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Molecular characterization of some virulence genes of *Staphylococcus aureus* isolated from different sources and human.

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Abstract: This study aims to provide information about the genetic structure of *Staphylococcus aureus* strains isolated from (40) samples from humans (mouth, pharynx and hand), (70) samples from poultry and poultry byproducts, (70) samples of milk and milk byproducts. (20) samples of human food. From the collected 200 samples 23 *S. aureus* isolates were identified by morphological, biochemical and molecular methods. Additionally, the strains were screened for virulent genes as (nuc, spa, coa, icaA, Sea, Seb, Sec) enterotoxin production. When testing PCR for these group of genes that were identified and searched in 11 isolates showed a positive result for the genes (nuc, spa, coa) in a rate of 100%, except the gene (Sea) which had a negative result in all isolates, Where the (Seb) gene with a high rate of 100%, while the gene (Sec) gave a positive result in (1) isolate with a percentage of (0.09 %). The genes (icaA) was positive in all isolates with a rate of (90%) except in the two isolates. the severity of enterotoxin virulence gene in *S. aureus* strains leading to complications to human and animal life, including intestinal poisoning accompanied by diarrhea, nausea, vomition, dehydration and hypotension. Some of these virulent genes resulting in pneumonia, meningitis and septicemia and death

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Keywords: *Staphylococcus aureus*; virulence genes; *nuc, spa, coa, ica*A, S*ea,* S*eb,* S*ec* gene; human, poultry, food and milk.

1. Introduction

Staphylococcus is widely distributed in water, soil and air, in addition to its harmfull infection of various animal species and poultry ***(Goetz et al., 2006).*** *S. aureus* an important food borne pathogen, a major cause of food poisoning cases and out breaks worldwide ***(Wang etal., 2012).*** *Staphylococcus* was firstly detected by a Scottish surgeon Sir Alexander Ogston in 1881. Then, *S. aureus* was also firstly determinated by a German scientist Friedrich Julius Rosenbach in 1884 ***(Khan, et al; 2017).*** The coagulase-positive *S. aureus* genus inducing infections in humans and animals, spciatialy food intoxication. This genus including the most important species of *S. aureus*, ***(Betley and Mekalanos, 1988); (Bystroń et al 2005) and (Cremonesi et al; 2007***). The genus *Staphylococcus* including 62 species and 30 subspecies based on 16S rRNA sequences ***(LPSN Bacterio; 2020).*** Contamination ofpoultry meat by *Staphylococcus aureus* can be occurred from different retail sites and appliances such as cages, bleeding knife, drum , wooden log , cutting knife, polyethylene bag , hand swab of butcher , weighing balance , retail outlet wall and retail outlet floor ***(Vaidye, et al., 2009). El-Nagar et al. (2017)*** on samples collected from the poultry production chain, the enterotoxin genes (S*eb* and S*ec*) were ilustrated in five strains (23.8%) of *S. aureus*, with a percentage of 9.5% for S*eb* and S*ec* while S*ec* was found in six (20.6%) isolates.

Cell proliferation and biofilm formation are known virulence factors mediated by the presence of the *ica* locus, this gene *ica*A, was organized in an operon (*ica*ADBC) with the regulatory gene *ica*R. This operon is responsible for expression of PIA, present in the cell wall ***(Cucarella et al., 2004)***. Biofilm production of *S. aureus* isolated from cases of clinical mastitis and with the presence of icaA, in these species was screened by polymerase chain reaction ***(SalinaF et al., 2020)***. The Amplification of thermonuclease (*nuc*) gene was carried out by Polymerase Chain Reaction that used for the genotypic characterization of isolated *S. aureus* strains. All tested strains yielded amplification of the

(*nuc)* gene of 270 bp for all examined *S. aureus* isolates ***(Abo-Shama, 2014).*** The prevalence of *S. aureus* virulence factors such as enterotoxin E (S*ea*) or leukocidin E (*lu*kE) was detected that lukE may be less common ***(Peterson et al., 2019 and Klos et al., 2019).*** 12 individual *spa* types identified. t012 (n = 3, 18%) and t021 (n = 3, 18%) were the most commonly identified among this group. Overall, *spa* typing revealed greater diversity among ocular *S. aureus* strains ***(William et al., 2021).***
Goetz, T., & Frenzel, A. C. (2006). Pha ¨nomenologie schulischer
1318 Langeweile [Phenomenology of boredom at school]. Zeitschrift
1319 fu¨ r Entwicklungspsychologie und Pa¨ dagogische Psychologie,
1320 38(4), 149–153. doi:10.1026/0049-8637.38.4.149
Goetz, T., & Frenzel, A. C. (2006). Pha ¨nomenologie schulischer
1318 Langeweile [Phenomenology of boredom at school]. Zeitschrift
1319 fu¨ r Entwicklungspsychologie und Pa¨ dagogische Psychologie,
1320 38(4), 149–153. doi:10.1026/0049-8637.38.4.149
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1318 Langeweile [Phenomenology of boredom at school]. Zeitschrift
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1320 38(4), 149–153. doi:10.1026/0049-8637.38.4.149
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1318 Langeweile [Phenomenology of boredom at school]. Zeitschrift
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1320 38(4), 149–153. doi:10.1026/0049-8637.38.4.149
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1318 Langeweile [Phenomenology of boredom at school]. Zeitschrift
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1320 38(4), 149–153. doi:10.1026/0049-8637.38.4.149

2-Materials and Methods

Sample collection: -

A total of 200 samples of human samples (40), poultry and poultry byproducts (70), milk and milk byproducts (70) and ready to eat food (20) samples, were collected aseptically using sterile scissor and forceps in separate sterile plastic bag for each sample. each sample was subjected to bacteriological examination for *S. aureus* isolation ***(Sneath et. al., 1986)*** and (***Monecke et al., 2012*)**.

Preparation of samples (pre-enrichment):-

The collected samples from (poultry and poultry byproducts, milk and milk byproducts and ready to eat food). Ten grams of each sample were transferred into a flask containing 90 ml of 0.1% sterile peptone water to get a dilution of 10^{-1}, incubate at 37 ^{o}C for 24 hrs under aerobic condition.

Bacterial Isolates and culture media:

The samples were collected aseptically for detection of *S. aureus* strains that differentiated into coagulase positive and coagulase negative *Staphylococcus* isolates.

The coagulase positive *Staphylococcus* isolates were identified by conventional methods, including morphological characters as Gram staining, blood haemolysis, catalase, coagulase and anaerobic fermentation of mannitol tests ***(Swayne et al., 1998)* and *(Koneman et al., 2001)***. All isolated strains were stored on suitable maintenance media in the National Laboratory for Biochemical tests.

Laboratory center for PCR (*Sambrook et al., 2001) and (WHO, 2002)*:

The stored isolated strains were re-cultivated on brain heart infusion agar plates and broth for an extraction of DNA to be used in PCR technique.

Molecular biology technique (PCR): *(Riffon et al., 2001)*

PCR amplification was performed with PTC-100 programmable thermal cycler (Peltier Effect cycling, MJ, Research, INC, UK) in a volume of 50 ml consisting of: 12.5µl of Emerald Amp GT PCR master mix (2x premix), 1 µl of 20 pmol of each primer for one sample, 6 µl of the DNA template and water, nuclease-free up to 25 µl in uniplex PCR. While, 25µl of Emerald Amp GT PCR master mix (2x premix), 1 µl of 20 pmol of each primer for one sample, 10µl of the DNA template and water, nuclease-free up to 50 µl in PCR. Primer sequence and PCR amplification cycles of oligonucleotide primers among the selected isolates are illustrated.

3-Result:

S.aureus were detected 11.5% as follow 23 from 200 total sam ples were staphylicocus aureus positive samples of poultry products 10 % (7/70) had been examined ,human samples 17.5%(7/40), milk

and milk byproducts 8%(6/70) and ready to eat food 15 %(3/ 20) samples).

Isolation of *S. aureus* from the examined samples:

S. aureus isolates were Gram positive coccoi arranged in clucters. They had yellow colonies surrounded by yellow medium due to mannitol utilization on mannitol salt agar medium **(Figure 1)**. The isolates had black shiny convex colonies surrounded by a clear zone extended onto the opuque medium on baired parker agar medium **(Figure 2)**.

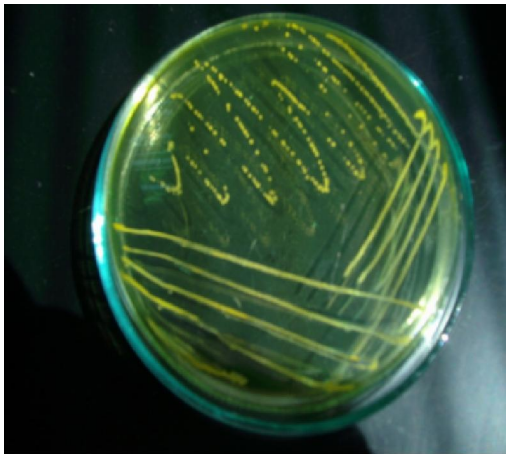

Figure 1: mannitol salt agar showed yellow colonies shiny convex surrounded by yellow medium

Figure 2: Baired parker agar showed black colonies surrounded by a clear zone

Table (4): The prevalence of *S.aureus* strains in the collected samples:-

Sample:	**Number of Examined Samples**	***S. aureus* isolation:**	
		+ve	**%**
Poultry and poultry by product	**70**	**7**	**0.1%**
Human	**40**	**7**	**17.5%**
milk and milk by products	**70**	**6**	**8%**
Ready to eat food	**20**	**3**	**15%**
Total:	**200**	**23**	**11.5%**

Table (5): The incidence rates of some virulent genes for the isolated *S. aureus* stains in the collected samples by the using of PCR technique:-

Sample	**N*uc***	**S*pa***	**C*oa***	***ica*A**	**S*ea***	**S*eb***	**S*ea***
1	+	+	+	+	-	+	-
2	+	+	+	-	-	+	-
3	+	+	+	+	-	+	-
4	+	+	+	+	-	+	-
5	+	+	+	+	-	+	-
6	+	+	+	+	-	+	-
7	+	+	+	+	-	+	-
8	+	+	+	+	-	+	-
9	+	+	+	+	-	+	-
10	+	+	+	+	-	+	+
11	+	+	+	+	-	+	-

Eleven isolates showed a positive result in the following virulent genes (nuc, Spa, coa and Seb) with a percentage of 100%. While the gene (Sea), had a negative result in all.

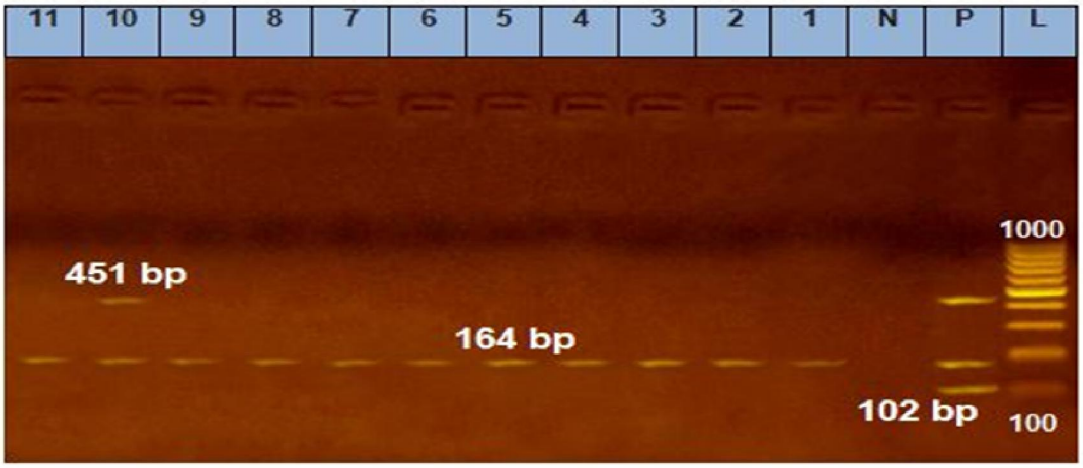

Fig.3. Agarose gel photo documentation for detection of thermo-nuclease toxin Virulence factor encoding genes (*Sea, Seb and Sec*) of *S. aureus* a genotyping identification of the isolates.
Lane: 100-1000 bp DNA ladder. Pos: **negative** control (at 102 bp).
Lanes 1,2,3,4,5,6,7,8,9,10 and11 ***S.aureus* (*Sea)gene* negative.**
Lane: 100-1000 bp DNA ladder. Pos: positive control (at 164 bp).
Lanes 1,2,3,4,5,6,7,8,9,10 and11 ***S.aureus* (*Seb)gene* positive.**
Lane: 100-1000 bp DNA ladder. Pos: positive control (at 164 bp).
Lanes 10 ***S.aureus* (*Sec)gene* positive.**

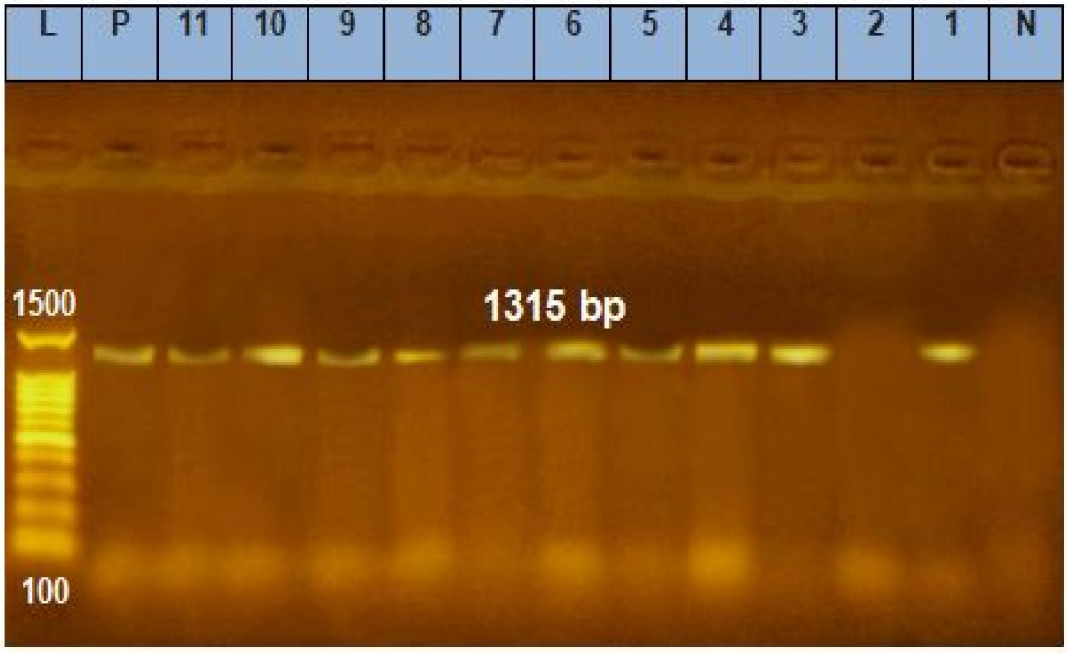

Fig.4. Agarose gel photo documentation for detection virulence factor encoding gene (*icaA*) of *S.aureus* as a genotyping identification of the isolates.
Lane: 100-1500 bp DNA ladder. Pos: positive control (at 1315 bp).
Lanes 1,2,3,4,5,6,7,8,9,10 and11 ***S.aureus* (*icaA)gene* positive.**

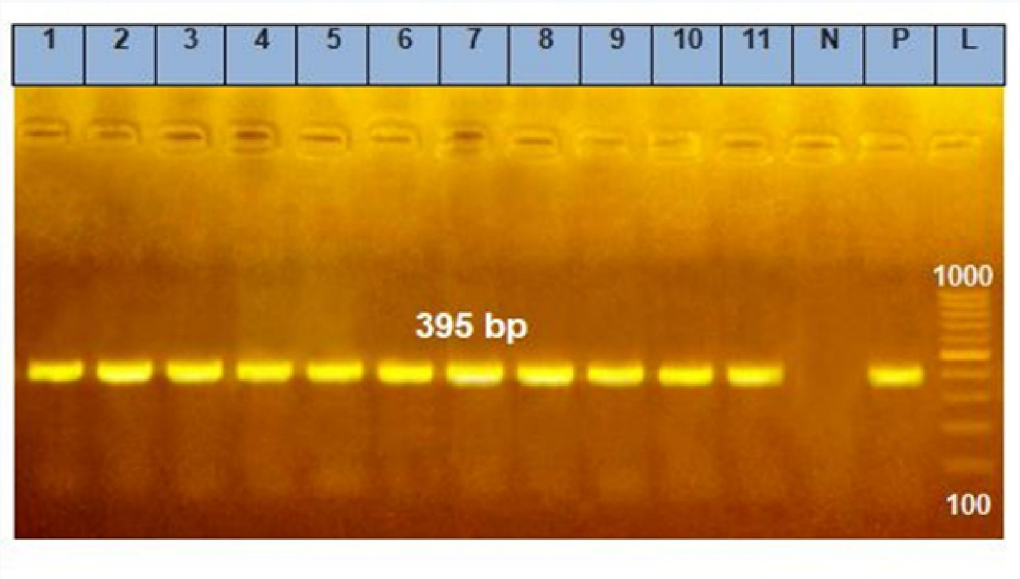

Fig.5. Agarose gel photo documentation for detection of thermo- nuclease toxin Virulence factor encoding gene (*nuc*) of *S.aureus* a genotyping identification of the isolates.
--Lane: 100-1000 bp DNA ladder. -Pos: positive control (at 395 bp).
-Lanes 1,2,3,4,5,6,7,8,9,10 and11 ***S.aureus* (*nuc*)*gene*** positive**.**

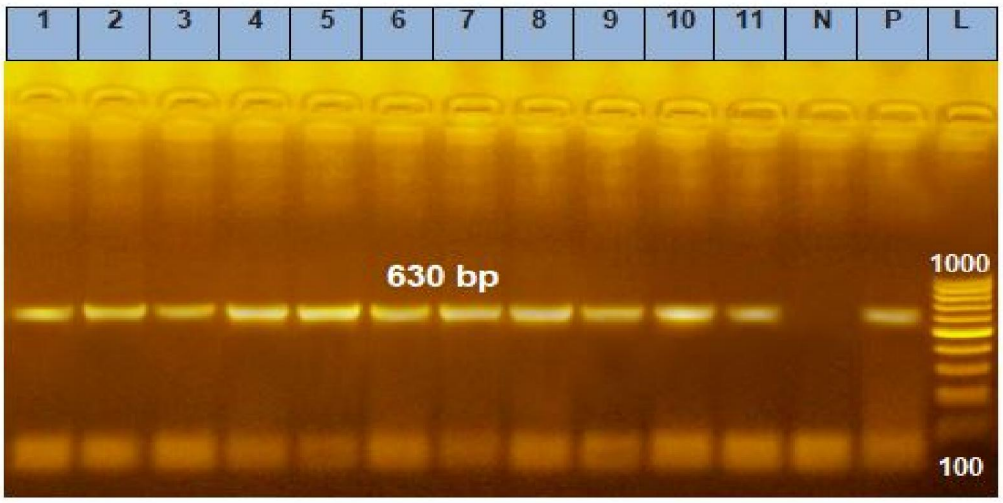

Fig.6. Agarose gel photo documentation for detection of thermo- nuclease toxin Virulence factor encoding gene (*Coa*) of *S.aureus* a genotyping identification of the isolates.
-Lane: 100-1000 bp DNA ladder. -Pos: positive control (at 630 bp). ---
-Lanes 1,2,3,4,5,6,7,8,9,10 and11 ***S.aureus* (*Coa*)*gene*** positive**.**

4. Discussion:-

To accomplish this study, 200 samples were taken from different sources, where we obtained 23 isolates (10.1%) of ***S. aureus***, and this corresponds to some extent with each of reported that only 3 (7.5%) *S. aureus* strains by **(*Pereira et al; 2018*).** These results were aggregated. While this result was disaggregated *with* ***(Emine Kayili and Pınar Sanlibaba, 2020)***, as the positive samples of *S. aureus* was 25.93% in Korea,**(*Jang et al., 2018*)** 87.32% in Serbia, **(*Bulajic et al., 2017*)** 73.6% in Brazil, **(*Pereira et al., 2018*)** 50% in Italy, ***(Spanu et a., 2012)*** 45% in Norway, **(*Mehli et al., 2017*)** 27.7% in China, ***(Liu et al; 2017)*** 50% in Poland, **(*Rola et al; 2016*)** and 25% in Iranian, (***Arefi et al., 2014).***

Eleven isolates showed a positive result in the following virulent genes (***nuc, spa, coa and Seb***) with a percentage of 100%, these results may be due the

collected samples from endemic area with *S.aureus* enrtotoxigenic strains and bade hyiegenic measures. The tabulated results were disagreed with the results of ***El-Nagar et al. (2017)*** and **(*Salina et al., 2021*).** While the virulent gene (**Sea**), had a negative result in all isolates, this is may be due to the compition between the different virulent genes and the low execration from the *S.aureus* strains. But this corresponds somewhat to **both (*Afsaneh Mozafarianari et al., 2019*)** who found the gene (S*ea)* at 6%. It does not agree with El-***Nagar et al. (2017), Peterson et al. (2019) and Klos et al. (2019)***. Moreover, ***(Pourmand et al., 2009)*** studied the prevalence of the S*ea* gene of *S. aureu*s was 46.9%. Other investigators defined the rate of enterotoxin gene-positive *S. aureus* isolates among the food handler in central Iran and explained that the rate of *Sea* was at food (***Fooladvand et al., 2019***).

The gene (***ica*A,**) were positive in most of the isolates by (90%), and this corresponds to some extent with, ***(Aguilar et al., 2001; Azara et al., 2017a)*** Percentage (100%). and different with ***(Salina et al., 2021).*** Percentage (68%), and The *ica*AD gene was detected in 16(32%) *staphylococcal* isolates ***(Rasha et al; 2012).***

While the gene (**Sec**) gave a positive result in one isolate with a percentage of (0.09%), and this is identical with (***El-Nagar et al. (2017)***

It does not match with ***(Argudín et al., 2012)*** Where the results showed that the gene (S*ec*) by (%16.1)**.**

The results of these studies are inconsistent with our results. Another researcher screened the *S. aureus* nasal strains to detect toxin genes and showed that 15% and 13% of the genes were *TST* and *Sec*, respectively ***(Piechowicz et al., 2011).*** This bacterium might induce allergic inflammatory responses via secreting enterotoxin (*Se*) and could lead to toxic shock syndrome toxin 1 (TSST-1). Humans are the main storage for *S. aureus* in nature. Approximately 30% of healthy people are involved in a bacterium *(*K*lotz* ***at al., 2003; Ludwig et al., 2019) and Cucarella et al., 2004****)*.

Conclusion

PCR assumed to be rapid, sensitive and accurate with the ability to deal with large number of samples for studying of Genotyping characters of virulence factors encoding genes of *S. aureus* that isolated from humans and animals samples. Finally results of antibiotics and sensitivity tests improved the treatment of mastitis and decrease the time and costs of mastitis treatment. Also by the using of PCR technique we determined the rate of virulence genes (***nuc, spa, coa and* Seb**) with a percentage of 100%**.** While the virulent gene (**Sea**), had a negative result in all isolates**.**

It is possible to prospectively define potential *S. aureus* virulence factors or other genetic determinants that may inducing infection in specific disease settings, thereby deepening our understanding of this important animal and human pathogen.

References

[1]. Abo-Shama, U.H (2014) Prevalence and Antimicrobial Susceptibility of *S. aureus* Isolated from Cattle, Buffalo, Sheep and Goat`S Raws Milk in Sohag Governorate, Egypt. Assiut Vet. Med. J. Vol. 60 No. 141 April 63.

[2]. Afsaneh Mozafarianari, Ashraf Kariminik, Mahnaz Tashakori (2019) Evaluation of the Frequency of Enterotoxin A (SEA) and Enterotoxin B (SEB) Genes in Clinical Isolates of S. aureus in Rafsanjan, Iran. Avicenna J Clin Microbiol Infect, 6(4), 118-121; DOI:10.34172/ajcmi..21

[3]. Aguilar, B., B. Amorena, and M. Iturralde. 2001. Effect of slime on adherence of S. aureus isolated from bovine and ovine mastitis. Vet. Microbiol. 78:183–191.

[4]. Arefi, F.; Mohsenzadeh, M.; Razmyar, J. Isolation, Antimicrobial Suspectibility and Meca Gene Analysis Of Methicillin-Resistant S. aureus In Iranian White Cheeses. Iran. J. Vet. Res. 2014, 15(2), 127–131.

[5]. Argudín M. A, M. C. Mendoza, M. A. González-Hevia, M. Bances, B. Guerra and M. R. Rodicio (2012) Genotypes, Exotoxin Gene Content, and Antimicrobial Resistance of S. aureus Strains Recovered from Foods and Food Handlers. Appl environ Microbiol.; 78(8): 2930–2935. doi: 10.1128/AEM.07487-11.

[6]. Azara, E., C. Longheu, G. Sanna, and S. Tola. 2017a. Biofilm formation and virulence factor analysis of S. aureus isolates collected from ovine mastitis. J. Appl. Microbiol. 123:372–379. https://doi.org/10.1111/jam.13502.

[7]. Betley M.J., Mekalanos J.J.: Nucleotide Sequence Of The Type A Staphylococcal Enterotoxin Gene. J Bacteriol 1988, 170, 34–41.

[8]. Bulajic, S.; Colovic, S.; Misic, D.; Djordjevic, J.; Savic-Radovanovic, R.; Asanin, J.; Ledina, T. Enterotoxin Production And Antimicrobial Susceptibility In Staphylococci Isolated From Traditional Raw Milk Cheeses In Serbia. J. Environ. Sci.

Health Part B. 2017, 52(12), 864–870. Doi: 10.1080/03601234.2017.1361764

[9]. Bystroń J., Molenda J., Bania J., Kosek-Paszkowska K., Czerw M.: Occurrence of Enterotoxigenic Strains of Staphylococcus Aureus in Raw Poultry Meat. Pol J Vet Sci 2005, 8, 37–40

[10]. Cremonesi P., Perez G., Pisoni G., Moroni P., Morandi S., Luzzana M., Brasca M., Castiglioni B.: Detection Of Enterotoxigenic S. aureus Isolates In Raw Milk Cheese. Lett Appl Microbiol 2007, 45, 586–591

[11]. Cucarella, C.; Tormo, M.A.; Úbeda, C.; Trotonda, M.P. Et Al.(2004) Role Of Biofilm-Associated Protein Bap In The Pathogenesis Of Bovine S. aureus. Infect. Immun., V.72, P.2177-2185.

[12]. El-Nagar S., El-Azeem M.W.A., Nasef S.A., Sultan S.: Prevalence Of Toxigenic And Methicillin Resistant Staphylococci In Poultry Chain Production. J Adv Vet Res 2017, 7, 33–38.

[13]. Emine Kayili & Pınar Sanlibaba (2020) Prevalence, Characterization And Antibiotic Resistance Of Staphylococcusaureus Isolated From Traditional Cheeses In Turkey, International Journal Of Food Properties, 23:1, 1441-1451, Doi: 10.1080/10942912.

[14]. Fooladvand S, Sarmadian H, Habibi D, Van Belkum A, Ghaznavi-Rad E. High (2019) Prevalence Of Methicillin Resistant And Enterotoxin Gene-Positive S. aureus Among Nasally Colonized Food Handlers In Central Iran. Eur J Clin Microbiol Infect Dis; 38(1):87-92. Doi: 10.1007/S10096- 018-3398-0. [Crossref]

[15]. Goetz, AnilRupasinghaaStephan J. DavidFreshwater (2006).The production of social capital in US counties Author links open overlay pane. The Journal of Socio-EconomicsVolume 35, Issue 1, February 2006, Pages 83-101.

[16]. Jang, K.; Lee, J.; Lee, H.; Kim, S.; Ha, J.; Choi, Y.; Oh, H.; Yoon, Y.; Lee, S. Pathogenic Characteristics And Antibiotic Resistance Of Bacterial Isolates From Farmstead Cheeses. Korean J. Food Sci. Animal Resour. 2018, 38 (1), 203–208. Doi: 10.5851/Kosfa.2018.38.1.203.

[17]. Khan, M. F.;. Brief History of Staphylococcus Aureus: A Focus to Antibiotic Resistance. E-Cronicon Microbiol. 2017, 5(2), 36–39.

[18]. Klos M, Pomorska-Wesolowska M, Romaniszyn D, Chmielarczyk A, Wojkowska-Mach J. Epidemiology, Drug Resistance, And Virulence Of Staphylococcus Aureus Isolated From Ocular Infections In Polish Patients. Pol J Microbiol. 2019; 68(4):541–8. Https://Doi.Org/10.33073/Pjm-2019-056 Pmid: 31880897

[19]. Klotz M, Opper S, Heeg K, Zimmermann S. Detection Of Staphylococcus Aureus Enterotoxins A To D By Real-Time Fluorescence Pcr Assay. J Clin Microbiol 2003;41(10):4683-7. Doi: 10.1128/Jcm.41.10.4683-4687.2003. [Crossref]

[20]. Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schreckenberger, P.C.; Winn Junior, W.C. Diagnóstico microbiológico. Texto e Atlas Colorido. 5 ed. Rio de Janeiro: MEDSI, 2001. 1465p

[21]. Liu, H.; Li, S.; Meng, L.; Dong, L.; Zhao, S.; Lan, X.; Wang, J.; Zheng, N. Prevalence, Antimicrobial Susceptibility, And Molecular Characterization Of Staphylococcus Aureus Isolated From Dairy Herds In Northern China. J. Dairy Sci. 2017, 100(11), 8796–8803. Doi: 10.3168/Jds.2017-13370.

[22]. LPSN Bacterio.net, List of Prokaryotic Names with Standing in Nomenclature. https://lpsn.dsmz.de/search?word =Staphylococcus Accessed May 15, 2020.

[23]. Ludwig S, Jimenez-Bush I, Brigham E, Bose S, Diette G, Mccormack Mc, Et Al. Analysis Of Home Dust For Staphylococcus Aureus And Staphylococcal Enterotoxin Genes Using Quantitative Pcr. Sci Total Environ 2017;581-582:750- 5. Doi: 10.1016/J.Scitotenv.2017.01.003. [Crossref].

[24]. Mehli, L.; Hoel, S.; Thomassen, G. M. B.; Jakobsen, A. N.; Karlsen, H. The Prevalence, Genetic Diversity and Antibiotic Resistance of Staphylococcus Aureus In Milk, Whey, And Cheese From Artisan Farm Dairies. Int. Dairy J. 2017, 65, 20–

[25]. Mehrotra, M.; WANG, G. and, Johnson, W.M. (2000): Multiplex PCR for Detection of Genes for Staphylococcus aureus Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. Journal Of Clinical Microbiology. Vol. 38, No. 3.

[26]. Pereira, G.; Gracindo, A.; Tinoco, A.; Oliveira, P.; Rangel, A., 2018. Fatty Acid Profile Of Milk Of Goats Fed With Growing Levels Of Flor-De-Seda Hay. Revista Caatinga, 22 (3): 206-210

[27]. Peterson Jc, Durkee H, Miller D, Maestre-Mesa J, Arboleda A, Aguilar Mc, Et Al. Molecular Epidemiology And Resistance Profiles Among Healthcare- And Community-Associated Staphylococcus Aureus Keratitis Isolates. Infect Drug Resist. 2019; 12:831–43. Https://Doi.Org/10.2147/Idr.S190245 Pmid:31043797.

[28]. Piechowicz L, Garbacz K, Wiśniewska K, Dąbrowska-Szponar M (2019). Screening Of Staphylococcus Aureus Nasal Strains Isolated From Medical Students For Toxin Genes. Folia Microbiol (Praha) 2011;56(3):225-9. Doi: 10.1007/S12223-011-0041-1. [Crossref]

[29]. Pourmand Mr, Memariani M, Hoseini M, Bagherzadeh Yazdchi S. High Prevalence Of Sea Gene Among Clinical Isolates Of Staphylococcus Aureus In Tehran. Acta Med Iran. 2009:47(5):357-61.

[30]. Rasha A.Nasr,Hala M.AbuShady,Hussein S.Hussein (2012) Biofilm formation and presence of icaAD gene in clinical isolates of staphylococci. Egyptian Journal of Medical Human Genetics Volume 13, Issue 3, October 2012, Pages 269-274.

[31]. Riffon, R., Sayasith, K., Khalil, H., Dubreuil, P., Drolet, M., Lagace, J. 2001. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J.Clinical Microbiol. 39: 2584- 2589.

[32]. Rola, J. G.; Czubkowska, A.; Korpysa-Dzirba, W.; Osek, J. Occurence Of Staphylococcus Aureus On Farms With Small Scale Production Of Raw Milk Cheeses In Poland. Toxins. 2016, 8, 62. Doi: 10.3390/Toxins8030062.

[33]. Salina, ÁlvaroFernández-Montero and YangDing (2021) Mucosal immunoglobulins of teleost fish: A decade of advances. Author links open overlay panel J. Oriol Sunyer Volume 121, August 2021, 104079.

[34]. Salinaf.F. Guimarãesv.B. Richini Pereirab.D Menozziv.L.M. Rallh. Langoni (2020) Detection Of Icaa, Icad, And Bap Genes And Biofilm Production In Staphylococcus Aureus And Non-Aureus Staphylococci Isolated From Subclinical And Clinical Bovine Mastitis. Communication • Arq. Bras. Med. Vet. Zootec. 72 (03) • May-Jun 2020 • Https://Doi.Org/10.1590/1678-4162-11284

[35]. Sambrook. Joseph, Russell. Davids W (2001): Includes bibliographical references and index. ISBN 978-1-936113-41-5 (cloth) – ISBN 978-1-936113-42-2

[36]. Sneath, P.H.A; Mair, N. S.; Elisabeth Sharpe, M.; Holt, j. g.; Murray, R. G. E.; Chairman; Brenner, D.J.; Bryant, M.P.; Krieg, N. R.; Moulder, J.W.;Pfennig, N. and Staley, J. T. (1986): Section 12 gram-positive cocci, Bergey's Manual of Systemic Bacteriology, Vol. 2, Williams. USA

[37]. Spanu, V.; Spanu, C.; Virdis, S.; Cossu, F.; Scarano, C.; De Santis, E. P. Virulence Factors And Genetic Variability Of Staphylococcus Aureus Strains Isolated From Raw Sheep's Milk Cheese. Int. J. Food Microbiol. 2012, 153(1–2), 53–57. Doi: 10.1016/J.Ijfoodmicro.2011.10.015.

[38]. Swayne, D.E.; Chairman; G; isson, J.R.; Jackwood, M.W; Pearson, J.E and Reed, W.M. (1998): Staphylococcosis, A laboratory manual for the isolation and identification of avian pathogens, fourth edition. Am. Assoc. Avian Pathol.

[39]. Vaidya, V. S., Ford, G. M., Waikar, S. S., et al. (2009) A rapid urine test for early detection of kidney injury. Kidney International 76, 108-114

[40]. Wang, X.; Tao,X.; Xia,X.; Yang,B.; Xi,M.; Meng, J.; Zhang , J. and Xu,B. (2012): Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in retail raw chicken in China. Food Control 29 ,103-106.

[41]. WHO (2002): World Health organization. Department of communicable diseases surveillance and response.

[42]. William L. JohnsonI Michael B. Sohn, Samantha Taffner, Payel Chatterjee, Paul M. Dunman, Nicole Pecora, Rachel A. F. Wozniak(2021) Genomics of Staphylococcus aureus ocular isolates PLoS ONE 16(5): e0250975. https://doi.org/10.1371/journal.pone.0250975 .

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