

BACTERIOLOGICAL ISOLATION AND EVALUATION OF THE ANTIMICROBIAL RESISTANCE OF ENTEROBACTERIAL ISOLATES FROM GALL BLADDER OF SLAUGHTERED CATTLE.

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ABSTRACT

The increasing prevalence of antimicrobial resistance in meat-producing animals, especially ruminants, represents a major problem for human and animal and also could increase the patient's morbidity and mortality. The gallbladder may be a site of persistence and a source for fecal shedding of certain enteric food-borne pathogen resistant to many antimicrobial agents. In the current study 80 samples (32 bile, 48 epithelium) were examined to isolate the enteric pathogen; All samples were cultured on primary and selective. The frequency of isolation of microorganisms was (60%) in the epithelium and (40%) in the bile. The major pathogen isolated were (68.57%) *Proteus spp.* (21.25%) *E-coli* (7.5%) *Citrobacter* (1.25%) *Psudomonas* and (1.25%) *Klebsiella*. The antibiotic resistance was determined by Kirby-bauer disc diffusion method using 10 of routine and practical antibiotics. In antimicrobial testing from both bile and gallbladder epithelium showed sensitivity to the following antimicrobial : amikacin, cefoxitin, chloramphenicol, gentamycin, kanamycin and ciprofloxacin.

In conclusion , the current study provide helpful insights into the prevalence of food source pathogens. High level of antibiotic resistance in *proteus spp* and *Ecoli* that could transmit to humans through meat and meat products need for monitoring system on the incidence and antimicrobial susceptibility of enteric pathogens in meat animals in slaughterhouses.

INTRODUCTION

Bile is a digestive secretion that plays a major role in the dispersion and absorption of fats. The composition of bile is complex, but can be thought of as lipid rich and protein poor (1). Pathogenic microorganisms can reside transiently or permanently in the gallbladder of cattle . Thus during slaughter of cattle more attention should be given to the gastrointestinal tract, especially to the accessory organs (2), Although the bile acids possess potent antimicrobial activity, recent researches has

shown that the pathogenic bacteria is capable of tolerating high level of bile by biofilm formation(3).Cholysititis occurs due to obstruction or inflammation . The tissue of gallbladder is weakness which leads to bacteria which find normally in gallbladder and intestine to inflammation. This bacteria usually Gram negative bacteria,(4). Some of bacterial microorganism had the ability to librated toxins which turn might lead to severe emphysema followed by emphysematous cholysititis resulting in cell damage and necrotic processes, therefore using specific antibiotic were advised , More over supplicative microorganism will causes suppuration and might contaminate most body tissues(5). This study aimed to characterized the bacteria present in bile and gallbladder epithelium of cattle slaughtered in slaughtering house under sanitary condition and to evaluate the antimicrobial resistance without surgery.

MATERIALS AND METHODS

Sample collection:

During a period started from April 2016 to November 2016` a total of 100 samples of bile and epithelium (50 foreach) were collected from gall bladder of the local cattle breeds from slaughtered house of Basra. All samples were collected into sterile containers and immediately transported to the clinical pathology laboratory, under refrigeration using an insulated ice bag . The gall bladder was opened under a septic conditions and bile collected . The billiard epithelium was then sectioned with the aid of sterilized instrument (6). Bile and gallbladder epithelium were analyzed separately for total enterobacteriaceae.

Bacterial identification:

Microbiological analysis was performed at same day. Oneml of each sample was pipette and spread with 9 ml sterile double strength PBS on nutrient , MacConkey broth and peptone water (BPW) (Himedia,India;Bioscience,India). In aratio of 1:10 (w/v) (7).

Isolation of bacteria was carried out using different media such as,SS agar, EMB agar, brilliant green agar,and red bile glucose agar (Himedia,India) (7).All the plates were incubated at 37Cfor 24-48 h. and the number of growth colony was determine then suspected colonies were subculture and further identified by biochemical test to identify the colonies. Furthermore, different tests such as gram stain, motility, nitrite reduction, oxidase activity,catalase activity, oxidation, fermentation of glucose, pigment production and citrate utilization were applied, at the end isolated colonies were frozen in BHI broth containing 20% glycerol for later susceptibility test(8).

Table (1): Biochemical tests of bacterial species isolated from bile and epithelium.

Isolates	gram stain	Motility	Fermentation of glucos	Pigment production	Nitrat Reduction	Oxidas activity	Catalas activity	oxidation	Citrate utilization	Methyle red	vp
<i>Proteus spp</i>	-	+	+	-	+	-	+	+	+	+	-
<i>E coli</i>	-	+	+	-	+	-	+	+	-	+	-
<i>Citrobacter</i>	-	+	+	-	+	-	+	-	+	+	-
<i>Pseudomonas</i>	-	Motil, non motil	+	+	+	-	+	-	+	-	-
<i>klebsella</i>	-	-	+	-	+	-	+	-	+	-	+

In the detection of *E coli* the fluorocult LMX broth modified was used as described previous (7). In the tubes with indication of *E coli*, the kovac's reagent was added. The positive tubes were streaked with eosin methylen blue(EMB)agar(9)

Antibiotic Susceptibility Test:

The Kirby-baure disc diffusion technique was used to determine the resistant isolates. After overnight incubation at 37°C, the inhibition zone was measured and categorized as resistant as described previous(6). Isolates were tested against (10 of routin and practical antibiotics)ampicilllin(10 µg /disk),amikacin (30 µg /disc), cefixime (5µg/disc), colistin(10µg/disc), ceftriaxone (30µg/disc),

ciprofloxacin (5µg/disc), chloramphenicol (30µg/disc), gentamycin(10µg/disc), kanamycin (30µg/disc) and tetracycline(30µg/disc). The discs were purchased from an international company (7). Isolates, which were resistant to four or more antibiotics, were determined as multi antibiotic resistant.(MAR).

RESULTS AND DISCUSSION

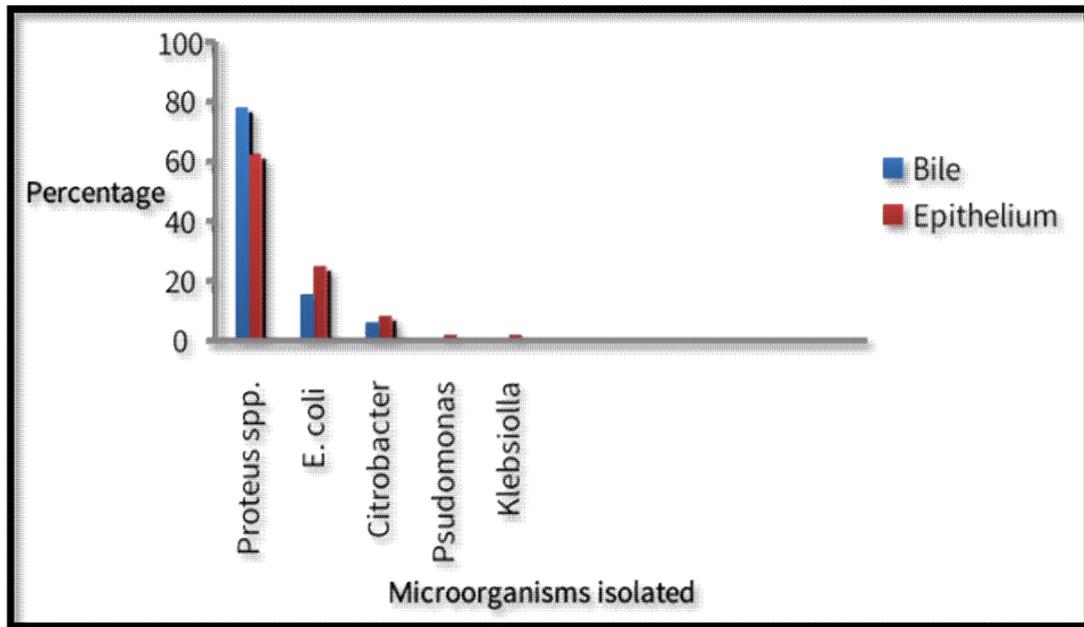
The relative and absolute abundance of live stock considerably differs by country and geographic area. Today, live stock plays a major role in agriculture, economy, and meat production system in Iraq. The distribution of enteric pathogens among ruminants varies greatly overtime, and initially sensitive food borne pathogens have become resistant to the clinically important antibacterial drugs. These resistant pathogens are mainly transmitted to human through direct contact and shedding by animals. Also the presence of these organisms in meat animals and raw meat products has relevant public health implication. (10,11).

From 100 bile and epithelium samples examined in the present study, 7 sample revealed negative, 13 sample revealed mixed culture of three or more environmental bacterial and according considered contamination. The remaining 80 sample revealed positive culture of which 5 species of bacteria. In the current study, Eighty bacteria were collected which belong to these genera; *proteusspp.*(68.75%),*Ecoli*,(21.25%),*Citrobacter*,(7.5),*pseudomonasspp.*(1.25%) and*klebsiellaspp.* (1.25%)

(Table 2),Figure(1).

Table (2):Types and frequencies of microorganisms isolated from the bile and gallbladder epithelium of 80 slaughtered cattle.

<i>Type of Bacteria isolates</i>	No of isolate in percentage		
	Bile N(%)	Epithelium N(%)	Total
<i>Proteus spp.</i>	25(78.125)	30(62.5)	55(68.75)
<i>E.Coli</i>	5(15.625)	12(25)	17(21.25)
<i>Citrobacter</i>	2(6.25)	4(8.33)	6(7.5)
<i>Pseudomonas</i>		1(2.08)	1(1.25)
<i>Klebseilla</i>		1(2.08)	1(1.25)
<i>Total</i>	32(40)	48(60)	80(100)



Figure(1): Frequency (%)of bacterial isolated present in the bile and epithelium of gallbladders from 80 slaughtered cattle.

The total obtained in the epithelium samples were higher than those of bile samples, (40%in bile and , 60% in epithelium). This finding showed that the magnitude of contamination of food establishments and slaughter houses which may be due to food associate diseases (11,12). The isolation of bacteria in the bile normal coincides with the presence of bacteria in the epithelium . The rate of isolate in the epithelium is higher than the isolate from bile. This finding may be attributed to the fact that the bacteria reside in the gall bladder, the storage sit for bile(13). In the current study, *proteusspp.* isolated from of bile and epithelium samples were(68.75) it has been found the presence of *Proteus* in gallbladder of ruminants(14,15,16 ,17). Thus, the responses of *proteuse* to bile are important for virulence and also for the establishment of chronic infection or carrier states in the host (18).

The high frequency of *proteus spp* should be considered seriously. *Proteus spp* was also isolated from food sample and stools of patient with gastroenteritis, thus, the role of *proteus spp.* as food pathogen should be investigated (19). Resistance of pathogens to the antibiotics used for the animal or human medicine is of major concern in clinical settings, and will be important in the future (20). Although great part of *E. coli* was adhered to the epithelium,(21, 25%) of *E coli* was isolated. This finding is in agreement with previous studies (,17 ,21,22),in which *Ecoli* can reside in the gallbladder of cattle (25%). Thus during slaughter great attention must be given to the gastro-intestinal tract of cattle specially the gall bladder because the gall bladder may be a sit of and a source of gastro intestinal toxin-producing by *Ecoli* which can contaminate beef products. Other bacteria including *citrobacter*(7.5%),*Psudomonas*(1,25%), and *Klebsiella*(1.25%),were also isolated but

in lower proportion compared with other bacteria, This finding in agreement with those reported by (17,23), in which *Klebsiella* was (3.8%), and *Pseudomonas*(7.5)

According to the standardization of techniques for cattle slaughtering, in the post mortem examination of cattle liver, the gallbladder is compressed and incised as need because gallstones, have considerable market value as research samples, it is common to open the gallbladder at the slaughterhouse. As a result, bile is frequently spilled, often on the line (5). Therefore, evisceration should be done carefully and the bile duct should be tied before removing the gallbladder to prevent cross contamination with bile because bacteria are aggregated in larger number in the epithelium, there is the occurrence of a low metabolic rate of cells due to low availability of nutrients (24), which fits the gallbladder and becomes an interfering factor of antimicrobial resistance in the microbial population. Considering the results obtained in this study, it can be affirmed that the environment for the microorganism in the gallbladder is common. The bacteria can be isolated in the bile as well as in the epithelium, but in larger quantities in the epithelium. The permanence of the microorganism in the gallbladder due to its adherence to the epithelium being its habitat. Although due to the organ's contractility, there is a detachment of the bacteria in the epithelium and migration to word the bile. Intestinal pathogens are also able to adhere to and subsequently from bacterial communities, micro colonies and even mature biofilms on epithelium cells.(2,3).

In present study,antibiogram profile of different bacteria isolated from both bile and epithelium indicate that resistance to more than four antibiotics.Amikacin,cefoxitin,chloramphenicol,gentamycin,kanamycin and ciprofloxacin provid to most effective antimicrobial against bacteria table(3 , 4 ,5),(Figur2).

Table(3): Antibioqram classification of studied Bacterial isolated.

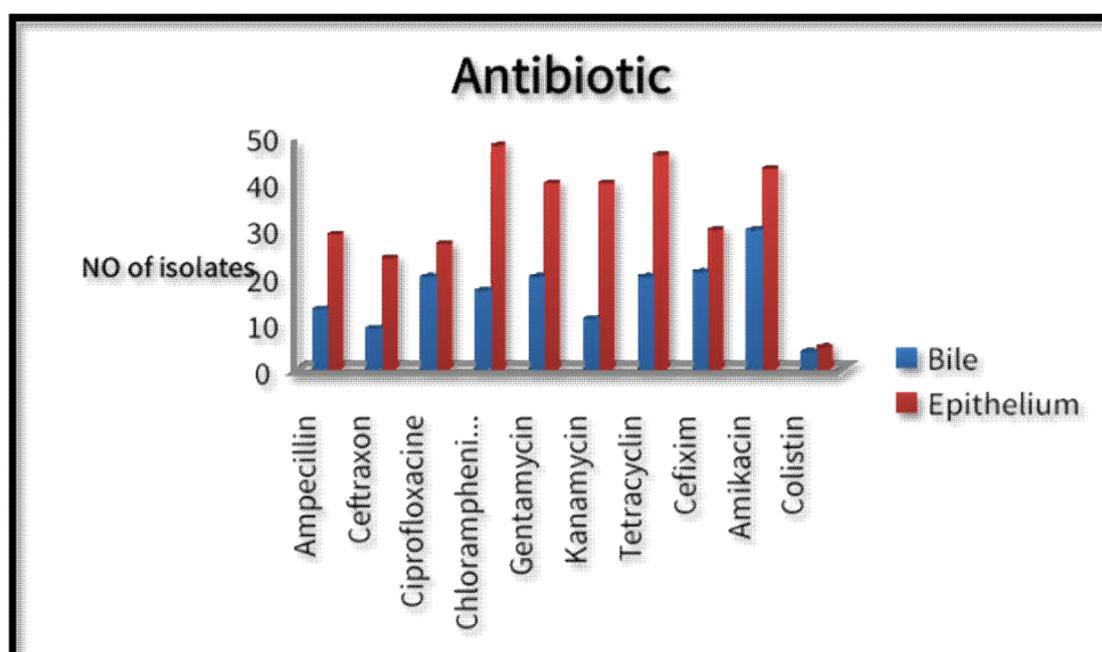
Antibiotic resist Typing	Protenssp p (55)	E-coli (17)	citrobacter(6)	Psudomonas (1)	Klebsiolla (1)	Total
Ampecillin	27	9	6	----	----	42
Ceftraxon	20	11	2	----	-----	33
Ciprofloxacin	35	6	5	-----	1	47
Chloramphenicol	50	7	6	1	1	65
Gentamycin	45	9	4	1	1	60
Kanamycin	40	5	4	1	1	51
Tetracyclin	55	5	4	1	1	66
Cefixim	40	5	4	1	1	51
Amikacin	55	10	6	1	1	73
Colistin	—	5	2	1	1	9

Table(4): In vitro percent sensitivity of different antibiotics against bacterial isolated.

NO.Antibiotic	NO.of isolates sensitive	Sensitivity %
Ampicillin	42	52.5
Amikacin	73	91.25
Cefixime	51	63.75
Colistin	9	11.25
Ceftriaxone	33	41.25
Ciprofloxacin	55	68.75
Chloramphenical	68	85
Gentamycin	60	75
Kanamycin	51	63.75
Tetracycline	63	78.75

Table(5): Distribution of Antimicrobial Isolated From The Bile and Epithelium of gallbladder of slaughtered cattle .

Antibiotic	Bile(32)	%	Epithelium(48)	%
Ampecillin	13	40.62	29	60.41
cefatraxon	9	28.12	24	50
Ciprofloxacin	20	62.5	27	56.25
Chloramphenicol	17	53.12	48	100
Gentamycin	20	62.5	40	83.33
Kanamycin	11	34.37	40	83.33
Tetracyclin	20	62.5	46	95.83
Cefixim	21	65.62	30	62.5
Amikacin	30	93.75	43	89.58
Colistin	4	12.5	5	10.41



Figure(2)

This finding is in agreement with previous studies (17, 24, 25) ,in which amikacin ,cefoxitin, gentamycin ,and ciprofloxacin as most effective drugs. .

The *proteus spp.* present in the epithelium were more susceptible to drugs because bacteria are aggregated in larger number in the epithelium. The occurrence of a low metabolic rate of cells due to low availability of nutrient which fits the gallbladder and becomes an interfering factor of antimicrobial resistance in the microbial population (24)

CONCLUSION

The gallbladder support a high number of micro organisms .The ability of bacteria to survive the harsh environment of the gallbladder and become resistant to antimicrobials highlights the need for special care to be taken during the raising and slaughter of cattle to prevent contamination.Moreover,the gallbladder of cattle should be recognized as reservoir of some pathogens that are multidrug resistant and great important to public health.

الكشف عن بعض الجراثيم المعوية المعزولة من مرارة العجول المذبوحة وتقدير مقاومتها
للمضادات الحيوية

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الخلاصة

تزايد مقاومة البكتريا للمضادات الحيوية في الحيوانات المنتجة للحوم وخاصة المجترات يمثل مشكلة كبرى للطب البشري والبيطري كما بإمكانه رفع مستوى الإصابات والهلاكات . ربما تكون المرارة مكان دائم ومصدر لذرف البكتريا المعوية المنقولة عبر الغذاء والمقاومة للعديد من المضادات الميكروبية . فحصت ٨٠ عينه (٣٢ صفراء، ٤٨ نسيج طلائي) لعزل المسببات المرضية . كانت نسبة الجراثيم المعزولة من النسيج الطلائي للصفراء اكبر من النسبة المعزولة من الصفراء بواقع ٦٠% من النسيج الطلائي و ٤٠% من الصفراء، أكثر نسبة من المسببات الجرثومية كانت لجراثيم (*ProtusSpp*) (٦٨,٧٥%)، تليها *E- coli* (٢١,٢٥%)، *Citrobacter* (٧,٥%)، *Psudomnas* (١,٢٥%) و *Klebsiella* (١,٢٥%) .

أظهرت فحوصات حساسية الجراثيم المعزولة للمضادات الحيوية إن استخدام Amikacin, Cefoxitin, Chloramphanicol, Gentamycin, Kanamycin& Ciprofloxacin قد يكون نافعا لعلاج التهاب المرارة في العجول .

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