

Isolation, characterization and sensitivity test of organism found in bile obtained from sheep and cattle.

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Abstract:

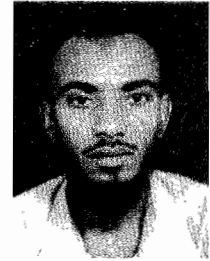
Objectives: The purpose of our study was to see if there were any pathogenic bacteria present in the raw bile of sheep and cattle used as an appetizer in some Sudanese food and to determine the sensitivity of the bacterial isolates to the common antibiotics used in the Sudan.

Specimens and Methods: This study was conducted in Oudurman, Sudan in the period from Nov 2003 to May 2005. A total of 210 specimens from 108 sheep and 102 cattle were examined for the presence of pathogenic bacteria. The isolates were identified by conventional methods.

Results: Thirty-five pathogenic bacteria were isolated from the bile of slaughtered clinically healthy sheep and cattle. Seven isolates from sheep were classified as *Escherichia coli*, *Pseudomonas stutzeri*, *Staphylococcus aureus* and *Acinetobacter lwoffii*. Twenty eight isolates from cattle were classified as *Escherichia coli*, *Escherichia hermannii*, and *Escherichia vulneris*, *Proteus mirabilis*, *Pasteurella haemolytica* var *urea*, *Pseudomonas maltophilia*, *Pseudomonas cepacia*, *Pseudomonas alcaligenes*, *Bacillus cereus* and *Bacillus mycoides*. Antibiotic sensitivity tests were done for each isolate. All isolates were found to be sensitive to pefloxacin (100%), ofloxacin (100%), ciprofloxacin (97%) and most of them were resistant to cefotaxime (30.6%). All of the gram-negative isolates were sensitive to chloramphenicol (100%), piperacillin (96.8%) ceftizoxime (90.3%), and amikacin (96.8%) and gram positive isolates was sensitive to ciprofloxacin (100%). All of the gram positive isolate were resistant to co-trimoxazole, (100%), cefotaxime,(100%) and cephaloxin (100%).

Conclusion: This study shows that raw bile may serve as a potential source for infection to people who use it as food appetiser.

Key words: gallbladder, bacteria, gram staining, antibiotic, slaughtered



Introduction

The Sudan is one of the richest African countries in animal resources. It is estimated to have more than one hundred and thirty million heads. Some Sudanese use raw bile from slaughtered sheep and cattle as an appetizer in some foods (MRARA). Researchers from different parts of the world reported that the gallbladders of cattle and sheep contain a spectrum of pathogenic bacteria¹⁻⁵. We have studied the presence of pathogenic bacteria in the bile of slaughtered sheep and cattle in Khartoum, tested their sensitivity to commonly used antibiotics and compared that with reports from elsewhere¹⁻⁵.

Materials and methods

Study area: Samples were collected from Alsabloga Slaughter House located at Omdurman province, during the period from November 2003 to May 2005.

Specimens: Bile was collected from the gallbladders of 210 apparently healthy cattle and sheep immediately after being slaughtered. Sterile plastic syringes and containers were used and the samples were taken directly to the laboratory.

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Identification of the isolates:

The specimens were inoculated in blood agar and MacConkey agar media, incubated aerobically at 37°C for 24 hours. Identification of isolates were done using gram stain and the following biochemical test: indole, urease, citrate, methayle red, Vojase proskar, oxidase, coagulase, sugar fermentation test and catalase test as recommended in Cowan⁶.

Antimicrobial Susceptibility Test:

Antibiotic sensitivity tests were done by the conventional methods and interpretation of the results for each isolate was carried out according to the Kirby-Bauer method⁷. The antibioticstested were: ofloxacin, Ceftizoxime, cotrimoxazole, tetracycline, cefotaxime, ciprofloxacin, lincomycin, piperacillin, gentamicin, chloramphenicol, amikacin, pefloxacin, co-trimoxazole, cloxacillin and cephalixin.

Results

Bacteriological examination:

A total of two hundred and ten samples of bile were collected from cattle (102) and sheep (108). They were subjected to culture and bacteriological examination. 35 (16.67%) samples showed bacterial growth and the rest one hundred and 71 (83.33%) samples did not revealed any growth of bacteria.

Bacteria isolated from the bile samples:

The isolated bacteria from aerobic growth were shown in table1. The number of isolated bacteria from both animals was 35 isolates, [28 isolates were from cattle and seven isolates were from sheep].

Sensitivity tests:

All isolates were found to be sensitive to pefloxacin (100%), ofloxacin (100%) and gram-positive isolates were resistant to co-trimoxazole (100%), Cephalexin (100%), Cefotaxime (100%) as shown in table2 and 3.

Table [1] bacterial species isolated from the bile collected from cattle and sheep:

Bacteria isolated	No of isolates	Isolation percentages
<i>Escherichia spp</i>	24	11,90%
<i>Pseudomonas spp</i>	5	01,90%
<i>Bacillus spp</i>	2	00,95%
<i>Proteus spp</i>	1	00,50%
<i>Pasteurella spp</i>	1	00,50%
<i>Staphylococcus spp</i>	1	00,50%
<i>Acinotobacter Spp</i>	1	00,50%
Total	35	16,67%

Table{3} Sensitivity of bacterial speceis isolated from bile sample collected from cattle and sheep (gram positive anti biotic)

Code number of isolates	Bacterial species isolated	No .of Isolates	Sensitivity test result									
			CP	PF	OF	BA	PR	TE	CF	LM	GM	
22	<i>Bacillus</i>	Cattle	S	S	S	R	R	R	R	R	R	
	<i>Mycoides</i>											
52	<i>Staphylococcus</i>	Sheep	S	S	S	R	R	S	R	R	S	
	<i>Aureus</i>											
21	<i>Bacillus cerues</i>	Cattle	0	0	0	0	0	0	0	0	0	

Discussion

The purpose of our study was to look for the presence of pathogenic bacteria in the bile of slaughtered sheep and cattle as raw bile is used as an appetizer in some food of Sudanese people - which may be a potential health risk. The results obtained showed that the bile of cattle contains more bacteria than sheep. The commonest organism isolated was *Escherichia .spp* accounting for 11.4% which agrees with Hang¹.

The other species isolated were *Pseudomonas spp*, *Bacillus spp*, *Staphylococcus spp*, *Acintobacter spp*, *Proteus spp* and *Pasturella spp* in small percentage, these species were not isolated in the previous studies which had targeted certain organisms¹⁻⁵. *Salmonella spp* were not isolated in our study which may be due to the rare presence of these organisms. However, this contrasted a report from Nigeria where the organism was isolated in 1% of the sample⁴.

Table {2} Sensitivity test of some bacterial species isolated from the bile sample collected from cattle and sheep. (gram negative anti biotic)

Code number of isolates	Bacterial species isolated	Type of animal	Sensitivity test result										
			TE	BA	CF	PC	CH	CP	CL	PF	OF	GM	AK
6	<i>E.coli</i>	Sheep	S	S	R	S	S	S	R	S	S	S	S
6	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
8	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
28	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
30	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
62	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
34	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
71	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
87	<i>E.coli</i>	Sheep	S	S	S	S	S	S	S	S	S	S	S
97	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
98	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
100	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
101	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
102	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
27	<i>E.coli</i>	Cattle	R	S	R	S	S	S	S	S	S	S	S
29	<i>E.coli</i>	Sheep	R	R	S	R	S	S	S	S	S	S	S
30	<i>E.coli</i>	Sheep	S	S	S	S	S	S	R	S	S	S	S
41	<i>E.coli</i>	Cattle	S	S	S	S	S	S	S	S	S	S	R
45	<i>E.coli</i>	Cattle	S	S	R	S	S	S	S	S	S	S	S
56	<i>E.coli</i>	Cattle	R	S	S	S	S	S	R	S	S	S	S
72	<i>E.coli</i>	Cattle	R	S	S	S	S	S	S	S	S	S	S
93	<i>E.coli</i>	Cattle	R	S	S	S	S	S	S	S	S	S	S
4	<i>E.hermannii</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
25	<i>E.vulneris</i>	Cattle	S	R	R	S	S	S	S	S	S	R	S
99	<i>Pseudomonas</i>	Cattle	S	R	S	S	S	R	S	S	S	S	S
20	<i>Pseudomonas</i>	Cattle	S	S	R	S	S	S	S	S	S	S	S
23	<i>Pseudomonas</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
26	<i>Pseudomonas</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S
22	<i>Pseudomonas</i>	Sheep	*0	0	0	0	0	0	0	0	0	0	0
100	<i>Acinetobacter</i>	Sheep	S	S	S	S	S	S	S	S	S	S	S
3	<i>Pasturella</i>	Cattle	S	R	R	S	S	S	S	S	S	S	S
90	<i>P. mirabilis</i>	Cattle	S	S	S	S	S	S	S	S	S	S	S

* means organism not viable before antibiotic test carried out

Also *Campylobacter* spp were not isolated in this study which does not go with previous reports from the same locality and else where⁵. This variation in organisms isolated may be due to change in the prevalence of organisms; the previous study was conducted in Sudan four decades ago. Another large study will be of great significance to validate our results.

The results of antibiotic sensitivity testing in this study showed different responses to different antibiotics. All isolates were found to be sensitive to both ofloxacin and pefloxacin almost scoring 100%, these results are in agreement with the previous results⁸. Similar to other reports⁹, 100% of gram positive isolates were found to be resistant to co-trimoxazole, cefotaxime, lincomycin and cephalixin; co-trimoxazole and cephalixin. While 18%, 12% and 15% of gram negative isolated were resistant to cefotaxime, co-trimoxazole respectively [table 2]. This is in concordance with Celia C and others findings^{10, 11}.

Conclusion

This study revealed that pathogens like *Escherichia* spp, *Pseudomonas* spp, *Bacillus* spp, *Proteus* spp, *Pasteurella* spp, *Staphylococcus* spp and *Acinetobacter* Spp. were found in the raw bile of slaughtered sheep and cattle. 16.67% of Consumers of this raw bile were at risk of infection with these pathogens. If infection took place, then pefloxacin and ofloxacin may be used as first line treatment.

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