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Isolation of Some Pathogenic Bacteria from Cow Raw Milk in Relation to Public Health at Khartoum State, Sudan.

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Abstract

This study was conducted to isolate some pathogenic bacteria from raw milk and to assess the risk factors associated with milk consumption in the seven localities of Khartoum state (Khartoum, Jabal Awlia, Bahry, Sharg alnil, Omdurman, Ombadda, Karary) . Atotal of 102 samples of raw cow milk were collected in winter , summer and autumn seasons from December 2018 to October 2019 from milk equipment , udders of milking cows, and venders, the samples were analyzed and examined for detection of coliform bacteria and some pathogens contaminate raw milk causing milk borne diseases and effect on public health .The isolates were identified morphologically and biochemically and the results revealed that examined samples showed growth of Gram negative bacteria (233 isolates) and recorded , *Escherichia* spp. *75(32.19%), Klebsiella spp. 30(12.88%) Salmonella spp.15(6.44%), Shigellaspp. 20(8.58%), Proteus merabilis 37(15.88%), Proteus* spp *29(12.45%).and Pseudomonas* spp.*7(11.58%)* . According to the source of collection the isolates were *65(27.9%)* from the udders , *93(40%)* from equipment , and *75(32.1%)* from vendors and this indicated high contamination in milk equipment . According to the seasons the isolates were 35(15.0%) in winter, 66(28.3%) in summer , and 132(56.7%) in autumn respectively .In conclusion the contamination of milk by bacteria is high because of this dairy workers need more awareness about sanitary measures.

Keywords: pathogenic bacteria, cattle , raw milk.

Introduction

Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans, as can be easily contaminated during milking and handling (Addo *et al*., 2011). Human may be infected with milk-borne pathogens through consumption of infected raw or unpasteurized milk and milk products (Bertu *et al.,* 2010; Pal and Jadhav, 2013). Bacterial organisms which may gain entry into milk can multiply and cause spoilage, rendering raw or proccessed milk unsuitable for human consumption due to rancidity, musty odour, or toxin production (Nanu *et al*., 2007). Organisms in milk indicate inadequate milking procedures, poor storage conditions, or unhygienic productions (Gran *et al.,* 2003; Girmaud *et al*., 2009). Bacterial contamination of raw milk can originate from different sources including air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008).

Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal, the secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment and water supplies used in sanitary activities (Pal and Jadhav, 2013).

Fresh milk drawn from a healthy cow normally contains low microbial load and generally contains less than 1000 cfu/ml of total bacteria, but the load may increase once it is stored at normal temperature(Wallace, 2008 ; Salman and Hamad, 2011) . Many milk-borne epidemics of human diseases are spread through milk contamination (Bertu *et al*., 2010).

Unpasteurized milk and dairy products manufactured from raw milk, have been implicated in many outbreaks (Maher *et al*., 2001). In Sudan, the raw milk distributed for consumption is not subjected to proper quality control measures which are needed (Mohamed and El Zubeir, 2007).

Most of milk producers in Khartoum State are unaware of the effect of animal health and environmental conditions on producing safe milk due to absence of full certification of employees, absence of technical staff, retardation of milk production and processing system and lack of training and extension programs (Abdalla and Elhagaz, 2011)

The detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used (Olson and Mocquot ,1980, Bonfoh *et al*., 2003).

Detection of coliform bacteria or pathogenes in milk can be used as an indicator for udder infection (mastitis) , contamination in milking utensils or water supply (Yuen *et al* ., 2012). The presence of total coliforms in foods of animal origin indicates environmental sources of contamination (Mhone *et al*., 2011). Amongst the coliforms, Escherichia coli is the most common contaminant of raw and processed milk (Quinn *et al*., 2002).

E. coli is the most common species of facultative anaerobe found in the gastrointestinal tract of both man and animals and the most commonly encountered pathogen in the Enterobacteriaceae family, therefore the presence of such organism in foods is indicative of fecal pollution (Soomro *et al*., 2002 ; Benkerroum *et al*., 2004).

Salmonellosis is one of the most important zoonotic bacterial pathogen of food-borne infection all over the world. The most important serotypes of *Salmonella* are *Salmonella typhimurium* and *Salmonella enteritidis* (Fashae *et al*., 2010 ; Hendriksen *et al*., 2011). Salmonella spp can cause gastrointestinal disease. The main sources of transmission are water, eggs and raw foods (Karns *et al*., 2005).

Salmonella and Escherichia coli are Pathogens that have been frequently involved in foodborne outbreaks associated with the consumption of milk and their presence in milk emerged as major public health concerns, especially for those individuals who still drink raw milk (Ryser, 1998). Food borne Salmonellosis has been recognized due to consumption of raw or improperly pasteurized milk and milk products (Karshima *et al*., 2013).To protect public health against milk borne infections, there are regulations that require proper hygiene handling of milk and its pasteurization, but in developing countries such regulations are not usually adhered, hence milk borne health risk is higher in these countries (Donkor *et al*. 2007). Lack of knowledge about clean milk production , use of unclean milking equipment and lack of potable water for cleaning purposes were some of the factors which contributed to the poor hygienic quality of raw milk in the study farms (Godefay and Molla, 2000). This study was aimed to isolate some pathogenic bacteria from raw milk and to assess the risk factors associated with milk consumption in Khartoum state

Materials and Methods:

Area of Study:

The study was done in Khartoum state which localized between latitude 15.08 and 16.39 north , and longitude 31,36 and 34,25 east .Livestock production systems (Milk, Meat , and Poultry) are

operational within and around Khartoum city. The climate is hot and dry with rains in summer , cool and dry in winter

Collection of milk samples:

Atotal of 102 raw cow milk samples were collected from the seven localities of Khartoum state from December 2018 to October 2019 covering all seasons in the state (22 milk samples in winter , 32 in summer and 48 in autumn) . These samples were taken as follow : 24 samples from the udders directly, 42 from milk utensils inside the farms and 36 from the vendors in the seven localities (Table 1,2)

Table (1) :Collection of raw milk samples seasonally in Khartoum State

Season	Locality							
	Khartoum	Jabal Awlia	Bahry	Sharg alneel	Umdurman	Umb adda	Karary	Total sample number
Winter (December 2018 , January, February 2019)	8	3	6	6	5	4	4	34(33.33%) 1-34)(
Summer (March, April, May, June 2019)	5	2	5	4	5	8	3	34(33.33%) 35-68)(
Autumn (July, August, September,Octo-ber 2019)	7	3	4	6	7	3	4	34(33.33%) 67-102)(
Total	20	8	15	16	17	15	11	102

Bacteriology :

MacConkey's agar , Xylose lysine deoxycholate agar (XLD) ,and Nutreint agar were prepared as solid media for the growth of colonies.

Gram`s stain technique was used to descript the pathogenic organisms morphologically (Barrow and Feltham,1993) , and biochemical tests that applied were Indole test, Citrate test, Urease test , Methyle test , Voges Proskaur test ,Catalase test , Oxidase test , Kligler iron agar test , and Motility test. (Barrow and Fethman, 1993)

Results

From 102 cow raw milk , 233 of them were G-negative bacteria (*Enterobacteriaceae* family and *Pseudomonas* spp.) were isolated

75 (32%) *Escherichia* spp , 30(12.88%) *Klebseilla* spp., 15(6.44%) Salmonella spp., 20(8.58%) *Shigella* spp. , 37(15.88%) *Proteus* sp.., 66(54.90%) and *pseudomonas* spp. 27 (11.58%) table (2)

Table 2 : Number of isolated bacteria from cow raw milk (n-=233) in Khartoum state

olated bacteria	otal number and (%)
Escherichia spp.	75 (32.19%)
Klebseilla sp.	30 (12,88%)
Salmonella sp.	15 (6.44%)
Shigella sp.	20 (8.58%)
Proteus sp.	66 (28.32%)
Pseudomonas spp.	27 (11.58%)
Total	233(100%)

Table (3) : Numbers of isolated bacteria from cow raw milk samples according to the sources of collection .

Isolates	ources dder	arms equipment	enders containers	otal
Escherichiaspp.	20(26.7%)	29(38.7%)	26(34.6%)	75
Klebseilla	6(20.0%)	11(36.7%)	13(43.3%)	30
Salmonella	4(26.7%)	4(26.7%)	7(46.6%)	15
Shigella	11(55.0%)	5(25.0%)	4(20.0%)	20
Proteus spp.	14 (21.6%)	32(48.6%)	20 (29.8%)	66
Pseudomonas	9(33.4%)	12(44.4%)	6(22.2%)	27
Total	65(27.9%)	93(39.9%)	75(32.2%)	233

From table (3) **Escherichia** *spp*. , *Proteus spp* , were highest from equipment (38.7% , 48.6%) and lowest from the udder(26.7% , 21.6%) respectively. *Kleseilla spp* and *Salmonella spp* were high from vender containers (43.3% , 46.6%). But, *Shigella spp* was higest in udder samples (55.0%) and lowest in vender containers (20.0%), while *Pseudomonas spp* was highest in farm equipment (44.4%) and lowest in vender containers (22.2%)

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Table 4: Distribution of isolated bacteria from cow raw milk in Khartoum state

Isolations	ocality Khartoum	bal Awlia	Bahry	Sharg alneel	Umdurman	Umbadda	Karary	Total
Escherichia spp.	12	7	11	14	12	13	6	75
Klebseilla	4	1	1	6	7	7	4	30
Salmonella	1	1	2	5	2	2	2	15
Shigella	6	2	4	1	3	2	2	20
Proteus spp.	7	1	14	14	14	9	7	66
Pseudomonas	5	2	4	2	3	4	7	27
Total	35 (15 %)	14 (6%)	36 (15.5%)	42 (18%)	41 (17.6%)	37 (15.9%)	28 (12%)	233 (100%)

Table (4) showed that Sharg alnil locality represented high percentage (18.0%) , whereas, Karary represented lower percentage (12.0%).

Table 5: Isolated bacteria isolated from cow raw milk seasonally in Khartoum State

Isolations	Seasons Winter (December 018 , January, February 2019)	Summer March, April, May, June 2019)	Autumn(July ,August, September, October 2019)	Total
Escherichiaspp.	7 (9.4%)	28(37.3%)	40(53.3%)	75
Klebseilla	7(23.3%)	18(60%)	5(16.7%)	30
Shigella	12(60%)	5(25%)	3(15%)	20
Salmonella	2(13.3%)	1(6.7%)	12(80%)	15
Proteus spp.	3(4.54%)	5(7.58%)	58(87.88%)	66
Pseudomonas	4(14.8%)	9(33.3%)	14(51.9%)	27
Total	35(15.0%)	66(28.3%)	132(56.7%)	233

As shown in table 5 the contamination of milk by bacteria was highest in autumn season (56,7%) , medium in summer season (15.0%) and lowest in winter (28.3%).

Discussion:

The results of this study revealed that all isolated organisms were gram.negative (233 isolates from 102 cow raw milk samples (table 2). This indicated that contamination of milk in Khartoum state by **Escherichia spp** organism was more than other organisms, , while Donkor *et al.*(2002) in Ghana isolated **(2.1%) Escherichia coli**, (16.7%) **Klebsiella** spp , (7.3%) **Proteus** spp ,and they recorded that most of the organisms identified were enterobacteria indicating probable faecal contamination of the milk as a result of poor hygiene. Generally, the organism *E.coli* considered more member of coliforms that contaminate raw milk (Soomro *et al.* , 2002 ; Zeinhom and Gihan , 2014 ; Robert et al., 2014 . But Haftay *et al.* (2018) .who isolated *E. coli* in highest percentage than other organisms .

Rundasa *et al.* (2019) reported that the prevalence of the major problems of dairy cows in milk production that reduced the quality of milk is the distribution of this bacterial pathogen in the herd which indicates the economic impact of the diseases.

According to the source of collection (Table 4), the isolated pathogens were 65(27.9%) from the udders , 93(40%) from equipment , and 75(32.1%) from vendors`s conainers and this indicated that milk of equipment was highly contaminated and this agreed with Jayarao and Wang (1999) in eastern South Dakota and western Minnesota who found same results, and not agree with Rajeev and Amit, (2010) who found that out of all milk samples examined. The highest contamination was recorded from the milk collected from vendors 26% followed by dairy farms 20%. In this results , temperature and humidity were lead to increasing in the growth of bacteria in milk collected from Khartoum state (table 5) and this was in accordance with previous studies (Harmon, 1994 ; Adams *et al.* ,1999 ; McCarthy *et al.* ,(2001) ; and Bernabuccie *et al.* , 2010).
From the above results it can concluded that, the contamination rate of milk is high, which means the lack of knowledge and awareness among dairy workers about health aspects. This calls for developing plants to aware and educates dairy workers, take strict health measures, and impose laws that guarantee the safety of milk and the safety of dairy workers and consumers.

References:

Abdalla, M.O.M and Elhagaz, F.M.M. (2011) ; The impact of applying some hygienic practices on raw milk quality in Khartoum State, Sudan. *Res. J, Agri. and Bio. Sci.*. 7:169-173

Adams , R. , McCar ,l. B. , Segerson, K . , Rosenzweig, C. , Bryant, KJ. , Dixon, BL., Conner, R. , Evenson, R.E., Ojima, D. (1999). The economic effects of climate change on US agriculture. ; In: Mendelsohn R, Neumann J, editors Cambridge University Press; Cambridge, UK:. pp. 18–54.

Addo, K.K., Mensah, G.I., Aning, K.G., Nartey, N., Nipah, G.K., Bonsu, C., Akyeh, M.L. and Smits, H.L. (2011). Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana . *Trop.Med. Int. H.J.*, 16: 227 – 232.

Barrow, G,I.. and Feltham, R.K.A.(1993). Cowan and Steel,s Manual for Identification of medical bacteria.. London : Cambridge University press

Benkerroum, N., Y. Bouhal, A. EI-Attar and A. Marhaben,(2004) . Occurrence of Shiga toxinproducing *E. coli* 0157:H7 in selected diary and meat products marketed in the city of Rabat, Morocco. *J. Food. Prot.*
67: 1234-1237.

Bertu, W.J., Dapar, M., Gusi, A.M., Ngulukun, S.S., Leo, S. and Jwander, L.D. (2010) ; Prevalence of Brucella antibodies in marketed milk in Jos and environs. *Afr. F. Sc.J.*, 4: 062 - 064

Bonfoh, B., A. Wasem, A.N. Traore, A. Fane, H. Spillmann, C.F. Simbe, I.O. Alfaroukh, J. Nicolet, Z. Farah and J. Zinsstag, (2003) ; Microbiological quality of cow's milk taken at different intervals from the udder to the selling point in Bamako (Mali). *Food Control*, 14: 495-500

Coorevits, A., De Jonghe, V.; Vandroemme, J. ; Reekmans, R. ; Heyrman, J. ; Messens, W. ; De Vos, P. ; Heyndrickx M. (2008). Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms. *System of Applied Microbiology*. 31(2):126-40.doi: 10.1016/j.syapm.2008.03.002. Epub 2008 Apr 10.

Donkor , ES., Aning, KG. , Quaye, J., (2007); Bacterial contaminations of informally marketed raw milk in Ghana. *Ghana Med J* 41:58-61.

Fashae, K. F.; Ogunsola, F.M.; Aarestrup, R.S. Hendriksen .(2010). Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria *J Inf Dev Count*, 4 pp. 484-49.

Godefay B. and Molla B. (2000). Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa, *Berl. Münch Tierarztl Wschr.* 113:1–3.

Gran HM; Gadaga HT; Narvhus JA. (2003). Utilization of various starter cultures in the production of Amasi, a Zimbabwean naturally fermented raw milk product. *Int. J. of Food Microbiology*, 88(1): 19-2

Haftay, A. T.;Netsenet, B. G.; Kidane, W. ; Hagos. H. ; Seyfe, G.; Abrha B. and Habtamu T. (2018) . Antimicrobial Resistance Profile of *E. coli* Isolated from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia,.*J. Vet Med Int.* 8903142.

Harmon RJ.(1994). Physiology of mastitis and factors affecting somatic cell counts. *J Dairy Sci*.;77:2103–2112

Hendriksen R.S; Vieira A.R.; Karlsmose S.; Lo Fo Wong, D.M.; Jensen A.B.; Wegener, H.C. (2011). Global monitoring of *Salmonella* serovar distribution from the world health organization global food borne infections network country data bank. *Food Borne Path Dis*, 8 (8) pp. 1-14

Jayarao. B.M,; Pillai .S.R..; Sawant. A.A.; Wolfgang, DR.; Hegde, NV.(2004). Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. *J Dairy Sci.;*87:3561–3573

Karns JS.;Van Kassel JS.;McKluskey BJ.; Perdue M. (2005) . Prevalence of Salmonella enteric in bulk tank milk from US dairies as determined by Polymerase Chain Reaction. *J Dairy Sci*. 88:3475–9.

Karshima N. S.; Pam V. A.; Bata S. I.; Dung P. A. and Paman N. D. (2013). Processed Milk Products Traded for Human Consumption and Associated Risk Factors in Kanam, Plateau State, Nigeria. *J. Anim Prod. Adve*. (3) 3 :
69-74

Maher M . M.; Jordan K.N .; Upton M.E.; Coffey A. (2001) . Growth and survival of *E. coli* O157 :H7 during the manufacture and ripening of a smear ripened cheese produced from raw milk *J Appl Microbiol*, 90, pp. 201–207

McCarthy, J .J.; Canziani O.F.; Leary N.A.; Dokken D.J.; White K.S. (2001). Climate Change Impacts, adaptation, and vulnerability, contribution of working group II to the third assessment report of the Intergovernmental Panel on Climate Change (IPCC) Cambridge University Press; Cambridge, UK.

Nanu, E.; Latha, C.; Sunil, B.; Thomas, PM.; Menon, KV .(2007). Quality assurance and public health safety of raw milk at the production point. *Am. J. Food*

Olson, Jr.J. C and Mocquat, G. (1980). Milk and milk products,. In.Silliker, J.H.; Ellitto, R. P. Baird; A.C. Parker, F.L. Bryan J.H. Christion, D.S. Clark, Olson J.C , and Roberts T.A. (eds.). *Microbial ecology of foods*, vol.1 Academic Press, N. Y. USA.pp.470-520

Pal, M. and Jahdav, V.J. (2013). Microbial contamination of various Indian milk products. *Bev.:Food World*, 40: 43-44

Quinn, PJ.; Markey, BK.; Carter, ME.; Donnelly, WJC.; Leonard, FC. (2002). Veterinary microbiology and microbial diseases. Blackwell Science, Oxford

Rajeev, K..; Amit, P. (2010). Detection of *E. coli* and *Staphylococcus* in milk and milk products in and around pantnagar .*Vet World*, 3 (11) pp. 495–496

Robert, L.; Francis, S.; Athanasia, M.(2014). Prevalence of **Salmonella spp.** and **Escherichia coli** in raw milk value chain in Arusha, Tanzania. **Am . J. Res. Comm.**. 2(9):1–13.

Rundasa, M.; Mesfin, M .; Haben, F. (2019). Isolation and Identification of *Escherichia coli* from Dairy Cow Raw Milk in Bishoftu Town, Central Ethiopia *Sch. Vet.Med. Wolaita Sodo Univ, V. 1. I. 1*

Ryser, ET. (1998). Applied Diary Microbiology; Steele Edition. New York: Mercell Dekker, Inc; *Public Health Concerns*; pp. 263–404

Salman, A. M. A. and. Hamad, I. M, (2011). Enumeration and identification of Coliform bacteria from raw milk in Khartoum State, Sudan .*J .Cell .Anim. Bio*. Vol. 5(7), pp. 121-128.

Soomro, A.H.; M.A. Arain; M. Khaskheli and B. Bhutto,(2002). Isolation of Escherichia coli from raw milk and milk products is relation to public health sold under market condition at Tandojam. Pakistan. *J. Nutr*., 1: 151-152

Wallace RL. (2009). Bacteria Counts In Raw Milk. Available from: URL:http://en.engormix.com/MA-dairy- cattle/management/articles/bacteria-counts-raw-milk-t1285/124-p0.htm

Yuen, S. K.;Yee C. F. and Yin. F. H. (2012). Microbiological quality and the impact of hygienic practices on the raw milk obtained from the small-scale dairy farmers in Sabah, Malaysia. *Int. J. Agr. Food Sci*. 2 (2): 55-5

Zeinhom, M.A ; Gihan K. A. (2014). Public health risk of some milk borne pathogens, *J . Basic and appl. sci*. 1 – 7.