Antimicrobial Activity of Coagulase-Positive *Staphylococcus Aureus* in Raw Cow's Milk at Damietta Governorate

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Abstract:

Staphylococcus aureus (S. aureus) is one of the most important pathogens involved in food borne infections from consumption of milk and dairy products. Antibiotics are used to treat livestock commonly, resulting in a problem of antibiotic resistance of *Staphylococcus aureus* which has a huge risk for public health all over the world. Because of its toxin-mediated pathogenicity, antibiotic resistance, and invasion, *Staphylococcus aureus* consider one of the most significant pathogens. The present study was performed on a total of 150 raw cow's milk from different sources at Damietta Governorate. The examined samples were taken in ice box within an hour for bacteriological examination. The results revealed that 48 out of 150 isolates (32 %) were positive for *S. aureus*. Out of those 48 isolates, 25 (52.08%) were coagulase positive and the other 23 (47.92%) were coagulase negative. Detection of antibiotic sensitivity of *S. aureus* isolates by disk diffusion method and confirmed by viteck2 compact method revealed that against 10 antimicrobial discs *S. aureus* show resistance against penicillin G, oxacillin and tetracycline antibiotics by 24% followed by 16% resistance against ciprofloxacin and show good sensitivity against amikacin, chloramphenicol and erythromycin antibiotics. These results obtained according to CLSI (2016). This study aimed to isolate and identify the multi drug resistant coagulase positive *S. aureus* from raw cow's milk at different regions of Damietta governorate and evaluate its antimicrobial activity against different groups of antibiotics.

Key words: *Staphylococcus aureus*, Antibiotic resistance, Coagulase positive

Introduction:

Staphylococcus aureus (S. aureus) consider a common commensal bacterium present on skin and mucosa of ruminants, and it is linked to clinical and subclinical mastitis, which can be transferred to people through contaminated dairy products and milk ***(Swetha et al., 2017)***. *S. aureus* can colonize animal and human bodies and cause a lot of infections differ in severity ***(Kalayu et al., 2020)***. In humans, *S. aureus* can cause different infectious diseases ranging from mild infections in skin such as cellulitis, boils, abscesses, pimples or scalded syndrome of skin to more serious illnesses like meningitis, pneumonia, endocarditis, toxic shock syndrome and bacteremia. *S. aureus* is linked to bovine mastitis and bumble foot disease in poultry. ***(Ali Y et al., 2017)***. Certain kinds of food contaminated with *S. aureus*, such as dairy products, milk, canned meat, sausage, and salads, are considered a potential vector for spreading *S. aureus* to people. ***(Dehkordi et al., 2017).*** Many studies discussed that many *S. aureus* strains contain enterotoxins, which contaminate milk and milk products and cause milk-borne illness ***(Saadat et al., 2014)***. Because Staphylococcal enterotoxin (SE) resist freezing, drying, heat, decreasing PH and proteolytic enzymes, it can cause its effects in the digestive tract at low dose ***(Hennekinne et al., 2012)***. There is an association between the incidence of *Staphylococcus aureus* in milk and the previous exposure to mastitis. ***(Lakech Tibebu et al., 2021)***. Mastitis is a disease that affects dairy cattle and result in reduced milk quality and quantity. Mastitis-affected dairy cows produce 30% less milk every quartile, which can result in a 15% reduction in milk production this causing mastitis to be one of the most serious issues influencing the industry of dairy animals globally ***(Abutarbush, 2010)***. Two types of mastitis are present known as clinical and subclinical mastitis and It was found that the incidence of subclinical mastitis is higher than the incidence of clinical mastitis in dairy animals ***(Mbindyo et al., 2020)***. Humans are subjected to adverse consequences and mortality as a result of food-borne illnesses caused by milk and milk products ***(Painter et al., 2013)***. The main cause of food poisoning, invasiveness and antibiotic resistance among bacterial pathogens is *S. aureus* ***(Gundogan and Avci, 2014)***. Antibiotics are

now widely used in nutrition to enhance growth in farm and pastoral animals, resulting in the problem of antibiotic resistance ***(Onicuiuc et al., 2017)***. MRSA become of great concern for public health because it is the main pathogen that causing infection to human beings from livestock animals ***(Dooulgeraki et al., 2017)***. Multidrug resistance has been increased all over the world that is considered a public health threat. Several recent investigations reported the emergence of multidrug-resistant bacterial pathogens from different origins including humans, birds, cattle, and fish that increase the need for routine application of the antimicrobial susceptibility testing to detect the antibiotic of choice as well as the screening of the emerging MDR strains ***(Algammal et al., 2020; Algammal et al., 2021; Enany et al., 2020; Makharita et al., 2020; and Said et al., 2020)***.

Material and methods:

Sampling:

Between October 2019 and March 2021, 150 milk samples from dairy cows were collected in Damietta and New Damietta cities, Egypt. In centrifuge tubes, dairy milk was obtained directly from the milkman. 1 mL of milk is aseptically put into a glass sterile 10 mL tube containing 4 mL of buffered peptone water and incubated at 37°C for 24 hours ***(Thaker et al., 2013).***

Bacterial isolation and identification:

Following enrichment media incubation, cultured on mannitol salt agar, and Baird Parker agar ***(Quinn et al., 2002)*** plates and incubated at 37°C for 24-48 hours then colonies were identified. Suspected colonies of *S. aureus* were examined morphologically and biochemically according to ***(FDA, 2001)*** and microscopically according to ***(Ryan and Ray, 2004).*** Identification was accomplished through morphological and cultural features analysis, followed by microscopic examination using the gram staining method. The coagulase and catalase tests were used in a biochemical examination to confirm the *S. aureus* species. The catalase test was carried out by dropping 3 percent hydrogen peroxide onto a clean glass slide and mixing it with 1 bacterial colony loop inoculum ***(APHA, 1992).*** Coagulase testing can be done in two ways: (a) using a coagulase slide, and (b) using tube coagulase ***(Mackie and McCartney, 1996)*** which done by adding fresh pure culture to sterile agglutination tubes containing 5 ml of brain heart infusion broth and incubated at 37 ^{0}C overnight 0.1 ml was transferred to tubes containing

0.3 ml of sterile citrated rabbit plasma. Inoculated tubes were incubated at 37 ^{0}C and examined for clot formation after 2 hours. The positive reaction was indicated by clot formation. The negative tubes were left for another 20 hrs. at room temperature before final results were recorded.

Antimicrobial sensitivity test:

S. aureus isolates were subjected to antibiotic sensitivity tests against different antibiotics using disc diffusion method ***(Finegold and Martin, 1982)*** and all tested isolates were confirmed by viteck2 compact method. The fully automated Vitek2 equipment (bioMérieux, Marcy l'Etoile, France) was used to assess the antimicrobial susceptibility of the *S. aureus* isolates. The distinct Gram-positive specific bacterium card was used in this study. This card is included a total of 10 antimicrobial discs as shown in table (1). The Vitek2 apparatus classified the isolates as sensitive, intermediate, or resistant to the antimicrobials tested. The multi drug resistance (MDR) phenomenon was assessed based on acquired resistance to at least one antibiotic in three or more antimicrobial classes ***(Magiorakos et al., 2012),*** and isolates tested intermediate to a specific medication were declared resistant. The Clinical Laboratory Standards Institute (CLSI) guideline ***(CLSI, 2016)*** was used to identify resistance breakpoints.

Table (1): *Antimicrobial discs*

Antimicrobial discs	Symbol	Conc.
Penicillin G	P	10 units
Amikacin	AK	30 mg
Chloramphenicol	C	30 mg
Oxacillin	OX	30 mg
Tetracycline	TE	30 mg
Ciprofloxacin	CIP	5 mg
Gentamycin	CN	10 mg
Ceftaroline	Rx	30 mg
Erythromycin	E	15 mg
Trimethoprim- sulfamethoxazole	SXT	1.25/23.75

Figure (1): VITEK 2C: Automated solution for bacterial identification and antibiotic sensitivity.

Results:

Coagulase positive *S. aureus* was identified by morphological and culture characters as well as identical biochemical tests as:

Morphological characteristics of colonies:

On mannitol salt agar, colonies were yellow color surrounded by yellow halo with yellow colored medium as *S. aureus* ferment mannitol salt convert color from pink to yellow. On Baired Parker medium colonies appear black large in size surrounded by a clear zone and opalescent ring immediately in contact with colonies.

Microscopic examination:

Using gram staining method which show gram positive cocci bacteria arranged as grape like clusters under light microscope.

Biochemical reactions:

Table (2): *Results of catalase test*

No. of Isolates	Catalase test
48	+ve
102	-ve

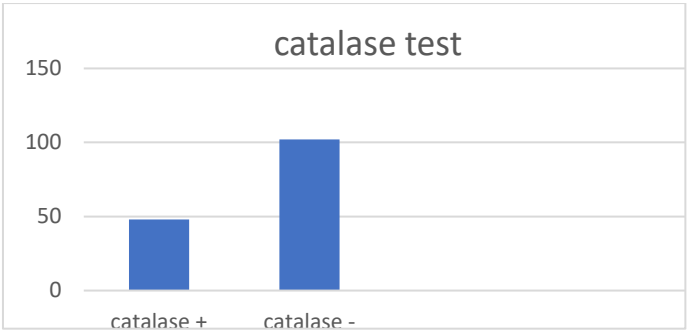

Figure (2): Result of catalase test

Table (3): *Result of the coagulase test:*

No. of sample	No. of positive	No. of negative
48	25	23
Percent	52.08 %	47.92 %

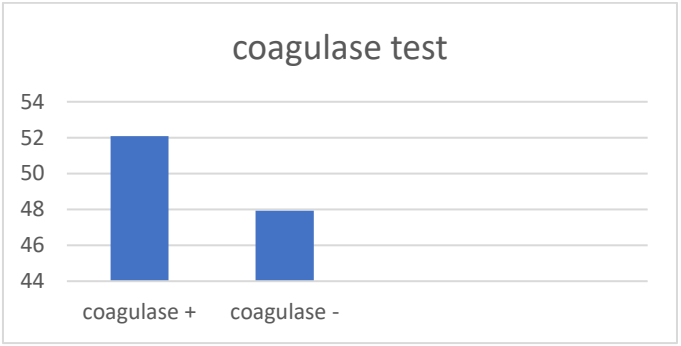

Figure (3): results of coagulase test

Table (4)*: Result of culture on Mannitol salt agar (MSA):*

No. of positive	No. of positive growth on MSA	No. of Mannitol fermenter isolate
48	20	28
Percentage	41.77 %	58.33%

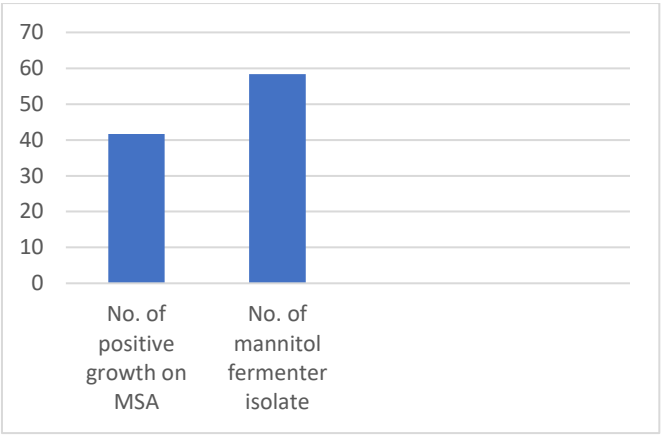

Figure (4): Result of Mannitol fermentation

Table (5): *Result of tellurium reduction and lipase activity on Baired Parker Media:*

No. of sample	Tellurite reduction	Lipase activity
48	48	28
Percentage	100%	58.33%

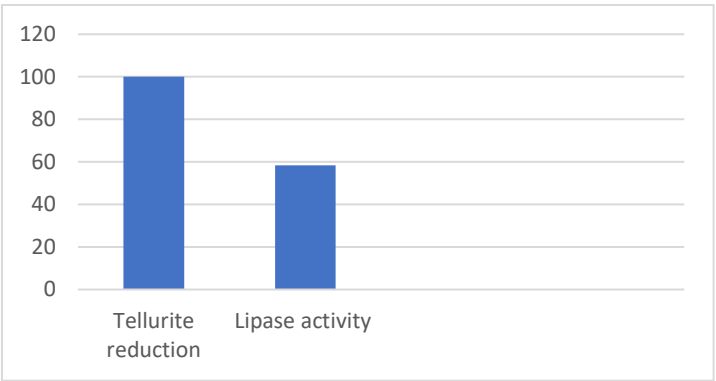

Figure (5): *Result of tellurium reduction and lipase activity on Baired Parker media*

Table (6): *Incidence of Staphylococcus aureus in the examined raw cow's milk:*

No. of milk sample	*Staphylococcus aureus* isolates	
150	**No. of positive**	**Percentage**
	48	32 %

Table (7): *Result of the sensitivity tests for the isolated coagulase positive S. aureus*

Antimicrobial discs	Sensitive		Intermediate		Resistant	
	No.	Percent.	No.	Percent.	No.	Percent.
B-Lactam						
Penicillin G	14	56%	5	20%	6	24%
Oxacillin	14	56%	5	20%	6	24%
Aminoglycosides						
Gentamycin	15	60%	10	40%	-	-
Amikacin	20	80%	5	20%	-	-
Tetracyclines						
Tetracycline	14	56%	5	20%	6	24%
Fluoroquinolones						
Ciprofloxacin	13	52%	8	32%	4	16%
Trimethoprim sulfamethoxazole						
SXT	14	56%	11	44%	-	-
Macrolides						
Erythromycin	16	64%	9	36%	-	-
Phenicols						
Chloramphenicol	18	72%	7	28%	-	
Cephems						
Ceftaroline	12	48%	13	52%	-	-

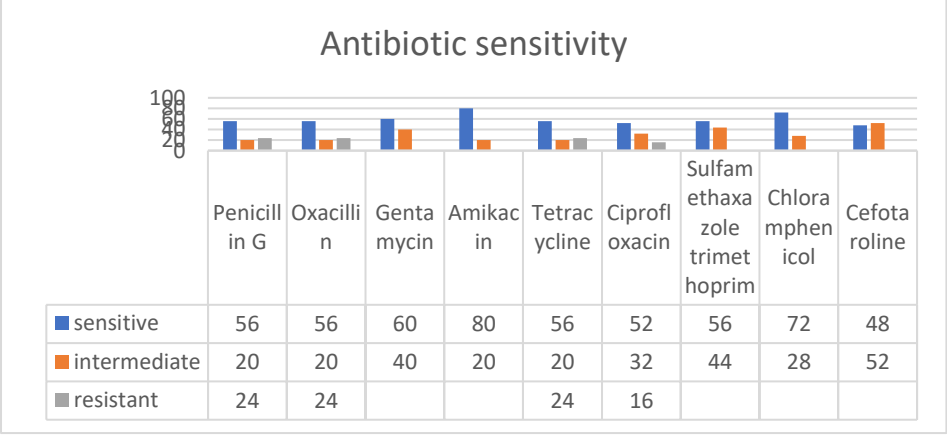

Figure (6): *S.aureus* shows different percentage of sensitivity to the used antibiotic discs

Discussion:

S. aureus is a pathogenic bacterium that can cause a variety of infectious illnesses, ranging from cutaneous to systemic infections, all of which can be fatal. ***(Decline et al., 2020).*** In the recent study, a

total of 150 raw cow's milk samples were examined bacteriologically to investigate the prevalence of staphylococci. Forty-eight (48) staphylococci isolates were isolated from 150 milk samples with a percentage of 32 %. These results differ from results conducted by ***(Patel et al., 2018)*** that showed the incidence of *S. aureus* isolated from raw milk in India is 10.16 % which higher than other studies in India as that done by ***(Thaker et al., 2013)*** who reported 6.25% and lower than ***(Kumar and Prasad, 2010)*** who reported 26% in other study. In raw milk samples, ***(Lingathurai and Vellathurai, 2011)*** found 61.7 % prevalence of *S. aureus* that higher than this study. Results of this study is nearly similar to other studies in different areas ***as (Bendahon et al., 2008; Farhan and Salk, 2007; Jahan et al., 2015 and Erhan Keyvan et al., 2020)*** found prevalence rates of 40%, 36.9%, 25.53 % and 37.32% in Morocco, Palestine, and Bangladesh and Turkey respectively. Researchers from Switzerland, Iran, and Brazil, on the other hand, found lower levels of *S. aureus* than those found in the current study ***(Jamali et al., 2015; Lee et al., 2012).*** Because of sample size, antibiotic use in animal husbandry, and hygienic procedures among dairy cows, prevalence results differed from place to place and region to region around the world. The significant prevalence of *S. aureus* is a sign of poor hygienic practices in food manufacturing, distribution, and handling ***(Vyas et al., 2015).*** In this study, all *S. aureus* isolates were Gram-positive cocci arranged in grape like clusters shown under microscope by Gram staining and they were coagulase producer as coagulase test is the main characteristic and most reliable phenotypic method for identification of *S. aureus*. These results came parallel with ***(Howard and Kloss, 1993; Wladimir et al., 2000)*** who found that *S. aureus* strains were Gram-positive cocci. Different biochemical assays were performed on all isolates, including coagulase, catalase, oxidase, vogos proskauer, methyl red, citrate utilization, and triple sugar iron agar tests. By yellow color of slant and buttom, all tested isolates were found to be coagulase positive, catalase positive, oxidase negative, indol test negative, methyl red test positive, vogos proskauer test positive, citrate utilization test negative, and triple sugar iron test positive without production of H2S gas. These findings agreed with findings of other studies ***(Habib et al., 2015; Reddy et al., 2015 and Ramya et al., 2017)*** who subiected *S. aureus* isolates to the same biochemical tests and found the same results. In this study *S.*

aureus give characteristic black-centered colonies surrounded by a halo zone onto Baired Parker medium. Also, Mannitol salt agar is a selective and indicator medium contain mannitol 1% and sodium chloride 7.5% with phenol red as indicator of acid production *S. aureus* ferment mannitol form colonies that turn the indicator to yellow colour. The same was confirmed by ***(Quinn et al., 1994; Colle et al., 1996 and Mackei and Mccarthey, 1996)*** who reported that Mannitol salt agar and Baired Parker medium are used and specifically in food microbiology. *S. aureus* gives characteristic black-centered colonies surrounded by an area of clearing, sometimes with an area of opacity within onto Baired Parker and mannitol salt agar is selective and indicator medium for *S.aureus*.

Penicillin G and oxacillin are B-lactam antibiotics, according to the results of antibiotic sensitivity tests. In the treatment of mastitis in dairy cows, B-lactam antibiotics are commonly utilized. The interaction of three heavy molecules and one light molecule in penicillin binding proteins gives this antibiotic its activity in *S. aureus*. Penicillin binding protein has an impact on peptidoglycan cell wall production and cell proliferation. Penicillin binding protein (PBPs), an enzyme for peptidoglycan production, is inhibited by B-lactam antibiotics ***(Effendi et al., 2019).*** There have been a slew of studies on *S. aureus* multi-resistance in recent years (resistance to other antibiotics as well as B-lactams) ***(Brennan et al., 2016; Ganai et al., 2016; Gopal et al., 2017)*** and this agreed with this study as *S. aureus* isolates show resistance against penicillin G, oxacillin, tetracycline and ciprofloxacin antibiotics. A study done by ***(Harish et al., 2019)*** reported that all *S. aureus* isolates show variable resistance to antibiotics tested. Maximum resistance was observed for penicillin G (74.9%) followed by gentamycin (27.2%), erythromycin (21%), cefoxitin (15.2%), ciprofloxacin (11.5%), tetracycline (7.8%), cotrimoxazole (5.8%) and chloramphenicol (0.5%) and these results agreed with results of ***(Hanson et al., 2011; Fawzy et al., 2017)*** who reported 77.7% and 73.6% penicillin resistance respectively and this differ from these findings. In this study resistance to tetracycline was found to be 24% among *S. aureus* isolates and this differ from results of a study reported by ***(Shamila-Syuhada et al., 2016)*** who revealed that there is 5% resistance to tetracycline among *S. aureus* isolates in Penang, Malaysia. Higher resistance to tetracycline among *S. aureus* isolates than this study was observed in others

(Mirzaei et al., 2012; Jackson et al., 2013; Feng et al., 2016) who reported 23%, 11.84% and 25% resistance respectively and only 1% tetracycline resistance was observed among *S. aureus* in a study conducted by ***(Wang et al., 2018)*** which is lower than the results of this study.

Conclusion:

Recently, the rise of multidrug resistant pathogenic infections in livestock has become a global concern. The rapid proliferation of drug-resistant strains aided by intrinsic or genetic features, is concerning since it complicates chemotherapy and diagnosis. Multidrug resistant strains can be transferred from livestock to people and vice versa. Each institution developed for multidrug resistant pathogens control in animals depend on its infection in humans and animals, particularly dairy animals and this information is critical for the development of specialized multidrug resistant pathogens control guidelines in veterinary practice.

References:

(APHA) American Public Health Association (1992): Compendium of methods for microbiological examination of foods. 2nd Ed. American Public Health Association, Washington, DC, USA.

(FDA) Food and Drug Adminstration (2001): Food Drug Adm. Bact. Analytical manual,.Chapter 12 *Staphylococcus aureus*. Reginald, W.B. and Gayle A.L.

Abdelazeem M Algammal, Hany R Hashem, Amenah S Al-Otaibi, Khyreyah J Alfifi, Esraa M El-Dawody, Eman Mahrous, Helal F Hetta, Ali W El-Kholy, Hazem Ramadan, Reham M El-Tarabili (2021): Emerging MDR-*Mycobacterium avium* subsp. avium in house-reared domestic birds as the first report in Egypt. BMC Microbiol. 2021 Aug 26;21(1):237.

Abdelazeem M Algammal, Helal F Hetta, Amr Elkelish, Dalal Hussien H Alkhalifah, Wael N Hozzein, Gaber El-Saber Batiha, Nihal El Nahhas, Mahmoud A Mabrok (2020): Methicillin-Resistant *Staphylococcus aureus* (MRSA): One Health Perspective Approach to the Bacterium Epidemiology, Virulence Factors, Antibiotic-Resistance, and Zoonotic Impact. Infect Drug Resist. 2020 Sep 22;13: 3255-3265.

Abutarbush, S. M. (2010): Veterinary medicine-a textbook of the diseases of cattle, horses, sheep, pigs and goats. The Canadian Veterinary Journal, 51(5), 541.

Ali, Y.; Islam, A.; Muzahid, N. H.; Sikder, O. F.; Hossain, A.; Marzan, L. W. (2017): Characterization, Prevalence and Antibiogram Study of *Staphylococcus Aureus* in Poultry. *Asian Pac. J. Trop. Biomed.* 2017, *7*(3), 253–256.

Bendahou A., Labbadi M., Ennanei L., Essadqui F.Z., and Abid M. (2008): Characterization of staphylococcus species isolated from raw milk and milk products, Iben and jben in northern Morocco.J. Infected Developing countries, 2(3): 218-225.

Brennan, G. I., Abbott, Y., Burn, A., Leonard, F., McManus, B. A., O'Connell, B., Coleman, D. C., & Shore, A. C. (2016): The emergence and spread of multiple livestock-associated clonal complex 398 methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains among animals and humans in the Republic of Ireland, 2010–2014. PLoS One, 11, 1–11.

CLSI (2016) Performance Standards for Antimicrobial Susceptibility Testing. 2016, 26th ed. CLSI supplement M100S.

Colle J.S., Granser A.G., Marmion B.P. and Simmons A. (1996): Practical Medical Microbiology (Mackie and McCartnery). Edited by Intrnational Student Ed. Of 14th edition. Chapter 11-C.USA. By Churchill Livingstone.

Decline V, Effendi MH, Rahmaniar RP, Yanestria SM, and Harijani N. (2020) Profile of antibiotic-resistant and presence of methicillin-resistant *Staphylococcus aureus* from nasal swab of dogs from several animal clinics in Surabaya, Indonesia, Int. J. One Health, 2020; 6(1): 90-94

Dehkordi, S. F.; Gandomi, H.; Basti, A. A.; Misaghi, A.; Rahimi, E. (2017): Phenotypic and Genotypic Characterization of Antibiotic Resistance of Methicillin-resistant *Staphylococcus Aureus* Isolated from Hospital Food. *Antimicrob.l Resist. Infect. Control*. 2017, *6*, 104.

Doulgeraki, A. I., Di Ciccio, P., Ianieri, A., & Nychas, G. E. (2017): Methicillin-resistant food-related *Staphylococcus aureus*: A review of current knowledge and biofilm formation for future studies and applications. *Research in Microbiology*, 168(1), 1-15.

Effendi M. H., Hisyam M. A. M., Hastutiek P., Tyasningsih W. (2019) Detection of coagulase gene in *Staphylococcus aureus* from several dairy farms in East Java, Indonesia, by polymerase chain reaction, *Vet. World*, 2019. **12**(1): 68-71.

Erhan Keyvan, Ozen Yurdakul, Ahu Demirtas, Halil Yalcin, Nuket Bilgen (2020): Identification of Methicillin-Resistant *Staphylococcus aureus* in Bulk Tank Milk. Food Sci. Technol, Campinas, 40(1): 150-156, Jan.-Mar. 2020.

Farhan, M. and Salk, S. (2007): Evaluation of bacteriological contamination in raw (unprocessed) milk sold in different regions of Lahore (Pakistan). *J. Agric. Soc. Sci.*, 3: 1813-2235.

Fawzy R, Samy AA, Salam HS, Khairy EA, Koraney AA. (2017): Polymerase chain reaction detection of genes responsible for multiple antibiotic resistance *Staphylococcus aureus* isolated from food of animal origin in Egypt. Veterinary world. 2017; 10(10):1205- 1211.

Ganai, A. W., Kotwal, S. K., Wani, N., Malik, M. A., Jeelani, R., Kour, S., & Zargar, R. (2016): Detection of *mec*A gene of methicillin resistant *Staphylococcus aureus* by PCR assay from raw milk. Indian Journal of Animal Sciences, 86(5), 508–511.

Gopal, S., & Divya, K. C. (2017): Can methicillin-resistant *Staphylococcus aureus* prevalence from dairy cows in India act as potential risk for community-associated infections: A review. Veterinary World, 10(3), 311–318.

Gundogan, N., & Avci, E. (2014): Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. *International Journal of Dairy Technology*, 67(4), 562-569.

Habib F, Rind R, Durani N, Bhutto AL, Buriro RS, Tunio A. (2015): Morphological and cultural characterization of *Staphylococcus aureus* isolated from different animal species. Journal of Applied Environmental and Biological Sciences. 2015; 5(2):15-26.

Hanson BM, Dressler AE, Harper AL, Scheibel RP, Wardyn SE, Roberts LK. (2011): Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. Journal of infection and public health. 2011; 4(4):169-174.

Hennekinne, J. A., de Buyser, M. L. and Dragacci, S. (2012): *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiology Reviews*. 2012; 36: 815–836.

Howard B.J. and Kloss W.E. (1993): Staphylococci. Chapter 12.

In: Clinical and pathogenic Microbiology, 2nd edition 243-256.

Jackson CR, Davis JA, Barrett JB. (2013): Prevalence and characterization of methicillin resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. Journal of Clinical Microbiology. 2013; 51(4):1199-1207.

Jamali, H., Paydar, M., Radmehr, B., Ismail, S., & Dadrasnia, A. (2015): Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control*, 54, 383- 388.

K Harish, A Jagadeesh Babu, T Madhava Rao, B Somasekhara Goud and Y Suresh (2019): A study on the tetracycline resistance *Staphylococcus aureus* from milk. The Pharma Innovation Journal 2019; 8(9): 316-323.

Kalayu AA, Woldetsadik DA, Woldeamanuel Y, Wang S-H, Gebreyes WA, Teferi T (2020): Burden and antimicrobial resistance of *S. aureus* in dairy farms in Mekelle, Northern Ethiopia. BMC Vet Res. 2020;16(1):20.

Kumar, R. and Prasad, A. (2010): Detection of *E. coli* and *Staphylococcus* in milk and milk products in and around Pantnagar. *Veterinary world. 3 (11):495-496.*

Lakech Tibebu, Yerega Belete, Eyasu Tigabu, and Wondewossen Tsegaye (2021): Prevalence of *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* and Potential Risk Factors in Selected Dairy Farms at the Interface of Animal and Human in Bishoftu, Ethiopia. Vet Med (Auckl). 2021; 12: 241–251.

Lee, S. H. I., Camargo, C. H., Gonçalves, J. L., Cruz, A. G., Sartori, B. T., Machado, M. B., & Oliveira, C. A. F. (2012): Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. Journal of Dairy Science, 95(12), 7377-7383.

Lingathurai, S. and Vellathurai, P. (2011): Bacteriological quality and safety of raw cow milk in Madurai, South India. *Webmed. Cent. Microbiol*, 1: 1-10.

Mackie and McCartney (1996): Practical Medical Microbiology. Intrnational student Ed., of 14th edition, Chaptr 11. Distributed in U.S.A. BY Churchill Livengtone.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard

definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.

Mbindyo, C.M., Gitao, G.C. and Mulei, C.M. (2020): Prevalence, etiology, and risk factors of mastitis in dairy cattle in Embu and Kajiado Counties, Kenya. Vet. Med. Int., 2020: 8831172.

Mirzaei H, Farhoudi H, Tavassoli H, Farajli M, Monadi A. (2012): Presence and antimicrobial susceptibility of methicillin resistant *Staphylococcus aureus* in raw and pasteurized milk and ice cream in Tabriz by culture and PCR techniques. African Journal of Microbiology Research. 2002; 6(32):6224-6229.

Mohamed Elsayed Enany, Abdelazeem Mohamed Algammal, Gehan Ibrahim Shagar, Amr Mohamed Hanora, Wael Kamel Elfeil, Noha Mohamed Elshaffy (2018): Molecular typing and evaluation of Sidr honey inhibitory effect on virulence genes of MRSA strains isolated from catfish in Egypt. Pak J Pharm Sci. 2018 Sep;31(5):1865-1870.

Oniciuc, E. A., Nicolau, A. I., Hernández, M., & Rodríguez-Lázaro, D. (2017): Presence of methicillin resistant *Staphylococcus aureus* in the food chain. Trends in Food Science & Technology, 61, 49-59.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013): Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerging Infectious Diseases Journal*,19(3),407-415.

Quinn, P.J.; Cater, M.E.; Markey, B.K. and Cater, G.R., (1994): Clinical Veterinary Microbiological Mosby-Year Book Europe Limited. Staphylococcus species: 118-127 and Aeromonas, plesiomonas, Vibrio spp: 243-248.

Quninn, P.J.; Markey, M.E.; Carter, W.J.; Donnelly, F.C.; Leonard and Maguire, D. (2002): Veterinary Microbiology and Microbial Disease. 1st published, Oxford: Blackwell Science Ltd.

R.K. Patel, R. Kumar, C.V. Savalia and N.G. Patel (2018): Isolation of *Staphylococcus aureus* from Raw Cattle Milk and their Drug Resistance Pattern International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706.

Rabab R Makharita, Iman El-Kholy, Helal F Hetta, Moahmed H Abdelaziz, Fatma I Hagagy, Amera A Ahmed, Abdelazeem M Algammal (2020): Antibiogram and Genetic Characterization of Carbapenem-Resistant Gram-

Negative Pathogens Incriminated in Healthcare-Associated Infections. Infect Drug Resist. 2020 Nov 4; 13:3991-4002.

Ramya P. (2017): Prevalence of *Staphylococcus aureus* in raw milk samples. International Journal of Chemical Studies. 2017; 5(5):1351-1353.

Reddy MS, Babu AJ, Ramya P, Swetha CS. (2015): Molecular characterization of methicillin resistant *Staphylococcus aureus* from goats, pigs and their handlers. International Journal of Microbiology Research. 2015; 7(3):648-655.

Ryan, K. J. and Ray C.G. (2004): Sherris Medical Microbiology 4th ed. McGraw Hill. ISBN 0-8385-8529-9.

Saadat YR, 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 microbiology. 2014 Oct;6(5):345.

Said Kamal Abolghait, Amal Gamal Fathi, Fatma Mohamed Youssef, Abdelazeem Mohamed Algammal (2020): Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. Int J Food Microbiol. 2020 Sep 2; 328:108669.

Shamila-Syuhada AK, Rusul G, Wan-Nadiah WA, Chuah LO. (2016): Prevalence and antibiotics resistance of *Staphylococcus aureus* isolates isolated from raw milk obtained from small-scale dairy farms in Penang, Malaysia. Pakistan Veterinary Journal. 2016; 36(1):98-102.

Swetha, C. S., Supriya, R. A., Goud, S. S., Babu A. J. and Rao, T. M. (2017): A study on the prevalence of zoonotic important methicillin resistant and vancomycin resistant *Staphylococcus aureus* (MRSA & VRSA) and coagulase negative Staphylococci (MR-CNS & VR-CNS) in raw milk samples of Tirupati, Andhra Pradesh. *The Pharma Innovation Journal*., 2017; 9: 17-24.

Thaker, H.C., Brahmbhatt, M.N. and Nayak, J.B. (2013) Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet. World, 6(1): 10-13.

Vyas A., Sharma, M., Kumar, S., Kumar M. and Mehra, S. K. A. (2015): comparative study of oxacillin screen agar, oxacillin disc diffusion and Cefoxitin disc diffusion, oxacillin E-test method for routine screening of methicillin resistant *Staphylococcus aureus*.

International Journal of Current Research., 2015; 7: 55-60.

Wang W, Lin X, Jiang T, Peng Z, Xu J, Yi L (2018): Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy cows with mastitis in Beijing, China. Frontiers in microbiology. 2018; 9(1123):1-16.

Wladimir Padiha da Sliva; Maria Teresa Destro; Mariza Landgraf; Bernadette D.G.M Franco. (2000): Biochemical characteristics of typical and atypical *Staphylococcus aureus* in mastitic milk and environmental samples of Brazilian dairy farms. Brazilian Journal of Microbiology, 31:103-106.

الملخص العربي

المكورات العنقودية الذهبية (*S.aureus*) هي واحدة من أهم مسببات الأمراض التي تدخل في العدوى التي تنقلها الأغذية من استهلاك الحليب ومنتجات الألبان. تستخدم المضادات الحيوية لعلاج الماشية بشكل شائع ، مما يؤدي إلى مشكلة مقاومة المضادات الحيوية للمكورات العنقودية الذهبية التي تنطوي على مخاطر كبيرة على صحة الأفراد في جميع أنحاء العالم. تعتبر المكورات العنقودية الذهبية واحدة من أهم مسببات الأمراض بسبب قدرتها على التسبب في المرض بوساطة السموم ، ومقاومة المضادات الحيوية ، والغزو. أجريت الدراسة الحالية على 150 عينة من اللبن البقري الخام من مصادر مختلفة في محافظة دمياط. وأخذت العينات المفحوصة في صندوق ثلج خلال ساعة للفحص البكتيريولوجي. أظهرت النتائج أن 48 عزلة من أصل 150 (32٪) كانت موجبة لبكتريا(*S.aureus*). من بين تلك الـ 48 عزلة ، كانت 25 عزلة (52.08٪) موجبة لتجلط الدم و 23 عزلة أخرى (47.92٪) كانت سلبية لتجلط الدم. بالكشف عن الحساسية للمضادات الحيوية لعزلات المكورات العنقودية الذهبية بطريقة الانتشار القرصي وتأكيدها بالطريقة المدمجة viteck2 أنه مقابل 10 أقراص مضادة للميكروبات من بكتريا المكورة العنقودية البرتقالية تظهر مقاومة ضد مضادات البنسلين جي وأوكساسيلين والتتراسيكلين بنسبة 24٪ تليها مقاومة 16٪ للسيبروفلوكساسين وتظهر حساسية جيدة ضد المضادات الحيوية amikacin و chloramphenicol و erythromycin. تم الحصول على هذه النتائج وفقًا لـ CLSI (2016). هدفت هذه الدراسة إلى عزل والتعرف على بكتيريا العنقودية الذهبية الموجبة المقاومة للأدوية من حليب البقر الخام في مناطق مختلفة من محافظة دمياط وتقييم نشاطها ضد مجموعة مختلفة من المضادات الحيوية.