



Decoding *Staphylococcus aureus*: Properties, Mechanisms & Proteins

Authors: Jean Reichhold, Tim Holland, Merlin Steiner, Chinmay Jansen, Miguel Rodriguez, Renske de Vries

Abstract

This case study investigates an unknown DNA fragment that was traced back to the organism *Staphylococcus aureus* (*S. aureus*) using BLASTN. To further explore the genetic and functional contents of this fragment, various bioinformatics tools like NCBI BLAST, KBase, Benchling, etc. were used. The results are presented in this poster and documented in the corresponding logbook.

Introduction

Staphylococcus aureus is a facultative anaerobic, gram-positive bacterium that exists both as a commensal organism (microbiota, upper respiratory tract and skin) and as a pathogenic bacterium (bloodstream). Its circular genome ranges from 2,7 to 2,9 Mb and encodes for numerous virulence factors, including toxins. These toxins are particularly dangerous if the bacterium enters the bloodstream through a break in the skin, or in some cases through respiratory droplets. Of particular concern are methicillin-resistant *S. aureus* (MRSA) strains, which are resistant to many available antibiotics. MRSA can cause severe hospital-acquired infections, such as aggressive skin infections and high fever, which are especially difficult to treat in immunocompromised individuals. Overall, this makes *S. aureus*, especially MRSA strains, a significant pathogen in healthcare settings and a major challenge for clinical treatment.

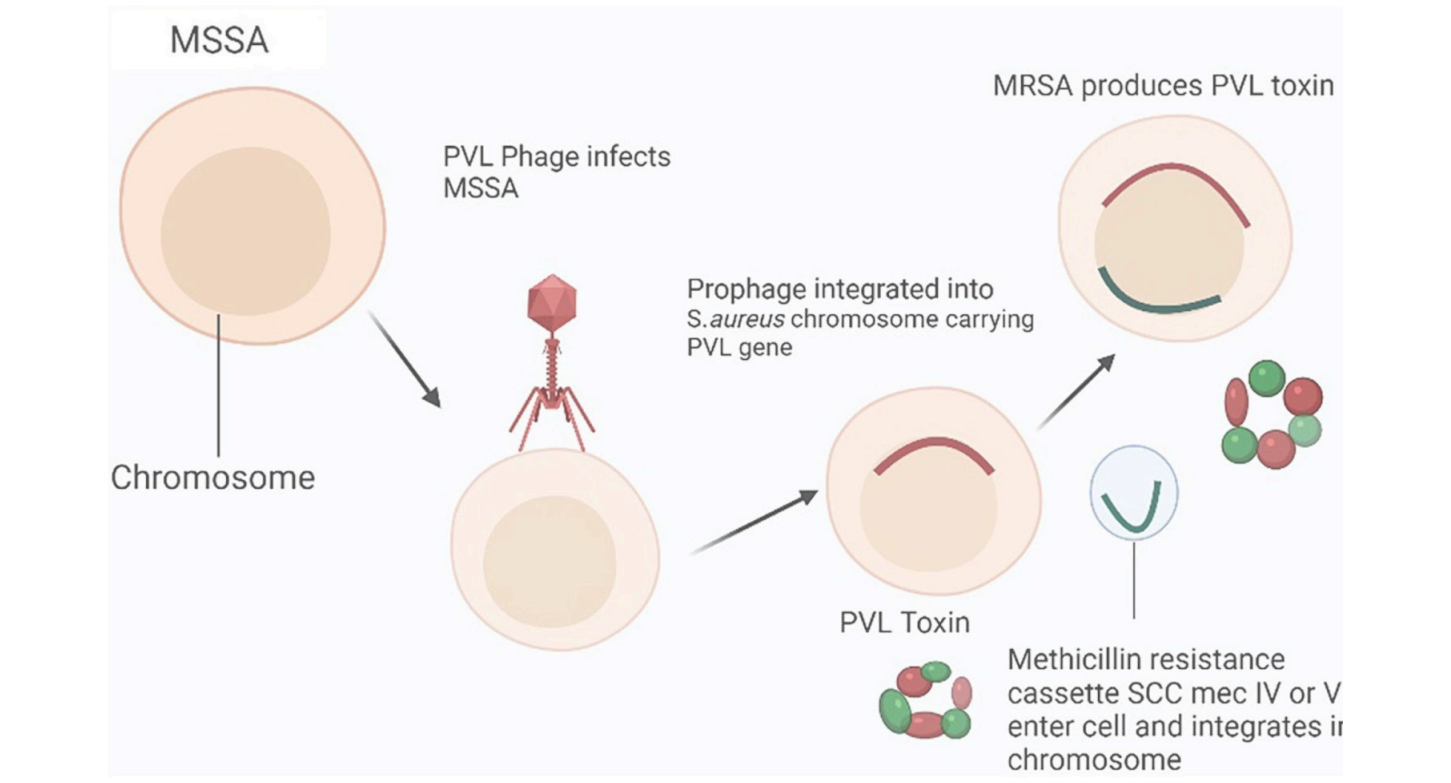


Fig. 1: Depiction of an MSSA strain (Methicillin-sensitive *S. aureus*), that firstly get infected trough a PVL phage, carrying the methicillin resistance. During lysogenic infection, the PVL toxin integrates itself into the host chromosome, now called MRSA strain. This MRSA strain is then able to produce the PVL toxin, increasing its virulence and making it highly antibiotic-resistant.

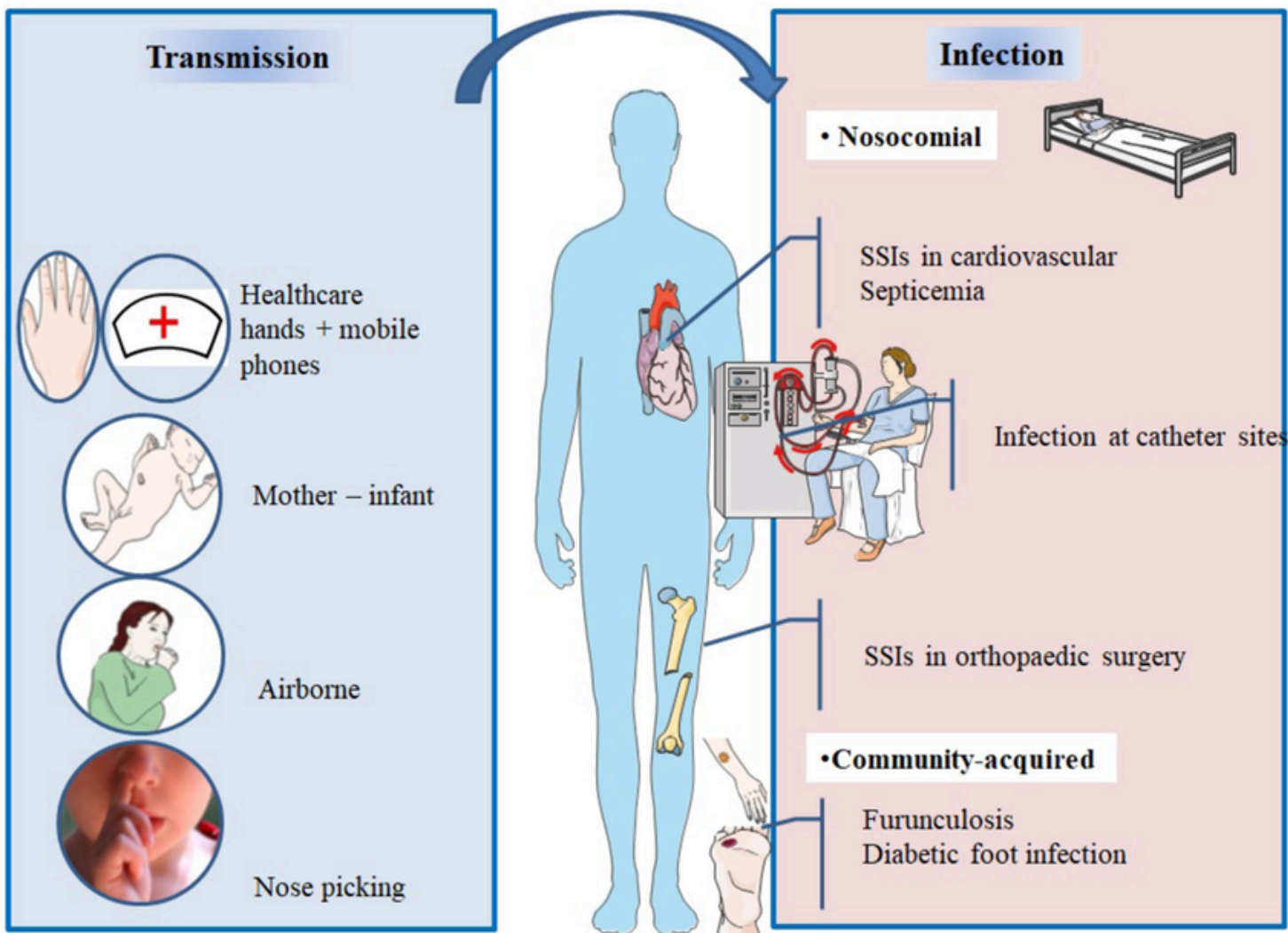


Fig. 2: Main transmission mechanisms of *S. aureus* and modes of infections.

Methods

- Sequence analysis:** BLASTN analysis identified the unknown DNA fragment as *S. aureus*. ORFs were predicted using ORFfinder, and Prokka (KBase) provided genomic annotation.
- Protein analysis:** DNA topoisomerase I was characterized using Clustal Omega/Jalview alignment across species and the Protein DataBase/Google AlphaFold3 for structural analysis.
- Organism analysis:** Phylogenetic analysis was performed using PhyloT/iTOL. Operon structure and KEGG pathway proteins were determined with FCoP. Protein-protein interactions were visualized with the STRING database.
- In silico overexpression:** XerC gene specific primers with NcoI and HindIII restriction sites designed with Primer-BLAST, followed by in silico ligation of the XerC gene into the pBAD/His vector using Benchling.
- Pathogenicity assessment:** Literature research was performed, examining the pathogenicity, cause of infection, toxic mechanisms, transmission pathways, pathogen significance and global impact of *Staphylococcus aureus*.

Description / Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident (%)	Acc. Len	Accession
Staphylococcus aureus subsp. aureus MSSA476	1425	1425	100%	0	99.85	689	Q6G9W3.1
Staphylococcus aureus subsp. aureus USA300	1423	1423	100%	0	99.56	689	Q2FHI8.2
Staphylococcus aureus	1422	1422	100%	0	99.56	689	Q06AK7.1
Staphylococcus aureus RF122	1419	1419	100%	0	99.42	689	Q2YXL8.2
Staphylococcus haemolyticus JCS1435	1308	1308	100%	0	90.13	689	Q4L5V2.1
Staphylococcus epidermidis RP62A	1285	1285	100%	0	88.82	689	Q5HPU2.1
Staphylococcus saprophyticus subsp. saprophyticus	1219	1219	100%	0	86.5	688	Q49X35.1
Halalkalibacterium halodurans C-125	982	982	99%	0	67.05	690	Q9KA23.1
Bacillus subtilis subsp. subtilis str. 168	964	964	100%	0	67.44	691	P39814.1
Lactococcus lactis subsp. lactis II1403	897	897	100%	0	61.73	710	Q9CG80.1

Fig. 3: The NCBI table showing the PBLAST results based on the second largest ORF (53), using the Swissprot database.

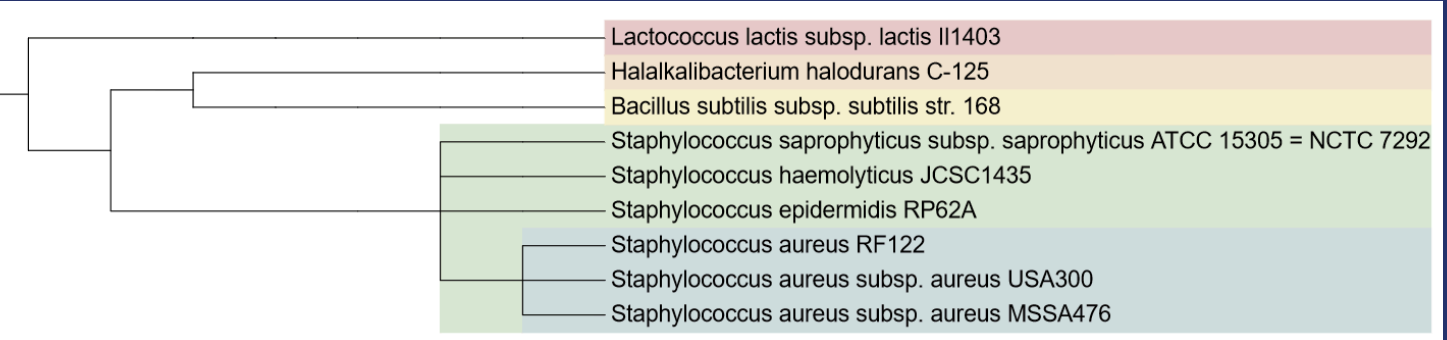


Fig. 4: Phylogenetic tree of the top BLASTP hits specific to organisms encoding the Topoisomerase I enzyme.

Type	Function	Aliases	Start	Strand	Length	Contig
CDS	Signal recognition particle receptor FtsY	ftsY	52	+	1,095	fragment_1
CDS	hypothetical protein		1,133	+	333	fragment_1
CDS	Succinate-CoA ligase [ADP-forming] subunit beta	sucC	10,898	+	1,167	fragment_1
CDS	Succinate-CoA ligase [ADP-forming] subunit alpha	sucD	12,086	+	909	fragment_1
CDS	hypothetical protein		13,318	+	873	fragment_1
CDS	DNA topoisomerase 1	topA	14,364	+	2,076	fragment_1
CDS	Methylenetetrahydr ofolate--tRNA-(ura cil-5-)-methyltransf erase TrmFO	trmFO	16,595	+	1,308	fragment_1
CDS	Tyrosine recombinase XerC	xerC	18,319	+	897	fragment_1
CDS	ATP-dependent protease subunit HsIV	hsIV	19,212	+	546	fragment_1
CDS	Signal recognition particle protein	ffh	1,491	+	1,368	fragment_1

Fig. 5: Top 10 results from KBase analysis of fragment 1, ordered by coding sequences (CDS) on the forward strand (+). The table provides the basis for the following investigated protein topoisomerase I and XerC gene.

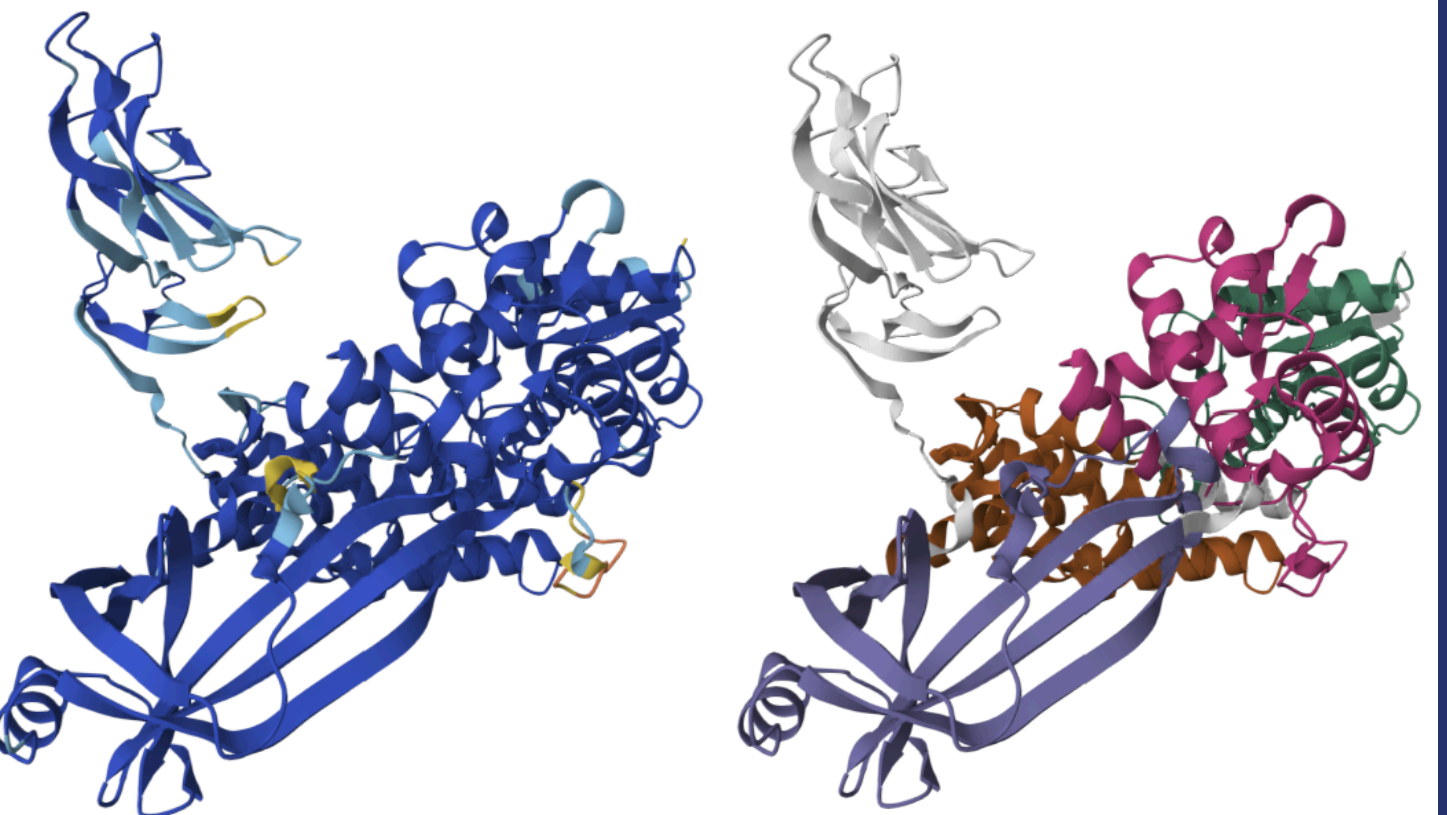


Fig. 6: AlphaFold prediction of DNA topoisomerase I (left). Model confidence scores: Dark blue (Very High pLDDT > 90), light blue (High pLDDT > 70), yellow (Low 70 > pLDDT > 50) and orange (Very low pLDDT < 50). The enzyme's 4 highly conserved central domains (right) differentiated by colors; green, pink, orange and purple.

Results

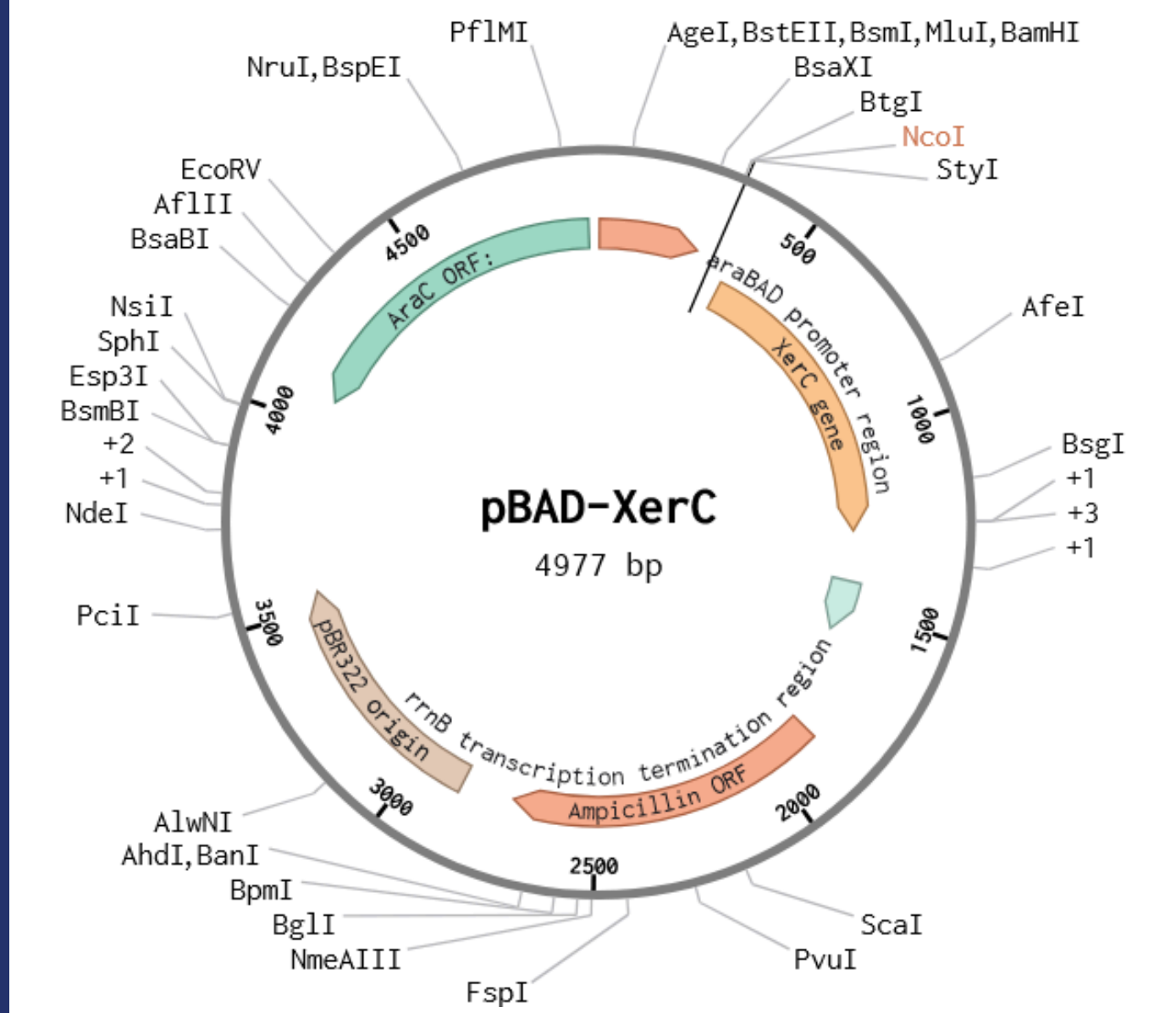


Fig. 7: In silico design of the overexpression vector of tyrosine recombinase XerC. Using primers and restriction sites, the XerC gene was isolated from the genome, and the restriction sites were used to create complementary sticky ends in the plasmid / gene of interest for ligation.

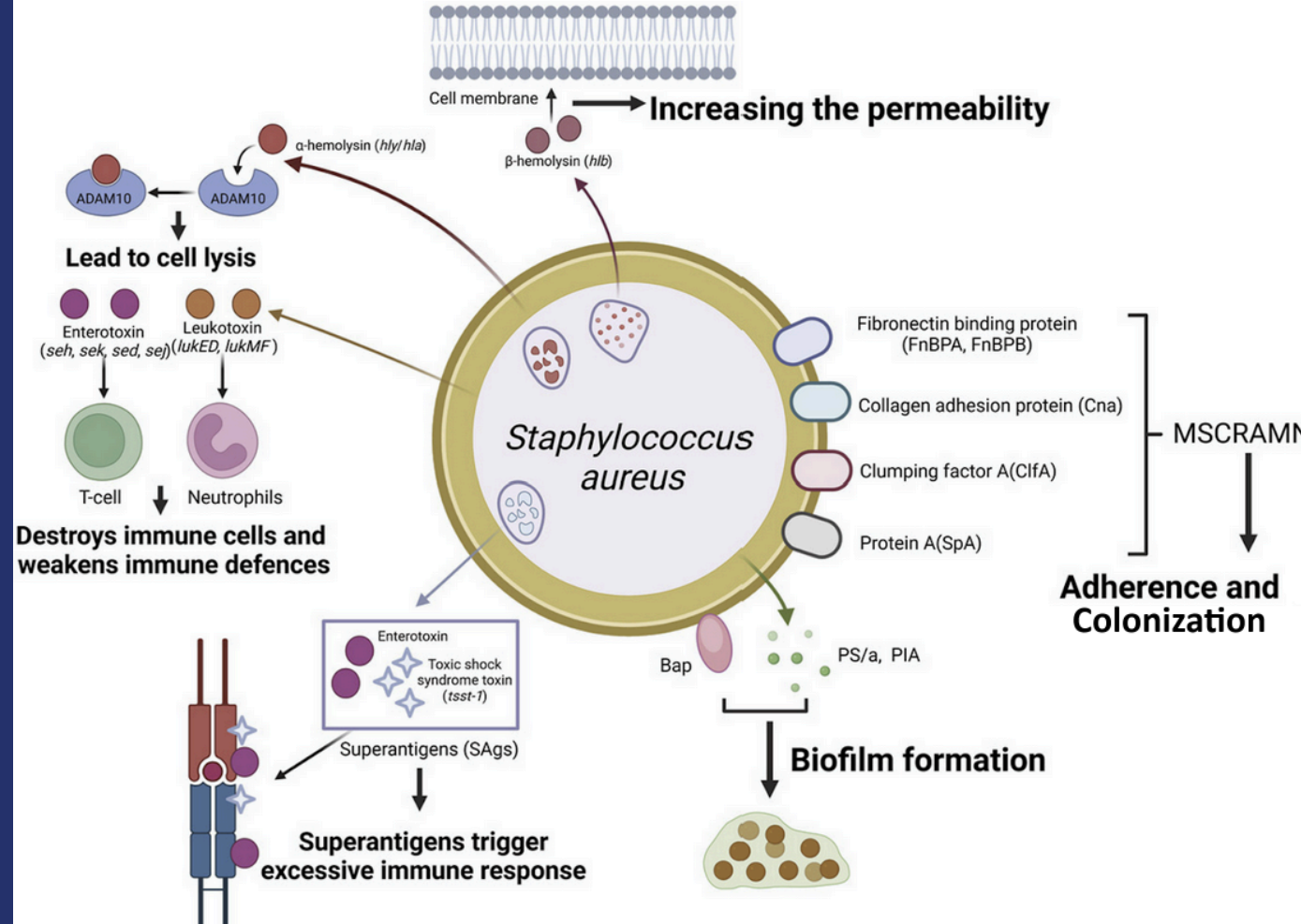


Fig. 8: Virulence factors of *Staphylococcus aureus*, including surface proteins (MSCRAMMs), α -hemolysin-mediated cell lysis, leukotoxins and enterotoxins for immune evasion, superantigens triggering excessive immune responses, and secreted proteins promoting biofilm formation.

S. aureus genome and pathogenic impact:

- Its genome contains ~2830 genes, from which ~2750 are protein-coding.
- It is one of the leading causes of hospital acquired infections and deaths related to several toxins that are resistant to antibiotics.
- The global burden of MRSA has more than doubled over three decades: while MRSA infections resulted in 57,200 attributable deaths worldwide in 1990, this number increased to ~130,000 deaths in 2021.

Conclusion

The unknown sequence was traced back to *Staphylococcus aureus*. Using tools like NCBI blast, ORF finder, KBase, phyloT, iTOL, Benchling and AlphaFold, related genes and proteins were analyzed. Using literary research papers, we show the pathogenicity and virulence of *S. aureus*. Due to its surface proteins for adherence and colonization, and toxins like enterotoxin and leukotoxin, *S. aureus* can easily infect a host. The specific protein retrieved from fragment 1 was DNA topoisomerase I, an enzyme that removes supercoils during replication. The XerC gene was chosen for the in-silico design, to over express and generate primers for restriction analysis.

References

Tong, X. et al. (2025) Virulence of bacteria causing mastitis in dairy cows: A literature review, MDPI. Available at: <https://www.mdpi.com/2076-2607/13/1/167>

Siddiqui, A.H., K., Janak (2023) Methicillin-resistant *Staphylococcus aureus*, StatPearls [Internet]. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK48221/>

Staphylococcus aureus basics (2024) Centers for Disease Control and Prevention. Available at: <https://www.cdc.gov/staphylococcus-aureus/about/index.html>

Shoib, M. et al. (2025) MRSA compendium of epidemiology, transmission, pathophysiology, treatment, and prevention within one health framework, Frontiers. Available at: <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.1067284/full>

GBD 2021 Antimicrobial Resistance Collaborators (2024) Global burden of bacterial antimicrobial resistance 1990-2021: A systematic analysis with forecasts to 2050, Lancet (London, England). Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC11718157/>

Linz, M.S. et al. (2023) Clinical impact of staphylococcus aureus skin and soft tissue infections, Antibiotics (Basel, Switzerland). Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC10044708/>

G. Ram, R.P Novick (2017) Staphylococcal Pathogenicity Islands-movers and shakers in the genomic firmament, Current opinion in microbiology. Available at: <https://pubmed.ncbi.nlm.nih.gov/29100762/>

Zubair, S. et al. (2015) Complete genome sequence of *Staphylococcus aureus*, strain IIR_eymole1/1, isolated from a Kenyan dromedary camel - environmental microbiome, BioMed Central. Available at: <https://environmentalmicrobiome.biomedcentral.com/articles/10.1186/s40793-015-0098-6>

Tong, S. Y. C., et al. (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661. <https://doi.org/10.1128/CMR.00134-14>

Thingstad, T. F., et al. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. Virulence, 12(1), 2175-2192. <https://doi.org/10.1080/21505594.2021.1878688>

Tong, S. Y. C., et al. (2025). The virulence toolkit of *Staphylococcus aureus*: a comprehensive review of toxin diversity, molecular mechanisms and clinical implications. European Journal of Clinical Microbiology & Infectious Diseases, 44(5), 95-108. <https://doi.org/10.1007/s10096-025-05148-y>