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A COMPARATIVE APPROACH TO UNDERSTANDING BACTERIAL PATHOGENICITY: THE ROLE OF ENZYMATIC AND HEMOLYTIC VIRULENCE FACTORS

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Abstract: This comparison study has aimed to identify the impact of enzymatic and hematogenic virulence factors on the pathogenesis of bacteria using S. aureus (ATCC BAA-976) and E. coli (strain O157) as examples. Protease and lipase activity levels were measured using enzyme tests; the hemolytic activity was measured by examining the growth of the bacteria, with non-hemolytic colonies producing a zone around them. A two-tailed t-test was used to build this statistical relationship. The results showed that S. aureus and E. coli had significantly different levels of enzyme activity. Protease and lipase activity levels were higher in S. aureus than in E. coli. It also revealed significant differences in hemolysis zone widths between the two strains, with S. aureus having a greater hemolysis zone diameter than E. coli. Hemolytic virulence factors are crucial for bacterial pathogenic action, allowing them to thrive within host tissues, gain nutrition from red blood cells, and evade the host's defense mechanism. This study can help develop targeted strategies for fighting infections and improving patient outcomes during infection transmission.

Keywords: Bacterial pathogenicity, Enzymatic factors, Hemolytic factors, Virulence factors, Staphylococcus aureus, Escherichia coli, Enzye activity, Hemolysis.

Introduction

Disease mechanisms within the body are conceivable as a virus of a certain kind has made its way into the body and begun to change how the body normally functions. The more changes made to the body's cells, the more symptoms and hallmarks of the disease appear. When bacteria enter their host and develop in close proximity to the host's tissues, infection takes place. Distinguishing between infection and illness is crucial, as the latter is a morbid process that can occasionally transpire in the absence of an infection (diabetes, for instance, is a disease that lacks a recognized cause). Bacteria can be the source of a wide range of infections, from non-existent to intense.

A bacterium's relative pathogenicity is reflected in its ability to cause disease. Bacteria can be categorized into three main classes based on this. When primary or frankin' pathogens are identified from a patient, they are considered likely agents of disease (e.g., when the laboratory isolation of Salmonella spp. from feces is used to identify the source of diarrheal sickness). Pathogens classified as opportunistic are those that are isolated from patients with impaired host

defenses (Leitão, 2020). They might be the disease's agents (e.g., in individuals who have been catheterized and are therefore vulnerable to urinary tract infections with Escherichia coli). Last but not least, some bacteria—like Lactobacillus acidophilus—are regarded as non-pathogens as they sporadically or never result in illness in humans. However, their classification as non-pathogens may change because of bacterial adaptation and the detrimental impact of modern chemotherapy, radiation therapy, and immunotherapy on resistance mechanisms. It's true that some bacteria that were once thought to be non-pathogens can actually cause illness. For instance, the ubiquitous soil bacterium Serratia marcescens may lead to bacteremia, pneumonia, and urinary tract infections in vulnerable hosts (M & Singh, 2017).

The term "virulence" refers to an organism's capacity to infect its host and spread illness. The bacterium gains entry into the host's cells with the aid of chemicals referred to as virulence factors. These elements fall into one of three categories: secretory, membrane-related, or cytosolic. Cytosolic factors enable the bacteria to quickly adapt by altering its shape, metabolism, and physiology. The bacteria are assisted in adhering to and escaping the host cell by the virulence factors associated with the membrane. Secretory factors are crucial components of the bacterial armory that help them maneuver both the host's adaptive and innate immune systems (Głowacka et al., 2018). Secretory virulence factors collectively have extracellular infections that lead to the death of host cells. E. coli species are incredibly versatile organisms as far as the functions and roles they play within the microbiota of animals and humans. Once this benign commensal bacterium acquires several various complete movement mobile genetic components that contain genes coding for virulence factors, it can turn become a “revenue generating” human bacterial pathogen that can cause a spectrum of intestinal and extra-intestinal ailments. Nine different variety of pathogenic E. col K series have been existing and has been defined as a cause of variety of health problems from simple diarrhea to more complicated ones like urinary tract infection. To regulate when and how the pathogen enters the host cells, the patho-type utilizes different types of virulence factors and effectors that either breach or disturbs the activities of the host cells (Pontes et al., 2020).

Pathogen virulence describes how virulent an organism is. The capacity to cause disease in the host organism despite of that it has a defense system is strongly correlated with the high virulence of a pathogen. Bacteria need to be capable of infecting the host firstly, which can be achieved in a plurality of ways depending on the particular mode of entry, secondly, the bacteria should produce elicitors which are means to trigger the protective responses of the body, thirdly, bacteria should involve virulence attributes which are only triggered when a highly specific set of conditions inflict the body, and finally the virulence of the bacteria. The dose of bacteria needed to produce a desired outcome within a set time frame following its administration by a particular method reflects the power of a pathogen to kill animals and/or inflict disease or lesions in an experiment. Thus, calculations of a fatal dosage which impacts 50% of an animal population (LD50) or a therapeutic dosage that generates a disease symptom in 50% of an animal population (ED50) are useful for comparing the corresponding virulence of a number of microorganisms (Liu et al., 2022).

Many virulence factors, or phenotypic traits connected to a microorganism's level of virulence, are involved in the complex process of bacterial pathogenicity. The ability of the pathogen to multiply in vivo, harm host cells, impede or elude host defense mechanisms, and endure on or break down skin and mucous membranes are just a few of the mechanisms that these virulence factors—which can be hemolytic or enzymatic—improve. Toxins are metabolites

produced by bacteria which remain as an active threat on the environment after the death of the bacteria. This shows the activity of the enzymes. Toxins of any kind can induce a variety of disorders and diseases, not only in intestines but also in extra intestines, such as HUS, depending on the interaction. To the contrary, the hemolytic virulence mechanism entails the red blood cells getting lysed accompanied by hemoglobin release as a consequential effect. Anemia, jaundice, and acute renal damage may also develop during this process. A hemolysis effecting pathogenic factor, like cytolysin or hemolysin, significantly boosts cell quick entrance through an individual hence causing harm to the host (Głowacka et al., 2018).

The enteric commensal bacteria E. coli harbour the capability to become a medicinal pathogen when it crosses the urinary tract, which is a sterile tissue area. The uropathogenic E. coli (UPEC) isolates have opined hemolysin (toxin that forms anionic pores) as the major contributing virulence factor amongst the diverse ones. A typical example of cytolysins is hemolysin with a (HlyA) (repeats-in-toxin) family arbitrator. The hlyA tranistration of the commensal E. coli isolates numbers roughly 15% by the sequencing study. Remarkably, UPEC isolates' expression of HlyA is correlated with a higher severity of urinary tract infection (UTI) presentations in the clinic (Cobo-Simón et al., 2023). Since UPEC encodes hemolysin in 31–48% of cystitis cases, quantifying HlyA expression among UPEC strains shows a significant rise in the operon's existence throughout the genome. Up to 78% of infectious isolates found in patients with bacteremia or pyelonephritis encode hemolysin. Although lysing red blood cells is how HlyA was discovered and given its name, it also exhibits cytotoxic activity against a variety of other species and cell types. Though research has long focused on the genomic organization and control of hemolysin expression in vitro and in vivo, more recent studies have shifted their attention to comprehending the mechanism and effects of hemolysin action. It is believed that high concentrations of HlyA cause a target cell's membrane to become permeable, which causes the cell to lyse (Sora et al., 2021).

S. aureus is a dangerous pathogen that can infect a wide range of vertebrate species, involving humans and livestock, in various body locations. Staphylococcus aureus possesses an excess of virulence factors, which regulate both the innate and adaptive immune responses of the host, contributing significantly to the pathogen's success. Exo-enzymes, toxins discharged into the environment, and cofactors required for host zymogen activation are a few of the numerous immune-modulating risk factors. When used effectively, highly inflammatory produced toxins, such as superantigens and pore-forming toxins can kill leukocyte cells by clonal deletion and cytolysis (Rasheed & Hussein, 2021). Collagenases and staphylokinases are examples of cofactors that take over the host's coagulation system. Exo-enzymes, like nucleases and proteases, break down and render inactive a variety of immune defense and surveillance components, including complement factors, antimicrobial peptides, and surface receptors essential for leukocyte chemotaxis. Furthermore, by cell lysis and junction protein cleavage, a few of these released toxins and exoenzymes can damage endothelium and epithelial barriers. One distinctive observation about the spectrum of virulence factors released by S. aureus is the evident functional redundancy displayed by most toxins and exoenzymes. But a closer look at each virulence factor showed that they all had distinct qualities with significant functional implications (Castro et al., 2018).

Overall, Enzymatic and hemolytic factors are just two of the many virulence factors that contribute to the intricate process of bacterial pathogenicity. In vulnerable people, these virulence factors augment one or more of the processes that comprise the phases of pathogenicity, resulting in the onset of clinical illness. Efficient anti-virulence therapy and preventive measures against bacterial infections depend on our ability to comprehend these virulence factors and their pathogenic pathways.

Objectives

To detect the comparing enzymatic and hemolytic activities between different bacterial strains and their role in bacterial pathogenicity

Methods

This research examined the importance of both enzymatic and hemolytic virulence factors in the pathogenesis of bacteria. Two bacterial strains were used:

Staphylococcus aureus (ATCC BAA-976): The highly virulent strain identified as the leading cause of different manifestations, i.e. infections.

Escherichia coli (strain O157: PG- strain reportedly a cause of many ailments including food poisoning.

Both S. aureus and E. coli O157: The last three (H7) viruses are great human pathogens producing meaningful research, as it is concerned with understanding and fighting infectious diseases.

Collection and preservation of blood cells

In order to rule out any anomalies related to red blood cells, nine patients underwent laboratory testing, including hemoglobin, packed cell volume (PCV), and peripheral smear inspection. All of the volunteers shared the same blood type. An institutional ethical committee (IEC) approved the study, and the subjects gave their informed permission. To avoid coagulation, ten milliliters of each patient's blood were aseptically removed and placed in a citrated tube. To create a pack of red cells, the materials were centrifuged for five minutes at 5000 rpm. The RBCs were rinsed five times with phosphate-buffered saline (PBS) at a pH of 7.2 after discarding the supernatant plasma. These cells were then suspended in freshly prepared autoclaved Alsever's solution, which had the same concentrations of sodium citrate (0.8%), dextrose (2.05%), citric acid (0.055%), and chloride (0.42%) as before. As needed, a small amount of RBCs maintained in Alsever's solution was moved into a test tube. When the RBCs were centrifuged, they were rinsed three times with pH 7.2 PBS. A working concentration of 1% V/V was then created for hemolysis testing, and 3% V/V for hemagglutination experimentation.

Collection of bacterial isolates

Utilizing standard bacteriological techniques, clinical specimens (blood, urine, pus, and other body fluids) collected from patients hospitalized under numerous specialties of this hospital were analyzed, and the isolates were determined. For additional research, these isolates were kept in semi-solid nutrient agar.

Growth conditions:

Working with bacterial cultures, Tryptic Soy Broth (TSB) at 37°C was used for overnight incubation in shaken flasks for 24 hours. Next, the cultures were diluted to a turbidity of 0.600 at 600 nm wavelength to make sure cultures were still in a uniformly exponential growth phase before further applications.

Enzyme assays:

Protease activity: Protease assay through casein degradation assay was performed. Briefly, the cultures of bacteria were tried with casein as a substrate which they incubated for a predefined period. In the next step, the casein was allowed to be digested. After that, the supernatant was measured by spectrophotometrically at 280 nm. The absorbance values higher demonstrate the increased presence of protease activity.

Lipase activity: The rate of hydrolysis of p-nitrophenyl palmitate by lipase was analyzed with a spectrophotomtir assay. Bacterial cultures were incubated for half an hour; after that time they were

incubated with p-NPP substrate. The color of the compound released p-nitrophenol was measured spectrophotometrically in the wavelength of 405 nm Measuring values of high absorbance suggest higher levels of lipase activity.

Hemolysis assays:

Hemolytic activity was demonstrated by the translucent halo with well-defined borders around colonies, and stated by an index. The hemolytic index (Hi), which is determined by dividing the colony diameter by the colony diameter plus the hemolysis zone, was also used to indicate hemolytic activity. The isolates underwent a 48-hour incubation period at 37°C in 5 milliliters of brain-heart infusion (BHI) broth. Each well of a micro-titer plate was filled with 200 microliters of culture. An equivalent volume of 1% concentration of cleaned human red blood cells from various blood groups was then added. The plate was then sealed with cellophane tape, incubated for 24 hours at 37°C, and hemolysis was monitored. After 24 hours, if there was still no hemolysis, the plate was placed in a cool environment (2–6 °C) and checked for hemolysis again. 200 microliters of sterile BHI broth with an equivalent volume of cleansed human red blood cells from various blood types made up the controls. The plates were checked for the presence of a clearing zone surrounding the bacterial colonies, which would indicate hemolysis, after incubation. Hemolytic activity was assessed by measuring the clearing zone's diameter.

Statistical analysis:

All experiments were performed in triplicate. A two-tailed t-test was used to evaluate the hemolytic zone widths and enzyme activity of the two bacterial strains on spss 28. Less than 0.05 was the threshold for statistical significance.

Results and Discussion

Table 1: Enzyme Activity (AU)

Bacteria	Mean (AU)	Standard Deviation	p-value
S. aureus (Protease)	0.53	0.05	< 0.05
S. aureus (Lipase)	0.79	0.03	< 0.05
E. coli (Protease)	0.30	0.02	< 0.05
E. coli (Lipase)	0.15	0.02	< 0.05

Table 1 shows the significance of the enzymatic activity of S. aureus and E. coli for both protease and lipase with greater activity of protease. S. aureus displayed the higher enzyme mean activity with protease (mean = 0.53 AU) and lipase (mean = 0.79 AU) in comparison with E. coli with lower enzyme mean activity with protease (mean = 0.30 AU) and lipase (mean = 0.15 AU). These results demonstrate S. aureus' greater ability to synthesize and secrete these hydrolytic enzymes, which are known toxins and effector molecules responsible for microbial pathogenicity. The enzymatic virulence factor shows to be a key element in bacterial pathogenicity, as evidenced by the p-values (< 0.05) obtained from the comparison of enzyme activity between two bacterial species, which has revealed substantial differences between the groups. Through the higher enzyme levels of S. aureus, the microbe may display an enhanced capability to undermine the host tissues and evade the immune response of the host. Proteases can help a pathogen by cleaving the host's proteins thus affecting the host's tissue invasion and spread. Lipases, in turn, are lipid-hydrolyzing enzymes that can thus convert them into a nutritive source for the bacteria, which increases the chances of the bacteria's survival and propagation within the host.

Table 2: Hemolysis zone diameter:

Bacteria	Mean (mm)	Standard Deviation	p-value
S. aureus	12.10	0.29	< 0.05
E. coli	7.90	0.29	< 0.05

As Table 2 outlines there were several statistical differences in the diameters of the zones of hemolysis between S. aureus and E. coli. S. aureus baptized a more extensive mean hemolysis zone diameter of 10.10 mm, while E.coli had a smaller mean hemolysis zone diameter of 7.90 mm. This shows that S. aureus exhibits a bigger tendency for hemolysis in comparison with E.coli. The p-values (< 0.05) demonstrate the statistical significance of the observed variations in hemolysis zone diameter. This implies that bacterial pathogenicity is significantly influenced by hemolytic virulence factors. The findings highlight the contributions of such virulence factors with hemolytic elements in the development of bacterial infection. Really, bacterial hemolysis is the key mechanism that helps them both take their nutrients from the RBCs and stay active in the blood of the host. Such bacteria would then move to different organs of the host's body. In general, the more the hemolysis zone is formed, the more the red blood cells are lysed. The mechanism induces the bacteria in closing in on host tissues and in evading the host advantageous responses, ending up in an infection and the illness.

Enzymatic and Hemolytic activities are the key virulence strategies, employed by the bacteria, in initiating the pathogenicity. The bacteria, E. coli, on the other hand, recorded the lowest protease and lipase activities, implying that the protease and lipase activity of S. aureus are superior in terms of host tissue breakdown and nutrient utilization. The pathogenicity, or harmful impact, of S. aureus is largely due to the effect of its enzymatic virulence components. Furthermore, the hemolysis zone of Hemolysis of S. aureus astonished larger than the hemolysis zone of E. coli, which appears in a higher level ability to form hydrotroposis. Bacteria do hemolysis as the major issue is to invade the sites of injury, receive nutrients, and to avoid one's immune system answer. Therefore, the S. aureus`a increased hemolytic activity shows that chances it could cause disease.

These data are further supported by a comparative analysis of hemagglutination and hemolytic capacity of a number of bacterial isolates against various human blood groups showed that most isolates agglutinated to red blood cells (A group) and B and AB group cells are following in terms of agglutination, and red blood cells (O group) are the least agglutinated. The agglutination and the hemolysis were examined on human A, B, AB, and O group red cells of 135 bacterial clinical specimens, such as urine, pus, blood, and other body fluids. Escherichia coli accounted for 81 isolates out of the 150, followed by Klebsiella pneumoniae (18), Pseudomonas aeruginosa (19), Pseudomonas spp. (10), Proteus mirabilis (6), and Staphylococcus aureus (16). The isolates that showed hemolytic and hemagglutinating properties against A group cells were 46 percent; the isolates that showed same properties against B and AB group cells were 28.6% and 21.3%, respectively. The fewest number of isolates, 32 (21.3%), showed both traits when they were tested against O group cells. The isolates' hemolytic and hemagglutination characteristics against various combinations of human blood type cells varied greatly. The study emphasizes how crucial it is to choose the right type of cells, particularly when using human red blood cells to examine the hemolytic and hemagglutination activities of bacterial isolates, as these are traits that are thought to be specific to pathogenic strains (Rajkumar et al., 2016).

In (de Melo et al., 2015)`s study sought to to investigate the in vitro production of virulence factors in Candida spp., such as proteinase, phospholipase, deoxyribonuclease (DNase), and

hemolytic activity. Candida albicans (15), Candida tropicalis (15), Candida parapsilosis (10), Candida glabrata (5), and Candida krusei (5) were the fifty clinical isolates whose virulence factors were examined. On Sabouraud dextrose agar plates with 3% glucose and 7% sheep red blood cells, hemolytic activity was measured. DNase, phospholipase, and proteinase activities were measured in culture medium containing agar-base DNA, egg yolk, and bovine albumin, correspondingly. Of the 50 isolates, 48 (96%) showed hemolytic activity; DNase, proteinase, and phospholipase tests yielded positive results in 10 (20%), 19 (38%), and 16 (32%), cases respectively. Differences in proteinase synthesis between species were statistically significant ($p<0.05$) and phospholipase ($p<0.0001$). It is determined that hemolytic activity was present in every species. All species excluding Candida glabrata, Candida albicans, Candida tropicalis, and Candida parapsilosis have proteinase, phospholipase, and DNase activity.

In an additional study, the hemolytic activity, hyaluronidase, chondroitin sulphatase, proteinase, and chondroitin dubliniensis and 30 C. albicans strains derived from different sources were investigated (Linares et al., 2007). Upon review of their results, the researchers found that the hyaluronidase, chondroitin sulfate and hemolytic activities pertaining to C. albicans strains were similar to each other, whereas the proteinase activity was significantly lower in C. dubliniensis strains. The application of the apparatus, which is very sensitive in detecting a very wide range of chemiluminescent sensing techniques, had been able to separate different bacterial species and strains by the enzymatic activity profiling method. The research showed that the dual-probe system, which uses pyroglutamyl aminopeptidase and leucine aminopeptidase, was the more accurate almost to the extent of 97%. This was for S. aureus, E. coli, and P. aeruginosa (Shelef et al., 2024)

Phospholipases is a class of libolytic enzymes which numerous bacterial pathogens utilize during the infection process, to begin with, and throughout. Host membranes become permeabilized that can lead to a high level of host cell death. This phenomenon has been proven by a number of studies. Nevertheless, the new studies offer an insight into the biochemical mechanism responsible for phospholipase from bacterial cells to exploit the intracellular signaling pathways of the host. So, utilization of lipolysis is the key to their success and enables them to adapt to the conditions that brought them inside the cell during their battle for dominance (Liu et al., 2022).

The virulence of S.aureus is related to different mechanisms including the presence of lysins, lipases, proteases, catalase, and DNase. As well as bacterial pathogenicity is known to involve the effect of the lipase and protease in many researches so far (Malik, 2018; Cheung et al., 2021). The protease and lipase activities of S. aureus strains obtained from bio samples of meat, chicken and meatballs have not been properly investigated in this regard. The result of that research work by Suaifan et al. (2019) is that skim milk agar has been used as a method for measuring the isolates' protease, and S. aureus strains found in healthy chicken are mostly protease-negative, while those in sick chicken are usually protease-positive. The treatment of S. aureus strains isolated from chickens that the experienced dermatitis and necrotic and edematous dermatitis caused significant proteolytic activity was observed.

S. aureus can utilize S. aureus lipases 1 and 2, which are encoded SAL1 and SAL2. This system has two of the lipases as signal peptidase I pre-proenzymes and the third that has a specific preference for substrates. SAL1 will perform well at pH 6.0 and be stable in acidic conditions, whereas SAL2 performs the best at pH 8.0 and is stable only in alkaline conditions. Particularly notable is the fact that lipases are highly resistant to staphylococcal species. This finding is indicative of lipases being crucial for the microbial adaptation in evolution. It seems that lipases and FAMEs additionally help staphylococci to better cope with abscesses environement. Lipases cleave the

triglycerides that render the FAME agents immobilized; however, FAME utilizes those ions released when the lipases break down the molecules to aid the staphylococci. Not surprisingly, FAME activity happens in most of the S. aureus strains with a lipase-encoding gene, and these strains express strong symptoms of infection. As far as lipase is concerned in S. aureus, it can be FAME, phospholipase, and lipase, all of which are included in the process of maintaining microbial life and development of disease. Sometimes methicillin-resistant S. aureus (MRSA) is present in the patient, wherein it will stay alive for several months or longer without causing any signs of disease. It is just like the carriers of the organisms that do not show any symptoms of the disease and serve merely as a reservoir (Nguyen et al., 2018).

The main extracellular hydrolytic enzymes produced by both S. aureus and S. epidermidis are hemolysin, lipase, protease, and phospholipase whose roles are mentioned in a number of reports as they are crucial for persistence and pathogenicity of these bacteria strains (Alkhafaji et al., 2019; Angel, 2 The streptococcal alpha hemolysins are correlated with hemolysis, in addition to the target toxin in cow-associated mastitis and cytotoxic and dermonecrotic effects on cell lines (Iversen, 2021). The S. aureus isolate already in the food from the bacteria, the lipase has been created, which is understood because the bacteria perform a function in lipid metabolism and virulence (Alkhafaji et al., 2019). Thus, the human beings and animals can experience lots of disease range, the most active protease isolates are capable to do. This theory is quite well supported by Gundogan, et al. (2013), that of S aureus protease which is generally known to cause dermatitis in hens

Unlike bacterial species, staphylococci needs alpha hemolysin for the purpose of enabling the trigger of infections, which actually is leucocidal because it rapidly increases the number of the rest of toxins they produce (Divyakolu et al., 2019). Hemolysins represent a group of the toxin that damages red blood cells (RBCs). They usually target the immunological and signaling receptors on the RBCs' surface. Hemolysins are subdivided, according to their nature, into several classes, with α, β, and γ-hemolysins being the most common. PSM is another major group of peptides which are also known as a kind of phenol-soluble modulins. They can perform the hemolytic activity without any receptors. α-hemolysin is the staphylococcal hemolysin among which α-hemolysin has the established history of study. The small β-barrel pore-forming protein cytotoxin acts upon lysing leucocytes as well as (but not neutrophils) by binding to its protein receptor ADAM10 which is a disinterring and metalloproteinase (Escajadillo & Nizet, 2018).

It seems that ADAM10 deletion models are immune to this toxin's lethal effects. Research has shown that the combined effects of α-toxin on platelets and myeloid cells during sepsis result in the death of the host mice (Cheung et al., 2021). The toxin's receptor engagement results in pore development on cell membranes, which causes K+ efflux and Ca2+ influx. This disturbance of homeostasis ultimately causes necrotic cell death. β-hemolysin has been classified as a sphingomyelinase and is non-pore-forming. The toxin hydrolyzes monocytes and sphingomyelin, but it is not cytolytic to lymphocytes or granulocytes. It only lyses erythrocytes at low temperatures. The precise mode of action of the toxin remains unidentified even though the cells it targets are known. According to Yang et al. (2023), β-hemolysin may lead to instability of the cell's plasma membrane's bi-lipid layer and inconsistencies in the membrane's fluidity since it predominantly impacts sphingomyelin (Yang et al., 2023).

Leukocytes, including neutrophils, monocytes, granulocytes, and macrophages, exhibit membrane-damaging activity in addition to being hemolytic to rabbit erythrocytes. These hemolysins are classified as bi-component because they contain polypeptides with the names S (slow, HlgA or HlgC) and F (fast, HlgB). It is hypothesized that the S components affect the vulnerabilities of

different cell types to these toxins. HlgB (F component) will bind to phosphatidylcholine on the targeted cells as the compatible S element binds to host cell membranes, resulting in cell lysis (Astley et al., 2019). PSMs (δ-hemolysin, PSMα1-4, PSMmec, and PSMβ1-2) are multifunctional peptides implicated in the pathogenesis of staphylococci that are produced by a large number of S. aureus strains. They are hemolytic to spheroplasts, bacterial protoplasts, various organelles, and erythrocytes. The toxins have a strong affinity for lipids and are tiny and amphipathic. It has been demonstrated that S. aureus PSMα lyses neutrophils after phagocytosis and aids in the development of biofilms (Worku et al., 2023).

The hemolysis and aggregation of red blood cells are exists in pathogenic bacteria spectrums and the pathogenicity can be frequently determined. Red blood cells from a multitude of species, including the ones we commonly think of, such as humans, rabbits, guinea pigs, chickens, lambs, mice and several others, have been known to glom onto bacteria. Actually, research on the binding of red blood cells with bacteria has found as many as 14 different species of red blood cells bind this. Besides, the method was also utilized as a marker for epidemiological studies by hemagglutinin. It is these two activities, the hemolysis and enzymes production, of E. coli that chiefly account for the virulence or the ability to cause harm in this bacteria type. E. coli bacteria synthesize a toxin known as hemolysin that is located on the operon hlyCABD. The enzymatic function of hlyC makes an effect on post-transcriptionally heard secreted HlyA protein (of Tulla et al., 2023). Interestingly, HlyA's cytolytic activity occurs when plasmid-encoded acid addition to substances takes place, yielding a synthetic toxin which is more virulent α-hemolysin as a result resists E. coli killing which is found in several types of host cells. It is the pore-forming property of this agent that causes cell rupture and subsequent hemolysis in erythrocytes, above others. Besides hemolysin, Escherichia coli have other ways to add to their pathogenic abilities such as enzymatic activities as well. The type of E. coli is a viable example of how it challenges the treatment of E. coli infections because it produces β-lactamases which are a kind of enzyme that impart resistance to β-lactam antibiotics. The E. coli essentially use its capacity to cause hemolysis and its enzymatic activities as virulence factors to infect people and navigate the host defense system (Ramos et al., 2020).

Galido- Méndez (2020) applied in vitro system using human red blood cells of rabbits, lambs, and guinea pigs to evaluate 345 Escherichia coli strains for their ability to hemagglutinate. Such research work established that 17.9% of the strains of Escherichia coli attached to human cells; nevertheless, it does not highlight which type of human red blood cells was used. Certain strains of gram-negative bacterium E.coli cause hemolysis and hemagglutination types VI and is pathogenic to humans. Specifically, Hoyle et al. (2021), whose study concentrated on 611 strains of E.coli isolated from blood and urine samples, observed that 89% of those strains were hemolytic to human A cells, For completion of this research study by Barua et al. (2018), human A cells were conducted along with guinea pig and monkey cells addressing the EHV strains; however, there was no reporting on the determination of hemagglutination of human A cells. In 70% of the uropathogenic Escherichia coli isolates that (Shah et al., 2019) examined for their assessment of virulence variables, hemagglutination of human O cells was seen.

Conclusion

Enzymatic virulence agents, such as proteases, lipases, and toxins, contribute a lot to bacterial pathogenesis by destroying tissues, the immune system, and host cells. Bacteria can destroy host proteins, mess up with cell membranes, and mess up with host immune responses, thereby enabling their reproduction and further infection. Bacterial metabolic systems are among the hemolytic

virulence factors, such as hemolysins, that can aid in the germs' ability to obtain energy from host tissue, evade immune cell recognition, and mediate local tissue death. There are different types of bacterial toxins that different bacterial pathogens produce to cause the lysis of host cells and these vary in their extent and effects but they are the basis of the pathogenicity of the bacteria. The contrasting analysis of the factors identified above gives us critical information that helps us to understand the complicated processes of bacterial infectious processes and requires further research. Such research is highly important in advancing the knowledge of infectious diseases and treatment outcomes.

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