International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Target Identification for Bacteria: Bioinformatics Study

Edwina Jospiene. M[1], Dr. Subramaniam S[2], Dr. Shyama Subramaniam[3]

[1]Research Scholar, Regenix Super Specialty Laboratories Pvt., Ltd., (Affilated to University of Madras) No. 42, Loganathan Nagar, 100 feet road, Choolaimadu(vadapalani), Chennai- 600 094, Tamil nadu, India
Email ID: *edwina18[at]ymail.com*
Phone number: 9600056014

[2]Lab director and Professor, Regenix Super Specialty Laboratories Pvt., Ltd., (Affilated to University of Madras), No. 42, Loganathan Nagar, 100 feet road, Choolaimadu(vadapalani), Chennai- 600 094, Tamil nadu, India
Email ID: *subramaniam[at]regenix.in*
Phone number: 9841113331

[3]HOD and Professor of Biochemistry, Apollo Lab Services, Chennai, Tamil nadu, India
Email ID: *shyamasubramaniam[at]gmail.com*
Phone number: 9884213969

Abstract: ***Microbial - infections have a large impact on public health. The disease can occur at any body site and can be caused by the organism itself or by the body's response to its presence. Infectious diseases can be viral, bacterial, parasitic, or fungal infections. This bacterial infection is one of the top 10 deadly diseases announced by WHO. In this study, we have focussed on staphylococcus aureus which is gram-positive bacteria. SpA(Surface protein A) and Sbi (second immunoglobulin-binding protein) will serve as an efficient target for the point-of-care technique.***

Keywords: target identification, *staphylococcus aureus*, bacterial infection, genomics

1. Introduction

Microbial toxins promote infection and disease by directly damaging host tissues and by decreasing the immune system, the clinical significance of a toxin involves many factors, including the toxin's prevalence, virulence, and role in disease pathogenesis. Toxin production and release, interaction and entry into the host target cells, mechanisms of action and relevance, and clinical significance historically have been limited, with increasing utilization of genomics and proteomics techniques, there is a greater understanding of microbial pathogenesis and their role in clinical disease.

The most commonly occurring infections are bacterial infections, which occurs through an opening in your skin, such as a cut, a bug bite, or a surgical wound. Bacteria can also enter your body through your airway and cause infections like bacterial pneumonia and also transmitted through air, water, food, or living vectors. The microbial bodies will infect their surroundings by eliminating the toxins.

Target identification can be approached by direct biochemical methods, genetic interactions, or computational inference. In many cases, however, combinations of approaches may be required to fully characterize on-target and off-target effects and to understand mechanisms of small-molecule action. There are so many bioinformatics tools that help us to understand genomics and proteomics in a better way.

Bioinformatics can extract, analyse, and interconnect hidden information from sequences to structures as well as functional knowledge of nucleic acids and proteins to discover and identify diagnostic aids and new drug targets as well. This can potentially guide the design of therapeutic drugs that can activate or block the biological functions of biomolecules and help to construct various prediction models to aid virtual bioactive screening. This will, in turn, help to design and discover molecular markers or a diagnostic treatment for the patients at the point-of-care level.

Here in this study, we are targeting a highly pathogenic bacteria which is very common in nosocomial infection and gram-positive cocci. As per a statistical study done earlier, these bacteria are the top pathogenic organisms and it takes a minimum of 72 hours to see the report but using these bioinformatics tools we can able to identify the organism at the gene level and treat it accordingly which saves many lives. [1,2]

Global statistics:
Common bacterial infections were connected to one in eight global deaths in 2019 according to a study published in the *Lancet* by the Global Research on Antimicrobial Resistance Project represented the second top cause of death that year. The data collection is accommodated by the Centre for Tropical Medicine and Global Health, at the Nuffield Department of Medicine, UK. [6]

Bacterial infections lead to many complications one of the major and common problems while treating the patient with or without the antibiogram report broad-spectrum antibiotics might be given which increases the risk of antibiotic resistance which is one of the top ten deadly diseases announced by WHO(World health organization). The number of deaths associated with drug-resistant infections and sepsis found that infections remain a leading cause of death globally.

There were 7.7 million deaths in 2019 associated with 33 common bacterial infections, with five bacteria alone connected to half of these mortalities, according to the research by GRAM, a partnership between the Institute for Health Metrics and Evaluation (IHME) and the University of Oxford. The deadliest bacterial pathogens and types of infection varied by location and age.

A recent study estimated that there were more than 10 million sepsis-related deaths in 2017, indicating that infections were involved in more than 20% of deaths globally for that year. Reducing the number of deaths due to infections is a foundational principle in moving towards health equity because there is a disproportionate infectious burden in low-income and middle-income countries.

In 2019, the Centre for Disease Control and Prevention Antimicrobial Resistance Threats Report noted that dedicated prevention and infection control efforts in the U.S. reduced deaths from antimicrobial-resistant infections by 18% overall and by nearly 30% in hospitals. However, the CDC's 2022 special report highlighting the impact of COVID-19 on antimicrobial resistance in the U.S. found that much of that progress was lost, in large part, due to the effects of the pandemic. The pandemic pushed healthcare facilities, health departments, and communities near their breaking points in 2020, making it very hard to maintain progress in combating antimicrobial resistance.

The overall data is clearly explained in the below chart.

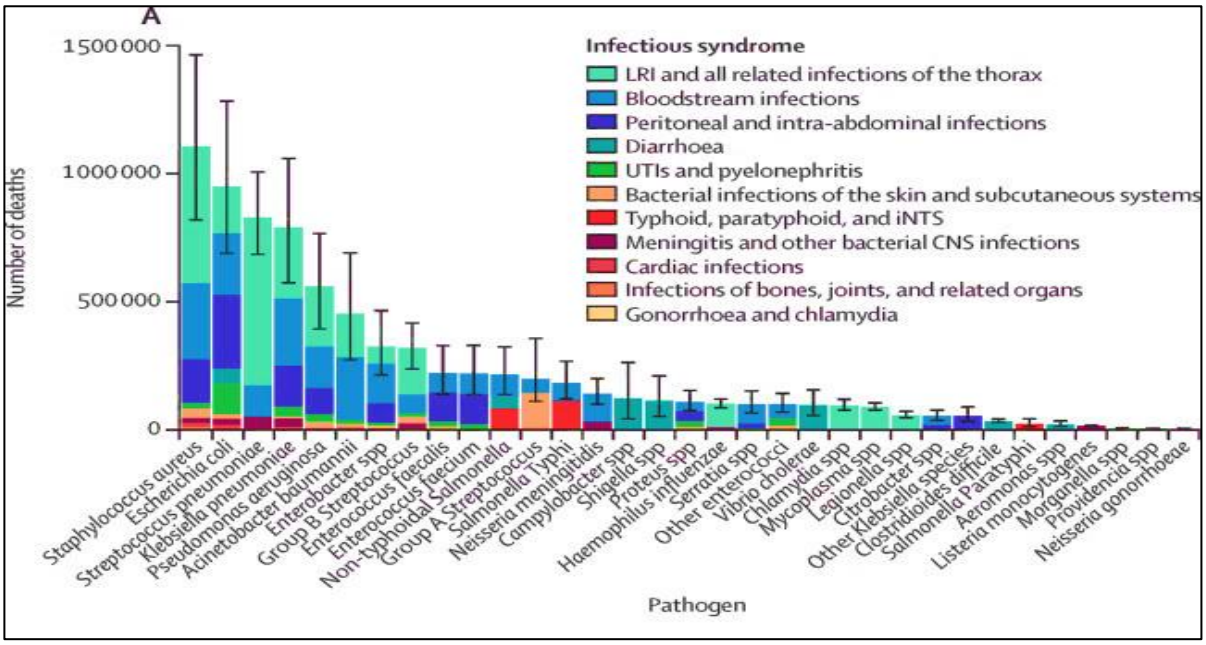

Figure 1: Global statistics show the number of deaths each year and the increase in pathogenic diseases

Computer-aided target identification and validation service use sophisticated chemistry simulation software to help identify and confirm therapeutic targets. With our powerful computer-aided platform, bioinformatics provides various gene level dates information such as gene expression, proteomics, and transgenic phenotyping. The computer-aided platform takes advantage of high accuracy and short turnaround time. The bioinformatical approach can be used to identify pathogenesis-associated genes and genes involved in interactions between infectious diseases and hosts. These genes may perform as potential drug targets. Besides identifying potential targets, bioinformatics can facilitate the prioritization of potential targets, elucidate complex metabolic pathways, aid in the identification of beneficial metabolites, and enhance the validation of targets.

2. Methods and Materials

The KEGG pathway was used to shortlist the target for the organism which helps us to develop and validate the diagnostic kit aid or for the drug delivery. There are so many target identification tools available online that help us to pick up the best target at the molecular level.

The activity of toxins in *Staphylococcus aureus*

The types of target identification involve drug target discovery, molecular markers identification and validation, diagnostic kit manufacturing, and genomic data storage.

Staphylococcus aureus is the most dangerous of all many common staphylococcal bacteria. These gram–positive, sphere-shaped (coccal) microbial bacteria often cause skin infections but can cause pneumonia, heart valve infections, and bone infections. Since it's highly pathogenic and contagious there is a niche for a study of a marker for quick diagnosis and treatment. These bacterial proteins are exported via a signal sequence (Randall and Hardy, 1989) and, as in the eukaryotic defaulting pathway, either attached to the cytoplasmic membrane (Wickner and Lodish, 1985; Davis and Model, 1985). In gram-positive bacteria, proteins are secreted into the surrounding medium, whereas in gram-negative bacteria, secretion occurs in the periplasmic space between the cytoplasmic and outer membranes (Model and Russel, 1990; Schatz and Beckwith, 1990). Gram-negative bacteria have developed a separate pathway of protein secretion into the medium (Pugsley and Reyss, 1990; Koronakis et al., 1991) and its export signal is rather complex (Koronakis et al., 1989; Stanley et al., 1991).

Infecion pathway:

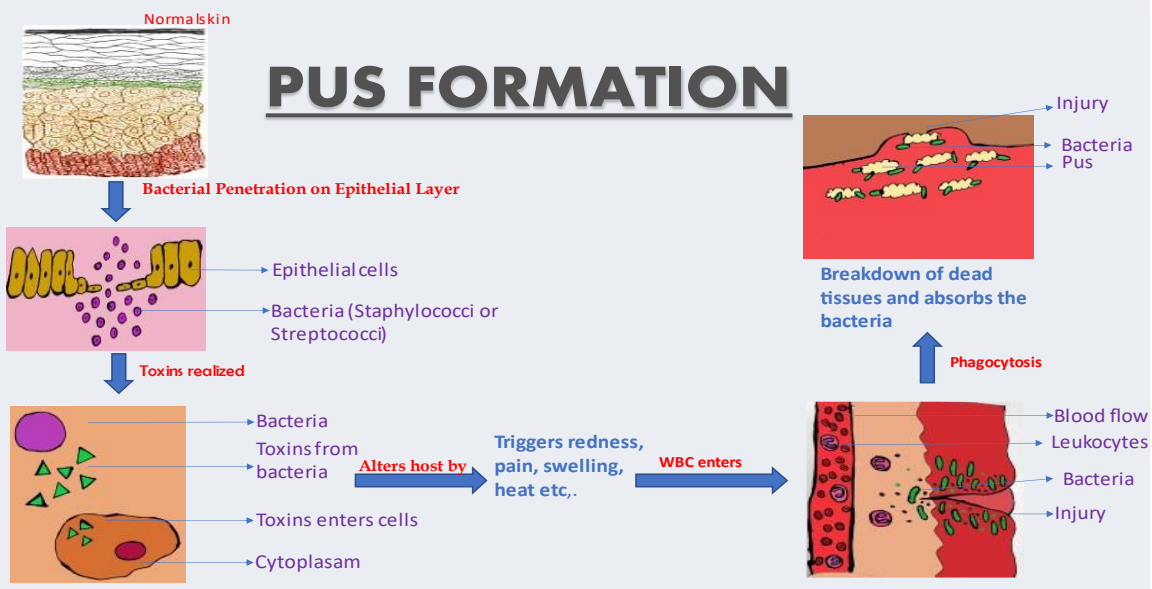

Figure 2: Toxin entry and Pus formation

Antibacterial drugs are the major weapons to suppress or kill bacteria or their activity. Due to the inevitable evolution of antibiotic resistance, the development of novel antibiotics is essential. Antibiotics work either by stopping bacterial growth or by killing the bacteria, without harming the human host. The successful use of antibiotics has been facing challenges because microbial pathogens are developing various forms of resistance in the last decades. Despite this critical situation, new drug development projects have been inadequate for reasons ranging from the bad selection of targets to reduced antimicrobial drug discovery efforts by pharmaceutical companies. Currently, it is accepted that the identification and validation of appropriate targets are critical steps for designing new drugs. In this sense, next-generation sequencing is increasingly aiding the evaluation of gene function, essentiality, and suitability for drug development.

3. Results

Target identification through Bioinformatics:

In this study, the staphylococcus aureus target is identified through bioinformatics tools and using this the target is identified SpA is the membrane protein which involves in the infection pathway. (https://www.genome.jp/pathway/hsa05150). It is shown that the YSIRK-G/S motif plays a role in the efficiency of the secretion of protein A, a cell wall-anchored surface protein of *S. aureus*. The YSIRK-G/S motif is, however, dispensable for the cell wall anchoring of surface proteins. Several models are discussed to account for the existence of two classes of signal peptides in gram-positive cocci.

Role of SpA and pathways involved

SpA protein comprises 90% of the total cell wall contents remaining 10% from the cytoplasm content of the bacteria. The five N-terminal repeated domains of protein A binds with the Fc portion of immunoglobulins. The main role of the SpA protein in the infection pathway is that it binds to the 'Fc' region of the immunoglobulin and thus helps with the significant exclusion of phagocytosis of the bacteria (Figure 4). SpA proteins are known to bind with the 'Fab' region of the membrane–anchored Ig's which in turn stimulates the B cells and consequently helps with lymphocyte clonal selection. The SpA promoter contains two transcriptional sites and three upstream *cis-elements* for the purpose of regulation. The evasion of the host immune response is the touchstone mechanism of pathogenicity of *Staphylococcus aureus* and is facilitated by the ability of the cell wall-associated protein A (SpA) to bind immunoglobulin G Fc fragments, thereby impeding phagocytosis and classical pathway complement fixation. The structure of the SpA protein contains 5 homologous binding sites for the human immune globulin. These domains are labelled E, D, C, B, and A. The SpA protein enhances the expression of osteoclast-specific genes, such as the tartrate-resistant acid phosphatase, matrix metalloproteinase-9, cathepsin K, calcitonin receptors, and d2 isoform of the vascular ATPase domain. The strength of binding of IgG to Fc is better than that of the spA ligand. This SpA contains 90% to 100% of *Staphylococcus aureus* strains. Therefore, protein A is used as a target for the detection and identification of *Staphylococcus aureus.*

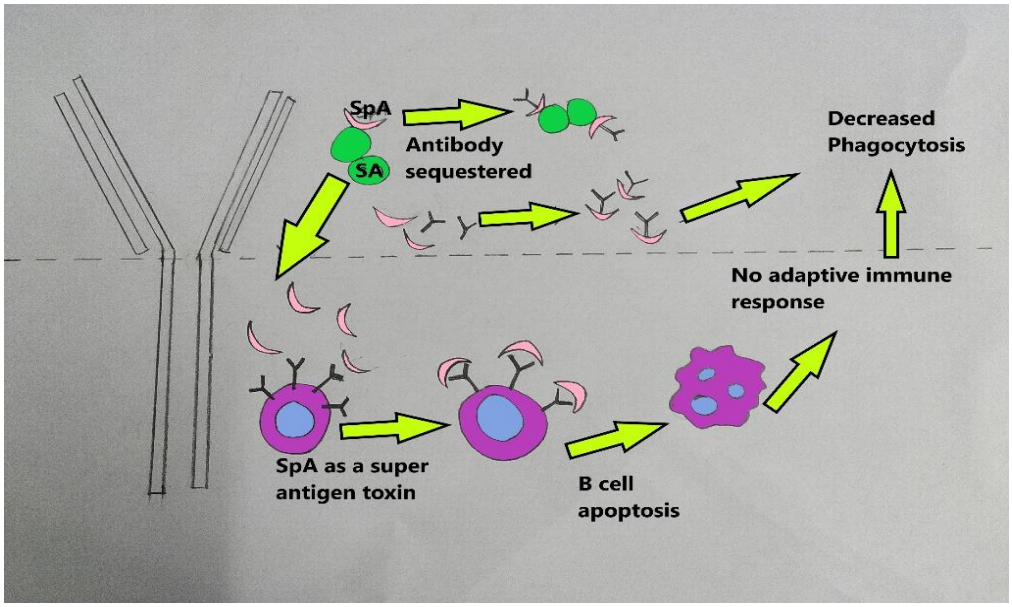

Figure 4: SpA-antibody reaction and phagocytosis

The abscess formation is highly influenced by *S.aureus* protein SpA and Sbi. SpA and Sbi induce inflammatory cytokines and chemokines production during infection. This initiates a cascade of events that lead to the early recruitment of neutrophils, modulate their lifespan in the skin and contribute to abscess formation and consequently bacterial eradication. Neutrophil recruitment to the site of infection is the hallmark of *S. aureus* infections and is required for bacterial clearance. *S. aureus* induced an increase in levels of IL-1β and IL-6. Studies indicate that inflammatory responses are higher during the expression of SpA protein and are positively correlated.

Toxin release by Staphylococcus Aureus:

Staphylococcus aureus produces many different virulence factors and manipulate the host's immune response for bacterial survival. Most of these toxins are exotoxins. Around 40 endotoxins are known of which many have high similarity structurally and functionally. The functions of the exotoxins are cytotoxins, superantigens, and cytotoxic enzymes. The cytotoxins help in the lysis of erythrocytes, platelets, endothelial cells, epithelial cells, and certain leukocytes and help with cytolysis by binding. A major superantigen is Staphylococcal protein A (SpA), the only known B-cell superantigen produced by *S. aureus*. A majority of clinical isolates contain SpA in the core genome.

Metagenomics for staphylococcus aureus:

The bacteria can travel through the bloodstream called bacteraemia and infect almost any site in the body, mostly endocarditis and bones like osteomyelitis. The bacteria also tend to accumulate on medical devices in the body, such as artificial heart valves or joints, pacemakers inside the heart, and catheters inserted through the skin into blood vessels. To understand the process at the gene level these metagenomic tools will provide all the necessary tools and help us to understand better.

Metagenomics is the study of genetic material recovered directly from environmental samples. It can be used to study the genetic diversity of microbial communities, including those containing Staphylococcus aureus bacteria. The gene sequence pathway is taken from KEGG analogue then the target is identified.

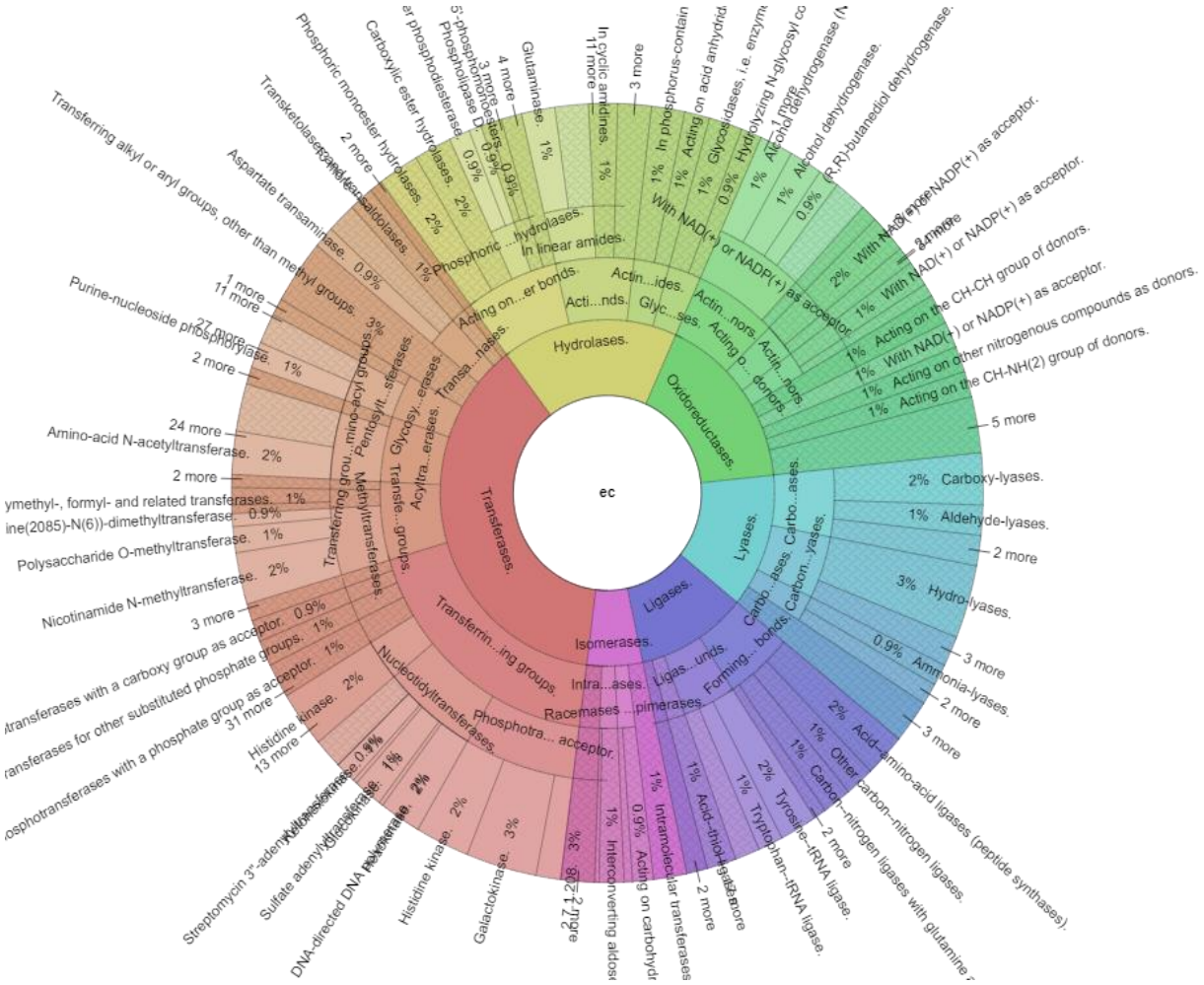

Figure 5: EC image of staphylococcus aureus – Metagenomics

Metagenomic techniques have revealed the presence of multiple S. aureus strains in a single environment, as well as the role of horizontal gene transfer in the evolution of antibiotic resistance. Additionally, metagenomics can be used to identify novel virulence factors and understand the transmission mechanisms. Finally, metagenomics can help in the development of diagnostic techniques and treatments for S. aureus infections.

Staphylococcus aureus can cause multiple forms of infections extending from superficial skin infections to food poisoning and life-threatening infections. The organism has several ways to divert the effectiveness of the immune system: secreting immune-modulating proteins that inhibit complement activation and neutrophil chemotaxis or lysis, modulating the sensitivity to cationic antimicrobial peptides (such as defensin) by increasing the positive net charge of its cytoplasmic membrane, and expression of superantigens that prevent the development of a normal immune response or cause an emetic response when ingested (figure 6).

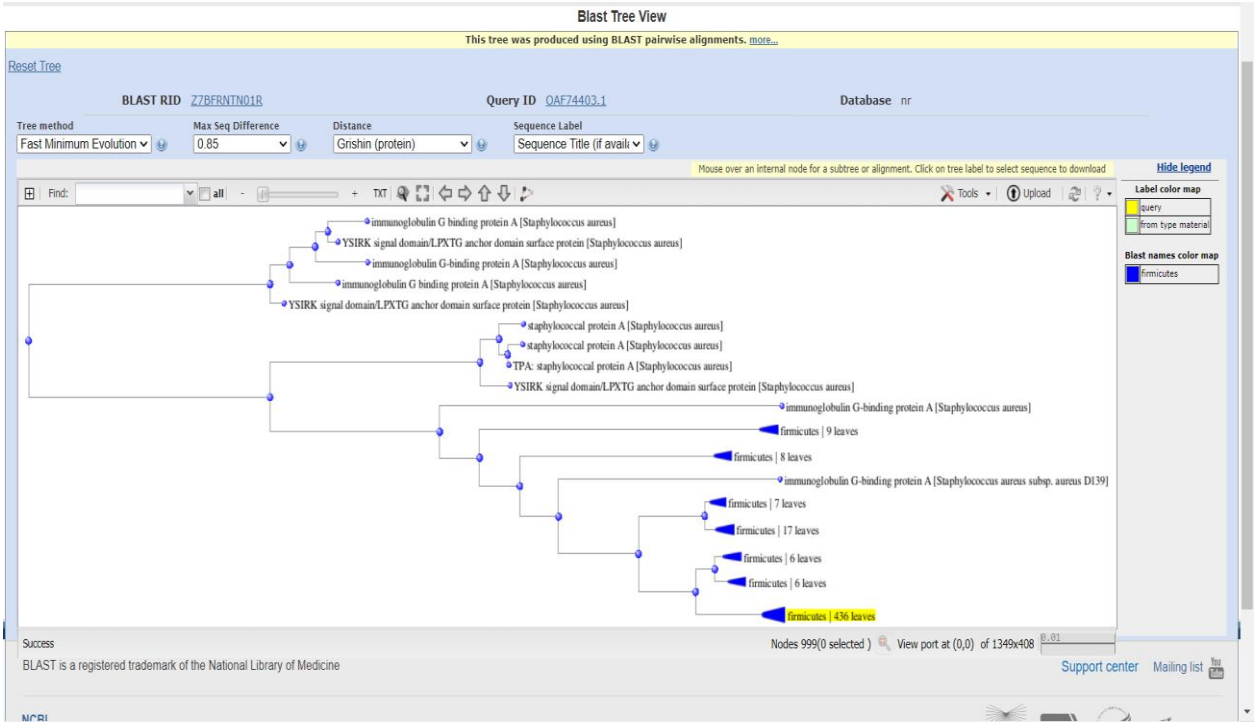

Figure 6: BLAST tree view from NCBI. This shows the phylogenic evolution of *staphylococcus aureus*

4. Discussion

Bioinformatics is the application of computational tools to the analysis of biological data. It can be used to identify potentially pathogenic bacteria by analyzing gene sequences, protein structures, and other biological features.[4] The most common approach is to compare the sequences of known pathogens with the sequences of unknown bacteria, looking for similarities that indicate a potential match. Other methods include sequence alignment and phylogenetic analysis, which can be used to infer the evolutionary relationship between different species of bacteria [5]. Additionally, machine learning algorithms can be used to classify bacteria based on their genetic features.

The bacteria themselves can also produce enzymes and toxins that damage tissues and promote the formation of pus. For example, some bacteria release proteases that break down proteins in the body, while others produce exotoxins that damage cells and disrupt the immune response says *Fierer et, al.* Similarly, if the infection is not treated, it can spread and lead to more severe symptoms. In some cases, the pus may need to be drained to help the body fight the infection. Antibiotics may also be prescribed to kill the bacteria causing the infection

Eamonn P Culligan et,al says that metagenomics can provide insight into the ecology and evolution of this pathogen, as well as its interactions with other bacteria and the environment.[3] Metagenomic analyses enable comprehensive investigations of microbial communities and provide unprecedented access to the genetic diversity therein. Likewise, an incredible amount of information has been gained in a relatively short time with regard to the taxonomic, phylogenetic, and genetic novelty within diverse metagenomes. The development of new technologies and the continual reduction in sequencing costs will expand this at an ever-increasing rate in the future, while the development of novel hosts and expression systems will increase hit rates and the variety of novel genes that can be discovered, thus helping to overcome some of the limitations of metagenomics. The development of novel and innovative screening assays will facilitate the discovery of previously unknown gene functions and novel therapeutic compounds.

The function YSIRK-G/S signal peptide motif using staphylococcal protein A as a model system. Both deletion of the YSIR sequence and/or replacements of the G and S residues significantly reduced signal peptide processing and the secretion of protein A. In contrast, mutational changes in the YSIRK-G/S motif did not affect the cell wall anchoring or the functional assembly of protein A. In the same way, *Taeok Bae et, al* says that the hypothesis that signals peptides contain information other than that for the default initiation into the secretory pathway has been previously examined. Comparative whole-genome sequencing revealed molecular evidence for host adaptation including gene decay and diversification of proteins involved in host–pathogen interactions.

5. Conclusion

The WHO stated that bacterial infections and antimicrobial resistance will be the top ten deadly diseases in the upcoming years. So, we need to find a quick solution for the redressal. It is pertinent to know the underlying mechanism of infection for both detections as well as treatment. The above study indicates the importance of the SpA and Sbi proteins in the establishment of a staphylococcus aureus infection and it's a target protein which will act as an efficient target. The formation of pus is also influenced by the SpA protein. SpA being the surface protein and one of the main proteins secreted at the time of infection is a good target for both identification and drug designing pertaining to

staphylococcus aureus infection. SpA protein has 5 homologous sequences and hence a reliable target.

Conflicts of Interest:
All authors declare no conflicts of interests. The graphical abstract has been designed by myself and the metagenomics done using KEGG pathway analysis.

References

[1] Edwina Jospiene M, S. Subramaniam, Shyama Subramaniam, "*Impact of Wound Infected Patients based on Demographic and Comorbidities*", International Journal of Science and Research (IJSR), Volume 12 Issue 1, January 2023, pp. 368-372, https://www.ijsr.net/get_abstract.php?paper_id=SR23103231731.

[2] Edwina Jospiene M, Dr. S. Subramaniam, Dr. Shyama Subramaniam, "*Prevalence of Wound Infections; Evaluation of its Growth Frequency and Antibiotic Resistance Pattern in Chennai Population*", International Journal of Science and Research (IJSR), Volume 12 Issue 1, January 2023, pp. 373-379, https://www.ijsr.net/get_abstract.php?paper_id=SR23103002502

[3] Martín, R., Miquel, S., Langella, P., & Bermúdez-Humarán, L. G. (2014). The role of metagenomics in understanding the human microbiome in health and disease. *Virulence*, *5*(3), 413–423. https://doi.org/10.4161/viru.27864

[4] Bayat A. (2002). Science, medicine, and the future: Bioinformatics. *BMJ (Clinical research ed.)*, *324*(7344), 1018–1022. https://doi.org/10.1136/bmj.324.7344.1018

[5] Clarridge J. E., 3rd (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical microbiology reviews*, *17*(4), 840–862. https://doi.org/10.1128/CMR.17.4.840-862.2004

[6] Nuffield department of medicine.-University of oxford

[7] Fierer, J., Looney, D., & Pechère, J. C. (2017). Nature and Pathogenicity of Micro-organisms. *Infectious Diseases*, 4–25.e1. https://doi.org/10.1016/B978-0-7020-6285-8.00002-2

[8] Culligan, E. P., Sleator, R. D., Marchesi, J. R., & Hill, C. (2014). Metagenomics and novel gene discovery: promise and potential for novel therapeutics. *Virulence*, *5*(3), 399–412. https://doi.org/10.4161/viru.27208

[9] Monteiro, A. S., Pinto, B. L. S., Monteiro, J. M., Ferreira, R. M., Ribeiro, P. C. S., Bando, S. Y., Marques, S. G., Silva, L. C. N., Neto, W. R. N., Ferreira, G. F., Bomfim, M. R. Q., & Abreu, A. G. (2019). Phylogenetic and Molecular Profile of *Staphylococcus aureus* Isolated from Bloodstream Infections in Northeast Brazil. *Microorganisms*, *7*(7), 210. https://doi.org/10.3390/microorganisms7070210

[10] Guinane, C. M., Ben Zakour, N. L., Tormo-Mas, M. A., Weinert, L. A., Lowder, B. V., Cartwright, R. A., Smyth, D. S., Smyth, C. J., Lindsay, J. A., Gould, K. A., Witney, A., Hinds, J., Bollback, J. P., Rambaut, A., Penadés, J. R., & Fitzgerald, J. R. (2010). Evolutionary genomics of Staphylococcus aureus reveals insights into the origin and molecular basis of ruminant host adaptation. *Genome biology and evolution*, *2*, 454–466. https://doi.org/10.1093/gbe/evq031

[11] Gonzalez, C. D., Ledo, C., Cela, E., Stella, I., Xu, C., Ojeda, D. S., ... & Gómez, M. I. (2019). The good side of inflammation: Staphylococcus aureus proteins SpA and Sbi contribute to proper abscess formation and wound healing during skin and soft tissue infections. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1865*(10), 2657-2670.

[12] Cheng, A. G., DeDent, A. C., Schneewind, O., & Missiakas, D. (2011). A play in four acts: Staphylococcus aureus abscess formation. *Trends in microbiology*, *19*(5), 225-232.

[13] Cheng, A. G., Kim, H. K., Burts, M. L., Krausz, T., Schneewind, O., & Missiakas, D. M. (2009). Genetic requirements for Staphylococcus aureus abscess formation and persistence in host tissues. *The FASEB Journal*, *23*(10), 3393.

[14] Kolar, S. L., Antonio Ibarra, J., Rivera, F. E., Mootz, J. M., Davenport, J. E., Stevens, S. M., ... & Shaw, L. N. (2013). Extracellular proteases are key mediators of S taphylococcus aureus virulence via the global modulation of virulence-determinant stability. *Microbiologyopen*, *2*(1), 18-34.

[15] Sugimoto, S., Iwamoto, T., Takada, K., Okuda, K. I., Tajima, A., Iwase, T., & Mizunoe, Y. (2013). Staphylococcus epidermidis Esp degrades specific proteins associated with Staphylococcus aureus biofilm formation and host-pathogen interaction. *Journal of bacteriology*, *195*(8), 1645-1655.

[16] Cruz, A. R., Boer, M. A. D., Strasser, J., Zwarthoff, S. A., Beurskens, F. J., de Haas, C. J., ... & Rooijakkers, S. H. (2021). Staphylococcal protein A inhibits complement activation by interfering with IgG hexamer formation. *Proceedings of the National Academy of Sciences*, *118*(7), e2016772118.

[17] Hong, X., Qin, J., Li, T., Dai, Y., Wang, Y., Liu, Q., ... & Li, M. (2016). Staphylococcal protein A promotes colonization and immune evasion of the epidemic healthcare-associated MRSA ST239. *Frontiers in microbiology*, *7*, 951.

[18] Crosby, H. A., Schlievert, P. M., Merriman, J. A., King, J. M., Salgado-Pabón, W., & Horswill, A. R. (2016). The Staphylococcus aureus global regulator MgrA modulates clumping and virulence by controlling surface protein expression. *PLoS pathogens*, *12*(5), e1005604.

[19] Foster, T. J. (2019). Surface proteins of Staphylococcus aureus. *Microbiology spectrum*, *7*(4), 7-4.