Sensitivity of bacterial isolates from mastitic She-camel (*Camelus dromedaries*) to antibiotics

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Abstract: This study was carried out to identify the causative bacterial agents of mastitis in she-camel and to determine the sensitivity of these bacterial causes to antibiotics. Milk samples were collected from 25 mastitic lactating she-camels. Bacterial cultures were prepared from milk samples according to a standard culture technique. The results of the bacterial culture showed that the most predominant cause of she-camel mastitis is *Staphylococcus spp.* (80.30%). The remaining causes were Bacillus cereus (9.09%), *Pasteurella haemolytica* (6.06%), *Corynebacteria bovis* (6.06%), and *Streptococcus dysaglactiae* (1.52). The *Staphylococcus* isolates were *S. aureus* (22.75%), *S. epidermidus* (12.12%), *S. intermediatius* (7.56%), *S. haemolyticus* (6.06%), *S. simulans* (6.06%), *S. kloosii* (4.55%), *S. hyicus* (3.03%), *S. delphini* (3.03%), *S. lentus* (3.03%), *S. lugdunensis* (3.03%), *S. saprophyticus* (3.03%), *S. chromogenes* (1.52%), *S. sacchrolyticus* (1.52%) and *S. carnosus* (1.52%). The sensitivity of these bacterial isolates to antibiotics was done using oxoid discs impregnated with ciprofloxacin (CIP), gentamycin (GN), ofloxacin (OFF), cephalexine (CFX), tetracycline (TE), co-trimoxazole (SXT), ampicillin (AM), cefotaxime (CTX), coloxacillin (CLX) and lincomycin (LN). The results of the sensitivity test showed that the sensitivity of *Staphylococcus spp.* to CIP was 96.6 3%, OFF 89.48%, GN 84.92%, CFX 76.79%, TE 66.87%, SXT 51.19%, AM 38.39%, CTX 22.02%, LN 14.4% and CLX 7.14%. The *Streptococcus dysgalactiae* sensitivity percent to CIP, OFF, GN, CFX, TE and SXT was 100% and it was resistant to AM, CTX, CLX, and LN. The sensitivity of *Corynebaterium bovis* to CFX, CIP, OFF and GN was 100%, whereas it was completely resistant to AM, SXT, CLX and LN. The *Bacillus cereus* was high sensitivity to CIP, GN (100%), followed by SXT, TE and OFF (80%) and it was resistant to the remaining antibiotics. Finally, *Pasteurella haemolytica* was high sensitivity to AM, TE, CIP, OFF and GN (100%). It is concluded that the most dominant cause of mastitis in she-camels of North Kordofan State of Sudan is *Staphylococcus spp.* and the most effective antibiotics against most of the isolated organisms are CIP, OFF, GN, CFX and TE.
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1. Introduction

Mastitis has been estimated to affect more that 25% of lactating she-camel (Alamin, et al., 2013; Saleh, et al., 2011). Mastitis is also known to cause approximately 70% losses in milk production (Fazhani et al., 2011). The abuse use of antibiotics; for treatment of mastitis without in vitro testing of the sensitivity of the causative agents to antibiotics; leads to failure of mastitis treatment (Saxena et al., 1993). The practice of mastitis treatment without conducting sensitivity test augments the economic losses and increases the number of microbes that developed drug resistance (Fazhani et al., 2011). The bacterial resistance to the antimicrobial agents, including the major last-resort drugs become a very serious threat to public health, and the frequencies of resistance are increasing worldwide (Levy and Marshall, 2004; Mandal et al., 2009). The bacterial isolates from mastitic she-camel milk; *Staphylococcus aureus*, *Micrococci*, *Streptococci* and *Corynebacteria species*; vary in their susceptibility to antibiotic and some are drug resistant (Fazhani et al., 2011). The sensitivity of microbes isolated from the milk of mastitic cows to various antibiotics was extensively studied all over the world (Piret, et al., 2011; Botrel, et al., 2009; Sampimon, et al., 2009; Taponen, et al., 2009; Bagadi, 1966), however, few reports studied the sensitivity of microbes isolated from mastitic camel milk (Dewani 2000; Abdelgadir, 2001; Gentilini et al., 2002; Fazhani, et al. 2011).

Although the camel's population in the Sudan is 3 million heads (Mohamed et al., 1990, Yagoub, 2003); and the camel's milk is an essential source of protein for the nomadic tribes living in the desert and semi-desert areas (Schwartz and Dioli, 1992; Wilson, 1998); sparse information about the causative agents of mastitis in the camels and their sensitivity to antibiotics is available (Amel, 2003; Suheir, 2004). Thus, the objectives of the current study were to identify

the causative bacterial agents of mastitis in she-camel and their sensitivity to antibiotics.

2. Materials and methods

1- Study Area

This experiment was executed in North Kordofan State, Sudan, latitudes 9 -16.8°N and 27-32°E. The district is sandy with ridges. The area being desert and semi-desert, the camel's owners live a nomadic life, migrating from place to another searching for pasture and water. The area is covered with trees and seasonal grasses of different densities. The annual rainfall average is 300 mm in the North to 600 mm in the South of the state. The temperature range is between 10°C in the cool dry season (November to February) to 45°C in the hot dry season (March to June), while in the hot wet rainy season (July to October), the temperature varies according to humidity from 25°-45°C.

2- Milk Samples Collection

Hundred milk samples were collected from 25 mastitic lactating she-camels. The milk was aseptically collected. After getting rid of the first few squirts of milk, 5-10 ml of milk were collected in sterile tubes. The milk samples were transferred immediately in ice-boxes to Elobied Veterinary Research Laboratory to be prepared for bacteriological procedures.

3- Bacteriological procedures

The milk samples were thawed at room temperature and a bacteriological loop full of milk was streaked on 5% blood agar and Mac Conkey agar (Barrow and Feltham, 1993). The bacteriological plates were incubated aerobically at 37°C for 18-24 hours. Pure cultures were obtained by sub-culturing part of typical and well isolated colony on a corresponding medium. This method was repeated at least twice. The resulting growth was checked for purity by staining smear samples with Gram's stain. The pure isolates of bacteria were identified by using standard biochemical tests (Cowan and Steel, 1985).

4- Sensitivity test

4-1. Antibiotics and media

The sensitivity of the organisms to different antibiotics was done using oxoid discs impregnated with 10 mcg ampicillin (AM), 30 mcg cefotaxime (CTX), 2 mcg lincomycin (LN), 30 mcg tetracycline (TE), 25 mcg co-trimoxazole (SXT), 5 mcg ciprofloxacin (CIP), 10 mcg gentamycin (GN), 30 mcg cephalexine (CFX), 5 mcg cloxacillin (CLX) and 5 mcg ofloxacin (OFF) (Basingtok, Hampshire, England). The media used is Diagnostic Sensitivity Test (DST) medium containing protease peptone 10 gm, veal infusion solids 10 gm, dextrose gm 2, sodium chloride 3 gm, disodium phosphate 2 gm, sodium acetate 1 gm, adenine sulphate 0.01 gm, guanine hydrochloride 0.01 gm, uracil 0.01 gm, xanthine 0.01 gm, aneurine 0.00002 gm, ion agar No.2: 12. gm. According to manufacturer's instructions 40 grams of the media were dispensed in one liter of distilled water and dissolved by boiling in water bath, sterilized by autoclaving at 121°C for 15 minutes and poured into peteridishes in 15 ml portions, then stored at 4°C until use.

4-2.Test procedure

The method used was the standard disc diffusion method (Cruickshank et al., 1975). The organisms were sub-cultured onto blood agar and incubated at 37°C for 18-24 hrs, they were then suspended in 4 ml sterile normal saline tubes and 2 ml of the suspension were spread onto surface of nutrient agar and D.S.T. agar after drying. The excess fluid was aspirated by sterile pipette, and the plates were allowed to dry. The antimicrobial discs were placed to the surface of the medium and pressed gently using sterile forceps. They were incubated at 37°C for 24-48 hrs. The zones of inhibition were measured in (mm) to determine whether the organism was sensitive or resistant to the antibiotic according to Jacoby and Archer (1991) and the WHO standards table 1(1977). The percentage of sensitivity was calculated as described elsewhere (Bauer et al. 1966; Fazlani et al. 2011).

Table (1): Standard inhibition zone diameter interpretive chart (WHO, 1977).

Antibiotic	Disk contents	Zone of inhibition (mm)		
		Res. ≤	Inter.	Sens. ≥
AM	10 mcg	22	23-30	31
CFX	30 mcg	14	15-17	18
CIP	05 mcg	15	16-20	21
CTX	30 mcg	14	15-22	23
SXT	25 mcg	11	12-16	17
GN	10 mcg	12	13-14	15
LN	02 mcg	09	10-14	15
OFF	05 mcg	15	16-20	21
CLX	05 mcg	09	10-13	14
TE	30 mcg	14	15-18	19

Res. = resistant; Inter. = intermediate resistance; Sens. = sensitive

3. RESULTS

3-1. Mastitis causative agents

The predominant bacteria isolated were *Staphylococcus spp.* (80.30%), *Bacillus spp.* (9.09%), *Pasteurella haemolytica* (6.06%), *Corynebacterium spp*. (3.03%) and *Streptococcus dysgalactiae* (1.52%). The *Staphylococcus* isolated were *S. aureus* (22.75%), *S. epidermidus* (12.12%), *S. intermediatius* (7.56%), *S. haemolyticus* (6.06%), *S. simulans* (6.06%), *S. kloosii* (4.55%), *S. hyicus* (3.03%), *S. delphini* (3.03%), *S. lentus* (3.03%), *S. lugdunensis* (3.03%), *S. saprophyticus* (3.03%), *S. chromogenes* (1.52%), *S. sacchrolyticus* (1.52%) and *S. carnosus* (1.52%). The *bacillus* was *B. cereus* (9.09%), *corynebacterium* was *C. bovis* (6.06%), *Pasteurella* was *P. haemolytica* (6.06%) and *Streptococcus* was *S. dysgalactiae*.

3-2. Sensitivity test results

As shown in table (2) the *Staphylococci spp.* showed variable sensitivity to the tested antibiotics. However, the overall sensitivity percent of *Staphylococcus spp*. to ciprofloxacin is 96.63, ofloxacin 89.48, gentamycin 84.92, cephalexine 76.79, tetracycline 66.87, co-trimoxazole 51.19, ampicillin 38.39, cefotaxime 22.02, lincomycin 14.4 and cloxacillin 7.14. Accordingly *Staphylococci spp*. are high sensitive to ciprofloxacin, ofloxacin and gentamycin, respectively.

The *Streptococcus dysgalactiae* sensitivity percent to co-trimoxazole, cephalexine, tetracycline, ciprofloxacin, ofloxacin and gentamycin was 100. It was resistant to ampicillin, cefotaxime, cloxacillin and lincomycin (Table 3).

As in table 3, the sensitivity percent of *Corynebaterium bovis* to cephalexine, ciprofloxacin, ofloxacin and gentamycin was 100, whereas it was resistant to ampicillin co-trimoxazole, cloxacillin and lincomycin. The *Bacillus cereus* was high sensitivity to ciprofloxacin, gentamycin followed by co-trimoxazole, tetracycline and ofloxacin and it was resistant to the remaining antibiotics. Finally, *Pasteurella haemolytica* was high sensitivity to ampicillin, tetracycline, ciprofloxacin, ofloxacin and gentamycin.

Table 2. Sensitivity percentages of *Staphylococcus spp*. isolated from the milk of mastitic she-camel .to the different tested antibiotics.

Organisms	No. of isolates	Antibiotics tested									
		AM	SXT	CFX	TE	CTX	CIP	OFF	CLX	LN	GN
S. aureus	9	66.67	33.33	66.67	77.78	33.33	77.78	77.78	00.00	11.11	88.89
S. hyicus	2	50.00	00.00	100.00	50.00	50.00	100.00	00.00	00.00	00.00	100.00
S. intermidius	2	50.00	100.00	100.00	100.00	00.00	100.00	100.00	00.00	50.00	100.00
S. epidermidis	4	50.00	75.00	25.00	25.00	00.00	75.00	100.00	00.00	25.00	100.00
S. simulans	4	25.00	25.00	50.00	50.00	25.00	100.00	75.00	00.00	00.00	50.00
S. kloosii	3	00.00	00.00	00.00	66.67	00.00	100.00	100.00	00.00	00.00	100.00
S. lentus	3	66.67	66.67	100.00	100.00	66.67	100.00	100.00	00.00	33.33	100.00
S. haemolyticus	3	33.33	66.67	33.33	66.67	33.33	100.00	100.00	00.00	33.33	100.00
S. lugdunensis	2	50.00	50.00	100.00	50.00	00.00	100.00	100.00	00.00	50.00	50.00
S. saprophyticus	2	50.00	100.00	100.00	50.00	00.00	100.00	100.00	00.00	00.00	100.00
S. delphini	1	00.00	00.00	100.00	100.00	00.00	100.00	100.00	00.00	00.00	100.00
S. carnosus	1	00.00	100.00	100.00	00.00	00.00	100.00	100.00	00.00	00.00	100.00
S. chromogenes	1	00.00	100.00	100.00	100.00	00.00	100.00	100.00	00.00	00.00	100.00
S. sacchrolyticus	1	100.00	00.00	100.00	100.00	100.00	100.00	100.00	100.00	00.00	00.00
Mean percentage	-	38.69	51.19	76.79	66.87	22.02	96.63	89..48	7.14	14.48	84.92

S = Staphylococcus

Table 3. Sensitivity percentages of the remaining bacteria isolated from milk of mastitic she-camel to the different tested antibiotics.

Organisms	No. of isolates	Antibiotics tested									
		AM	SXT	CFX	TE	CTX	CIP	OFF	CLX	LN	GN
S. dysgalactiae	1	00.00	100.00	100.00	100.00	00.00	100.00	100.00	00.00	00.00	100.00
C. bovis	2	00.00	00.00	100.00	50.00	50.00	100.00	100.00	00.00	00.00	100.00
B. cereus	5	40.00	80.00	40.00	80.00	20.00	100.00	80.00	20.00	00.00	100.00
P. haemolytica	2	100.00	00.00	50.00	100.00	50.00	100.00	100.00	00.00	00.00	100.00

S = Streptococcus
C = Corynebaterium
B = Bacillus
P = Pasteurella

4. Discussion

The results of the present study indicated that *Staphylococcus spp*. predominate in she-camel mastitis. This result confirms the results of Suheir (2004), Abdurahman et al., (1995) and Amel (2003). Contrary, Saleh and Faye, 2011, reported that *Streptococcus spp*. predominate in mastitic she-camels. The *Staphylococcus spp.* might spread between she-camels due to the bad milking habits and/or the use of local anti-suckling devices (Alamin, et al. 2013). The she-camels examined suffered wounds on teats caused by the piece of wood and cloth used in the anti-suckling devices. Presumably *Staphylococcus spp.* invaded the mammary gland tissue through these wounds.

The percentage of infection with *Corynebacterium bovis* resembles the findings reported by Abdel Gadir (2001), Almaw and Molla, (2000), Salwa (1995), Amel (2003) and Abdurahman et al., (1995). This finding disagrees with that of Suheir (2004) who reported high percent of *Corynebacterium bovis* infection. The low incidence of *Streptococcus dysagalactiae* found in this investigation oppose that recorded by Suheir (2004) and Amel (2003) who reported high incidences (17.39% and 13.6%, respectively).

The percentage of mastitic she-camels infected with *Bacillus cereus* was very low contrasting the findings of Salwa (1995), Ramadan et al., (1987) and Hafiz et al., (1987) who found that the main cause of all types of she-camel mastitis is *Bacillus cereus*. Although *Pasteurella haemolytica* is considered as an uncommon pathogen that cause sporadic and sever mastitis (Radostits et al., 2000), the organism is isolated from the milk of the clinical and sub-clinical mastitic cases. This finding agrees with that of Bekele and Molla (2001) who were able to isolate the organism from subclinical mastitic cases. The *Staphylococci spp.* isolated in this study are high sensitive to ciprofloxacin, ofloxacin and gentamycin and less sensitive to the remaining antibiotic tested. This finding disagrees in part with the finding of McDonald and Anderson (1981) who reported that *S. aureus* is sensitive to cloxacillin. In the present study all *Staphylococcus spp.* are resistant to cloxacillin except *S. sacchrolyticus*. The results also showed that the sensitivity of *Staphylococcus spp.* to ampicillin and tetracycline is lower than that reported before (Obeid 1983, Abdel Gadir 2001, Amel 2003 and Suheir 2004). The discrepancies between these results are probably due to the resistance developed by the organisms due to frequent and/or faulty use of these antibiotics.

The *Bacillus cereus* isolated from camel mastitis is highly sensitive to ciprofloxacin and gentamycin and sensitive to tetracycline, co-trimixazole and ofloxacin. This finding agrees with the finding of Dewani (2000) who reported high sensitivity of *B. cereus* to gentamycin. However, our findings disagree with that of Fazlani et al., 2011 who observed high sensitivity of the organism to ampicillin, tetracycline and cephalexine. Our results for ofloxacin are in accordance with that of Fazlani et al. (2011).

The *Corynebacterium bovis* is highly sensitive to gentamycin and ofloxacin which differs from the finding of Fazlani et al. (2011) and Rind and Khan (2000) who tested the same drugs on *Corynebacterium pyogens*. Although they are from the same family they might differ in their susceptibility to the antibiotics. The *C. bovis* showed a moderate sensitivity to tetracycline which is less than that reported by Fazlani et al. (2011).

The *Pasteurella haemolytica* was highly sensitive to tetracycline, ofloxacin, gentamycin and ampicillin. This result agrees only with that of Fazlani et al. (2011) in case of tetracycline and it differs from their result of the remaining antibiotics. These differences may be attributed to the microorganism resistance that develops due to the frequent use of these antibiotics, which differs from place to another.

Streptococcus dysaglactiae is highly sensitive to most of the antibiotics used in this investigation except ampicillin, cloxacillin and lincomycin where it shows complete resistant. However, Hawari and Hassawi (2008) reported that *Streptococcus spp.* is highly sensitive to ampicillin. The differences between these studies are probably due to the variable resistance of these microorganisms to the antibiotics which is influenced by the faulty treatment of infected cases.

It is concluded that the most dominant cause of mastitis in she-camels of North Kordofan State of Sudan is *Staphylococcus spp.* and the most effective antibiotics against the isolated bacterial spp. are ciprofloxacin, ofloxacin, genatmycin, cephalexine and tetracyclin. Therefore we recommend the use of these antibiotics in combination to avoid the microbial resistance.

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