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

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Authors’ contributions

This work was carried out in collaboration between all authors. Author BAA designed the study, performed the statistical analysis wrote the protocol and the first draft of the manuscript. Author MKG collect the data of the study. Author AMA managed the literature searches and arrange tables and graphs. All authors read and approved the final manuscript.

ABSTRACT

During the past decades, many researches have investigated the microbiological quality of milk and milk products. Milk was found to be contaminated with several types of bacteria. Most of these bacteria have been found to show different antibiotic resistance patterns against several known antibiotics. Different characterization methods such as conventional biochemical tests and DNA-based methods have been applied. Therefore, the aim of this study was to review the recent studies about the microbiological quality of milk and milk products.

Keywords: Basrah; milk; cheese; antibiotics; genes.
1. 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 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. Illnesses due to the consumption of milk occur because of the bacterial pathogens such as *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter* sp., *Yersinia* sp. [3,4].

Milk is contaminated by the organisms found on the exterior surfaces of the animal and the surfaces of milk handling equipment such as milking machines, pipeline, and containers resulting in infections and threatening to consumer’s health by the illnesses such as tuberculosis, brucellosis, typhoid fever, and listeriosis [5,6]. The investigation demonstrates that dairy cattle are a reservoir of *E. coli* 0157:H7 and other Shiga-like-toxin-producing *E. coli* [7]. Milk of buffaloes constituting an important source of market milk has some different characteristics. The fat content in this milk can exceptionally be as high as 15% and the overall average may be 7%. 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 [8-10]. Enteropathogenic *E. coli* can cause severe diarrhea and vomiting in infants and young children [11]. Methicillin–resistant *S. aureus* (MRSA) has become an important acquired pathogen in hospitals and also livestock (LA-MRSA) in recent years. MRSA associated with (LA-MRSA) have been reported worldwide in many species [12-14]. MRSA produces a low affinity penicillin binding protein (PBP2 or PBP2a) in addition to the usual PBPs [15]. Furthermore, MRSA strains are resistant to gentamicin, kanamycin, tobramycin, tetracycline and fluoroquinolones. Thus, multiple resistance of *S. aureus* strains occurs [16-18]. The objective of the study was to review more than 30 papers and thesis that studied microbiological quality of milk or milk products in Basrah province. These studies have used conventional biochemical tests and different molecular techniques for the identification organisms isolated from different sample types and determined the antibiotic susceptibility patterns.

2. ANIMAL ORIGIN

Different animals have been studied for collecting samples. These include cows followed by buffaloes, sheep goat and camel. This may be because of the availability of these animals. In addition, the milk of cows and buffaloes was traditionally used in Iraq. Camel milk has less attention during the mentioned period because its use is limited in 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 is easy to collect and handle for laboratory analysis. In some cases, unpasteurized milk is used for the production of local cream and cheese (Table 1).

3. MICROFLORA AND DAIRY SAMPLES

As seen in Table 2, *E. coli* and *S. aureus* are the most prevalent organisms in this area. In addition, *Salmonella*, *Brucella*, *L. monocytogenes* have been isolated from these samples. The highest percentage, which refer to number of positive sample for isolation of microbes upon number of total collected samples, were found 62.66% for *E. coli* and 53% for *S. aureus*.

4. ANTIMICROBIAL SUSCEPTIBILITY

Most of the studies have determined the antibiotic susceptibility patterns of isolated microorganisms (Table 3). They showed resistance to one or more antibiotics. Common used antibiotics such as tetracycline, cloxacillin, erythromycin, ampicillin, chloramphenicol, gentamycin and vancomycin were extensively used during the studies.

5. STUDIED GENES AND VIRULENCE FACTORS

Since 2012, many studies investigated the presence of virulence genes and toxin genes by DNA-based methods because they cause diseases in both animals and humans (Table 4). These genes such as verotoxin genes (*vet*), coagulase genes (*coa*), Emetic toxin genes,
enterotoxigeni genes \( \text{(see, sea, sec, seb and sed)} \), and other types of genes for \( E \text{ coli} \) such as \( \text{pap, its, pai and icd gene} \) which used for species identification \([30,34,36,37,41,49]\).

Table 1. The most studied animals used for milk and dairy products collection

| Animal | Source of isolation | Microorganism(s) | References |
|--------|---------------------|------------------|------------|
| Cow    | Milk                | \( S. \text{ aureus, Streptococcus agalactia Streptococcus dysagalactia; Corynebacterium pyogenes, E. coli, K. pneumonia; Candida glabrata, Aspergillus fumigatus Candida albicans, Saccharomyces cerevisiae, Cryptococcus neoformans.} \) | [19]         |
| Goat, buffalo unidentified cheese, cream |                        | \( E. \text{ coli} & S. \text{ aureus} \) | [20]         |
| Buffalo, sheep Milk |                        | \( \text{Brucella} \) | [21]         |
| Cow    | Milk                | \( S. \text{ aureus, Streptococcus spp, E. coli, Klebsiella spp, Salmonella sp, Aspergillus spp, Candida spp} \) | [22]         |
| Cow    | Milk, Cheese        | \( \text{Campylobacter spp.} \) | [23]         |
| unidentified Milk products |                        | \( \text{Brucella} \) | [24]         |
| unidentified Cheese |                        | \( S. \text{ aureus} \) | [25]         |
| unidentified Milk |                        | \( E. \text{ coli} \) | [26]         |
| Cow    | Milk                | \( \text{Staphylococcus Spp.} \) | [27]         |
| Cow, goat Milk |                        | \( \text{Burkholderia pseudomallei} \) | [28]         |
| Animal, unidentified Milk & milk products |                        | \( \text{Bacillus cereus} \) | [29]         |
| Animals Milk |                        | \( E. \text{ coli} \) | [30]         |
| Cow    | Milk                | \( E. \text{ coli} \) | [31]         |
| Animals Milk |                        | \( S. \text{ aureus} \) | [32]         |
| cow    | Milk                | \( B. \text{ cereus} \) | [33]         |
| Cow    | Milk                | \( L. \text{ monocytogenes} \) | [34]         |
| Cow    | soft cheese         | \( E.\text{coli} \) | [35]         |
| Animal | Milk                | \( E. \text{ coli} \) | [36]         |
| Animal | Milk                | \( S. \text{ aureus} \) | [37]         |
| Cow    | Milk                | \( S. \text{ aureus} \) | [38]         |
| Animals Milk, milk products |                        | \( B. \text{ cereus} \) | [39]         |
| Cow    | Milk                | \( S. \text{ aureus} \) | [40]         |
| Cow    | Milk                | \( E. \text{ coli} \) | [41]         |
| Camel  | Milk                | \( E. \text{ coli} \) | [42]         |
| Camel  | Milk                | \( E. \text{ coli} \) | [43]         |
| unidentified Milk |                        | \( \text{Salmonella} \) | [44]         |
| Cow    | Milk                | \( \text{Lactic acid Bacteria} \) | [45]         |
| Cow, unidentified Milk, yogurt, cheese |                        | \( \text{Coliform, E. coli & lactic acid bacteria} \) | [46]         |
| Animals White cheese |                        | \( B. \text{ cereus} \) | [47]         |
| Cow    | Milk                | \( S. \text{ aureus} \) | [48]         |
| unidentified Milk |                        | \( \text{Bacterial count} \) | [49]         |
| Cow, buffalo, sheep Milk |                        | \( L. \text{ monocytogenes} \) | [50]         |
Table 2. The percentage of recorded organisms during investigated period

| Source                | Product        | Isolated organisms | Percentage % | References |
|-----------------------|----------------|--------------------|--------------|------------|
| unidentified          | Chees          | E. coli            | 62.66        | [27]       |
| unidentified          | Milk           | Salmonella         | 6.1          | [45]       |
| Cow                   | Milk           | Lactobacilli       | 51           | [46]       |
| unidentified          | Milk           | Staphylococci      | 15.55        | [28]       |
| unidentified          | Milk product   | Campylobacter spp  | -            | [24]       |
| Cow milk              | Milk           | B. Pseudomalleli   | 33.33        | [29]       |
| Goat                  | Milk           |                    | 26.66        |            |
| unidentified          | Milk, White    | Bacillus cereus    | 30           | [48]       |
| Buffalo               | Milk           | S. aureus          | 22.2         | [52]       |
| unidentified          | milk           | Bacillus cereus    | 32.7         | [30]       |
| unidentified          | soft cheese    |                    | 16.66        |            |
| unidentified          | curls cheese   |                    | 18.00        |            |
|                      | yoghurt        |                    | 6.00         |            |
| Cow, buffalo, sheep   | Milk           | Brucella spp.      | 24.2         | [22]       |
| unidentified          | Cheese, cream, | Brucella spp.      | 8            | [25]       |
|                      | ice-cream      |                    | 1            |            |
|                      |                |                    | 0            |            |
| Camel                 | Milk           | E. coli            | 7.44         | [43, 44]   |
| unidentified          | soft cheese,   |                    | -            |            |
|                      | curls cheese,  |                    |              |            |
|                      | yoghurt, local |                    |              |            |
|                      | local cream    |                    |              |            |
| Cow                   | Milk           | S. aureus          | 48           | [49]       |
| Cow, buffalo, sheep   | Milk           | L. monocytogenes   | 7.3          | [51]       |
| Cow                   | Milk           | S. aureus          | 53           | [49]       |
| cow                   | Milk           | S. aureus          | 30           | [39]       |
| buffalo               | Milk           | S. aureus          | 27           | [41]       |
| unidentified          | Cheese         | S. aureus          | 39           | [26]       |
| unidentified          | Milk           | E. coli O157:H7    | 14.3         | [42]       |
| Cow                   | Milk           | S. aureus          | 33.12        | [19]       |
|                      |                | Streptococcus spp  | 24.84        |            |
|                      |                | E. coli            | 12.88        |            |
|                      |                | Klebsiella spp.    | 1.84         |            |
|                      |                | Salmonella sp      | 0.92         |            |
|                      |                | Aspergillus spp,   | 20           |            |
|                      |                | Candida spp        | 80           |            |

Table 3. Antibiotic susceptibility of isolated microorganisms

| Microorganism     | Antibiotic                          | Susceptibility | Reference |
|-------------------|-------------------------------------|----------------|-----------|
| E. coli           | Gentamycin, amikacin, Amoxicillin,  | S              | [37]      |
|                   | cepfoxitim, chloramphenicol,         |                |           |
|                   | rifampin, ciprofloxacin              |                |           |
| Salmonella        | Ampicillin, novobiocin + penicillin, | R              | [28]      |
|                   | ciprofloxacin, chloramphenicol,      | S              |           |
|                   | gentamycin                          |                |           |
| Staphylococci     | Kanamycin, ampicillin, erythromycin, | R              | [25]      |
|                   | metronidazole                        |                |           |
|                   | Gentamycin, ceproxiflaxitin          | S              |           |
| Campylobacter spp | Carbencillin, cephalothin, ampicillin| R              | [48]      |
| Bacillus cereus   | Erythromycin, gentamycin,            | S              |           |
|                   | chloramfenicol, nalidixic            |                |           |
| Microorganism       | Antibiotic                                      | Susceptibility | Reference |
|---------------------|-------------------------------------------------|----------------|-----------|
| S. aureus           | Sulfamethoxazole-trimethoprim.                  |                |           |
| Bacillus cereus     | Cloxacinil                                       | R              | [49]      |
|                     | Neomycin, chloramphenicol, gentamycin           | S              | [30]      |
|                     | Penicillin                                       | R              |           |
| E. coli O157:H7     | Cephalothin, cefoxitin, cefixime, trimethoprim,  | R              | [31]      |
|                     | amoxicillin, erythromycin, amikacin, Ciprofloxacin, | S              |           |
|                     | imipenem, nitrofurantoin, gentamycin            |                |           |
| Listeria monocyotosogenes | Cefotaxine, sulfamethoxazol, chloramphenicol,   | R              | [35]      |
|                     | tobramycin                                       |                |           |
|                     | Rifampicin                                       | S              |           |
| S. aureus           | Nitrofurantoin, chloramphenicol, tobramycin,    | R              | [39]      |
|                     | azithromycin                                     |                |           |
|                     | Ceftriaxone, cefotaxime                          | R              |           |

*S, sensitive; R, resistant*

Table 4. Genes present in organisms originated from animal samples

| Microbe       | Genes                  | Presence | Reference |
|---------------|------------------------|----------|-----------|
| E. coli       | Vet1, pap              | +        | [37]      |
|               | Vet2                   | -        |           |
| S. aureus     | Coa                    | +        | [41, 49]  |
| B. cereus     | cytK                   | +        | [30]      |
| B. cereus     | hbl, nhe               | +        | [40]      |
|               | bceT                   | -        |           |
| E. coli       | Its                    | +        | [43]      |
| E. coli       | Pai                    | +        | [44]      |
|               | Icd                    | +        |           |
| E. coli O157:H7 | Vt1, Vt2              | +        | [36]      |
| S aureus      | Sec                    | +        | [33]      |
|               | Sea, Seb, Sed, See     | -        |           |
| E. coli O157:H7 | Tem                   | +        | [42]      |
|               | Shv                    | +        |           |
| B. cereus     | Emetic toxin genes     | +        | [34]      |

5. CONCLUSION

From the above reviewed literatures, we can conclude that milk and its products at Basrah city are contaminated with different microorganisms. Most of them are infectious and can cause a disease for both humans and animals. In addition, many investigated microbes have multidrug resistance and harbor a virulence and toxin producing genes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Javaid SB, Gadahi JA, Khaskeli M, Bhutto MB, Kumbher S, Panhwar AH. Physical and chemical quality of market milk sold at Tandojam, Pakistan. Pakistan Vet. J. 2009;29(1):27-31.
2. Allore HG. A review of the incidence of the mastitis buffaloes and cows. Pakistan Vet. J. 1993;13:1-7.
3. Adesiyun AA, Webb L, Rahman S. Microbiological quality of raw cow’s milk at collection centres in Trinidad. J. Food Prot. 1995;58:139-146.
4. Hahn G. Pathogenic bacteria of raw milk situation and significance. Proceedings bacteriological quality of raw milk, IDF. Brussels (Belgium). 1996;(13-15):67-83.
5. Burton H. Microbiological aspects of pasteurized milk. Bulletin of the International Dairy Federation. 1986;200 (Chapter III):9–14.
6. Spreer E. Milk and dairy product technology. Ed. Marcel Dekker Inc. New York, NY. 1998:39.

7. Wells JG, Shipman LD, Gren KD, Sowers EG, Green JH, Cameron DN, Downers PP, Martin ML, Griffin PM, Ostroff SM, Potter ME, Tauxe RV, Wachsmuth IK. Isolation of *Escherichia coli* serotypes 0157: H7 and other shiga-like toxin-producing *E. coli* from dairy cattle. J. Clinical Microbiol. 1991;29:985-988.

8. Adesiyun AA. Bacteriological quality associated public health risk of pre-processed bovine milk in Trinidad. Int. J. Food Microbiol. 1994;21:253-261.

9. Aslam M, Hogan J, Smith KL. Development of a PCR-based assay to detect shiga toxin producing *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* in milk, J. Food Microbiol. 2002;20:345-350.

10. Soomro AH, Arain MA. Khaskheli M, Bhutto B. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market condition at Tandojam. Pak. J. Nutr. 2002;13:151–152.

11. Anonymous. *E. coli* enteritis. Lancet. 1975;1131–1132.

12. Persoons D. van Hoorebeke S, Hermans K, Butaye P, de Kruif A. Haesebrouck F, et al. Methicillin-resistant *Staphylococcus aureus* in poultry. Emerg. Infect. Dis. 2009;15:452-453.

13. de Neeling AJ, van den Broek MJ, Spalburg, EC, van Santen-Verheuvel MG, Dam-Deisz, WD, Boshuizen HC, van de Giessen AW, van Duijkeren E, Huijsdens XW. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet. Microbiol. 2007;122:366-372.

14. Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, et al. Methicillin-resistant *Staphylococcus aureus* strain ST398 is present in Midwestern U.S. swine and swine workers. PLoS One. 2008;4:4258.

15. Hartman B, Tomasz. A. Low-affinity penicillin binding protein associated with beta-lactam resistance *Staphylococcus aureus*. J. Bacteriol. 1984;158:513–516.

16. Chambers HF, Methicillin resistance staphylococci; Molecular and biochemical basis clinical implications. Clin. Microbiol. Rev. 1997;781-791.

17. Petinaki E, Miriagou V, Tzouvelekis LS, Pounaras S, Hatzis F, Kontos F, Maniati M, Maniatis AN. Methicillin-resistant *Staphylococcus aureus* in the hospitals of central Greece. International Journal of Antimicrobial Agents. 2001;18:61-65.

18. Maddox CW. Transfer of methicillin resistance and virulence factors between staphylococci. AAVL Bacteriology /Mycology Sub-Committee Mini-Symposium. Sponsorship generously provided by: Biolog. Inc. and Pfizer Animal Health. New York, USA; 2011.

19. Abdulla F, Khudor MH, Muhsen RK. Microbiological study of subclinical mastitis of cows in Basrah city. Al-Qadisiya J. of Vet. Med. Sci. 2002;1(2):44-49.

20. Faaz. et al. The role of goat and buffalo's milk allergens. Bas. J. Vet. Res. 2006;5:77.

21. Othman RM, Abdul Alwahid AT, Japer NN. The microbiological quality of some raw milk products. Bas. J. Vet. Res. 2008;7(1):35.

22. Abbas BA, Al-Deewan AB. Occurrence and epidemiology of *Brucella* Spp. in raw milk samples at Basrah province, Iraq. Bulgarian Journal of Veterinary Medicine. 2009;12(2):136-142.

23. Lyli A, Abdul Wadood E, Jassim AY. Isolation and identification of some species of microbes from cows milk and their sensitivity for antibiotics at Basra province. AL-Qadisiyah J. Vet. Med. Sci. 2009;8(1):41.

24. Majeed KR, Abdul Hussain R. Patterns Frequencies of *Campylobacter* Spp. isolated from food In Basrah province and its sensitivety to antibiotics. Basrah. J. Agric. Sci. 2011;24(1):141.

25. Abbas BA, Talei AB. Isolation, identification and biotyping of *Brucella* spp. from milk product at Basrah province. Bas. J. Vet. Res. 2010;9(1):152.

26. Japer NN. Isolation and biotyping of *Staphylococcus aureus* from white cheese in Basrah local markets. Bas. J. Vet. Res. 2011;11(2):55.

27. AL-Edany AA, Khudor MH, AL-Mousawi KS. Comparison of three indirect tests for the diagnosis of bovine subclinical mastitis caused by coagulase negative staphylococci with their susceptibility to
seven antibiotics. Bas. J. Vet. Res. 2012;11(1):74.
29. AL-Rodhan AM, Ibrahim HK. Isolation and identification of Burkholderia pseudomallei from cows, goat's milk and their surrounding environment in Basrah province. Bas. J. Vet. Res. 2012;11(2):145.
30. Khudor MH, Abbas BA, Saeed BMS. Molecular detection of enterotoxin (Cyt K) gene and antimicrobial susceptibility of Bacillus cereus isolates from milk and milk products. Bas. J. Vet. Res. 2012;11(1):164.
31. Khudaier BY, Abbas BA, Khleel KA. Detection of virulence and its susceptibility against Staphylococcus aureus from bovine and the detection of its coagulase gene (coa) using polymerase chain reaction (PCR). Sc. Res. Assays. 2014;9(20):864-868.
32. Abbas BA, Khudaier BY, Khleel KA. Molecular detection of β-lactamase (tem and shv) genes in Escherichia coli O157:H7 isolated from different sources in Basra, Iraq. Journal of Advanced Biomedical & Pathobiology Research. 2014;4(4):1-101.
33. Abbas BA, Khudaier BY, Anad IT. Sequencing of icd gene of Escherichia coli isolated from camel milk. Special Issue of Scientific and Practical Journal Veterinaryinya. 2015;#2(42):220.
34. Al-Hadithi HT, Ali, EK. Incidence of Bacillus cereus in food and environmental Sources in Basrah / Iraq. antibiotic resistance profiles of recovered isolates. Int. J. Curr. Res. Aca. Rev. 2016;4(10):117-127.
35. Alhelfi NA. Assessment of the efficiency of PETRI film method in study of bacteriological quality of some homemade dairy products in local market of Basra. Food Science and Quality Management. 2016;54:39-46.
antibiotic susceptibility and coa gene polymorphism in *Staphylococcus aureus* isolated from bovine. J. Vet. Med., Assiut Univ. 2016;17:33.

50. AL-Edany AA, Ghazi SS. Evaluation of raw milk from local markets and milk samples taken directly from cows in Basrah-Iraq. IOSR Journal of Agriculture and Veterinary Science. 2016;9(12):59-64.

51. Niamah AK. Detection of *Listeria monocytogenes* Bacteria in four types of milk using PCR. Pakistani Journal of Nutrition. 2012;11:1158-1160.

52. Khaleel DA, Othman RM, Khudaier, BY. Isolation and identification of *Staphylococcus aureus* from buffaloes milk with subclinical mastitis and milk workers. Bas. J. Vet. Res. 2016;15(2):304.