JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S7

Impact of Coagulase Enzyme Extracted from Staphylococcus aureus on Breast, Gastric Cancer Cell Models

Marwa Ali Athab[1], Sawsan Hassan AUthman[2], Mohammed Fadhil Abo Ksour[3]

1. Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.,
   Email: marwaali1997@uomustansiriyah.edu.iq
2. College of science/Biology department/Mustansiriyah University.
   Email: dr.sawsanh@uomustansiriyah.edu.iq
3. Microbiolgy department, College of Science, Mustansiriyah university,
   Email: m.aboksour@uomustansiriyah.edu.iq

Abstract
Background;Coagulase is an enzyme involved in blood clotting and is a pathogen that helps *Staphylococcus aureus* evade the immune system response. Research shows that this enzyme may affect the growth of breast and gastric cancer cells, which calls for studying its potential effects on cell division, migration, and cell death, and thus understanding the relationship between bacterial infection and cancer progression.**Aims of the study;**Study of how the enzyme coagulase extracted from *Staphylococcus aureus* affects the growth of breast and gastric cancer cells.**Methodology;** Two hundred samples of blood, biopsies, wounds, vaginal swabs, and urine were collected from Baghdad hospitals. The samples were cultured on selective medium for *S. aureus* isolation, where the medium was sterilized by pressure at 121°C. In the laboratory, the samples were cultured on mannitol saline medium for 24 hours at 37°C, and then transferred to nutrient agar to obtain pure colonies. *S. aureus* was identified using Bergey's guide. Coagulase tests were performed using slide and tube assay to confirm the activity. After enzyme extraction, its activity was measured using spectrophotometric techniques. Ion exchange chromatography and gel filtration were used to purify the enzyme. Breast and gastric cancer cells were cultured, and MTT assay was used to evaluate the effect of the enzyme on the cells.**Result;**The study showed that coagulase caused toxic effects on MDA-MB-231 breast cancer cells and MKN45 gastric cancer cells in a dose-dependent manner. The survival rate of MDA-MB-231 cells decreased from 92.41% at 1.75 mg/mL to 2.15% at 140 mg/mL, while that of MKN45 decreased from 88.48% to 1.59%. The results showed that MKN45 was more sensitive at intermediate concentrations. These results suggest that coagulase has the potential to be used as an anticancer agent, but further research is needed to understand its mechanism of action and safety.**Conclusions**;The results show that the enzyme coagulase has a toxic effect on breast and gastric cancer cells, indicating its potential as an anticancer agent, although further research is needed to evaluate its safety.
Key word: Coagulase enzyme, Breast cancer, Gastric cancer, Cytotoxicity, Anticancer agent, *Staphylococcus aureus*

Introduction:

Staphylococcus aureus is a Gram-positive bacterium living as a commensal on the skin, mouth and upper respiratory system, making it a risk factor for opportunistic and nosocomial infections [1].*S. aureus* is present in the environment and in the normal human flora. It is found on the skin and mucous membranes (primarily in the nasal area) of most healthy people. *S. aureus* does not usually cause infections on healthy skin. However, when these bacteria enter the bloodstream or internal tissues, they can cause a variety of potentially serious infections. Transmission usually occurs through direct contact. However, some infections involve other modes of transmission [2,3].

Staphylococcus aureus has a spherical shape , It is salt tolerant and facultatively anaerobic. It may grow in mannitol-salt agar medium with 7.5% of sodium chloride and give positive hemolysis, coagulase, and catalase reactions but negative oxidase reactions. It is non-spore-forming, non-motile, and rarely capsulated [4].

S. aureus secretes two proteins that promote coagulation: coagulase (Coa) and von Willebrand factor binding protein (vWbp). Both proteins activate prothrombin in a non-proteolytic manner. The N-termini of Coa and vWbp are each attached to the prothrombin precursor, completing the active site that is unique to thrombin. Notably, activation mediated by Coa and vWbp does not involve cleavage of prothrombin by fVa and fXa [5,6]. Coa is a protein of approximately 670 amino acids, which vary in length between strains. The N-terminal 282 amino acids of Coa contain the alpha-helical D1-D2 domain that binds to the C-terminus of the prothrombin β chain. The D1-D2 domain is followed by a 153-residue linker region, the function of which remains unknown. The C-terminus of Coa consists of 2–8 tandem repeats of a 27-residue peptide, called the fibrinogen binding domain, which is located at the

C-terminus of another staphylococcal fibrinogen binding protein. Coa mediates the coagulation of soluble fibrinogen, plasma, or blood due to its interaction with fibrinogen and prothrombin [7,8]..

The role of coagulase in disease has been the subject of much research. After the discovery that Staphylococcus aureus sequesters plasma or blood clots, microbiologists discovered a link between the virulence of H. aureus isolates and their ability to coagulate plasma. Injection of coagulase-negative staphylococci incubated with purified coagulase into the bloodstream of mice resulted in increased mortality, even when the bacteria were washed prior to injection. However, when coagulase was injected prior to infection with coagulase-negative staphylococci, survival of the animals was not affected. These results suggest that the pathogenic effect of coagulase is due to the proximity of staphylococci to the resulting coagulation products [9].

The negatively charged surface of the bacteria activates the contact-dependent intrinsic coagulation cascade, which results in the formation of thrombin. Transglutaminases (represented by fXIII in mammals) are the most conserved factors in the coagulation cascade. Both *S. aureus* and E. coli are cross-linked by transglutaminases in the fibrin matrix of Drosophila hemolymph and human plasma. The ability to cross-link bacteria into clots reduces the burden of infection in insects. In a subcutaneous mouse model of *S. pyogenes* infection, transglutaminase also reduced bacterial load. These experiments demonstrate that the coagulation cascade is a key component of the innate immune defense against bacterial infection[10].

The bacterial surface also activates the complement cascade via the lectin pathway. Activation of mannan-binding lectin-associated serine protease 2 (MASP-2), which converts prothrombin to thrombin more slowly than the prothrombinase complex, leads to fibrin deposition on the target surface. If the bacterial surface activates coagulation, leading to its capture, why would staphylococci secrete two molecules (Coa and vWbp) that elicit a similar response? If the fibrin polymerization products of thrombin and staphylococcal coagulase are identical (Coa·prothrombin and vWbp·prothrombin), it would be difficult to discern a benefit to S. aureus pathogenesis. The Coa·prothrombin complex cleaves the fibrinopeptide at the same site as thrombin[11]. However, the biochemical and physiological properties of fibrin produced by staphylococcal coagulase are thought to differ from those produced by thrombin. Staphylococcal coagulase cleaves fibrinogen much more slowly than thrombin, and staphylococcal clots dissolve faster than physiological clots. Thromboelastography shows that clots produced by staphylococcal coagulase are weaker than those produced by thrombin. These data support the hypothesis that fibrin clots produced by staphylococcal coagulase are mechanically and morphologically different from clots produced by endogenous thrombin activation [12].

Clinical evidence suggests a link between the development, progression, and treatment of cancer and staphylococci. In many cases, infection with certain staphylococci accompanies and promotes tumor susceptibility. Hattar, K. et al. reported that lipoteichoic acid, an inflammatory mediator of Staphylococcus aureus, promotes the proliferation of lung cancer cell lines (A549 and H226) in vitro. Staphylococcus aureus infection has been found to promote lung metastasis of breast cancer cells through the formation of extracellular neutrophil traps [13]. Therefore, some cancer-related interventions may be based in part on the pathogenesis of Staphylococci. For example, drug resistance of Staphylococci and tumors may be circumvented by regulating intracellular reactive oxygen species [14]. Interestingly, there is continued evidence that certain Staphylococci have inhibitory effects on the proliferation, migration, and other biological behaviors of certain tumors [15].

Methodology:

2.1. Collection of Sample

A total of two hundred samples from blood, biopsies, wounds, vaginal swabs, and urine were collected from hospitals in Baghdad are Al-Shahed Alsadder Teaching Hospital and Ibn Al-Baladi Hospital. All samples were cultivated on selective media for isolation of *S.aureus* The media (from Himedia and others) were autoclave sterilized at 121°C for 15 minutes. All solutions and other materials have been prepared in advance.

***2*.2. Isolation of Bacteria**

In the laboratory and under aseptic conditions, the collected samples were cultured directly on a suitable selective media; the selective media used mannitol salt agar for 24 hours at 37ºC. Then sub-culture on the nutrient agar plate by streaking and incubation at 37°C for 24 hours to obtain pure well-isolated colonies, for identification.

2.3.Identification of Bacteria

In identifying *S. aureus*, Bergey's textbook on systematic bacteriology proved to be crucial [3].

2.4 Phenotypic detection of Coagulase enzyme

The slide test: was conducted to isolate bound coagulase. This involved suspending a single, uncontaminated bacterial colony in a mixture of normal saline and human plasma, while gently stirring. The test is classified as coagulase-positive, and the formation of clumps within a few seconds is observed. The control group consisted of

regular saline and bacteria without the addition of plasma. This was done to verify that the bacteria did not form clusters spontaneously in normal saline.

Tube test: for this tube experiment, a solitary colony of the bacteria was mixed with milliliters of human plasma (diluted in normal saline at a ratio of 1:6) and incubated for 24 hours at a temperature of 37 °C. The process of testing the tubes required a duration of one to four hours. The result was affirmative when a clot formed, and negative when the tube was incubated for a further 24 hours and examined again.

2.3 Detection Enzyme Activity

after the crude enzyme was extract "Plasma 0.2ml is diluted with 1.8 ml physiological saline solution." and then 1ml of diluted plasma is mixed with the 1 ml crude enzyme, and the tubes are incubated in a shaker incubator under ideal conditions (37C, 200rpm, 4 hours) . The time of clot formation is recorded, then the tubes are transferred to a centrifuge at (4C, 6000 rpm for 5 minutes). The supernatant is taken, and the absorption spectrum is measured at a wavelength of 450 nm. The enzyme activity was determined based on ability the coagulase to catalyzes the rapid breakdown of fibrinogen into fibrin and formation clots.

Enzyme Activity Unitl/ ml $= Absorbanceat\ 450\ nm / 0.01 * 30 * 1$

0.001=The amount of enzyme given 0.001 increace in absorbance

1= enzyme Volume

30= Time (min)

Specific activity = enzyme activity/ protein concentration

Total activity= enzyme activity *Volume

Purification Fold= Specific activity for the step / Specific activity for the first step (Crude)

Yield= Total activity for the step /Total activity for the first step(Crude)*100

2.4. Enzyme purification

2.4.1. Enzyme Extraction

Heart infusion broth was used for coagulase production. Staphylococci were grown in heart infusion broth for 48h at 37 C on a rotary shaker. The organisms were removed by centrifugation at 20,000 rpm in a continuous flow centrifuge.

2.4.2. Enzyme dialyzes

The dialysis process has been carried out to the solution collected from the Precipitation step against 0.1 M Tris HCl buffer pH 8 for 24 h at 4 C with continuous stirring and then concentrated by sucrose. Protein concentration, DNase activity and specific activity were estimated

2.4.3. Ion exchange chromatography (DEAE-Cellulose)

After the dialysis step, add 10 mL of sample to the ion exchange column. Set the flow rate to 60 ml/h and collect the eluate in 5 ml fractions. Elute the enzyme bound to the gel with elution buffer and a gradient of NaCl concentrations (0.2-1M). Measure the absorbance of each fraction at 280 nm. Estimate the coagulase activity as described above and determine the protein concentration of the peak..

2.4.4. Gel filtration chromatography (Sephadex G-100)

Sephadex G-150 was prepared as recommended by Pharmacia Fine Chemicals. A certain amount of Sephadex G-150 was suspended in 0.1 M Tris-HCl buffer pH 8, degassed and packed in a glass column (1.5 x 80 cm) and equilibrated with 0.1 M Tris-HCl buffer pH 8. A certain volume of concentrated DNase (obtained from the ion exchange step and previously equilibrated with 0.1 M Tris-HCl buffer pH 8) was added to the matrix. Elution was achieved with the same equilibration buffer at a flow rate of 3 ml/fraction. The absorbance of each fraction was measured at 280 nm..

2.5 Molecular weight determination

The molecular weight was determined using gel filtration chromatography. The column used was Sephadex G-150 (2 x 40 cm) prepared and washed with 0.05 M phosphate buffer at pH 7. Alcohol dehydrogenase with a molecular weight of 150,000, albumin with a molecular weight of 66,000, carbonic anhydrase with a molecular weight of 29,000, and lysozyme with a molecular weight of 14,300 were used as molecular weight markers. The pore volume at 600 nm was measured using blue dextran. The elution volume of each reference protein was found using a UV-Vis Bio-Rad spectrophotometer at a wavelength of 280 nm. The molecular weight of coagulase was measured by comparison with the molecular weight and elution volume of established reference proteins..

2.6 Cell line culture

Cell line culture involves the maintenance and growth of cells under controlled conditions. Begin by thawing frozen cells in a 37°C water bath, then transfer them to a tube containing pre-warmed complete medium, such as DMEM with 10% FBS. Centrifuge the cells at 200 g for 5 minutes, discard the supernatant, and resuspend the pellet in fresh

medium. Seed the cells into a culture flask and incubate at 37°C in a humidified atmosphere with 5% CO_2. Monitor the culture daily for cell growth and contamination. Replace the medium every 2-3 days. When cells reach 70-80% confluence, remove the spent medium, wash the cells with PBS, and add Trypsin-EDTA to detach them. Incubate for 2-5 minutes at 37°C, neutralize the trypsin with fresh medium, and transfer the cell suspension to a tube. Centrifuge discards the supernatant and resuspends in fresh medium. Seed the cells at a 1:3 or 1:5 ratio into new flasks. For long-term storage, freeze cells in 90% FBS and 10% DMSO, and store the cryovials in liquid nitrogen.

2.7 MTT assay

MTT is a colorimetric assay based on the cleavage of the tetrazolium ring of MTT (3-(4,5-dimethyltriazol-2-yl)-2,5-diphenyltetrazolium bromide) by dehydrogenases in the active mitochondria of living cells as an estimate of cell viability (Twentyman and Luscombe, 1987). Both MDA-MB-231 and MKN-45 cells were seeded at approximately 4 × 104 cells/well in 96-well plates (cells/mL) and incubated at 37°C for 24 hours. After 24 hours, the medium was removed from the wells and an equal amount (100 ml) of growth medium was added to each well, followed by the addition of different concentrations of enzyme for 48 hours, and then 100 ml of MTT was added to each well. After 4 hours of incubation, the supernatant was removed and 100 mL of dimethyl sulfoxide (DMSO) was added to each well. The samples were then shaken for 15 minutes to dissolve the dark blue crystals. Optical density was measured at a wavelength of 570 nm using a microplate reader (Bio-Rad UK). All experiments were performed in triplicate..

Statistical analysis:

Statistical analysis is commonly used to analyze quantitative data and provides methods for data description and simply infer continuous and categorical data. The process involves collecting data that leads to testing the relationship between two statistical data sets. In this study, all data are expressed as frequencies and percentages. We used variables with a normal distribution distribution, and we used SPSS (version 26) and dependent t-test (two-tailed) and independent t-test (two-tailed). For variables without a normal distribution distribution, we used the Mann-Whitney U test, Wilcoxon test, and chi-square test. $M<0.05$ was considered statistically significant.

Ethical approval:

The study was approved by the Ethics Committee of Al-Imamain Alkadhimain Medical City. All the people who participated in the study were informed about it and asked to sign a consent form. The patient was also assured that his information would be kept confidential..

Results

In this study, the cytotoxic effects of the coagulase enzyme were evaluated on two cancer cell lines: MDA-MB-231 (human breast cancer) and MKN45 (human gastric cancer) by measuring the survival fraction at varying concentrations. The data reveal a dose-dependent reduction in cell viability for both cell lines, with notable differences in sensitivity. The survival fraction of MDA-MB-231 cells significantly decreased with increasing concentrations of the coagulase enzyme, from 92.41% at 1.75 mg/ml to as low as 2.15% at 140 mg/ml. Similarly, MKN45 cells showed a reduction in viability, from 88.48% at 1.75 mg/ml to 1.59% at 140 mg/ml. Both cell lines demonstrated heightened sensitivity at higher enzyme concentrations, with MKN45 cells exhibiting slightly higher sensitivity at moderate concentrations (28 mg/ml: 34.02% vs. 57.35% in MDA-MB-231). At the highest concentration of 140 mg/ml, survival fractions were reduced to around 2% and 1.6% for MDA-MB-231 and MKN45 cells, respectively, indicating that coagulase has substantial cytotoxic effects on both cell types.

The observed dose-dependent cytotoxicity suggests that the coagulase enzyme possesses significant anticancer potential. At concentrations above 46 mg/ml, cell viability drops dramatically, especially in MDA-MB-231 cells, where survival fractions reach below 20%. This drastic reduction at high concentrations indicates that coagulase may interfere with essential cellular processes, possibly by inducing apoptosis or disrupting cellular membranes, leading to cell death. Such mechanisms are common in enzyme-based therapies, where enzymes alter the tumor microenvironment or interfere directly with cancer cell metabolism.

Interestingly, MKN45 cells exhibited higher sensitivity at lower concentrations compared to MDA-MB-231 cells, with a 28.28% survival fraction at 46 mg/ml (vs. 19.45% in MDA-MB-231). This differential response could reflect variations in the cell lines' inherent resistance mechanisms, such as membrane composition or metabolic activity. The lower overall survival fraction in MKN45 cells across concentrations could indicate that gastric cancer cells may be more susceptible to coagulase-induced damage than breast cancer cells.

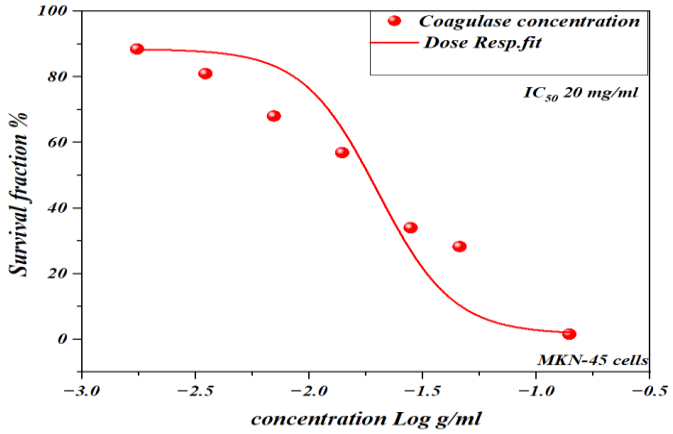
The significant reduction in cell viability at higher enzyme concentrations, particularly at 140 mg/ml, highlights coagulase's potential as an anticancer agent. However, while these in vitro results are promising, further research is necessary to understand the enzyme's mechanism of action and to determine its safety profile. One potential concern is coagulase's role in coagulation, which could lead to adverse effects such as thrombosis when applied in a therapeutic context. Future studies should focus on in vivo models to confirm the enzyme's efficacy and explore

methods of safely targeting coagulase to tumor sites, possibly through nanotechnology or antibody-conjugated delivery systems.

there is no widely recognized study that explicitly investigates the use of coagulase as an anticancer agent in mainstream cancer research. Most research involving coagulase has focused on its role in bacterial infections, particularly those caused by *Staphylococcus aureus*, where it functions by inducing blood clotting to protect bacteria from the host's immune system. but there is a study that explores the use of coagulase as an anticancer agent. Researchers have developed a novel approach by targeting coagulase to tumor vasculature using an NGR (Asn-Gly-Arg) motif. This targeted delivery system aims to induce selective thrombosis and infarction in tumor-feeding vessels, effectively arresting cancer cell growth. The study demonstrated that a genetically engineered fusion protein, consisting of truncated coagulase (tCoa) and the NGR motif, selectively accumulated in tumor sites and significantly reduced tumor growth in mouse models. This reduction was accompanied by massive thrombotic occlusion of tumor vessels, leading to tumor infarction and cell death[1]

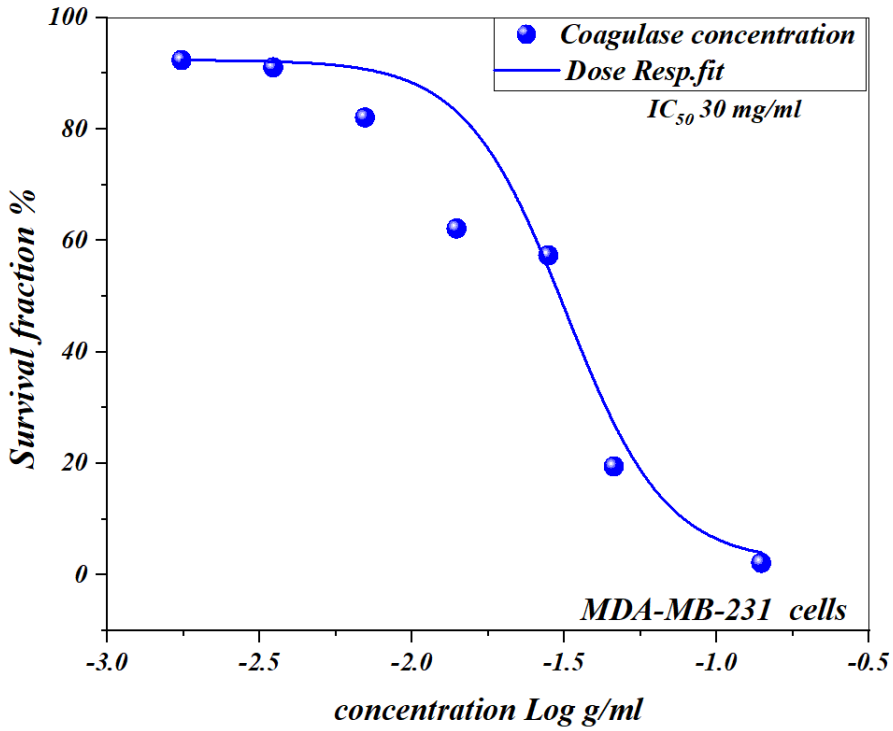
This study demonstrates that coagulase enzyme induces significant cytotoxicity in both MDA-MB-231 and MKN45 cancer cell lines in a dose-dependent manner. The results suggest that coagulase may have potential as an anticancer agent, but further investigation is needed to assess its therapeutic applicability and safety in vivo.

MKN45 cell line (Gastric cancer cell line) 48hrs (treatment)					
	O.D 1	**O.D.2**	**O.D.3**	**Mean**	**Survival Fraction**
Untreated	2.202	2.143	2.1725	2.1355	**100.00**
140 mg/ml	0.081	0.061	0.071	0.034	**1.59**
46 mg/ml	0.641		0.641	0.604	**28.28**
28 mg/ml	0.837	0.69	0.7635	0.7265	**34.02**
14 mg/ml	1.258	1.246	1.252	1.215	**56.90**
7 mg/ml	1.428	1.553	1.4905	1.4535	**68.06**
3.5 mg/ml	1.964	1.569	1.7665	1.7295	**80.99**
1.75 mg/ml	2.129	1.724	1.9265	1.8895	**88.48**



341

MDA-MB-231 Cell line (breast cell line cancer) 48 hrs treatment						
	O.D 1	O.D.2	O.D.3	Mean		**Survival Fraction**
untreated	0.957	0.946	0.949	0.950667	0.91	**100.00**
140 mg/ml	0.057	0.054	0.059	0.056667	0.02	**2.15**
46 mg/ml	0.235	0.245	0.164	0.214667	0.18	**19.45**
28 mg/ml	0.554	0.549	0.58	0.561	0.52	**57.35**
14 mg/ml	0.579	0.543	0.693	0.605	0.57	**62.17**
7 mg/ml	0.795	0.861	0.705	0.787	0.75	**82.09**
3.5 mg/ml	0.895	0.871	0.842	0.869333	0.83	**91.10**
1.75 mg/ml	0.907	0.891	0.846	0.881333	0.84	**92.41**
blank	0.037	0.035	0.04	0.037333		



Discussion:

The exploration of coagulase's impact on breast and gastric cancer is an emerging area of research that highlights the complex interplay between coagulation processes and tumor biology. The literature on this topic reveals significant insights into how coagulase and the broader coagulome contribute to cancer progression and therapeutic strategies [16].

In 2018, presented a foundational understanding of coagulase's mechanism, emphasizing its unique ability to bind to thrombin and prothrombin, which leads to the formation of proteolytically active complexes that generate fibrin without activating other clotting factors. This localized action of coagulase, which avoids a systemic coagulation response, positions it as a promising candidate for cancer therapy [17]. The study's functional analyses demonstrated that the targeted delivery of coagulase to tumor vasculature could effectively mediate its activity, suggesting a potential therapeutic avenue for inhibiting cancer cell growth [18].

Building on this foundation, Lottin et al., 2022, expanded the understanding of the tumor coagulome, particularly in the context of oral squamous cell carcinoma (OSCC) [19]. Their work highlighted the equilibrium between coagulation and fibrinolysis within tumors, revealing that the overexpression of pro-coagulant factors, such as tissue

factor (F3), is a pivotal event in establishing a hypercoagulable state in tumors [20]. The study further explored how thrombin's interaction with protease-activated receptor-1 (PAR-1) can modulate cancer cell growth and influence the tumor microenvironment (TME). This interaction underscores the multifaceted role of coagulation factors in cancer progression and the potential for targeting these pathways for therapeutic benefit [21].

Most recentlyexamined the relationship between biofilms and cancer, elucidating how the TME, which includes coagulase and other factors, contributes to tumor heterogeneity and immune evasion [22]. Their findings indicate that biofilms, formed through microbial interactions, can alter the extracellular matrix (ECM) and promote epithelial-to-mesenchymal transition (EMT), a critical process in cancer metastasis. The study highlights the role of the TME in modulating immune responses and the metabolic adaptations of cancer cells, such as the Warburg effect, which are essential for tumor survival and growth [23].

Collectively, these articles illustrate a nuanced understanding of how coagulase and the broader coagulation landscape influence cancer biology. The interplay between coagulation factors, the TME, and tumor progression reveals important implications for developing novel therapeutic strategies targeting coagulation pathways in breast and gastric cancer [24,25].

Conclusion:

The results show that the enzyme coagulase from *Staphylococcus aureus* has toxic effects on breast cancer (MDA-MB-231) and gastric cancer (MKN45) cells in a dose-dependent manner. As the concentration increased, the cell viability decreased significantly, suggesting the potential of coagulase as an anticancer agent. The study also showed that MKN45 cells were more sensitive than MDA-MB-231 cells at intermediate concentrations. Despite the promising results, further research is needed to understand the mechanism of action and evaluate the safety and potential therapeutic applications of the enzyme in biological models, especially considering its potential effects on coagulation.

References

1. Khwen, N. N., Authman, S. H., & AL-Marjani, M. F. (2021). Purification and Characterization of Thermo Stable DNase of Staphylococcus Aureus Isolated from Different Clinical Source. *Medico-Legal Update*, *Vol.*21,No.2,Apr2021.
2. Centers for Disease Control and Prevention (CDC). Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections--Los Angeles County, California, 2002-2003. MMWR Morb Mortal Wkly Rep. 2003 Feb 07;52(5):88.
3. Boucher HW, Corey GR. Epidemiology of methicillin-resistant Staphylococcus aureus. Clin Infect Dis. 2008 Jun 01;46 Suppl 5:S344-9.
4. Hato, A. F., & Authman, S. H. The effect of purified lysine from Staphylococcus aureus and their effected on human blood clotting.,Bulletin of National Institute of Health Sciences,vol .140,no 01, pp 1-2,Apr 2022.
5. Friedrich R, Panizzi P, Fuentes-Prior P, Richter K, Verhamme I, Anderson PJ, Kawabata S, Huber R, Bode W, Bock PE. Staphylocoagulase is a prototype for the mechanism of cofactor-induced zymogen activation. Nature. 2003;425:535–539.
6. Kroh HK, Panizzi P, Bock PE. von Willebrand factor-binding protein is a hysteretic conformational activator of prothrombin. Proc Natl Acad Sci USA. 2009;106:7786–7791.
7. Watanabe S, Ito T, Takeuchi F, Endo M, Okuno E, Hiramatsu K. Structural comparison of ten serotypes of staphylocoagulases in Staphylococcus aureus. J Bacteriol. 2005;187:3698–3707.
8. Cheung AI, Projan SJ, Edelstein RE, Fischetti VA. Cloning, expression, and nucleotide sequence of a Staphylococcus aureus gene (fbpA) encoding a fibrinogen-binding protein. Infect Immun. 1995;63:1914–1920.
9. Chapman GH, Berens C, Peters A, Curcio L. Coagulase and hemolysin tests as measures of the pathogenicity of staphylococci. J Bacteriol. 1934;28:343–363.
10. Wang Z, Wilhelmsson C, Hyrsl P, Loof TG, Dobes P, Klupp M, Loseva O, Morgelin M, Ikle J, Cripps RM, Herwald H, Theopold U. Pathogen entrapment by transglutaminase – a conserved early innate immune mechanism. PLoS Pathog. 2010;6:e1000763.
11. Adams RL, Bird RJ. Coagulation cascade and therapeutics update: relevance to nephrology. Part 1. Overview of coagulation, thrombophilias and history of anticoagulants. Nephrology. 2009;14:462–470.
12. Krarup A, Wallis R, Presanis JS, Gal P, Sim RB. Simultaneous activation of complement and coagulation by MBL-associated serine protease 2. PLoS One. 2007;2:e623.
13. Sheweita, S.A.; Alsamghan, A.S. Molecular Mechanisms Contributing Bacterial Infections to the Incidence of Various Types of Cancer. Mediat. Inflamm. 2020, 2020, 4070419.
14. Dharmaraja, A.T. Role of Reactive Oxygen Species (ROS) in Therapeutics and Drug Resistance in Cancer and

Bacteria. J. Med. Chem. 2017, 60, 3221–3240.
15. Terman, D.S.; Serier, A.; Dauwalder, O.; Badiou, C.; Dutour, A.; Thomas, D.; Brun, V.; Bienvenu, J.; Etienne, J.; Vandenesch, F.; et al. Staphylococcal entertotoxins of the enterotoxin gene cluster (egcSEs) induce nitrous oxide- and cytokine dependent tumor cell apoptosis in a broad panel of human tumor cells. Front. Cell. Infect. Microbiol. 2013, 3, 38.
16. Seidi, Farzad, et al. "Saccharides, oligosaccharides, and polysaccharides nanoparticles for biomedical applications." Journal of controlled release 284 (2018): 188-212.
17. Seidi, Khaled, et al. "NGR (Asn-Gly-Arg)-targeted delivery of coagulase to tumor vasculature arrests cancer cell growth." Oncogene 37.29 (2018): 3967-3980.
18. Morioka, Shinichiro, et al. "Coagulase-negative staphylococcal bacteraemia in cancer patients. Time to positive culture can distinguish bacteraemia from contamination." Infectious Diseases 50.9 (2018): 660-665.
19. Costa, Silvia F., et al. "Colonization and molecular epidemiology of coagulase-negative Staphylococcal bacteremia in cancer patients: a pilot study." American journal of infection control 34.1 (2006): 36-40.
20. Mulanovich, Victor E., et al. "Emergence of linezolid-resistant coagulase-negative Staphylococcus in a cancer centre linked to increased linezolid utilization." Journal of antimicrobial chemotherapy 65.9 (2010): 2001-2004.
21. Najm, Mohammed Jasim. Molecular and Pathological Aspects of Staphylococcus aureus Isolated from Breast Tumors of Women and Dogs in Baghdad. Diss. University of Baghdad, 2020.
22. Najm, Mohammed Jasim. Molecular and Pathological Aspects of Staphylococcus aureus Isolated from Breast Tumors of Women and Dogs in Baghdad. Diss. University of Baghdad, 2020.
23. Salih, Hanaa Naji. Production, Purification and Characterization of Collagenase produced from locally isolated Staphylococcus aureus HN77. Diss. College of Science/University of Baghdad as a Partial Fulfillment of the Requirement for Degree of M. Sc. in Biotechnology By Hanaa Naji Salih B. Sc. in biotechnology, Department of Biotechnology, University of Baghdad, 2022.
24. Mempin, Maria, et al. "Gram-negative bacterial lipopolysaccharide promotes tumor cell proliferation in breast implant-associated anaplastic large-cell lymphoma." Cancers 13.21 (2021): 5298.
25. Podkowik, M., et al. "Enterotoxigenic potential of coagulase-negative staphylococci." International journal of food microbiology 163.1 (2013): 34-40.