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Molecular detection and characterization of *Staphylococcus aureus* from buffalo milk from Muzaffargarh, Punjab, Pakistan

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Abstract

This study was designed to identify and characterize *Staphylococcus aureus* (*S. aureus)* in the raw buffalos' milk in district Muzaffargarh by microscopic, biochemical and molecular studies. Moreover, we evaluated different antibiotics on *S. aureus* to check the susceptibility of drug resistant bacteria, which were isolated from raw buffalos' milk. A total of 100 milk samples were collected from different city markets of Jutoi, Alipur, Kotadu, Murad Abad, Choak Qureshi, Ahmadpur, Ruhilan Wali, shahjamal, Khanpur and Muzaffargarh city of district Muzaffargarh, Punjab, Pakistan. Milk samples were cultured onto various culture media for the isolation of bacteria. The isolated bacteria were identified by studying staining characteristics, cultural properties on different selective media, biochemical tests, catalas, coagulase, methyl red and voges prockauer, and finally by PCR. Out of 100 samples, 11 (11%) milk samples were found positive for *S. aureus*. 11 *S. aureus* isolates were amplified by 16S rRNA gene based PCR. Antimicrobial sensitivity test was carried out to ascertain the susceptibility of the organism to various antibiotics. This study was indicated the potential of staphylococcal food poisoning and health risks in district Muzaffargarh. Poor hygienic conditions effect the bacterial growth and *S. aureus* availability also related to the hygienic conditions.

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Introduction

Milk and milk products obtained from the buffalos milk is an important dietary source for the urban and the rural population. But milk production often does not full fill requirements of the county due to its contaminations. There are many factors which cause this deficiency. One of these factors is the mastitis which is a disease of the mammary glands of cattle's. These factors reduce the production of milk and milk products (Wubishet *et al.,* 2013).

Milk is an important food item and raw milk is used in number of dairy products. The tryptophan residues protein concentration which has great nutritional value is in good amount in cow milk as compared to buffalo milk. They are also resistant to drying and can survive in dry conditions. Colonies of S. aureus colonized the skin, also colonizes the pharynx of animals and human (Torimiro *et al.,* 2012). The colonies are 2-3mm in diameter. *S. aureus* is gram positive bacteria which colonizes in the nasal passage and found on skin surface of almost 50% of the healthy persons (Arciola *et al.,* 2012; Wubishet., 2013). In all over the world, contaminated food became the main source of many communicable and non communicable diseases. These diseases put a burden in human life and economy of country is also affected (Bianchi *et al.,* 2014). The concentration of magnesium and calcium is about 1.5 more than cow milk. Vitamin A concentration is high in buffalo milk. Buffalo milk full fill the human body requirement of high quality of protein which has all essential and major amino acids (Abbas *et al.,* 2014; Khedkar *et al.,* 2016). Various microorganisms which contaminate the milk and milk products can cause the discoloration, decomposition, rotting, stickiness and many other defects (Abbas *et al.,* 2017). Pneumonia, septicima and arthritis like chronic diseases are caused by *S. aureus* (Mohammad *et al.,* 2013). *S. aureus* has ability to grow abundantly and rapidly under aerobic conditions (Farley *et al.,* 2015). The raw milk have tendency to provide the growth medium for different kinds of bacteria (Jahan *et al.,* 2015). *S. aureus* can contaminate the milk or milk products and some other dairy products can act as the transmitter for *S. aureus* associated infection in mammals (Bharathy *et al.,* 2015). Patients of cystic fibrosis (CF) are vulnerable to many infections by number of pathogens and *S. aureus* is one of them (Garland *et al.,* 2013; El-Ashker *et al.,* 2015). Bacteria are the major cause of Infective endocarditis and *S. aureus* is one of them which cause this kind of infection (Salgado-Pabón *et al.,* 2014). In the United States almost 40–50% cases of Infective endocarditis are reported (Werdan *et al.,* 2014; Tong *et al.,* 2015). One of the globally spread nosocomaial infections is associated with Methicillin-resistant *S. aureus* (MRSA) strains. The injuries which are open and sometimes urinary tract are also the places where these stains can also be found in human and animals (Manal *et al.,* 2013; Marwa *et al.,* 2014). *S. aureus* is commonly colonized in hospitals environment and this colonization rates vary from 10% to 20% (Gleeson *et al.,* 2016). Methicillin-resistant *S. aureus* (MRSA) is also a main source of infections which are very severe in nature (Basil *et al.,* 2017). Novel treatments for *S. aureus* biofilm according to new researches can be possible by the use of silver nano particles, sometimes bacteriophages and also some plant-derived antibiotic agents can be used for treatments of these kinds of infections. These all things show inhibitory effects on the *S. aureus* in biofilms forms (Hogan, *et al.,* 2017). There are many diseases affecting the milk yield of buffaloes, main of these, mastitis is at the top. Mastitis is swelling of mammary gland of cattle which is one of the most dangerous diseases. An increased resistance of *S. aureus* isolated from dairy cattle and buffalos, with mastitis against antibiotics were reported by many workers (Jeykumar *et al.,* 2013). Buffalo milk analysis show that the presence of the *S. aureus,* which can cause infections, is 22.2%. This percentage is proved by other researchers (Khaleel *et al.,* 2016).The cattle which become infected by the Staphylococcal mastitis have significant problems and economically the greatest burden in dairy farming (Duguma *et al.,* 2018).

S. aureus has many kinds of virulence factors which can be establish silently and produce toxins. As the

pathogen microbes enter into the skin the inflammatory response occurs and the host cells cover *S. aureus* cells to form specific structures (Scott *et al.,* 2015). *S. aureus* are normally present in human in the respiratory tract, on the skin and in the gut track. They can colonized and act as symbiotic but if they take over the tissues they occupy the other tissues and then they act as pathobionts (Schenck *et al.,* 2016; Sergelidis *et al.,* 2017). *S. aureus* can spread infection through the skin to skin contact with pus from infected wound. *S. aureus* can spread infection through the objects (towels, sheets and cloths) which used by the infected person (Wollina *et al.,* 2017).

The rate of prevalence of *S. aureus* may vary with the geographical presence of the area where the infected species are present. The high concentration of the bacterial contamination in milk relates to poor hygiene conditions and practices (Bhattacharya *et al.,* 2015). Infections caused by *S. aureus* are more harmful in diabetics, drug users and heart patients. These factors act as enhancer of toxins is infection of patients (Kavanaugh *et al.,* 2016). The main factor is the poor hygiene condition of udder and another factor is previous mastitis occurrence record can affect the invasion of this type of infection (Elemo *et al.,* 2017; Duguma *et al.,* 2018).

There are more than a few methods for detection of *S. aureus*, therefore, PCR is proves to be the more specific and accurate as a detector (Torimiro *et al.,* 2012). The rapid and standardized detection of *S. aureus* is most significant for the proper treatment for the infection. Reliable identification of S. aureus is necessary for the correct clinical microbiological diagnostics (Saadat *et al.,* 2014). There are different genes which are used to detect the S. aureus but the nuc gene, which encodes thermonuclease, is most and widely used gene. The nuc gene is reliable as a specific target for the identification of *S. aureus* by PCR (Najimaana *et al.,* 2015). Rapid and accurate identification of *S. aureus* can only through PCR method (Leke *et al.,* 2017). The PCR technique is used as extremely exact one of the procedure for direct recognition of *S. aureus* (Ahmed *et al.,* 2017).

S. aureus mediated infections can be treated with a combination of rifampand, gentamicin, vancomycin. Antibiotics are effective in combination but an important fact is that the use of these antibiotics is not very safe (Hoegh *et al.,* 2014; Peacock *et al.,* 2015). Vancomycin resistance has not been detected in several countries but the frequently use of vancomycin after infections has caused a decrease in vancomycin sensitivity (Gleeson *et al.,* 2016). Adjunctive rifampicin was historically used in the treatment of *S aureus* bacteraemia, but it has shown that there are no overall good results of standard antibiotic therapy. The combination of the antibiotics is more effective in the therapy for these kinds of infections (Keclik *et al.,* 2017).

This study was designed to identify and characterize *S. aureus* in the raw buffalos' milk in district Muzaffargarh by microscopic, biochemical and molecular studies. Moreover, we evaluated different antibiotics on *S. aureus* to check the susceptibility of drug resistant bacteria, which were isolated from raw buffalos' milk.

Materials and methods

Sample collection

A total of 100 buffalo's raw milk samples were collected from different markets of Alipur, Jutoi, Murad Abad, Kotadu, Ruhilan Wali, Choak Qureshi, Ahmadpur, khanpur, Shahjamal and from the main city of Muzaffargarh district by using falcon tube. The samples were collected from December 2017 to July 2018 and investigation was carried out following collection. The collected milk samples were immediately transported on ice to the Microbiology Laboratory of Multan vu campus for bacteriological analysis.

Isolation and identification of S. aureus

The collected milk samples were inoculated onto Mannitol salt agar (MSA) agar by pour plate method and incubation at 37 ºC for 24 hours. Then sub cultured onto eosin methylene blue (EMB) agar by streaking to obtain pure culture. These isolates were preserved for further bacterial identification. The

isolates were identified as *S. aureus* on the basis of Gram staining, colony morphology on MSA agar, biochemical characterization of the isolates by using sugar fermentation test, methyl red (MR) test, voges proskauer (VP) test, catalase and coagulase tests. Further the isolates were confirmed by amplification of *S. aureus* specific 16S rRNA gene.

Bacterial genomic DNA extraction

A pure bacterial colony of *S.aureus* was mixed with 100 µl of distilled water which were boiled for 10 minutes then immediately kept on ice for ice shock. Finally centrifugation was done at 10000 rpm for 10 minutes. The supernatant were collected and used as DNA template for PCR (Abbas *et al.,* 2014).

Identification of S. aureus by PCR

Identification of *S. aureus* can be done by using PCR amplification of 16S rRNA gene (nuc gene 447bp) of *S. aureus*. Two different primers pairs were used for this purpose, 21-nucleotide forward primer: SAS2F (5`- GCGATTGATGGTGATACGGTT -3`) and 24-nucleotide reverse primer: SAS2R (5`-AGCCAAGCCTTGACGAACTAAAGC -3`) used (Shortle *et al.,* 1983). Each 20 µl reaction mixture consists of 3 µl genomic DNA, 10 µl PCR master mixtures, 1 µl of each of the two primers with the final volume adjusted to 20 µl with 5µl of nuclease free water. Amplification was done by initial denaturation at 95°C for 5 minutes, followed by denaturation at 94°C for 45 sec, annealing temperature of primers was 55°C for 45 sec and extension at 72°C for 1 minutes. The final extension was conducted at 72°C for 5 minutes. The total reaction was performed at 30 cycles (Ahmed *et al.*, 2017). The amplified PCR products were resolved by electrophoresis in 8% agarose gel at 100v for 30 minutes, stained with ethidium bromide and finally visualized under UV trans-illuminator (Saadat *et al.*, 2014).

Results

Out of 100 samples, 11% were contaminated with *S. aureus*. In MSA agar plates, the samples showed golden yellow color indicated the presence of *Staphylococcus* species, the colonies looked irregular clusters (Fig. 1). The microscopic examination of Gram's stained smears from MSA agar showed that the isolated bacteria were Gram positive, the colonies looked grape-like irregular clusters purple colored, small circular shaped organisms arranged in pairs or short clusters (Fig. 2). *S. aureus* showed positive result to catalase, coagulase, methyl red and voges proskauer tests.

Table 1. Results of PCR for identification of *S. aureus* from the milk samples collected from buffalo milk in district Muzaffargarh.

Sr#	Alipur	Jutoi	Murad Abad	Kotadu	Ruhilan Wali
1	-	-	-	-	-
2	-	+	-	-	-
3	-	-	-	-	-
4	+	-	+	-	+
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	+	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
persentage	10%	20%	10%	0%	10%

PCR was specific and valuable method for detection of *S. aureus*. Identification of *S. aureus* done by using amplification of DNA of reference and field buffalo milk samples yielded the PCR fragments of 447bp. In the early phase of this research PCR were done by using the DNA extract of reference bacterial samples were taken from institute of microbiology and biotechnology of BZU Multan to check the specificity

of PCR assay. To visualizing the proper amplification of PCR product was run on agarose gel. Only the positive control sample showed the band which was the verification of the specificity of the PCR assay (Fig. 3).

Table 2. Results of PCR for identification of *S. aureus* from the milk samples collected from buffalo milk in district Muzaffar Garh.

Sr#	Muzaffargarh city	Choak Qureshi	Khanpur	Ahmadpur	Shahjamal
1	-	-	-	+	-
2	-	-	-	-	-
3	-	-	-	-	-
4	+	-	+	-	+
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	+	-
8	+	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
%	20%	0%	10%	20%	10%

To find out the sensitivity PCR assay were carried out for *S. aureus* detection in reference sample. Reference samples were prepared by mixing appropriate concentrations of 0.01ng to 50ng of *S. aureus* (Fig. 4).

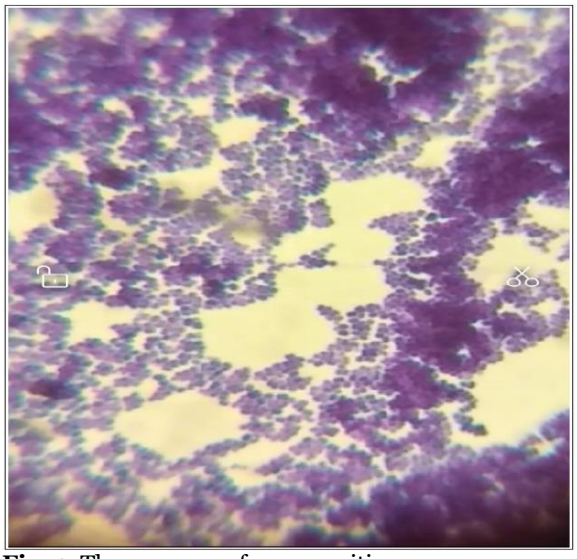

Fig. 1. The presence of gram positive sp.

The application of PCR assay for identification of *S. aureus* had been explained in the Table 1 and Table 2. The results showed that 11 samples showed positive results for the detection of *S. aureus* (Fig. 5).

Discussion

Out of 100 samples, 11 samples were revealed the positive result for *S. aureus*. *S. aureus* was identified and confirmed by cultural examination, morphological studies, staining characters and biochemical tests and finally PCR were performed for the amplification of specific gene (16s rRNA gene) of isolated bacteria. Milk is the best media for the growth of many bacteria in which some of them are pathogenic.

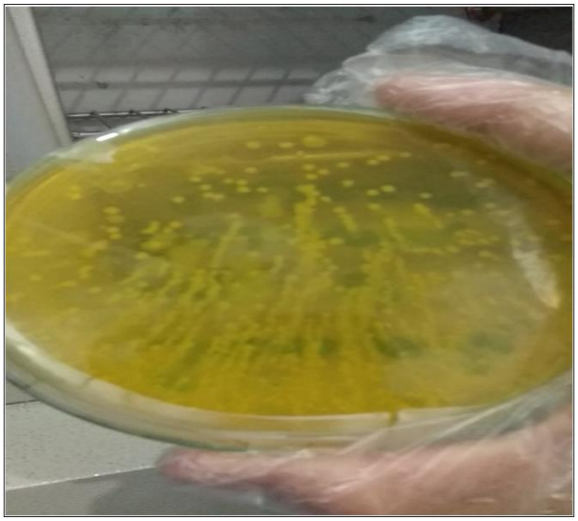

Fig. 2. Mannitol salt agar turned yellow.

As we know fresh milk is enriched with pathogenic and non pathogenic bacteria which can be transmitted to human by milking and consumption of milk. Coliform bacteria present in the fresh raw milk might be hazardous if proper boiling of milk is not done during consumption.

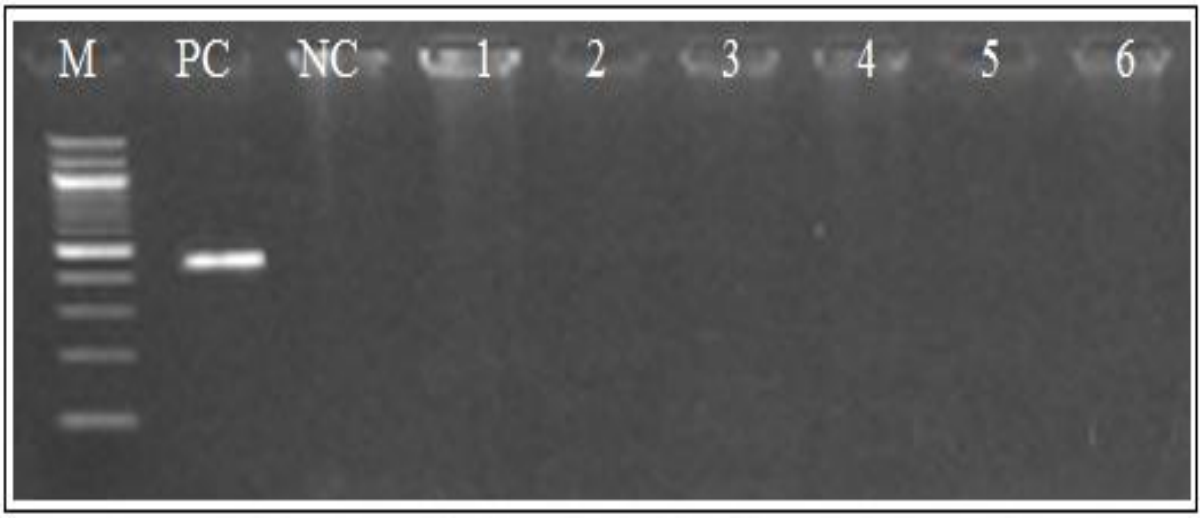

Fig. 3. Specificity of PCR assay of DNA from *Staphylococcus aureus* sample: M: marker, 100bp, NC: negative control (reagents with primers without DNAs) PC; *Staphylococcus aureus* DNA, (1) *Streptococcus pyogenes*, (2) *Bacillus anthracis*, (3) *Salmonella typhimurium*, (4) *Escherichia coli*, (5) , *Streptococcus agalactiae* (6) *Staphylococcus epidermidis*.

It causes disease if proper hygienic procedure is not maintained during milking. PCR was selected for detection of *S. aureus* due to its accuracy and rapidness, because rapid and proper detection of pathogen is necessary. PCR is the rapid method for detection of *S. aureus*. There was a need to set up a speedy and exact PCR method for the diagnosis of *S. aureus* in buffalo raw milk (Abo-Shama *et al.*,2014; Ali *et al.,* 2014; Ahmed *et al.,* 2017). The PCR assay not requires any specific expertise, not unique equipments or expensive chemicals and no need any additional processing aids (Howlin *et al.,* 2015).

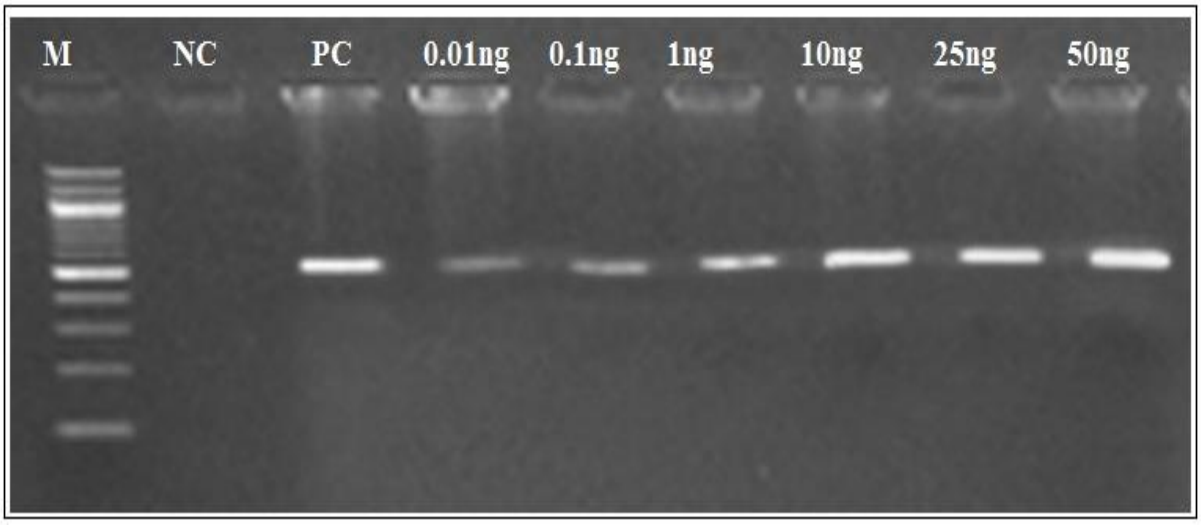

Fig. 4. Evaluation of PCR assay sensitivity for *Staphylococcus aureus* DNA sample; M: marker 100 bp. NC: negative control (reagents with primers without DNAs) PC; *Staphylococcus aureus* DNA, (1) 0.01ng (2) 0.1ng, (3) 1ng, (4) 10ng, (5) 25ng, (6) 50ng.

The specificity of PCR report was essential to avoid the error in the results. In other studies of specificity of PCR assay for detection of *S. aureus* and *E. coli* the specificity of PCR assay was documented as 97% and 99% respectively (Abbas *et al.,* 2014; Al-Zogibi *et al.,* 2015). The detection limit of this particular assay on reference *S. aureus* samples was 10ng that showed the method was highly sensitive as well as reliable. As

compared to our study, other researchers showed lower detection limits for detection of *S. aureus*. A minimum detection limit of 25ng for bacterial samples was found in different literatures (Ali *et al.,* 2014; Ahmed *et al.,* 2017). Some other researchers showed 50ng detection limits in their experiments of PCR (Burghardt *et al.,* 2016).

These *S aureus* positive samples were collected from the areas which had unsfaatisfactory hygienic conditions. Samples collected from Kotadu and Choak Qureshi showed negative results for detection of *S. aureus*. In Kotadu and choakfa Qureshi the condition of the areas, from where the samples were collected, had better hygienic conditions than other areas. So, we can conclude that the contamination of raw milk of buffalo caused by the main factor is the poor hygiene condition of herd and some other factors. There are many large size of herd in which cattle with different kinds of infection can also become the cause of prevalence of *S. aureus* (Elemo *et al.,* 2017). Comparison of results showed variations due to different reasons. The percentage of *S. aureus* infection occurrence results that recorded 10.9% by (Abd El-Hamid *et al.,* 2013) and by Khudaier *et al.,* (2014) were 10.23%. While the highest incidence of buffaloes subclinical mastitis were 34.6% by Torky and Kotb, (2013) reported, 78.12% reported by Hamed and Zaitoun, (2014), and 48% was reported by Sarkar *et al.,* (2014).

The frequency of *S. aureus* isolated was 16.66% from hand swabs of milk dealers, these results were reported by El-Gedawy *et al.,* (2014), 27% reported in samples of milk and milk products by Saadat *et al.,* (2014), 12% reported by Abo-shama *et al.,* (2014), 58.33% by Prabhu *et al.,* (2015) and 25.53% by Jahan *et al.,*(2015), 24% reported by Najimaana *et al.,* (2015) and the presence of the *S. aureus* in buffalo milk samples was reported 22.2% by Khaleel *et al.,* (2016). *S. aureus* were isolates from subclinical buffalo's mastitis 44% by Elemo *et al.,* (2017). Infected udders which provide medium to survive *S. aureus* in the udder shed in to milk during milking and establish chronic infections (Duguma *et al.,* 2018).

Conclusion

Out of 100 samples, 11 (11%) milk samples were found positive for *S. aureus*. 11 *S. aureus* isolates were amplified by 16S rRNA gene based PCR. Resistant pattern against antibiotic is an alarming situation and the main factor to contaminate the raw milk of buffalo is the poor hygiene condition of herd and some other factors which needs special consideration. The results of this study concluded that raw milk available for consumers have a not very high *S. aureus* contamination but the infections associated to *S. aureus* have adverse effects. Thus, the results of the present study warn the need for more precaution.

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References

Abbas BA, Ghadban MK, Alghanim AM. 2017. M.icrobial Evaluation of Milk and Milk Products during a Past Two Decades, in Basrah Southern Iraq: A Review **14(2),** 1-8.

Abbas BA, Khudor MH, Hanoon BM. 2014. Isolation and identification of *Staphylococcus aureus* from bovine and the detection of its coagulase gene (coa) using Polymerase Chain Reaction (PCR). Scientific Research and Essays **9(20),** 864-868.

Abd El-Hamid MI, Bendary MM. 2013. Association between agr Alleles and Toxin Gene Profiles of *S. aureus* Isolates from Human and Animal Sources in Egypt. International Journal of Advanced Research **8(1),** 133-144.

Abo-Shama UH. 2014. Prevalence and antimicrobial susceptibility of Staohylococcus aureus isolated from cattle, buffalo, sheep and goats raw milk in sohag governorate, Egypt. Assiut Veterinary Medical Journal **60(141),** 63-72.

Ahmed HS. 2017. Direct detection of "methicillin

resistant *Staphylococcus aureus*" from buffalo raw milk in Al-Qadissiya province using PCR assay. Kufa Journal For Veterinary Medical Sciences **8(1),** 38-44.

Al-Zogibi OG, Mohamed MI, Hessain AM, El-Jakee JK, Kabli SA. 2015. Molecular and serotyping characterization of shiga toxogenic Escherichia coli associated with food collected from Saudi Arabia. Saudi J Biol Science **22(4),** 438-442.

Ali R, Achkar KA, Mariri AA, Safi M. 2014. Role of PCR (PCR) in the detection of antibiotic-resistant *Staphylococcus aureus*. The Egyptian journalof medical human genetics **15(6),** 293-298.

Basil A, Abbas, Bassam Y, Khudaier Amaal M, Khudaier. 2017. Studies on mecA gene in methicillin resistant *Staphylococcus aureus* isolates. Jokull Journal **67(6),** 58-65.

Bharathy S, Gunaseelan L, Porteen K, Bojiraj, M. 2015. Prevalence of *Staphylcoccus aureus* in Raw Milk: Can it be a Potential Public Health Threat? International Journal of Advanced Research **2(3),** 801-806.

Bianchi DM, Gallina S, Bellio A, Chiesa F, Civera T, Decastelli L. 2014. Enterotoxin gene profiles of *Staphylococcus aureus* isolated from milk and dairy products in Italy. Letters in Applied Microbiology **58(2),** 190-196.

Burghardt EL, Flenker KS, Clark KC, Miguel J, Ince1 D, Winokur P, Ford B. 2016. Rapid, Culture-Free Detection of *Staphylococcus aureus* Bacteremia. Journal pone **11(6),** 1-17.

Duguma A, Wirtu A, Abunna F. 2018. Isolation and Identification of *Staphylococcus aureus* from Dairy Farms in Bishoftu Town, Ethiopia. JOJ Pub Health **3(1),** 555604.

El-**Ashker M, Gwida M, Tomaso H, Monecke S, Ehricht R, El-Gohary F, Hotzel H.** 2015. *Staphylococci* in cattle and buffaloes with mastitis in Dakahlia governorate, Egypt. J Dairy Science. **98(11),** 7450-9.

El-Gedawy AA, Ahmed HA, Awadallah MAI. 2014**.** Occurrence and molecular characterization of some zoonotic bacteria in bovine milk, milking equipments and humans in dairy farms, Sharkia, Egypt. International Food Research Journal **21(5),** 1813-1823.

Elemo KK, Sisay T, Shiferaw A, Fato MA. 2017. Prevalence, risk factors and multidrug resistance profile of *Staphylococcus aureus* isolated from bovine mastitis in selected dairy farms in and around Asella town, Arsi Zone, South Eastern Ethiopia. *Afr*ica Journal of Microbiological Research **11(45),** 1632-1642.

Farley JE, Hayat MJ, Sacamano PL, Ross T, Carroll K. 2015. Prevalence and risk factors for methicillin- resistant *Staphylococcus aureus* in an HIV- positive cohort. American Journal of Infection Control **43(4),** 329-35.

Garland AL, Walton WG, Coakley RD. 2013. Molecular basis for pH-dependent mucosal dehydration in cystic fibrosis airways. Proc Natl Acad Sci U S A. **110(40),** 15973–15978.

Gleeson A, Larkin P, Walsh C, Sullivan NO, 2016. "Methicillin resistant *Staphylococcus aureus*: Prevalence, incidence, risk factors, and effects on survival of patients in a specialist palliative care unit: a prospective observational study", Palliative Medicine **30(4),** 374–381.

Hamed MI, Zaitoun A. 2014. Prevalence of *Staphylococcus aureus* Subclinical Mastitis in Dairy Buffaloes Farms. International Journal of Livestock Research **4(3),** 21-28.

Hoegh SV, Skov MN, Boye K, Worning P, Jensen TG, Kemp M. 2014. Variation in the *Staphylococcus aureus* specific nuc gene can potentially lead to misidentification of meticillin

susceptible and resistant *S. aureus*. Journal of Medical Microbiology **63(5),** 1020-1022.

Hogan S, Zapotoczna M, Stevens NT, Humphreys H, O'Gara JP, O'Neill E. 2017. "Potential use of targeted enzymatic agents in the treatment of *Staphylococcus aureus* biofilm-related infections". The Journal of Hospital Infection **96(2),** 177– 182.

Howlin RP, Brayford MJ, Webb JS. 2015. Antibiotic-loaded synthetic calcium sulfate beads for prevention of bacterial colonization and biofilm formation in periprosthetic infections. Antimicrob Agents Chemother **59(1),** 111–120.

Jahan M, Marzia Rahman M, Parvej S, Chowdhury Z, Haque E, Talukder AK, Ahmed, S. 2015. Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. Journal of Advanced Veterinary Anim. **2(1),** 49-55.

Jeykumar M, Vinodkumar G, Bashir BP, Krovvidi S. 2013. Antibiogram of mastitis pathogens in the milk of crossbred cows in Namakkal district, Tamil Nadu. Veterinary. World **6(6),** 354-356.

Kavanaugh JS, Horswill AR. 2016. Impact of Environmental Cues on Staphylococcal Quorum Sensing and Biofilm Development. Journal of Biological Chemistry **291(24),** 12556-64**.**

Keclík M, Huslarová A, Bednár B. 2017. "Liver structure in patients with choledochoduodenostomy". Digestion **2(4),** 230–236.

Khaleel DA, Othman RM, Khudaier BY. 2016. Isolation and identification of *Staphylococcus aureus* from buffaloes milk with subclinical mastitis and milk workers Basrah Journal Veterinary Research **15(2),** 304.

Khedkar CD, Kalyankar SD, Deosarkar S, 2016. Buffalo milk. The Encyclopedia of Food and Health, **1(5),** 522-528.

Khudaier BY, Anad IT, Abbas BA. 2014**.** Isolation of *Staphylococcus aureus* from Buffalo Milk in Basra Governorate and Detection of Their Antibiotic Susceptibility.Basrah Journal of Veterinary Research **1(1),** 235-245.

Leke A, Goudjil S, Mullie C, Grognet S, Biendo M. 2017. PCR detection of staphylococcal enterotoxin genes and exfoliative toxin gene in mithicillin-susceptible *Staphylococcus aureus* stains from raw human breast milk. *Clinical* nutrition experimental. **14(1),** 26-35.

Manal H, Ghaffoori K, Ali H. 2013. Detection of methicillin or multidrug resistant *Staphylococcus aureus* (MRSA) in locally produced raw milk and soft cheese in Baghdad markets. Iraqi Journal of Veterinary Medicine **37(2),** 226 -231.

Marwa HA, May TF, Majeed AS. 2014. Methicillin resistance and enterotoxigenicity of Staphylococci isolated from milk and white cheese in Iraq. Iraqi Journal of Science **55(1),** 40-49.

Mohammad RS, Javad A, Alireza S. 2013. Antibiotic Susceptibility of *Staphylococcus aureus* in isolates of the patients with osteomyelitis. World Journal Medicine Science **9(3),** 180-183.

Najimaana W, Rizwan J. 2015. Detection of mecA gene of methicillin resistant *Staphylococcus aureus* by PCR assay from raw milk. Indian Journal of Animal Science **86(5),** 508– 511.

Percival SL, Hill KE, Williams D W. 2012. A review of the scientific evidence for biofilms in wounds. Wound Repair Regen. **20(5),** 647–657.

Saadat YR, Imani Fooladi AA, Shapouri R, Hosseini MM, Khiabani ZD. 2014. Prevalence of enterotoxigenic *Staphylococcus aureus* in organic milk and cheese in Tabriz, Iran. Iranian Journal of

Microbiol. **6(5),** 345-349.

Salgado-Pabón W, Schlievert PM. 2014. Models matter: the search for an effective *Staphylococcus aureus* vaccine. Nat Rev Microbiol. **12(8),** 585–591.

Sarkar P, Mohanta D, De S, Debnath C. 2014. *Staphylococcus aureus* in Dairy Animals and Farm Workers in a Closed Herd in Karnal, North India: Assessment of Prevalence Rate and Coa Variations. International Journal of Innovative Research in Science, Engineering and Technology **3(4),** 10962-10972.

Schenck LP, Surette MG, Bowdish DM. 2016. Composition and immunological significance of the upper respiratory tract microbiota. FEBS Letters. **590(21),** 3705-3720.

Scott D, Kobayashi Malachowa N, Frank R, De Leo. 2015. Pathogenesis of *Staphylococcus aureus* Abscesses. The American Journal of Pathology **185(6),** 1518-1527.

Sergelidis DAS, Angelidis AS. 2017. Methicillin-resistant *Staphylococcus aureus*: a controversial food-borne pathogen. Letters in Applied Microbiology **64(3),** 409 – 418.

Shortle D. 1983. A genetic system for analysis of *Staphylococcal* nuclease. Gene. **22(3),** 181-189.

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews **28(3),** 603-661.

Torimiro N, Torimiro SEA. 2012. Antibiotic resistance profile in community-associated *Staphylococcus aureus* strains isolated from a Nigerian peri-urban community. African Journal of Biotechnology **11(94),** 16071-16076.

Torky HA, Kotb SEA. 2013. The impact comparison of *Staphylococcus aureus* mastitis on the level of milk nitric oxide, immunoglobulin A and complement 3 between cows and buffaloes. New York Science Journal **6(11),** 90-95.

Werdan K, Dietz S, Löffler B. 2014. Mechanisms of infective endocarditis: pathogen-host interaction and risk states. Nat Rev Cardiol. **11(1),** 35–50.

Wollina U. 2017. Microbiome in atopic dermatitis. Clinical, Cosmetic and Investigational Dermatology. **10,** 51-56.

Wubishet Z, Ararsa D, Alemayehu L. 2013 Bovine Mastitis in selected districts of Borena zone, southern Ethiopia Bull. Anim Hlth Prod Afr. **61(1),** 285-291.

Zakary E, Marionette M, Nassif Z, Mohammed GM. 2011. Detection of *Staphylococcus aureus* in Bovine milk and its Product by Real Time PCR Assay. Global Journal of Biotechnology & Biochemistry, **6(4),** 171-177.