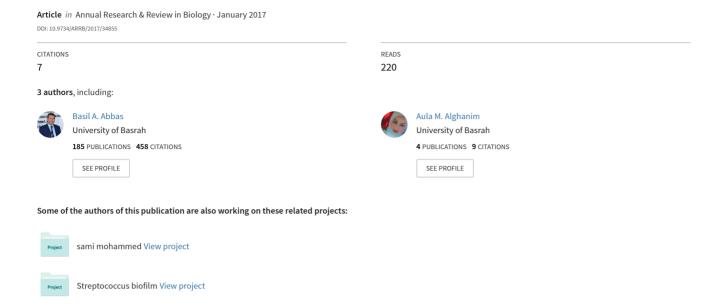
Microbial Evaluation of Milk and Milk Products during a Past Two Decades, in Basrah Southern Iraq: A Review



Microbial Evaluation of Milk and Milk Products During a Past Two Decades, in Basrah Southern Iraq, a Review

Basil A. Abbas1*, M. Khalid Ghadban1 and A. M. Alghanim1

¹Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq.

Abstract:

During the past decades many researches have been done for investigation of milk and milk products microbiologically. Milk was found to be contaminated with several types of bacteria. Studies also shows that most bacteria have different antibiotic resistance patterned towered several known antibiotics. Investigation were done using different methods for investigation. Methods have been developed during this period from conventional biochemical test to using different molecular

techniques for identification such as polymerase chain reaction or 16rDNA sequencing.

Key words: Basrah; Milk; cheese; antibiotics; genes.

Introduction:

Milk is one of the most important foods for human beings. It is universally recognized as a complete diet due to its essential components (1). Milk available is lower in food value due to high prevalence of mastitis in dairy animals (2). Milk is a major component in human diet all over the world, but it also serves as a good medium for growth of many microorganisms. Thus, the quality of milk is considered essential to the health and welfare of a community. Also, all cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include Salmonella sp., Listeria monocytogenes, Staphylococcus aureus, Campylobacter sp., Yersinia sp. (3,4).

Milk normally has a varied micro flora arising from several sources, such as the exterior surfaces of the animal and the surfaces of milk handling equipment such as milking machines, pipeline, and containers (5). Therefore, milk is susceptible to contamination by many pathogenic microorganisms, which result in infection and threat to consumer's health. Additionally, there is the potential that the diseases of causative such as tuberculosis, brucellosis, typhoid, and listeriosis can be transmitted (6). Microbial load in fresh milk is although very low i.e. less than 10-3 CFU/L but this level may increase up to 100 fold if this milk is stored for many days at normal temperature(7).

Milk of buffaloes constituting an important source of market milk has some unusual qualities. The fat content can exceptionally be as high as 15 percent and the overall average may be 7 %. Milk is also an excellent medium for the growth of a large variety of bacteria.

Microorganisms are always undesirable in milk and its products. These are capable of causing deterioration in flavor, physical appearance of milk and transmission of infectious diseases to the consumers. The various organisms get into milk through unhygienic, carelessness and unsanitary practices of the farmers, processors and distributors. The important genera of bacteria normally found in milk are, Microbacterium, Micrococcus, Streptococcus, Staphylococcus, Lactobacillus, Bacillus, Clostridium, Arthrobacter, Actinomyces, Coxiella, Pseudomonusetc. (8). Most of these organisms are free living, widely distributed in soil, feeds, cows, buffaloes, goats, dairy utensils etc. Contamination usually occurs at the farm where milk is produced. Escherichia coli and coliform bacteria can enter milk and milk products very easily and their presence in the milk is an indication of contamination of milk. The presence of E. coli is the indicator of fecal contamination as well as it indicates the presence of toxigenic or enteropathogenic bacteria which are the major public health hazard. (9). E. coli frequently contaminates food and it is agood indicator of fecal pollution (10). Presence of *E.coli* in milk products indicates the presence of microorganisms, which constitute public health hazard. Enteropathogenic E. coli can causesevere diarrhea and vomiting in infants and youngchildren (11). Methicillin-resistant Staphylococcus aureus (MRSA) has become important acquired pathogen in hospital and also livestock(LA-MRSA) in recent years. MRSA associated with (LA-MRSA) have been reported worldwide in many species (12,13,14). MRSA produce a low affinity penicillin binding protein (PBP2 orPBP2a) in addition to the usual PBPs (15). Furthermore, MRSA strains are resistant to gentamicin, kanamycin, tobramycin, microldes, tetracycline and fluoroquinolones. Thus, multiple resistance of S. aureus strains occur (16,17,18).

Methods:

This study reviewed more than 30 papers and thesis that studied milk or milk products microbiologically in Basrah province. The period extended from about 17 years during past two decades. Most researchs were don in college of veterinary medicine, college of agriculture and college of sciences. Methodsused such as conventional biochemical testor using different molecular techniques for identification likeDNA extraction and polymerase chain reactionor 16rDNA sequencing.

The investigated subjects are type of animals, source of sample, microbes, antibiotic susceptibility and studied gene.

Results and discussion:

The most studied animals:

Different animals have been studied for collecting samples. The most of studies conducted on cows followed by buffaloes, sheep and goat. This may be because of availability of these animals and cows and buffaloes milk was a traditional used milk in Iraq. Camel milk have less attention during the mentioned period. This because it limited to the desert area. Many milk samples have been taken from market without specification of animals.

Raw milk has a good chance of investigation since it was easy to collect and deal at laboratory for investigation. In addition, milk was added without pasteurization in some cases for milk products (Table 1).

The commonly isolated bacteria:

Many studies have been concentrated on few species of bacteria that commonly caused a disease in both human and animals. *E. coli* and *Staphylococcus aureus* are the most studied organisms. This because of these species were the most common pathogens in this area. In addition to that *Salmonella*, *Brucella*, *Listeria monocytogenes* and others were also studied during this period. Yeast and molds took less attention. Table (2), shows the recorded organisms during investigated period.

Antimicrobial Susceptibility:

Most the studies involved in determine the antibiotic susceptibility of isolated microorganisms. Many microorganisms showed resistance to one or more antibiotics. common used antibiotics such as Tetracycline, cloxacillin, erythromycin, ampicillin, chloramphenicol, gentamycine and vancomycin were extensively used during the studies. Methicilline resistance also indicated for some *S. aureus* studies. (Table 3).

Studies genes and virulance factors:

Starting from year 2012 many studies involved in detection of virulence factors and genetic studies. Molecular level of investigation for microorganisms increase the chance of detection the small number of microbes in milk samples. Toxin genes and virulence genes studied because its importance to create a disease in both animals and human (Table 1&4).

Table 1. The most studied animal from were milk is collected

Animal	Sourece of isolation	Microorganism(s)	Studied genes or target	Studied Antibiotics	Reference
Cow	Milk	Staphylococcus aureus		Tetracycline, cloxacillin,	19

		"Streptococcusagalactia Streptococcus dysagalactia; Corynebacteriumpyogenes, E, coli,Klebsiella pneumonia ;Candida glabrata, Aspirgillusfumigatus Candida albicans, Sacchromycescerevisiae,Cryptococcu sneoformans.		erythromycin, ampicillin, chloramphenicol, cephalosporin	
Goat and buffalo	Milk	Hypersensitivity	Hypersensitivity	-	20
Markets	cheese, cream	E. coli & S. aureus	-	-	21
Buffalo and sheep	Milk	Brucella	-	streptomycin, gentamicin, rifampin, trimethoprim, sulfamethoxazole, kanamycin, tetracycline, doxycline, cephalexin, cefotaxim,ampicillin , erythromycin	22
Cow	Milk	Staphylococcus aureus, Streptococcus spp, E. coli, Klebsiellaspp, Salmonella sp, Aspergillus spp, Candida spp	-	Erythromycin, gentamycin, neomycin, chloramphenicol, kanamycin	23
Markets	Milk products	Brucella	-	streptomycin, gentamicin, rifampin,trimethoprim,sulfametho xazole, , kanamycin, tetracycline. doxycline,cephalexin, ,cefotaxim ,ampicillin , erythromycin	24
Cow	Milk, Chees	Campylobacter spp.	-	Erythromycin , Kanamycin, Metronidazole , Ampicillin , Ciprofloxacin and Gentamycin	25
Markets	Chees	S. aureus	-	Erythromycin, Clindamycin, ciprofloxacin, Tobramycin, penicillin, Vancomycin, Cloramphenicol, Nitrofurantion, Tetracyclin, Gentamycin	26
Markets	Milk product	E. coli	-	-	27
Cow	Milk	Staphylococcus Spp.	-	Gentamycin, Ampicillin ,PencillinOxacillin ,Novobiocen ,Chlormphenicol ,Ciprofloxacin, Gentamycine	28
Cow,goat	Milk	Burkholderiapseudomallei	Virulence factors		29
Animal ,markets	Milk & milk products	Bacillus cereus	Enterotoxin genes (Sea-See), cytK	Penicillin, neomycin, streptomycin, erythromycin, chloramphenicol, gentamycin, tetracycline	30
Animals	Milk	E. coli	-	Amoxicillin,Amikacin ,Gentamycin , Cefixime, Cephalothin,Ciprofoxacin,Cefoxi tin, Amoxicillin/Clavlanic acid, Naldicxic acid, Nitrofurantoin, Imipenim, Tetracycline , Azithromycin, Trimethoprim.	31
Cow	Milk	E. coli		-	32
Animals	Milk	S. aureus		-	33
cow	Milk Milk	Bacillus cereus Listeria monocytogenes	Toxin emetic gene	Punicagranatum extract Vancomycin ,Lincomycin, Cefotaxime, Sulfamethoxazol, MethoxNitrafurantin, Rifampicin ,Chloramphenicol,Erythromycin ,Cloxacillin, Tobramycin	34 35
Cow	(soft chees)	E.coli	VT1, VT2, E. coli O157:H7	-	36
Animal	Milk	E. coli	Vt1, Pap, E coli	Amoxicillin,amikacin ,gentamycin , cefoxitim ,ciprofloxacin.	37
Animal	Milk	S. aureus	Enterotoxine	-	38
Cow	Milk	S. aureus		ceftriaxone ,cefotaxime ,ampecillin,vancomycincarbencil lin,oxacillin, lincomycin ,pencillin gentamycin, erythromycin,doxycillin,tecoplan ine ,clindamycin ,ciprofloxacin ,nitrofurantoin, chloramphenicol, tobramycin , azithromycin	39
Animals	Milk,milk products	Bacillus cereus	hbl, nheandbceT	-	40

Cow	Milk	S. aureus	Coa	-	41
cow	Milk	E. coli	Tem and shv	-	42
Camel	Milk	E. coli	Its	Amoxicillin/Clavulanic, Cephalothin, Cefixime, Cefoxitin, Amoxicillin, Trimethoprim, Azithromycin, Gentamycin, Imipenem, Amikacin	43
Camel	Milk	E. coli	Icd		44
market	Milk	Salmonella	16srRNA	Ampicillin,Amoxicillin, Azithromycin ,Ceftriaxone Chloramphenicol ,Ciprofloxacin ,Lincomycin, Rifampin Streptomycin,Trimthropin,Sulpha methoxide, Nalidixic acid, Vancomycin	45
Cow	Milk	Lactic acid Bacteria	each16s rRNA, Nisin, enti A, enti B.	-	46
Cow & market	Milk,yogurt, chees	Coliform, E. coli& lactic acid bacteria	-	-	47
Animals	White chees	Bacillus cereus	-	Erythromycin , Gentamycin , Tetracyclin, Streptomycin , Chloramphenicol , Cephalothin , Nalidixic acid Ampicillin Carbenicillin Sulfamethoxazole- trimethoprim .	48
Cow	Milk	S. aureus	Coa gene	Tobramycin , ciprofloxacin chloramphenicol , clindamycin oxacillin , vancomycin . erythromycin, gentamycin, nitrofurantoin, streptomycin, tetracycline, penicillin, cloxacillin,	49
Markets	Milk	Bacterial count	-	-	50
Cow, buffalo, sheep	Milk	Listeria monocytogenes	-	-	51

Table.2. The percentage of recorded organisms during investigated period.

Product	Microbe	Percentage %
Chees	E coli	62.66
Milk	Salmonella	6.1
Milk	Lactobacilli	51
Milk	Staphylococci	15.55
Milk product	Coliform	30 x 104cfu
Milk, meat	Campylobacter spp	4-26
Milk	Burkholderia	33.33
Milk	Pseudomaleli	26.66
Milk, White chees	Bacillus cereus	30&20
Milk	Staphylococcus aureus	22.2
milk	Bacillus cereus	32.7
soft cheese		16.66
curls cheese		18.00
yogurt		26.00
Milk	Brucella spp.	24.2
Cheese,cream,ice-cream	Brucella spp.	8, 1, 0
Milk	E. coli	7.44
soft cheese,	E. coli	90×104-212×104 cfu
curls cheese		35×103-21×104 cfu
yoghurt		17×102-55×103 cfu
local cream		35×103-111×103)cfu
Milk	E. coli O157:H7	13.7
Milk	S. aureus	48
Milk	Listeria monocytogenes	7.3
Milk	E. coli O157:H7	57.34
Milk	S. aureus	53
Milk	S. aureus	30&27
Milk	S. aureus	48.61
Chees	S. aureus	39
Milk	E. coli	14
Milk	Staphylococcus aureus Streptococcus spp E. coli, Klebsiellaspp, Salmonella sp Aspergillus spp, Candida spp	33.12 24.84 12.88 1.84 0.92 20 80
Milk & products	Bacillus cereus	12.9
	Chees Milk Milk Milk Milk Milk product Milk, meat Milk Milk Milk Milk Milk Milk Milk milk soft cheese curls cheese yogurt Milk Cheese, cream, ice-cream Milk soft cheese yoghurt local cream Milk Milk Milk Milk Milk Milk Milk Milk	Chees

Table 3. Antibiotic susceptibility of isolated microorganisms.

Microbe	Antibiotic	Susceptibility

E. coli	Gentamycin, Amikacin	S
	Amoxicillin, cefoxitim.	R
Salmonella	chloramphenicol, rifampin	R
	ciprofloxacin	S
Staphylococci	Ampicillin, Novbio. + Penicillin ,Oxcillin	R
	Ciprofloxacin, Chloramphenicol, Gentamycin	S
Campylobacter	Kanamycin, Ampicillin, Erythromycin, Metronidazole	R
spp	Gentamycin, ceproflaxacin	S
Bacillus cereus	carbencillin,cephalothin, ampicillin	R
	erythromycin, gentamycin, chloramphenicol, nalidixic	S
	sulfamethoxazole-trimethoprim.	S
Staphylococcus	Cloxacillin	R
aureus		
Bacillus cereus	neomycin,chloramphenicol,gentamycin,streptomycin	S
	erythromycin	S
	penicillin	R
E. coli	gentamycin,imipenem,andamikacin	S
	amoxicillin, trimethoprim,	R
	azithromycin,cefoxitin,amoxicillin/clavulanic, cephalothin,	R
	cefixime	
- 11 0.155 XX5	cephalothin ,cefoxitin ,cefixime, trimethoprim , amoxicillin,	R
E. coli O157:H7.	azithromycin, amoxicillin/clavulanic,	R
	ciprofloxacin, imepenim, nitrofurantoin, gentamycin ,	S
	amikacin	
Listeria	Cefotaxine, sulfamethoxazol, chloramphenical, tobramycin	R
mononcytogenes	Rifampicin	S
S. aureus	nitrofurantoin, chloramphenicol, tobramycin azithromycin	R
	ceftriaxone, cefotaxime	S

Table 4. Presence of gene(s) in investigated animals.

Microbe	Genes	Presence

E. coli	Vet 1, pap	+
	Vet2	-
Staphylococcus aureus	Coa	+
Bacillus cereus	cytK	+
Bacillus cereus	hbl, ,,nhe	+
	bceT	-
E. coli	Its	+
E. coli	Pai	+
	Icd	+
S aureus	Coa	+
E. coli O157:H7	Vt1, Vt2	+
S aureus	Sec	+
	Sea, Seb,Sed, See	-
Escherichia coli O157:H7	Tem	+
	Shv	+
Bacillus cereus	Emetic toxine gene	+

Conclusion: The literatures of investigated period revealed that milk and milk product collected from different animals or from markets were highly contaminated with microbes. It is not clear whether these microbes were infectious to animal or the milk is contaminated during the transport or handling. Most investigate microorganism were *E. coli* and *Staphylococcus aureus* followed by *Salmonella*, *Brucella*, and *Listeria monocytogenes*.

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