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Full Length Research Paper

Bovine Mastitis in Sudan: Antibiotic Susceptibility Patterns of Corynebacterum spp. from Kuku Dairy Farms

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A study on bovine mastitis due to *Corynebacterium spp.* showed that out of 2283 quarter milk samples, 224 (9.81%) gave positive reactivity to California mastitis test, while 600 (26.28%) and 1459 (63.9%) recorded as doubtful and negative respectively. Two hundred and five bacterial isolates were recovered from milk samples examined, the isolated bacteria were *Staphylococcus spp*. 107 (52.5%), *Streptococcus* spp. 25 (12.3%), *Enterobacterium* spp. 4 (2%) *Lactobacillus* spp. 4 (2%), *Coryneform bacteria* 27 (13.2%). The *Coryneform bacteria* tested was found to be 100% resistant to penicillin G except *Corynebacterium ulcer's* (60%). In contrast Gentamycin was the drug of choice for *Coryneform* (Resistant=0.0 %).

Key words: Bovine mastitis, *Corynbacterum* spp., antibiotics, Sudan.

INTRODUCTION

Mastitis remains the most common and the ambiguity disease of dairy cattle through out most of the word. It is estimated that one third of all dairy cows are infected with some form of mastitis in one or more quarters (Philpot et al., 1999). Mastitis is often the end result of the interact-tion of several factors such as: man, cow, environ-ment, microorganisms and management. Quarters infected with *Arcanobacterium pyogenes* always exhibit clinical symptoms and secrete a thick, foul-smelling, greenish fluid.

Infections result in a persistent form of mastitis, and invariably lead to loss of the quarter and culling of the cow because treatment is ineffective (Philpot et al., 1999). In lactating cows, infection may occur as a result of teat injuries or improper treatment procedure. No much data is available on mastitis caused by other *Corynebacterium.* Therefore, the study planned to deter-mine susceptibility of *Corynebacteruim* spp. cause bovine mastitis against commonly used antibiotics.

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MATERIALS AND METHODS

Study area

The study was conducted in East Nile Locality - Khartoum North (Hillat Kuku dairy farms), which is considered to be the largest milk producing and marketing area in Khartoum State, and regarded as semi-intensive system. Those farms previously belonged to Hillat Kuku dairy project, which consist of three barns distributed in vast space.

Study population and sampling methods

The dairy cows in Kuku area are mostly Frisian (cross between Frisian and local breeds namely kenana and Butana). Concerning sampling, One-stage sampling method was employed as described by Thrusfield (1995).

Collection of milk samples

A total of 2283 milk samples were collected from 585 animals. Before the collection of quarter milk samples from the tested cows, the udder was thoroughly cleaned with soap and water, rubbed dried, and the teats were disinfected with cotton wool moistened

with 70% ethyl alcohol; which been allowed to be air dried. The first few squirts of milk were discarded. 5-20 ml of milk was collected in a sterile universal bottle. The quarter milk samples were kept in ice container and transported as soon as possible to the laboratory at the Faculty of Veterinary Medicine, Khartoum University, (Shambat).

California Mastitis Test (CMT)

All collected milk samples were examined for mastitis using California mastitis test. (CMT) was carried out using the method described by Quinn et al. (1994). Briefly, equal volumes (5 ml) of commercial CMT reagent (avatar rapid mastitis test Kit-Alvetera Gmbh-Germany) and quarter milk were mixed and the changes in milk fluidity and viscosity were observed. The interpretation of the result was done according to the method described by Quinn et al. (1994). Negative (0) and trace (+/-) were considered as negative and different intensities of positive (1, 2 and 3) were considered as positive Table 1.

Culturing methods

The bacteriological culture was performed following the standard microbiological technique Quinn et al. (1994). One loop full of milk was streaked on 5% sheep blood agar and MacConkey agar to detect bacteria that could grow on this medium. The plates were incubated aerobically at 37°C for 24 - 48 h. The plates were examined for growth, morphologic features of the colonies, and hemolytic characteristic. Presumptive identification of bacteria on pure culture was done on the basis of colony morphology, heamolytic characteristics, Gram-stain and biochemical tests: co-agulase test, heamolyses, pigment production, fermentation of maltose (purple agar +1% maltose). Presence of *Streptococcus spp*. and *Enterococcus* spp. was determined according to CAMP reaction, type of heamolyses, growth characteristic on Edward medium and sugar fermentation.

Corynebacterium spp. and *Bacillus* spp. were identified based on heamolytic characteristics, catalase test, growth on 9% NaCl, CAMP reaction, and sugar fermentation tests. Gram-negative isolates were identified based on growth on Mac Conkey agar, catalase test, oxidase reaction, triple sugar iron agar (TSI), IMVIC test, urease and sugar fermentation tests. The differentiation of microbial isolates was carried out as summarized in. Identification of the isolated *Corynebacterium* to species level was done using commercial identification kit (API Coryne BIOMERIEUX, FRANCE)**.**

Determination of clinical and sub- clinical mastitis

Clinical mastitis was recognized by abnormal milk and signs of udder infection (abnormalities of the udder). While sub-clinical cases were based on California mastitis test (CMT) and bacterio-logical examinations.

Antibiotic susceptibility test

Susceptibility of *Corynebacteria* isolated from mastitic milk to commonly used anti-microbial (Table 2) were subjected using the disc diffusion susceptibility test (Kirby-Bauer method) (NCCLS, 1990, 1997; Quinn et al., 1994). Briefly, this was performed as follows:

The isolates were transferred to a tube containing 5 ml nutrient broth. The mixture was incubated at 37ºC until light visible turbidity appeared; t his was compared with the McFarland 0.5 turbidity standard.

The isolates were streaked onto Mueller-Hinton agar which contained 5% defibrinated blood.

McFarland 0.5 turbidity standard was prepared by mixing 0.5 ml of solution a (0.048 M $BaCl_2$) and 99.5 ml of solution B (0.36N H_2SO_4).

Solution A (0.048 M $BaCl_2$): 1.75 gram $BaCl_2X2H_2O$ was diluted in 100 ml distilled water.

Solution B (0.36N H_2SO_4): 1 ml H2SO4 was mixed with 100 ml distilled water

The antibiotic disc were incubated for 18 to 24 h at 37ºC and the diameters of growth-inhibition were measured in millimeters and reported as:

Susceptible: Refers to the response of the pathogens to the anti-microbial agent at the normal dosage.

Resistant: the pathogens were not inhibited by the usually achievable systemic concentration of the anti-microbial.

RESULTS

Out of 2283 quarter milk samples, 224 (9.81%) gave positive reactivity to California mastitis test, while 600 (26.28%) and 1459 (63.9%) recorded as doubtful and negative respectively. High prevalence (20.17%) of sub-clinical mastitis was obtained from farms, while low prevalence (3.58%) of clinical mastitis was reported (Table 3).

Bacteriological examination

The isolated bacteria were *Staphylococcus spp*. 107 (52.5%), *Streptococcus* spp. 25 (12.3%), *Enterobacterium* spp. 4 (2%) *Lactobacillus* spp. 4 (2%), *Coryneform bacteria* 27 (13.2%), *Micrococcus spp*. 10 (4.9%), *Pseudomonas* spp., 11 (5.9%), *Bacillus spp*., 10 (4.9%) and *Aercoccus* spp., (Table 4). In this study isolated *Corynebacterium* were *Corynebacterium striatum* 9 (33.3%), *Arcanobacterium pyogene* 4 (14.8%), *Corynebacterium Pseudotuberculosis* 2 (7.4%).

Antibiotic susceptibility

The *Coryneform* bacteria was found to be 100% resistant to penicillin G. In contrast Gentamycin was the drug of choice for *Coryneform* (Resistant= 0.00%). While *Corynebacterium pseudo tuberculosis* and *Corynebacterium bovis* were highly sensitive to Tetracycline, Ofloxacin and Gentamicin (100%). *Arcanobacterium pyogenes was* resistant to Penicillin G, (100%) and sensitive to Cephalexin and Gentamicin (100). All results are summarized in (Table 5).

DISCUSSION

Mastitis can be defined as an inflammation of the mammary glands caused by physical or chemical agent, but the

Table 1. Interpretation of the (C.M.T) results (Quinn et al., 1994).

Anti-microbial agent	Disc code	Disc conc.	Interpretation of zone size in mm			
			Resistant	Intermediate	Moderately	Susceptible
Ampicillin	AP	10 mg				
Staphylococci			≤28	-	-	≥29
Non-enteric Streptococci			≤21	-	22-29	≥30
Enterococci			≤16	-	≥17	-
Enterobacteriaceae			≤13	-	14-16	≥17
Other organisms			≤13	-	14-17	≥18
Erythromycin	E	15 mg	≤13	14-22	-	≥23
Kanamycin	K	30 mg	≤13	14-17	-	≥18
penicillin	PG	10 Units				
Staphylococci			≤28	-	-	≥29
Streptococci			≤19	-	20-27	≥28
Enterococci			≤14	-	-	≥15
Other Organisms			≤16	-	-	≥17
Streptomycin	S	10 mg	≤11	12-14	-	≥15
Tetracycline	T	30 mg	≤14	15-18	-	≥19
Ox tetracycline	OT	30 mg	≤14	15-18	-	≥19
Chloramphenicol	C	30 mg	≤12	13-17	-	≥18
Gentamicin	GM	10 mg	≤12	13-14	-	≥15
Nalidixic acid	NA	30 mg	≤13	14-18	-	≥119
Cephalothin	CF	30 mg	≤14 mm	-	15-17 mm	≥18 mm
Ciprofluxacin	CP	15 mg	≤15 mm	-	16-20 mm	≥21 mm
Cloxacillin	CX	10 mg	≤14 mm	15-20 mm	-	≥21 mm

Table 2. Zone size interpretation of anti-microbial agents used.

CMT score	Interpretation	Visible reaction
0	Negative	Milk fluid and normal
+/-	Trace	Slight precipitation
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickness with a gel formation
3	Strong positive	Viscosity greatly increased strong gel that is cohesive with a convex surface

Source: Modified from NCCLs (1990, 1997) and Quinn et al. (1994).

Table 3. The prevalence of clinical and sub-clinical mastitis in Kuku area.

Number examined	Prevalence (%)	
	Clinical	Sub-clinical
585	(21) 3.58	(118) 20.17

Clinical mastitis based on detection of udder and milk. Sub-clinical mastitis based on (CMT). Cut - off level of CMT: +++, ++, + ≡ + ve and ±, - ≡ - ve

majority of the infections are usually caused by bacteria (Quinn et al., 1994; Radostitis et al., 2000). Bovine mastitis is of great economic importance to diary industry world wide (Miller et al., 1984). This study on bovine mastitis was conducted in East Nile Province - Khartoum North (Hillat Kuku dairy farms), which is considered to be the largest milk producing and marketing area in Khartoum State, and regarded as semi-intensive system (small holder) of milk production. Those farms previously belonged to Hillat kuku dairy project. The area was chosen in accordance to the result that obtain from the Khartoum State Ministry of Agriculture and Animal Resources (2003) which conducted survey on milk hygiene in Kuku area at the farm level, bulk milk and venders. The survey proved that Kuku area is the mostly bad in this concern. Different Corynebacterial isolates showed varied degree of sensitivity to tetracycline, ofloxacin, ciprofloxacin, cloxacyline, cephalexin and ceflotoxine but most of them were found to resist penicillin (G), but gentamycin was found to be the most sensitive one (100%). On the other hand most of the *Corynebacterium*

Table 4. Gram positive and negative bacteria isolated from dairy farms of Kuku area.

Isolates	Mastitis (Frequency (%))		Total (Frequency (%))
	Clinical	Sub-clinical	
Staphylococcus spp.	8 (38.1)	99(53.8)	107 (52.5)
Streptococcus spp.	4(19)	21(11.4)	25(12.3)
Enter bacterium spp.	--	4(2.2)	4(2)
Lacto bacillus spp.	--	4(2.2)	4(2)
Coryneform bacterium	4(19)	23(12.5)	27(13.2)
Micrococcus spp.	1(4.8)	9(4.9)	10(4.9)
Pseudomonas spp.	3(14.3)	8(4.3)	11(5.9)
Bacillus spp.	1(4.8)	9(4.9)	10(4.9)
Aercoccus spp.	--	7(3.8)	7(3.2)
Total	21(100.0)	184(100.0)	205(100.0

Table 5. The percentage of resistance of some Coryneform isolates against some antimicrobial agents.

Isolates	No. tested	P 10 unit	BA 25 mg	PR 30 mg	TE 30 mg	CF 30 mg	CP 5 mg	PF 5 mg	OF 5 mg	CX 5 mg	E 15 mg	CD 2 mg	GM 10 mg
Corynebacterium striatum	9	100	75	44. 4	44.4	11.1	33.3	33.3	22.2	33.3	88.9	33.3	00
Arcanobacterium pyogenes	4	100	50	00	50	25	50	33.3	50	50	75	25	00
Corynebacterium pseudo tuberculosis	2	100	00	00	00	00	00	50	00	0	5	00	00
Corynebacterium ulcerans	5	60	60	40	40	00	00	50	00	20	30	40	00
Corynebacterium bovis	7	100	60	00	00	00	00	00	00	00	50	50	00

P≡penicillinG BA≡CO-Trimoxazole PR≡Cephalexin TE≡Tetracycline CF≡Cefotaxime CP≡Ciprofloxacin OF≡Ofloxacin E≡Erythromycin PF≡Pefloxacin CX≡Cloxacillin CD≡Clindamycin GM≡Gentamicin

found resistance to farmers in Kuku area for treatment of mastitis with wide use of antibacterial drug has resulted in occurrence of resistant species of bacteria among those bacterial popu-lations which were earlier suscep-tible, and found to be matching with the postulates established by Saluiemi (1980). Resistance of bacteria to Penicillin were known to developed through several mechanisms, one of them was that pro-duction of β. lactamase. Saluiemi (1980), added that bacterial resistant become dominant in countries where antimicrobials have been used for long times. On the other hand, Radostits et al. (2000) claimed that bacterial isolates from cases of summer mastitis are susceptible to penicillin (G) and other produced β.lactamase antimicrobial.

Conclusion

1. Different *Corynebacterium* species were isolated from the clinical cases of mastitis.
2. *Corynebacterium* species isolated, found to be highly sensitive to Gentamycin.

REFERENCES

Miller RH, Emanuelsn V, Bradund L, Person E, Funke H, Pillipson J (1984). relationship of current bacteriological status of the mammary gland to dairy milk yield and composition. Dairy Sci., 46: 6212.

Philpot WN, Stephen CN (1999). In mastitis counter attack a strategy to combat mastitis. Published by Westphalia. pp. 60-65.

Quinn PJ, Carter ME, Markey BK, Carter GR (1994). Clinicalveterinary microbiology. 1st\ ed. London: Wolfe publishing.

Radostitis OM, Gay CC, Blood DC, Hinchcliff KW (2000). Mastitis in Veterinary Medicne 9th ed., W . B. Saunders, London.

Saluiemi H (1980). Udder diseases in dairy cows-field observation on incidence, somatic and environmental factors and control, Finland. J. Sci. Agri. Soc., 52: 85-184.

Thrusfield M (1995). Veterinary epidemiology. 2ned. Blackwell Science Ltd. UK.