

SJMLS - 10(1) - 026

**Prediction of antibiotic target proteins of *streptococcus pyogenes* using network pharmacology approach**

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Author for Correspondence\*: aluefuafo@gmail.com. <https://dx.doi.org/10.4314/sokjmls.v10i1.26>**Abstract**

Many extracellular components secreted by Group A Streptococcus, also called *S. pyogenes*, are believed to be virulence factors. Therefore, this study aimed to predict antibiotic target proteins from *S. pyogenes*, rank the proteins based on their text-mining scores using the network pharmacology approach and provide an array of genes that could be identified following genome sequencing techniques. Cytoscape software version 3.9.1 was employed in this process. STRING; PubMed query was used as the data source to import networks from public databases. The species selected was *Streptococcus pyogenes*, and the PubMed query was Antibiotics Resistance Streptococcus. The confidence score was set at 0.7 while the maximum number of *S. pyogenes* target proteins to be downloaded was 50. The PubMed query returned 15,614 results, of which 9999 were downloaded, including target Proteins from *S. pyogenes* species widely mentioned in published abstracts. Nodes in networks represent the proteins, and edges indicate interactions between the proteins. The outcome displayed target proteins for *S. pyogenes*, including Pbp1A, gyrA, parC, MefE, gyrB-2, folP, adhP, GlcK, pheS, AKZ49992.1, and scpB, with high text-mining scores arranged in descending order. The text-mining score showed how frequently the target proteins are mentioned in the STRING database, with the most mentioned protein (pbp1A) having the highest score. Pbp1A, gyrA, parC, MefE, gyrB-2, folP, adhP, GlcK, pheS, AKZ49992.1, and scpB were predicted as proteins with high text-mining evidence in *S.*

*pyogenes* resistance using the network pharmacology approach.

**Keywords:** *S. pyogenes*, Resistance, Infection, Network pharmacology, Protein interaction

**Introduction**

*Streptococcus pyogenes* (*S. pyogenes*) is among the most significant Gram-positive human pathogens, with the skin and mucous membranes as the sole known reservoirs in nature (Passàli *et al.*, 2007; Yu *et al.*, 2023). According to WHO estimates, severe group A streptococcal infections specifically, invasive illness and the aftermath of acute rheumatic fever and subsequent rheumatic heart disease cause about 500,000 deaths per year (Jasim, Hatem and Mohammed, 2021).

Group A streptococcal infections cause roughly 9,600 cases and 1,100 - 1,300 fatalities annually in the United States, where the yearly rate of infection is approximately 3.5 cases per 100,000 people (Jasim, Hatem and Mohammed, 2021). Acute rheumatic fever and acute post-streptococcal glomerulonephritis are two nonsuppurative sequelae of group A Streptococcus that persist, especially in developing nations, in addition to acute infections (Vindebro, 2014; Cattoir, 2016; Ikebe *et al.*, 2021). According to Jasim, Hatem and Mohammed, (2021), developing nations account for over 95% of the estimated 294,000 fatal cases of rheumatic heart disease worldwide, and they also host the majority of other invasive group A streptococcal infections.

Group A *Streptococcus*, also known as *S. pyogenes*, secretes several extracellular products, many of which are thought to represent virulence factors (Ferretti, Stevens and Fischetti, 2016). Many of these proteins have been investigated in the past, either separately or in combination, to ascertain how they work to spread infection, elude host defenses, and whether they may be used as possible antigens in a vaccine against *S. pyogenes* (Ferretti, Stevens and Fischetti, 2016). Numerous antimicrobial medications have been developed to target the vulnerable proteins found in bacterial infections (Haenni *et al.*, 2010). Resistance in *S. pyogenes* is brought about by changes to these target proteins, leading to decreased affinity for the drug. These changes could arise from mutations and/or mosaics in the target protein genes of the extremely resistant isolates (Haenni *et al.*, 2010). Using genome sequencing techniques, the genes behind this pathogen's resistance to various antibiotics can be identified (Haenni *et al.*, 2010; Ahmadi *et al.*, 2022).

Proteins engage in a variety of functional partnerships and interactions that are fundamental to cellular processing, and their systematic characterization helps to provide context in molecular systems biology (Szkarczyk *et al.*, 2015). However, known and predicted interactions are scattered over multiple resources, and the available data exhibit notable differences in terms of quality and completeness. The STRING database (<http://string-db.org>) aims to provide a critical assessment and integration of protein-protein interactions, including direct (physical) as well as indirect (functional) associations (Szkarczyk *et al.*, 2015). Undoubtedly, the most beneficial networks for functional analyses are those that incorporate every kind of interaction, including information relay, transient binding, stable physical relationships, substrate chaining, and others. Such functional relationships between proteins are the focus of the global STRING database (Search Tool for the Retrieval of

Interacting Genes/Proteins) (Szkarczyk *et al.*, 2015). Significant insights into the roles of proteins and cellular processes have been gained from the statistical analysis of bacterial PPIs across multiple species (meta-interactomes) (Ahmadi *et al.*, 2022). Therefore, this present study was designed to use the network pharmacology approach to predict the antibiotic target protein in *S. pyogenes*, rank the proteins according to their text-mining scores, and provide an array of genes that could be identified following genome sequencing techniques.

## Materials and Methods

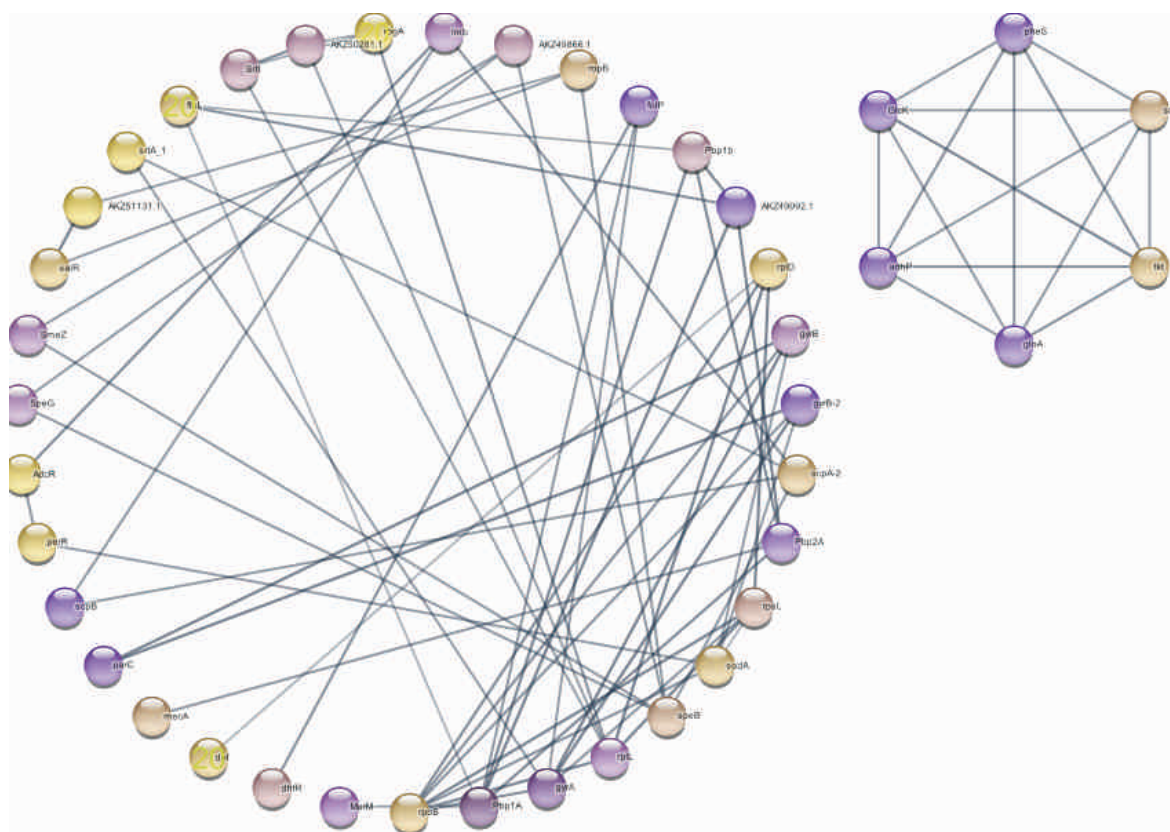
### Database Mining and Network Construction

Cytoscape software (v3.9.1) was employed in this process. From the App Store, StringApp (v2.0.1) was installed into Cytoscape. From file in cytoscape, we proceeded to import and then to network from public database. STRING; PubMed query was used as the data source to import networks from public databases. The species selected was *Streptococcus pyogenes*, and the PubMed query was Antibiotics Resistance *Streptococcus*. The confidence score was set at 0.7 while the maximum number of *S. pyogenes* resistance proteins to be downloaded was 50. The PubMed query returned 15,614 results, of which 9999 were downloaded, including target proteins from *S. pyogenes* species widely mentioned in published abstracts. Nodes in networks represent the proteins, and edges indicate interactions between the proteins.

## Results

String network for *S. pyogenes* resistance proteins depicting nodes and edges with their respective node label.

The network of *S. pyogenes* target proteins constructed from the string database is shown below (Figure 1). Proteins have been colored according to the text-mining evidence relating to antibiotic targets. Purple color represented nodes with high text-mining scores, and gold represented nodes with low text-mining scores.



**Figure 1. Merging of string data into a network to reveal *S. pyogenes* target proteins interactions. Purple color represented nodes with high text-mining scores, and gold represented low text-mining scores.**

**3.2. Ranking of *S. pyogenes* target proteins according to their text-mining scores**

The target proteins were ranked according to their text-mining scores using the Cytoscape software from the highest to the lowest with Pbp1A, gyrA, parC, MefE, gyrB-2, folP, adhP,

GlcK, pheS, AKZ49992.1, and scpB having high text-mining scores as shown in Table 1. The text-mining score showed how frequently the *S. pyogenes* target proteins are mentioned in the downloaded abstract, with the most mentioned protein (pbp1A) having the highest score.

**Table 1. Text-mining scores of *S. pyogenes* target protein.**

Names of Genes	Target proteins	Text-Mining Score	Names of Genes	Target proteins	Text-Mining Score
Pbp1A	Penicillin-binding protein	9.951378	rpsL	30S ribosomal protein S12	2.551635
GyrA	DNA gyrase subunit A	8.738247	aroE_2	Shikimate dehydrogenase	2.546044
ParC	DNA topoisomerase IV subunit A	6.378457	def2	Peptide deformylase	2.413671
MefE	Macrolide ABC transporter permease	6.005408	speB	Peptidase C10	2.397673

gyrB-2	DNA gyrase subunit B	5.237352	mecA	Adapter protein mecA	2.281634
FolP	Dihydropteroate synthase	5.033023	sdaAA	Serine dehydratase	2.24391
AdhP	Ethanol-active dehydrogenase	4.827964	ropB	XRE family transcriptional regulator	2.162123
GlcK	Glucokinase	4.826931	scpA-2	Peptidase C5	2.128116
pheS	phenylalanine-tRNA ligase	4.577933	ComEC	Competence protein ComEC	2.09728
AKZ499 92.1	Penicillin-binding protein	4.361144	tkf	Transketolase	2.091279
scpB	Segregation protein A	4.076285	rpoB	DNA-directed RNA polymerase subunit beta	2.042689
glnA	Glutamine synthetase	3.98336	sodA	Superoxide dismutase	2.029263
Pbp2A	Penicillin-binding protein	3.772604	def	Peptide deformylase	1.979262
MurM	UDP-N- acetylmuramoylpentap eptide	3.478547	ftsL	Cell division protein FtsL	1.979262
rplL	50S ribosomal protein L7/L12	3.346047	salR	Histidine kinase	1.963168
lmb	Adhesion protein	3.317815	rplD	50S ribosomal protein L4	1.897423
xpt	Xanthine phosphoribosyltransfer ase	3.289155	DexB	Glucan 1,6-alpha- glucosidase	1.761577
SmeZ	Exosortase	3.287504	perR	Fur family transcriptional regulator	1.741603
SpeG	Exosortase	3.276118	mrsA	Hypothetical protein	1.705181
gyrB	DNA gyrase subunit B	3.213681	cadX	ArsR family transcriptional regulator	1.682933
AKZ498 66.1	Hypothetical protein	2.945866	AdcR	MarR family transcriptional regulator	1.608977
AKZ502 81.1	Relaxase	2.934479	srtA_1	Sortase	1.607454
SrtI	Hypothetical protein	2.909163	rbgA	Essential GTPase	1.592429
Pbp1b	Transglycosylase	2.861868	cdsA	Phosphatidate cytidyltransferase	1.573479
dhfR	Dihydrofolate reductase	2.690801	AKZ511 31.1	SalK	1.565846

## Discussion

The STRING database (<https://www.stringdb.org>) is the source of PPIs. The reason STRING was selected is that it covers a wide range of bacterial species and may be extended to include other species (Ahmadi *et al.*, 2022). Bacterial antimicrobial resistance is typically genetically programmed. Antimicrobial resistance can be genetically encoded in a variety of ways, including overexpression or duplication of existing genes, point mutations, or the acquisition of wholly new genes via horizontal gene transfer (Boolchandani, DSouza and Dantas, 2019). Improvements in next-generation sequencing technologies and computational tools are allowing for faster identification and characterization of antimicrobial resistance genes in genomes and metagenomes. These emerging technologies and approaches complement existing culture-based methods for clinical and surveillance applications, allowing for rapid and sensitive resistance evaluations in both cultivable and uncultivable bacteria (Boolchandani, DSouza and Dantas, 2019). In this study, we ranked *S. pyogenes* target proteins according to their text-mining scores using the Cytoscape software from the highest to the lowest with Pbp1A, gyrA, parC, MefE, gyrB-2, folP, adhP, GlcK, pheS, AKZ49992.1, and scpB having high text-mining scores. The text-mining score showed how frequently the *S. pyogenes* target proteins are mentioned in the downloaded abstract, with the most mentioned protein (pbp1A) having the highest score. There is a report that there are three primary ways of resistance to beta-lactams, which include reduced affinity for penicillin-binding protein (PBP) binding, decreased access to PBPs, and the breakdown of antibiotics by beta-lactamases. The primary cause of Gram-positive organisms' resistance to beta-lactam antibiotics is target modification, which results in structural alterations to PBPs (Yu *et al.*, 2023). The capacity of PBPs to bind penicillin is affected by substitutions of amino acids, which have been shown to reduce susceptibility to penicillin in GAS. The identification and subsequent genetic research of strains that are resistant to antibiotics have shown that in comparison to susceptible strains, resistant strains have chimeric high molecular mass penicillin-binding proteins (HMM PBPs) (Yu *et al.*, 2023). Instead of single-event horizontal gene transfer of beta-lactamase or low beta-lactam affinity HMM PBPs, resistance Streptococci evolve from penicillin-susceptible to reduced susceptibility and ultimately to

non-susceptible through the progressive accumulation of amino acid substitutions in HMM PBPs (Yu *et al.*, 2023). From our findings, another protein with high text-mining score is the mef(E) proteins. The Mef proteins have 12 transmembrane segments (TMS) and are proton-dependent efflux pumps that are members of the major facilitator superfamily (MFS) (Yu *et al.*, 2023). The mef gene has been found to contain multiple allelic variations or subclasses, the most well-known of which are mef(A), first discovered in *S. pyogenes*, and mef(E), subsequently found in *S. pneumoniae*. The only resistance to 14- and 15-membered macrolides (the so-called M phenotype) is caused by active efflux caused by mef genes; resistance to 16-membered macrolides, lincosamides, and streptogramins is absent (Szklarczyk *et al.*, 2015; Dilucca, Cimini and Giansanti, 2021). The text-mining evidence for gyrA, parC, and gyrB-2 were also high in our findings. There is a report that the type II topoisomerases, DNA gyrase and DNA topoisomerase IV, which are both heterotetramers made up of GyrA2B2 and ParC2E2, are the targets of fluoroquinolone (FQ) compounds. The cause of FQ resistance in Gram-positive cocci is point mutations that alter the target. These mutations mainly occur in 120-bp conserved segments of the parC and gyrA genes, which are known as the quinolone resistance-determining regions (QRDRs) (Cattoir, 2016; Boolchandani, DSouza and Dantas, 2019). Chromosome point mutations in the dhps (folP) gene, which codes for the native DHPS, or the acquisition of plasmid-borne sulphonamide genes coding for resistant DHPS enzymes are the causes of bacterial resistance to sulfonamides (Cattoir, 2016).

## Limitations of the study

This insilico study ranked *S. pyogenes* target proteins derived from the STRING database according to their text-mining evidence. Where *S. pyogenes* resistance occurs, whether in vitro or in vivo, this study has provided an array of *S. pyogenes* genes and their text mining evidence that could be identified following genome sequencing techniques.

## Conclusion

Using the network Pharmacology approach, we predicted Pbp1A, gyrA, parC, MefE, gyrB-2, folP, adhP, GlcK, pheS, AKZ49992.1, and scpB as proteins with high text-mining evidence in *S. pyogenes* resistance.

### Declaration of patient consent

Patient consent is not required as there are no patients in this study.

**Financial support and sponsorship:** Nil.

### Credit Authorship Statement

The authors have made equal contributions to the paper.

### Conflicts of interest

There are no conflicts of interest.

### Ethical Waiver

An ethical waiver with clearance number PTAC/Sp/Arp/(RV)/65-24 dated Jan 28th 2024, was obtained from the ethical clearance committee of Usmanu Danfodiyo University, Sokoto, Nigeria.

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**Citation:** Omobhude Fidelis Aluefua, Abdulmajeed Yunusa, Ridwanu Zauro Abubakar. Prediction of antibiotic target proteins of streptococcus pyogenes using network pharmacology approach. *Sokoto Journal of Medical Laboratory Science*; 10(1): 247–252. <https://dx.doi.org/10.4314/sokjmls.v10i1.26>

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