses *(8 - 10)*. *Staph. aureus, E. coli, Proteus* spp. *Streptcoccus* spp. and *Corynebacterium* spp. were isolated *(5, 6, 7, 9, 11)*, and *Clostridium* spp. was recorded *(12)*. The bovine liver was microbiologically contaminated as heavy as raw meat, when found more than 50% of liver samples were contaminated with *Staph. Aureus (13)*. *Corynebacterium* spp., *Staph. aureus, E. coli* and *Pseudomonas aerogenosa* were isolated from the liver of sheep and goat *(14, 15)*.

Liver function tests may be used to establish a diagnosis in an individual animal or to detect subclinical liver damage following the bacterial infection and its circulating toxins, hence determining its economic value *(16)*.

The main purpose of this work was initiated to investigate the isolation and identification of possible causative bacterial agents of liver abscesses in buffaloes. On the other hand, antibiogram pattern of most bacterial isolates, as an aid to overcome this problem and reduce losses, and the biochemical changes associated with *Staph. aureus in* infected buffalo slaughtered in abattoir were our aim.

MATERIAL AND METHODS

Samples

75 apparently diseased liver samples (condemned buffalo liver) were collected from different abattoirs at El- Menoufiea Governorate, from slaughtered buffaloes of different ages. Liver appeared congestion, dark brown in colour, hard, firm, tough in consistency condemned and some samples affected with abscesses. Each samples kept separately in a sterile plastic ice bag for bacteriological examination in Animal Health Research Institute – Shebin El-Kom. Samples were divided into two portions and were submitted to aerobic and anaerobic examination.

Blood samples

Blood samples were collected in clean plastic centrifuge tubes and allowed to coagulate. The serum was separated by centrifugation at 3000 r.p.m for 10 minutes, Then the clear supernatant sera aspirated carefully into dry and sterile labeled vials.

Bacteriological examination

All collected samples were subjected to aerobic and anaerobic bacteriological examination.

1-Aerobic identification

Cultural methods were made from infected liver lesions by sterilizing the exposed surface of the liver and cutting down small piece from the deeper parts, and inoculated directly into nutrient broth, and aerobically incubated at 37°C for 24 hours, then subcultured onto nutrient agar, blood agar, MacConky bile salt lactose agar, crystal violet blood agar, mannitol salt agar media for isolation of *Staph. aureus,* and Eosin Methylene blue agar media for isolation of *E. coli,* all inoculated plates were incubated aerobically at 37°C for 24-48 hours.Suspected growing colonies onto the surface of these media were identified by studying characters of the colonies as well as Gram's stain, then identified morphologically according to the previously described methods *(17, 18)*. One single colony showed typical colonial appearance and morphological characters was picked up and streaked into semisolid agar media and incubated at 37°C for 24 hours, for further identification.

The pure colonies were biochemically identified *(19-22)*. The Gram negative bacteria included *Enterobacteriacae* family were typed *(23)*.

2- Anaerobic identification

A loopful from a small piece of the deeper parts of affected liver, was inoculated into tubes of freshly prepared Robertson's cooked

meat medium at 37°C for 24 hours. Loopful from each tubes was streaked onto the surface of 10% sheep blood agar, then incubated anaerobically at 37°C for 24 hours. The plates were examined for characteristic colonies of genus *Clostridium* . Subcultures from suspected colonies in cooked meat broth were made for further biochemical identification ***(20, 24, 25).***

Serum biochemical analysis

The sera of *Staph. aureus* infected buffaloes were used for estimation of Malnodialdehyde (MDA) ***(26),*** total protein ***(27),*** albumin ***(28).*** While total globulins calculated mathematically by substacting albumin from total protein ***(29),*** AST and ALT ***(30),*** urea ***(31),*** uric acid ***(32),*** and creatinine ***(33)*** were determined.

Antibiogram sensitivity pattern of isolates

Antibiogram was applied on most isolated strains using disc diffusion technique ***(20, 34)*** with Mueller Hinton agar, using the following chemotherapeutic and antibiotic discs (Oxoid) amoxycillin (10ug), cephalocin (10ug), chloramphenicol (30ug), ciprofloxacin (5ug), doxycycline (30ug), enrofloxacin (5ug), erythromycin (10ug), flumequine (30ug), gentamicin (10ug), kanamycin (30ug), norfloxacin (10ug), pencillin G (10U), streptomycin (10ug), tetracycline (30ug), and trimethoprim & sulphamethoxazole (1.25ug). The results were interpretated according to the manual supplied by Oxoid Company.

Statistical analysis

All data were subjected to statistical analysis ***(35).***

RESULTS

Table 1, showed the bacterial isolates from liver samples of buffaloes. Out of 75 samples of examined liver of buffaloes, 63 samples (84%) were positive. The bacteriological isolation of different types of bacteria from liver samples were *Staph. aureus, E. coli* (O157), *Streptococus pyogenes, C. perfringens* type "A", *Corynebacterium pyogenes, Klebsiella pneumoniae, Citrobacter freundi* and *Proteus vulgaris* with incidence of 30.6%, 12%, 10.7%, 8%, 8%, 6.7%, 5.3% and 2.7%, respectively.

The biochemical studies illustrated in Table 2 and 3, Proved that serum of *Staph. aureus* infected buffaloes showed, a highly significant increase of MDA, total globulins. While total protein and albumin recorded high significant decrease. On the other hand, a highly significant increase was found in the serum AST, ALT, urea, uric and creatinine.

I*n vitro* sensitivity of the most prevalent bacteria isolated from liver samples of buffalo were done against chemotherapeutic agents ***(15)*** as show in Table 4. All tested strains of *Staph. aureus* were highly sensitive to cephalocin, erythromycin, kanamycin, doxycycline, enrofloxacin, and trimmethoprim & sulphamethoxazole with activity percentage of 91.3%, 91.3%, 86.9%, 78.3% 73.9% 69.6% and 69.6%, respectively. Most of these strains were highly resistant to tetracycline, gentamicin, streptomycin, norfloxacin and penicillin G with activity percentage of 30.4%, 21.7%, 8.7%, 4.3% and 0%, respectively. Comparing the sensitivity of *E. coli* (O157) isolates, the majority of strains were highly sensitive to eryrhromycin, gentamicin, cephalocin, kanamycin, norfloxacin and doxycycline with activity percentage of 100%, 100%, 88.9%, 88.9%, 88.9% and 77.8%, respectively. The same strains were highly resistant to tetracycline, ciprofloxacin, enrofloxacin, chloramphenicol and flumequine with activity percentage of 33.3%, 22.2%, 11.1%, 0% and 0%, respectively. While the isolated strains of *Streptococus Pyogenes* were mainly highly sensitive to amoxycillin, ciprofloxacin, kanamycin, tetracycline, flumequine, and trimethoprim & sulphamethoxazole with activity percentage of 100%, 87.5%, 87.5%, 75%, 62.5% and 62.5%, respectively. As regards to *C. perfringens* type "A" isolates were highly sensitive to ciprofloxacin, enrofloxacin, chloramphenicol, flumequine, kanamycin and norfloxacin with activity percentage of 100%, 100%, 83.3%, 83.3%, 83.3% and 83.3%, respectively, but the same

strains were resistant to penicillin G, trimethoprim and sulphamethoxazole, cephalothin and tetracycline. While *Corynebacterium pyogenes* isolates were sensitive to amoxycillin, tetracycline, ciprofloxacin, kanamycin and enrofloxacin with activity percentage of 100%, 100%, 83.3%, 83.3% and 66.7%, respectively. Comparing the sensitivity of *Klebsiella pneumoniae* isolates, the majority of strains were highly sensitive to kanamycin, norfloxacin, ciprofloxacin, doxycycline and gentamicin with activity percentage of 100%, 100%, 80%, 80%, and 80%, respectively.

Table 1. Prevalence rate of different types of bacteria isolated from liver samples of buffalo.

Isolated microorganisms	Number of cases	%
Staph. aureus	23	30.6
E. coli (O157)	9	12
Streptococcus pyogens	8	10.7
C. perfringens type "A"	6	8
Corynebacterium pyogens	6	8
Klebsiella pneumoniae	5	6.7
Citrobacter freundi	4	5.3
Proteus vulgaris	2	2.7
Total	63	84

% was calculated according to the number of examined samples (75) .

Table 2. Serum MDA (n mol/ml) total protein (gm/dl), albumin (gm/dl), and total globulins (gm/dl) in both *Staph. aureus* infected group and control one.

Parameters	Control	*Staph. aureus* infected buffaloes
MDA	5.25 ± 0.15	7.72 ± 0.28*
Total protein	6.32 ± 0.17	5.20 ± 0.16*
Albumin	2.68 ± 0.053	1.27 ± 0.065*
Globulins	3.66 ± 0.117	3.83 ± 0.126*

Data are presented as mean + S.E. * Highly significant ($P < 0.001$)

Table 3. Liver and Kidney functions in both *Staph. aureus* infected group and control one.

Parameters	Control	*Staph. aureus* infected buffaloes
AST (Iu / L)	57.5 ± 1.72	88.1 ± 2.04 *
ALT (Iu / L)	18.8 ± 0.94	27.8 ± 1.7 *
Urea (mg / dl)	27.89 ± 0.77	41.1 ± 1.96 *
Uric (mg / dl)	0.95 ± 0.035	1.40 ± 0.079*
Creatinine (mg / dl)	1.51 ± 0.079	20.4 ± 0.078*

Data are presented as mean + S.E. * Highly significant ($P < 0.001$)

Table 4. Antibiotic sensitivity test of the most prevalent bacteria isolated from liver samples of buffalo using disc diffusion method.

Antibacterial agents	Symbol	Conc.	*Staph. aureus* (23)*		*E. coli* O157 (9)*		*Streptococcus pyogenes* (8)*		*C. perfringens* type "A" (6)*		Coryne. Pyogens (6)*		*Klebsiella pneumoniae* (5)*	
			S.	Activity %	S.	Activity %	S.	Activity %	S.	Activity %	S.	Activity %	S.	Activity%
Amoxycilln	AMLi	10ug	11/23	47.8	4/9	44.4	8/8	100	4/6	66.7	6/6	100	2/5	40
Cephalocin	KF	10ug	21/23	91.3	8/9	88.9	3/8	37.5	1/6	16.7	3/6	50	1/5	20
Chloramphenicol	C	30ug	16/23	69.6	0/9	0	3/8	37.5	5/6	83.3	3/6	50	1/5	20
Ciprofloxacin	CPR	5ug	13/23	56.5	2/9	22.2	7/8	87.5	6/6	100	5/6	83.3	4/5	80
Doxycycline	D	30ug	18/23	78.3	7/9	77.8	4/8	50	4/6	66.7	3/6	50	4/5	80
Enrofloxacin	ENR	5ug	17/23	73.9	1/9	11.1	3/8	37.5	6/6	100	4/6	66.7	3/5	60
Erythromycin	E	10ug	21/23	91.3	9/9	100	4/8	50	4/6	66.7	3/6	50	2/5	40
Flumequine	UB	30ug	12/23	52.2	0/9	0	5/8	62.5	5/6	83.3	4/6	66.7	3/5	60
Gentamicin	CN	10ug	5/23	21.7	9/9	100	1/8	12.5	3/6	50	0/6	0	4/5	80
Kanamycin	K	30ug	20/23	86.9	8/9	88.9	7/8	87.5	5/6	83.3	5/6	83.3	5/5	100
Norfloxacin	NOR	10ug	1/23	4.3	8/9	88.9	0	0	5/6	83.3	0/6	0	5/5	100
Penicillin G	P	10U	0/23	0	5/9	55.6	2/8	25	2/3	33.3	2/6	33.3	3/5	60
Streptomycin	S	10ug	2/23	8.7	5/9	55.6	0	0	3/6	50	1/6	16.7	3/5	60
Tetracycline	T	30ug	7/23	30.4	3/9	33.3	6/8	75	1/6	16.7	6/6	100	2/5	40
Trimethoprim& Sulphamethoxazole	SXT	1.25ug	16/23	69.6	4/9	44.4	5/8	62.5	2/6	33.3	4/6	66.7	1/5	20

*: Number of isolates. S: Sensitive.

%: Percentage of sensitive isolates in relation to tested isolates = Activity percentage.

Conc. : Concentration of antibiotic disc.

DISCUSSION

Buffalo liver is an important edible meat byproduct, but the liver which condemned at slaughter was unfit for human consumption because they harbour small numbers of intrensic bacteria and potential human pathogens *(7)*. Several causes of liver diseasae including ischemia induced by bacterial emboli, vitamin E-selenium deficiency and immune mediated disease *(36, 37)*

In this study, the bacteriological examination of diseased liver samples was done in an attempt to throw spotlights upon the incidence percentages of bacterial infection, *in vitro* chemotherapeutic susceptibility testing of the most prevalent bacterial isolates were detected and biochemical changes associated with *Staph. aureus* infected buffalo slaughtered in abattoir was also checked.

The obtained data in Table 1, proved that out of 75 diseased examined liver of buffalo, 63 samples proved to harbour bacteria with an incidence of 84%.

Previous studies *(7,9)*, on bacteriological examination of apparently normal hepatic abscesses, and telangiectatic liver in cattle recorded that about 82% of examined diseased liver were positive for several types of bacterial infection.

The identification of isolates in Table 1, proved that *Staph. aureus*, *E. coli* (O157), *Streptococus pyogenes*, *C. perfringens* type "A", *Corynebacterium pyogenes*, *Klebsiella pneumoniae*, *Citrobacter freundi* and *Proteus vulgaris* were present with incidence of 30.6%, 12%, 10.7%, 8%, 8%, 6.7%, 5.3% and 2.7%, respectively. These results agreement with the recorded work which indicated that *Corynebacterium pyogenes, Staphylococcus* spp. and *Streptococcus* spp. were the most prevalent bacteria recovered from liver abscess. More than 50% of market bovine liver samples were contaminated with *Staph. aureus* and these results indicated that the liver was microbiologicaly contaminated as heavy as raw meat *(13)*. *Corynebacterium pyogenes* was the most predominant isolates from liver abscesses of cattle, followed by *Staphylococcus* and *Streptococcus* spp *(9)*. *Staph. aureus*, *E. coli* (O157), *Streptococci* and *proteus* were also isolated from liver of cattle *(5)*. In addition *Corynebacterium* spp., *Citrobacter* and *Clostridium* spp. were isolated from diseased livers of cattle *(7,38)*.

MDA is an indicator of free radical production, and an increase in MDA may threrefore be due to oxidative stress. Also MDA is a major degradation production of lipid hydroperoxides and measurements MDA concentration is generally accepted as a marker for assessing the extent of lipid peroxidation *in vivo* ***(39, 40)***. Indeed MDA assay is the most popular and easiest methods used as an indicator of lipid peroxidation and free radical activity in biological samples ***(41)***. *Staph. aureus* infected buffaloes revealed a highly significant increase in serum MDA levels in comparison with control one which indicated oxidative stress. MDA produced by peroxidation can cause cross–linking and polymerization of membrane components. This can alter intrensic membrane properties such as a deformabilities, ion transport, enzyme activities and aggregation state of cell surface determinants. These lead to the removal from the circulation of cells exposed to the agents causing lipid peroxidation. MDA is diffusible, and react with nitrogenous bases of DNA. ***(42)***.

A highly significant drop in serum total protein and albumin which may be reffered to the state of inability of the liver to synthesis protein. The reduction of the protein level is attributed to the stress factors and the general unthriftiness which may affect worsely the hepatic parenchyma resulting in failure of protein synthesis *(43)*. Certain bacteria and its toxin cause increased capillary permeability and permit the escape of plasma proteins in the tissue fluids and decrease the osmatic pressure ***(44, 45)***. There was a significant increase in the serum globulin level in the diseased buffaloes. Which may be due to the stimulation of immune system by the infectious agents, to produce high amounts of immunoglobulins ***(46,47)***.

The obtained data concerning liver function tests, showed marked elevation of serum AST and ALT. Such elevation of the liver enzymes referred to the degnerative and necrotic changes of the liver following the bacterial infection and its circulating toxins ***(16)***. It has been shown that *Staph. aureus* affected buffaloes revealed focal area of necrosis ***(48)***.

Serum urea, uric and creatinine recorded highly significant increase. The increases may be due to protein catabolism ***(49)***.

The data presented in Table 4, indicated that, there was marked difference between the sensitivity to antibiotics between different bacterial isolates. Kanamycin, doxycycline, ciprofloxacin and trimethoprim & sulphamethoxazole is considered the antimicrobial agents of choice for treatment of bacterial liver affections. While variable results were recorded with the remaining used antibiotics. On the other hand penicillin G and streptomycin were ineffective chemotherapy for treatment of any isolates, because 40% of these bacteria were penicillinase positive ***(50)***. *Staph. aureus* strains were highly sensitive to cephalocin, erythromycin, kanamycin, doxycycline and enrofloxacin. On the other hand *Strept. pyogenes* were highly sensitive to amoxycillin, ciprofloxacin, kanamycin, tetracycline, flumequine, and trimethoprim & sulphamethoxazole. Similar findings were recorded by several investigators ***(51 -53)***.

All tested strains of *Corynebacterium pyogenes* were sensitive to amoxycillin, tetracycline, ciprofloxacin, kanamycin and enrofloxacin. These findings nearly agreed with that previously reported ***(54 - 56)***, and revealed that the majority of *Corynebacterium pyogenes* isolated from diseased liver of buffaloes were highly sensitive to tetracycline and tylosin. On the other hand it has been found that *C. perfringens strains* were resistant to tetracycline, lincomycine but not to erythromycin ***(57)***.

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الملخص العربي

الإصابات البكتيرية للكبد والتغيرات البيوكيميائية المصاحبة لها في الجاموس في محافظة المنوفية

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في هذة الدراسة تم فحص(٧٥) عينة من الكبد المصاب للجاموس من المجازر المختلفة بمحافظة المنوفية فوجد أن (٦٣) عينة إيجابية للفحص البكتيريولوجي بنسبة (٨٤%) .

وبالفحص البكتريولوجي للأكباد المصابة في الجاموس تم عزل الميكروب العنقودي الذهبي والأشريشيا القولونية والمكور السبحي الصديدي والكلوستريديم بيرفرنجينز نوع (أ) والكوريني الصديدي والكليبسيلا الرئوية والستروباكتر والبروتيوس فيلجارس بنسبة (٣٠,٦% ، ١٢% ، ١٠,٧% ، ٨% ، ٨% ، ٦,٧% ، ٥,٣% ، ٢,٧%) على الترتيب).

وبأجراء الفحوصات البيوكيميائية لعينات مصل الدم للحيوانات المعزول منها الميكروب الذهبي العنقودي ومقارنتها بالعينات السلبية إتضحت النتائج التالية:

١- وجود زيادة عالية المعنوية جدا فى المالون داي الدهيد والجلوبيولين الكلى مع نقص عالي المعنوية جدا فى البروتين الكلي والالبيومين .

٢- وجود زيادة عالية المعنوية جدا فى أنزيمات الكبد (ALT , AST) .

٣- وجود زيادة عالية المعنوية جدا في بولينا الدم وحمض اليوريك والكرياتنين.

وتم ايضا دراسة مدى حساسية العترات البكتيرية المعزولة للمضادات الحيوية فكانت معظم العترات من الميكروب العنقودي الذهبي أكثر حساسية للكيفالوسين والأريثروميسين والكاناميسين والدوكسى سيكلين و الأنروفلوكساسين و التراى ميثوبريم مع السلفاميثوكسازول.

كما أننا نلاحظ أن حساسية المضادات الحيوية تختلف باختلاف البكتريا المعزولة فنجد أن الكاناميسين والدوكسي سيكلين والسبروفلوكساسين و التراى ميثوبريم مع السلفاميثوكسازول من أفضل المضادات البكتيرية لعلاج الأصابات البكتيرية للكبد بينما البنسيلين والأستربتومايسين كانت غير مؤثرة كعلاج للبكتريا المعزولة .