Detection of Some Virulence Factors in Staphylococcus Aureus Isolated from Different Sources in Erbil City

Berivan Ali Ahmad Hasary [1] & Payman Ali Kareem[2]

[1&2] Food Technology Department, College of Agriculture Engineering Science, Salahaddin University, Erbil, Iraq
Correspondence: Berivan Ali Ahmad Hasary, Food Technology Department, College of Agriculture Engineering Science, Salahaddin University, Erbil, Iraq.
Email: berivan.ahmed@student.su.edu.krd

Doi: 10.23918/eajse.v8i3p320

Abstract: A total of 110 different samples were collected randomly from November 2021 to the end of February 2022 in Erbil city, in order to isolate different types of Staphylococcus, which are isolated from different sources like (milk, cheese, ice cream, meat, udder, hand worker, and nose worker). Staphylococcus aureus was examined for antibiotic susceptibility. The isolates were determined by the disk diffusion method. Twelve types of antibiotics have been chosen. S. aureus is more resistant to the Ciprofloxacin antibiotic and less resistant to the Streptomycin antibiotic. The virulence factors that the results have shown the ability of S. aureus to produce hemolycin in 59.7%, DNase in 17.7%, urease in 33.9%, lecithanase in 41.9%, lipase in 30.6%, B-Lactamease in 43.5%, coagulase enzyme in 61.3% and also protase in 61.3%. The biofilm forming ability of the isolates was investigated by the microtiter plate method. The production of biofilms by S. aureus isolates is 100%. 39.7% of the biofilm-positive S. aureus strains were strong producers, 35.5% were moderate producers, 17% were weak producers, and 7.5% were none.

Keywords: Staphylococcus Aureus, Virulence Factor, Antibiotics, Biofilm, Microtiter Plate Method

1. Introduction

Staphylococcus aureus is a gram-positive, aerobic bacterium that is a major cause of human infections. S. aureus is carried by approximately 30 percentage points of the healthy adult population. The perineum, anterior nares, skin, and pharynx are common sites of carriage (Verhoeven, et al., 2014).

Despite the fact that S. aureus has a wide spectrum of clinical diseases, including folliculitis, which is a very benign infection, it has the potential to cause invasive infections in society and in the medical environment (e.g., bloodstream infection). Hospital-acquired bloodstream infections (HA-BSIs), surgical site infections (SSIs), and pneumonia are all caused by S. aureus. These are key contributors to disease, mortality, and rising healthcare costs, all of which are linked to higher rates of morbidity and mortality (Troeman, et al., 2019).

Some virulent factors are produced by this bacterium, such as protein A, clumping factor, coagulase, hemolysin, nuclease, staphylococcal enterotoxin (SE), Panton-Valentine leukocidin, and toxic shock syndrome toxin-1. The bacterial toxin, SEs, is habitually implicated as the cardinal etiological agent of food poisoning in humankind (Hasan, & Hoshyar., 2019).

Received: June 1, 2022
Accepted: August 18, 2022
Hasary, B.A.A, & Kareem, P.A (2022). Detection of Some Virulence Factors in Staphylococcus Aureus Isolated from Different Sources in Erbil City. *Eurasian Journal of Science and Engineering*, *8*(3), 320-326.

Food handlers are a common and persistent source of sickness around the world. Many diseases are transmitted through food and are caused by living organisms. Raw foods, foods containing raw components, and foods bought from questionable sources have all been linked to severe gastroenteritis epidemics. Cooked moist protein-rich foods may become intoxication agents if they are kept for several hours without refrigeration or in containers (such as cans) without refrigeration (Argudín, et al., 2012). Staphylococcal defensive mechanisms and the ability of S. aureus to avoid clearance by the host's immune response may be to blame for the difficulties of treating prosthetic implant infections. S. aureus uses a well-developed biofilm to defy the host immune response and develop into a persistent infection as one of its most critical strategies. Bacterial cells adhered to a moist surface and embedded in a polysaccharide matrix constitute a biofilm (Prabhakara, et al., 2011).

It is common for prosthetic medical devices to become infected because they provide a good substrate for bacterial adhesion, colonization, and biofilm development, which alters bacteria's growth, gene expression, and protein production. Due to the enhanced susceptibility of bacteria in a biofilm to host defense and medications compared to those in a planktonic state, the eradication of S. aureus during prosthetic implant infection is particularly challenging (Basil, et al., 2014).

This study was a detection of some virulence factors in staphylococcus aureus isolated from different sources in Erbil City.

2. Materials and Methods

2.1 Sample Collection

A total of 110 different samples were collected randomly from November 2021 to the end of February 2022 in Erbil province, from (milk, cheese, ice cream, meat, udder, hand worker, and nose worker), of which 18 were milk, 17 were cheese, 11 samples of ice cream, and 16 samples from meat, 9 samples from the udder, 20 samples from hand worker, and 19 samples from nose. Samples were transported in a cooler box with ice packs (four to eight degrees) to the lab for examination in disposable sterile screw-cap containers. Others were taken by disposable swab. The samples were dispersed over Mannitol Salt agar and incubated at 37 degrees Celsius for 24 hours.

2.2 Isolation and Identification of S. Aureus

Each sample was incubated at 37°C for 24 hours on mannitol salt agar medium. There were a number of tests that were used to identify the bacteria: Gram staining; coagulase; catalase; hemolysis; glucose; mannitol fermentation. Test results can be confirmed with the Vitik 2 compact system (Basil, et al., 2014).

2.3 Determination of Virulence Factors

Testing was done on isolates that had been identified as methicillin-resistant by analyzing several virulence factors (hemolysin, DNase, urease, lecithinase, lipase, B-Lactamase, coagulase, protease) (Atiyea, et al., 2020).

2.4 Antimicrobial Susceptibility Testing

S. aureus– positive samples were selected for susceptibility tests. Antimicrobial susceptibility testing was done by the Kirby-Bauer disc diffusion method using muller hinton agar agar medium,

according to the Clinical Laboratory Standards Institute guidelines (CLSI,2020) Twelve antibiotics were examined in the current investigation, including Vancomycin(VA/30), Ciprofloxacin(CIP/5), Amikacin(AK/25), Gentamicin (CN/10), Amoxicillin (AX/25) , Amoxicillin/Clavulanic acid (AMC/30), Streptomycin (SP/10), Methicillin (MEC/10), Cefoxitin (FOX/30), Ceftriaxone (CRO/10), Imipenem (IPM/10), and Azithromycin (AZM/30).

2.5 Biofilm Formation by Microtiter Plate Method

This quantitative test described by (Christensen, et al., 1985). was regarded as the gold-standard method for detecting biofilm. The microtiter plate method was used to determine this. At 37 °C, each isolate was grown overnight in trypticase soy broth (TSB) with 0.25 percent glucose. The overnight growth was diluted in TSB-0.25 percent glucose at a 1:40 ratio. In sterile 96-well polystyrene Microtiter plates, 200 microliters of cell suspension were inoculated. After 24 hours, the wells were gently washed three times with 200 microliters of phosphate-buffered saline (PBS), dried inverted, and stained with 1% crystal violet for 15 minutes. To solubilize crystal violet, the wells were rinsed in 200 microliters of ethanol (95 percent). A microplate reader was used to calculate the optical density at 620 nm (OD 620). Each assay was carried out in triplicate, and the average optical density was used (Badave, & Kulkarni., 2015).

For biofilm determination, the following values were assigned:

Non-biofilm producer: OD < ODC-ve

Weak biofilm producer: ODC-ve ≤ OD<2 ODC-ve

Medium biofilm producer: 2 ODC-ve ≤ OD<4 ODC-ve

Strong biofilm producer: 4 ODC-ve <OD

3. Result and Discussion

Out of a total of 110 different samples that were collected, 62 isolates were isolated from Staphylococcus aureus and were identified using colonial morphology on culture media. The results shown in Figure 1 showed that 62 samples (56.4 %) produced bacterial growth, while 48 samples (43.6%) did not produce growth of bacteria, which could have been due to the use of antibiotics, the pathogen not being germinated and researched, or the pathogen being harder to identify in usual ways. Hand worker samples (11.8 %) were the most common source of Staphylococcus aureus. Nose worker samples were the second most common source (10 %), with cheese samples coming in third (8.2%), and after that, milk, meat, ice cream, and udder (4.5 %).

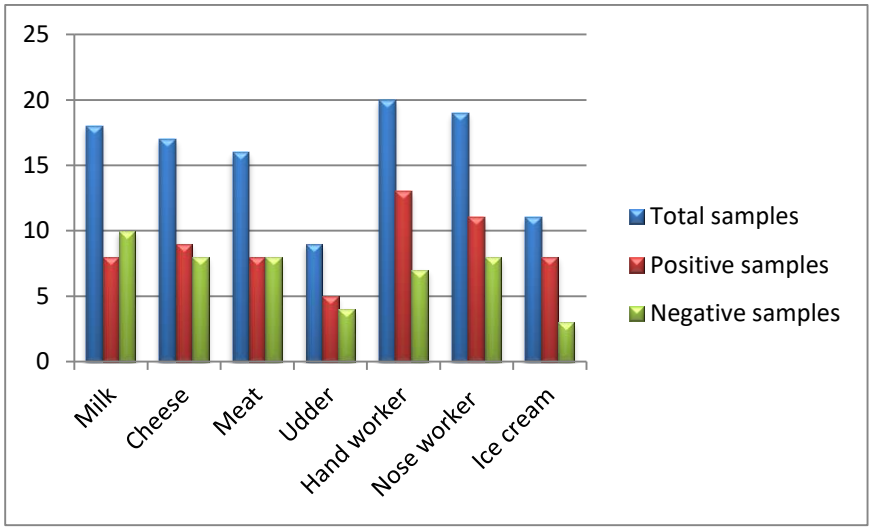

Figure 1: Numbers and percentages of isolated samples

For Staphylococcus aureus, the most important biochemical test results are listed in Table 1, which is (Catalase, Coagulase, Mannitol fermentation, Hemolysin, Gram stain, Glucose) were positive and Oxidase negative with the diagnostic step using Vitik 2, serving as a complement and confirmed diagnostic step (Konuku, et al, 2012).

Table 1: The biochemical tests of Staphylococcus aureus.

Test	Result
Oxidase	-
Catalase	+
Coagulase	+
Mannitol fermentation	+
Hemolysin	+ and
Gram stain	+
Glucose	+

Table 2 the outcomes of the identification of some virulence factors produced by Staphylococcal isolates showed that most of the isolates were producers of Hemolysin, DNase, urease, Lecithinase, lipase, B-Lactamase, coagulase, protease, and several others that aren't produced. These results are in accordance with those reported by other researchers (Akinjogunla & Enabulele, 2010).

Table 2: Relationship between virulence factors and different source of staphylococcus aureus.

Virulence factors	Milk	Cheese	Ice cream	Meat	Udder	Hand worker	Nose worker	Total
Hemolysin	5 (8.1%)	7 (11.3%)	6 (9.7%)	6 (9.7%)	0	6 (9.7%)	7 (11.3%)	37 (59.7%)
DNase	1 (1.6%)	1 (1.6%)	2 (3.2%)	3 (4.8%)	2 (3.2%)	2 (3.2%)	0	11 (17.7%)
Urease	4 (6.5%)	1 (1.6%)	2 (3.2%)	5 (8.1%)	0	7 (11.3%)	2 (3.2%)	21 (33.9%)
Lecithinase	6 (9.7%)	0	7 (11.3%)	3 (4.8%)	4 (6.5%)	5 (8.1%)	6 (9.7%)	26 (41.9%)
Lipase	4 (6.5%)	1 (1.6%)	4 (6.5%)	2 (3.2%)	3 (4.8%)	0	5 (8.1%)	19 (30.6%)
B-Lactamase	9 (14.5%)	1 (1.6%)	7 (11.3%)	0	1 (1.6%)	6 (9.7%)	3 (4.8%)	27 (43.5%)
Coagulase	9 (14.5%)	1 (1.6%)	9 (100)	8 (12.9%)	4 (6.5%)	7 (11.3%)	0	38 (61.3%)
Protease	3 (4.8%)	9 (14.5%)	4 (6.5%)	5 (8.1%)	0	9 (14.5%)	8 (12.9%)	38 (61.3%)

The virulence factors that the results have shown the ability of S. aureus to produce hemolycin in 59.7%, DNase in 17.7%, urease in 33.9%, Lecithanase in 41.9%, lipase in 30.6%, B-Lactamease in 43.5%, coagulase enzyme in 61.3% and also protase in 61.3%.

Table 3 revealed an exceptionally high rate of resistance to ciprofloxacin (87.5%) and amoxicillin (80%). While isolates are resistant to Vancomycin, Azithromycin, Amikacin, Ceftriaxone, Gentamicin, Imipenem,Methicillin, Cefoxitin, Amoxicillin/Clavulanic acid, and Streptomycin, the rates were 77.5%, 75%, 75%, 67.5%, 60%, 57.5%, 47.5%, and 37.5%, respectively. These results were in line with several other studies conducted around the world, and similar results were mentioned in (Hsu, et al., 2005 and Hasan & Hoshyar,.2019).

Table 3: Antibiotic Resistant of S. aureus.

Antibiotics	No.	Resistant (%)	Susceptible (%)	Intermediate (%)
CIP	40	35(87.5)	1(2.5)	4(10)
AX	40	32(80)	7(17.5)	1(2.5)
VA	40	31(77.5)	7(17.5)	2(5)
AZM	40	30(75)	5(12.5)	5(12.5)
AK	40	30(75)	10(25)	0
CRO	40	29(72.5)	7(17.5)	4(10)
CN	40	27(67.5)	8(20)	5(12.5)
IPM	40	24(60)	13(32.5)	3(7.5)
MEC	40	23 (57.5)	11(27.5)	6(15)
FOX	40	20(50)	13(32.5)	7(17.5)
AMC	40	19(47.5)	14(35)	7(17.5)
SP	40	15(37.5)	25(62.5)	0

The Microtiter Plate Method is used for determining the biofilm-forming ability of Staph. aureus from different sources. Figure 2 illustrates that 99.7 % of the isolates produced biofilms using the MTP method, though the level of production was diverse. The production of biofilms by S. aureus isolates is 100%. These findings are consistent with previous research (Aslantaş & Demir, 2016).

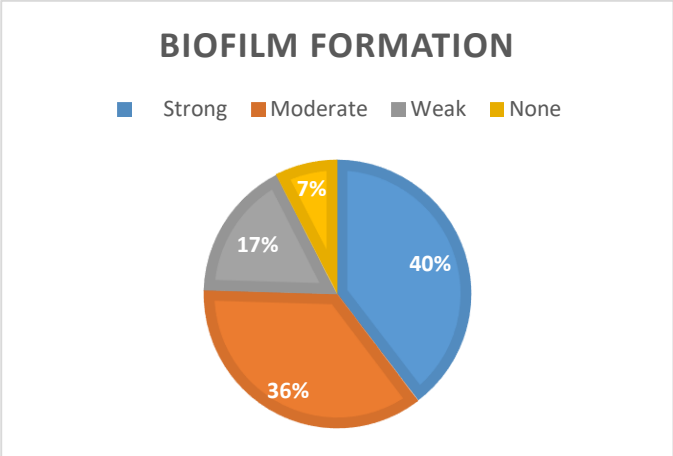

Figure 2: Correlation of biofilm production isolates from various samples by Microtiter plate (MP) method.

4. Conclusions

In terms of isolation, Staphylococcus aureus appears to be more prevalent in various sources, with the hand worker ranking first, followed by meat and the nose worker. Then comes cheese, milk and ice cream, poultry, bakery products, udder and cream high rate of resistance to Ciprofloxacin and a lower rate of resistance to Streptomycin, and the most of the isolates are produce virulence factor.

References

Akinjogunla, O. J., & Enabulele, I. O. (2010). Virulence factors, plasmid profiling and curing

analysis of multidrug resistant Staphylococcus aureus and coagulase negative Staphylococcus spp. isolated from patients with acute otitis media. *Journal of American science*, 6(11), 1022-1033.

Argudín, M. A., Mendoza, M. C., González-Hevia, M. A., Bances, M., Guerra, B., & Rodicio, M. R. (2012). Genotypes, exotoxin gene content, and antimicrobial resistance of Staphylococcus aureus strains recovered from foods and food handlers. *Applied and environmental microbiology*, 78(8), 2930-2935.

Aslantaş, Ö., & Demir, C. (2016). Investigation of the antibiotic resistance and biofilm-forming ability of Staphylococcus aureus from subclinical bovine mastitis cases. *Journal of Dairy Science*, 99(11), 8607-8613.

Atiyea, Q. M., Kareem, P. A., Aldowri, H. S., & Younis, R. W. MOLECULAR AND BACTERIOLOGICAL STUDY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM DIFFERENT SOURCES.

Badave, G. K., & Kulkarni, D. (2015). Biofilm producing multidrug resistant Acinetobacter baumannii: an emerging challenge. *Journal of clinical and diagnostic research: JCDR*, 9(1), DC08.

Basil, A. A., Mohammed, H. K., & Basim, M. H. (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.

Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *Journal of clinical microbiology*, 22(6), 996-1006.

Clinical Laboratory Standards Institute (CLSI). (2020). Performance standards for antimicrobial susceptibility testing 30th ed. CLSI supplement M100. Wayne,PA.

Hasan, N. S., & Hoshyar, D. F. (2019). Detection of enterotoxigenic Staphylococcus aureus strains in raw milk of cows reared in Erbil Province/Iraq. *Zanco Journal of Pure and Applied Sciences*, 31(4), 50-60.

Hsu, L. Y., Koh, T. H., Singh, K., Kang, M. L., Kurup, A., & Tan, B. H. (2005). Dissemination of multisusceptible methicillin-resistant Staphylococcus aureus in Singapore. *Journal of Clinical Microbiology*, 43(6), 2923-2925.

Konuku, S., Rajan, M. M., & Muruhan, S. (2012). Morphological and biochemical characteristics and antibiotic resistance pattern of Staphylococcus aureus isolated from grapes. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 2(1), 70.

Prabhakara, R., Harro, J. M., Leid, J. G., Harris, M., & Shirtliff, M. E. (2011). Murine immune response to a chronic Staphylococcus aureus biofilm infection. *Infection and immunity*, 79(4), 1789-1796.

Troeman, D. P. R., Van Hout, D., & Kluytmans, J. A. J. W. (2019). Antimicrobial approaches in the prevention of Staphylococcus aureus infections: a review. *Journal of Antimicrobial Chemotherapy*, 74(2), 281-294.

Verhoeven, P. O., Gagnaire, J., Botelho-Nevers, E., Grattard, F., Carricajo, A., Lucht, F., ... & Berthelot, P. (2014). Detection and clinical relevance of Staphylococcus aureus nasal carriage: an update. *Expert review of anti-infective therapy*, 12(1), 75-89.