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ARTÍCULOS ORIGINALES

In silico identification of auxiliary genes required for β-lactam resistance

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ABSTRACT

Staphylococcus aureus is a type of bacteria commonly found on the skin and in the nasal passages of healthy individuals. However, it can also cause a range of infections in clinical settings. One of the most concerning aspects of S. *aureus* is its ability to develop antibiotic resistance. Methicillin-resistant S. *aureus* (MRSA) is a strain of the bacteria that is resistant to many antibiotics and can be difficult to treat. The primary mechanism of methicillin resistance in MRSA is the presence of the *mecA* gene, which encodes for a modified penicillin-binding protein known as PBP2a. This protein has a lower affinity for beta-lactam antibiotics. Another gene, blaZ, is also present in MRSA and encodes for a beta-lactamase enzyme that can hydrolyse and inactivate beta-lactam antibiotics such as penicillin. In addition, there are several auxiliary factors that can contribute to beta-lactam resistance. They can include efflux pumps, enzymes that modify or degrade antibiotics, and bacterial cell wall modifications that reduce the affinity of antibiotics for their targets. In this study, with the aid of the *in silico* identification method, we identify the novel auxiliary factors *aux1, aux2, aux4, aux11, aux14, aux16* and *aux19*. Next, we show that *aux2, aux4, aux11, aux14* are not directly involved in β-lactam resistance, but may contribute through other mechanisms that decrease the efficacy of these antibiotics, whereas *aux16 and aux19* are directly associated with β-lactam and bacitracin resistance, respectively. Understanding the various auxiliary factors that contribute to beta-lactam resistance can help guide the development of new antibiotics and other therapeutic strategies.

Keywords: auxiliary factors, Staphylococcus aureus, β-lactam antibiotic, antibiotic resistance, in silico identification..

1. INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium that is commonly found on the skin and mucous membranes of humans and other animals. It is a leading cause of healthcare-associated infections, including bacteremia, sepsis, toxic shock syndrome, and skin and soft tissue infections (1). *Staphylococcus aureus* is able to acquire resistance to multiple antibiotics, including methicillin, through the acquisition of antibiotic resistance genes. This ability to resist antibiotics has made it difficult to treat *S. aureus* infections and has led to the emergence of methicillin-resistant *S. aureus* (MRSA). MRSA is highly transmissible and can spread through contact with infected persons or contaminated surfaces and, therefore, it poses a major challenge to public health and medical care (2).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is classified into three main types: healthcare-associated (HA-MRSA), community-associated (CA-MRSA) and livestock-associated (LA-MRSA) (3). CA-MRSA strains are usually acquired in the community and typically causes mild to moderate skin and soft tissue infections, such as boils and abscesses. One of the most important and common CA-MRSA strains is MW2 (4).

MRSA strains possess the *mecA* gene, which encodes the alternative penicillin-binding protein PBP2A. This protein confers resistance to β-lactam antibiotics by decreasing their ability to bind to the cell wall. Additionally, some MRSA strains may encode the beta-lactamase BlaZ, which hydrolyses β-lactam antibiotics (5).

Although the presence of *mecA* is necessary for the resistance to β-lactam antibiotics, it is not sufficient. Therefore, apart from *mecA* and *blaZ*, many other genes involved in resistance to β-lactam antibiotics have been identified and

named such as *fem* genes (factors essential for methicillin resistance) and *aux* genes (auxiliary genes). Both of these factors are not related to *mecA* gene (6). A list of currently known accessory and auxiliary factors is presented on Table 1.

Auxiliary factors may affect the ability of antibiotics to penetrate the bacterial cell wall or alter the expression of resistance genes (28). One such factor is the presence of efflux pumps, which are membrane proteins that can pump antibiotics out of the bacterial cell. This can reduce the concentration of antibiotics inside the cell and make it more difficult for the antibiotics to be effective (6). Another factor is the presence of enzymes that can modify or degrade antibiotics. For example, some bacteria produce beta-lactamases that can hydrolyse and inactivate beta-lactam antibiotics (29). Bacterial cell wall modifications, such as the addition of amino acids or sugars to the peptidoglycan layer, can also contribute to beta-lactam resistance by reducing the affinity of antibiotics for their targets (30).

Although the mechanisms of resistance to β-lactam used by *S. aureus* have been studied for many years, there are still many unclear mechanisms. Therefore, the aim of the present study is to identify novel putative auxiliary factors genes present in *S. aureus* using the *in silico* identification method.

Table 1: *List of auxiliary factors.*

Gene	Function
glmM	Phosphoglucosamine mutase: conversion of glucosamine-6-phosphate to glucosamine-1-phosphate during the early stage of peptidoglycan synthesis (7).
glmS	Glucosamine-fructose-6-phosphate aminotransferase: conversion of D-fructose-6-phosphate to D-glucosamine 6-phosphate (8).
murA	Transferase: conversion of UDP-GlcNAc to UDP-GlcNAc-enoylpyruvate (9).
murB	Reductase: conversion of UDP-GlcNAc-enoylpyruvate to UDP-MurNAc (9).
murC-F	Mur ligases: sequential addition of the aminoacids L-Ala, D-Glu, L-Lys, and D-Ala-D-Ala to build the tetrapeptide side chain (10).
glyS	Glycine tRNA synthetase: provides a glycine substrate for the assembly of the pentaglycine bridge (11).
femX	Peptidyltransferase: Incorporation of the first glycine of the pentaglycine bridge (12).
femA	Peptidyltransferase: Incorporation of the glycines 2 and 3 of the pentaglycine bridge (12).
femB	Peptidyltransferase: Incorporation of the glycines 4 and 5 of the pentaglycine bridge (12).
femC	Glutamine synthetase repressor: Participates in the tetrapeptide side chain amidation (13).
gatD/murT	Amidotransferase complex: amidation of lipid II (14).
SAV1754	Probably functions as a lipid II flippase during its translocation to the outer leaflet of the cell membrane (15).
ftsW	Proposed lipid II flippase (16).
pbp1	PBP, possesses transpeptidase activity (17).
pbp2	Bifunctional PBP with transglycosylase and transpeptidase domains (17).
pbp4	PBP, possesses transpeptidase and carboxipeptidase activities (18).
prsA	Probably required for posttranscriptional maturation of PBP2a (19).
fmtA	Accessory PBP, possesses transpeptidase activity and has low affinity to penicillin (20).
Llm (tarO)	Transferase: transfer of an N-acetylglucosamine-1-phosphate residue from UDP-N-acetylglucosamine to undecaprenyl-phosphate to produce the WTA intermediate lipid α (21).
tarA	Transferase: transfer of an N-acetylmannosamine residue to the lipid α to form the intermediate lipid β (21).
tarB	Transferase: transfer of an sn-glycerol-3-phosphate unit to generate the intermediate lipid φ.1 (22).
tarD	Cytidylyltransferase: production of CDP-glycerol for TarB during WTA synthesis (22).
tarL	Cytidylyltransferase: required for ribitol phosphate polymerisation of WTA (22).
tarI	CDP-ribitol synthase: Synthesis of CDP-ribitol for TarL during WTA synthesis (22).
tarS	Glycosyltransferase: Addition of β-O-GlcNAc to the WTA (22).
ltaS	LTA synthesis (23).
spsB	Signal peptidase enzyme: posttranscriptional regulation of the protein LtaS (24).
pknB	Eukaryote-like serine/threonine kinases: Associated with increased resistance to β-lactam antibiotics (25).
sigB	Alternative sigma factor: Involved in β-lactam resistance (26).
vraSR	Two-component regulatory system: activation of specific genes required for antibiotic resistance in response to cell wall stress (27).

2. METHODOLOGY

In silico gene identification

The sequence of the genes of interest was downloaded from the nucleotide databases of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/).

In silico protein characterisation

With the aid of the programme protein-protein BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins), the translated sequence of the genes was aligned with the sequence of amino acids of already known and studied proteins in order to predict the function of the protein encoded by the gene of interest. Several characteristics of the protein such as the isoelectric point (pI), molecular weight (mW), co-localisation with other genes, the transmembrane helices, homology or structure were predicted with the aid of the online available programmes: Expert Protein Analysis System (https://www.expasy.org/), DeepTMHMM (http://www.cbs.dtu.dk/services/TMHMM/) and Homology detection & structure prediction by HMM-HMM comparison (HHpred) (https://toolkit.tuebingen.mpg.de/tools/hhpred).

3. RESULTS

In 1999 the identification of 21 novel auxiliary genes was reported in the Microbial Drug Resistance journal (31). Unfortunately, after this, some of these genes have not been further characterised and the function of many of these auxiliary genes is still unknown. In this study, we decided to predict the function and properties of the proteins encoded by uncharacterised genes by using different bioinformatics tools.

The sequence data of the aux genes: *aux1, aux2, aux4, aux11, aux14, aux16* and *aux19*, was retrieved with the aid of NCBI using the accession numbers found in the afore-mentioned report. As these genes were discovered in *S. aureus* strain COL. In order to predict the preliminary name of the gene and the function of its product, the amino acid sequence was aligned with homolog sequences of other organisms using protein-protein BLAST. This and other information related to the protein were collected with the aid of the following online available softwares: ExPaSy, TMHMM Server v. 2.0 and HHpred. All the data is shown in Table 2. Our results suggest that, among all the genes described by De Lencastre et al. in 1999 (31), *aux16* and *aux19* were directly associated with antibiotic resistance.

Table 2: *Characteristics of new auxiliary genes generated in the background of S. aureus strain MW2.*

aux gene	Accession number	ORF	Putative gene	Length (aa)	pI/MW	Stoichiometry	Solubility	Encoded protein	Function
aux1	Y18639	1	prpC	247	5.10 / 28076	Monomer	Soluble	Phosphorylated protein phosphatase	Cellular regulation
aux2	Y13639	388	pknB	664	5.76 / 74363	Monomer	Trans-membrane	Protein kinase	Cellular regulation
aux4	Y18630	-	ccpA	329	5.58 / 36060	Monomer	Soluble	Catabolite Control Protein A	Transcriptional regulation
aux11	Y18632	-	lysA	421	5.58 / 47035	Homodimer	Soluble	diaminopimelate decar-boxylase	L-lysine biosynthesis
aux14	Y14324	271	-	303	5.64 / 34812	ND	Soluble	RNase adapter protein RapZ	Cellular regulation
aux16	AJ131754	1[1]	cutD	540	8.93 / 60254	Homotrimer	Trans-membrane	Osmoprotectant transporter	Choline and betaine/carnitine transporter
		2	bla_{MBL}[2]	228	5.68 / 26239	Monomer	Soluble	MBL[2]	Resistance to broad range of β-lactam resistance
		4	gbsA	496	4.96 / 54623	Homotetra-mer	Soluble	Betaine aldehyde dehydro-genase	Glycine, serine and threonine metabolism. Betaine biosynthesis via choline pathway
		5[1]	betA	569	7.08 / 63624	Monomer	Soluble	Choline dehydrogenase	Glycine, serine and threonine metabolism. Oxidation of choline to betaine aldehyde and betaine aldehyde to glycine betaine
aux19	Y18641	1	vraD	252	7.03 / 27775	Monomer	Soluble	Membrane transport and signal transduction	ABC transporter. Also part of a two component system. Associated to bacitracin
		2	vraE	626	9.65 / 70105	Homotrimer	Trans-membrane	Membrane transport and signal transduction	ABC transporter. Also part of a two component system. Associated to bacitracin resistance

ND, not determined; [1]Partial sequence analysis; [2]Metallo β-lactamase.

The DNA sequences *aux1, aux2, aux4* and *aux14* are associated with regulation mechanisms, whereas *aux11* is associated with biosynthetic pathways.

The genes *aux1, aux2* and *aux14* matched respectively the phosphorylated protein phosphatase with 100% probability, the protein kinase with 100% probability and RNase adapter protein RapZ with 100% probability (Figure 1).

Figure 1: *HHpred analysis of putative auxiliary factors aux1 (a), aux2 (b), aux4 (c), aux11 (d) and aux14 (e), and DeepTMHMM analysis of aux2, showing the presence of a membrane-bound domain.*

This suggests that all the three genes are involved in cellular regulation. Moreover, a DeepTMHMM analysis shows that *aux2* is a transmembrane protein (Figure 1). The gene *aux4* matched the catabolite control protein a with 99.66% probability (Figure 1), which suggests that this gene is involved in transcriptional regulation. Finally, the gene *aux11* matched the diaminopimelate decarboxylase gene with 100% probability (Figure 1). Diaminopimelate decarboxylase is involved in L-lysine biosynthesis (32).

The DNA sequence aux16 could be associated with β-lactam resistance

The DNA sequence *aux16* comprises 5 open reading frames (ORFs). Among these ORFs, the ORF2 was predicted with 99.85% probability to belong to the Subclass B3 metallo-beta-lactamases (MBL) (Figure 2), which are a type of bacterial enzymes that can inactivate many beta-lactam antibiotics, such as penicillins, cephalosporins, and carbapenems (33). Additionally, other ORFs associated with aux16 may be involved in glycine, serine and threonine metabolism, betaine biosynthesis via choline pathway and oxidation of choline to betaine aldehyde and betaine aldehyde to glycine betaine (Table 2). Interestingly, the ORF1 of aux16 was predicted to be a membrane-anchored protein (Figure 2) and act as a Choline and betaine/carnitine transporter.

Figure 2: *HHpred analysis of the putative proteins associated with the putative auxiliary factor aux16: cutD (a), blaMBL (b), gbsA (c) and betA (d), and DeepTMHMM analysis of putative protein cutD, showing the presence of membrane-bound domains.*

The DNA sequence aux19 was not directly associated with β-lactam resistance, but was associated with bacitracin resistance.

The DNA sequence of aux19 comprises two ORFs. These ORFs were predicted to act as ABC transporters and be part of a two component system (Table 2)(Figure 3). Although these ORFs were not predicted

to be directly associated with β-lactam resistance (Table 2)(Figure 3), they were predicted to be associated with resistance to bacitracin, which is an antibiotic that inhibits the synthesis of the cell wall (34).

The DNA sequence *aux19* was not directly associated with β-lactam resistance, but was associated with bacitracin resistance.

The DNA sequence of *aux19* comprises two ORFs. These ORFs were predicted to act as ABC transporters and be part of a two component system (Table 2)(Figure 3). Although these ORFs were not predicted to be directly associated with β-lactam resistance (Table 2)(Figure 3), they were predicted to be associated with resistance to bacitracin, which is an antibiotic that inhibits the synthesis of the cell wall (34).

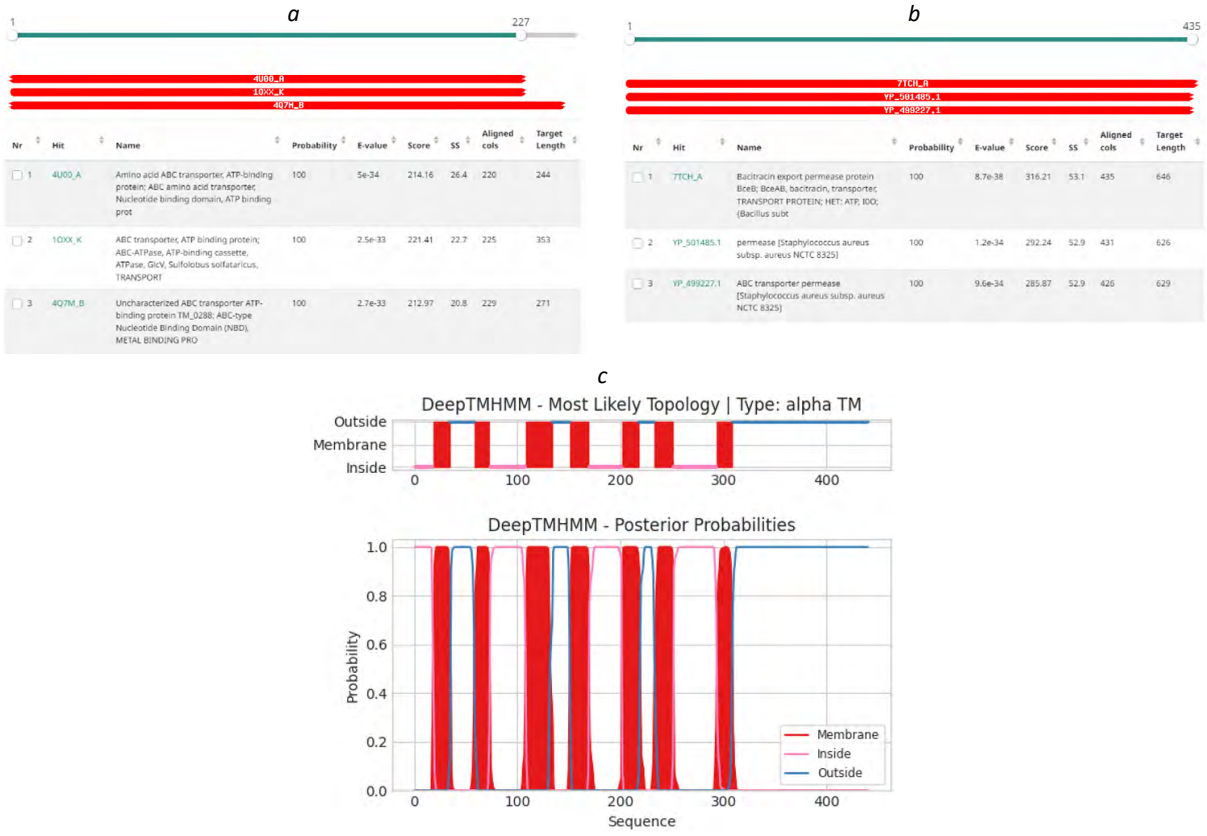

Figure 3: *HHpred analysis of the putative proteins associated with the putative auxiliary factor aux19: vraD (a) and vraE (b), and DeepTMHMM analysis of putative protein vraE, showing the presence of membrane-bound domains.*

4. DISCUSSIONS

As already mentioned, auxiliary factors significantly contribute to β-lactam resistance and during the last decades many of them have been described. These factors have been studied with the aid of transposon mutagenesis using the transposon (Tn551) (35). In 1994, a large Tn551 library produced in the background of *S. aureus* strain COL was screened for mutants with decreased levels of resistance to methicillin. The complete DNA sequence of the 21 new auxiliary genes derived from this library was published in 1999 (31).

In this study, seven of the 21 new auxiliary genes were characterised by in silico sequence analysis. As we can observe in Table 9, most of the genes are involved in metabolic pathways, transport and regulation of gene expression.

It is known that any kind of selective pressure, including antibiotic exposure, causes dramatic changes within the cell and this implies additional metabolic costs. Therefore, in order to withstand the damage caused by cell wall targeting antibiotics, the cell must produce additional

proteins required for cell wall maintenance (36). Notably, one of the aux genes – *aux11* has high homology with the gene *lysA*, which encodes diaminopimelate decarboxylase. This protein is required for the synthesis of the amino acid L-lysine (32), which is part of the tetrapeptide side chain of the PG (37). In consequence, it is possible to speculate that, in the presence of β-lactam antibiotics, enhanced expression of diaminopimelate decarboxylase is crucial to the cross-linking of PG, performed by penicillin binding proteins.

When resistant bacteria are exposed to antibiotics, before they synthesise antibiotic resistance proteins such as PBP2a, they temporarily become more vulnerable to osmotic stress. Therefore, the bacterial cell must activate genes required for the transport or synthesis of osmoprotectants or compatible solutes, which contribute to withstanding osmotic stress (38). Some putative genes that might play that important role have been identified in this study: *cutD, gbsA* and *betA*. All these genes were initially identified as *aux16*. The putative gene *cutD* was predicted to have transmembrane domains, which correlates with the predicted function of its product. The gene *cutD* may be required for the transport of osmoprectants such as betaine or precursors of other osmoprotectants such as choline (39). The putative genes *gbsA* and *betA* encode the proteins: betaine aldehyde dehydrogenase and choline dehydrogenase, respectively. Betaine aldehyde dehydrogenase mediates the biosynthesis of the osmoprotectant betaine, whereas choline dehydrogenase is responsible for the synthesis of the osmoprotectant glycine betaine (40).

β-lactam antibiotics induce the expression of operons that contain β-lactam resistance genes. Furthermore, there is evidence that β-lactam antibiotics also trigger a global stress response in bacterial cells. This occurs when the cell detects cell-wall synthesis perturbations with the aid of the specific VraS/R two-component system (27). In this study we identified two putative genes: vraD and vraE (initially identified as aux19), whose products might be part of VraS/R two-component system. The activation of this two-component system leads to massive overexpression of genes, many of which are crucial for β-lactam antibiotic resistance (27). For instance, VraS/R induces the expression of RelP and RelQ, which synthesises alarmones during the exponential phase of growth (41). Alarmones are generally produced during nutrient starvation (for instance, amino acid starvation) and play a principal role in stringent response, whereby the synthesis of rRNA, tRNA and amino acids available in the cell is repressed and synthesis of amino acids present in insufficient amounts within the cell is induced (42). The principal known alarmones are guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp). During nutrient starvation, these alarmones are produced by specific proteins such as RelA or SpoT, which become activated when uncharged tRNA molecules bind the ribosomal site A (43). There is evidence that this alarmones increase the resistance of MRSA to antibiotics (44).

The global stress response requires the participation of many different genes involved in cascade reactions such as protein kinases, phosphatases or transcriptional regulators. These molecules play similar roles in prokaryotes and eukaryotes (45). We identified three putative genes whose products may participate in this cascade: *prpC, pknB* and *ccpA* (initially identified as *aux1, aux2* and *aux4*, respectively). We can presume that the products of these genes may also be involved in the cascade reactions triggered by the *VraS/R* two-component system, but this proposed linkage must be verified experimentally.

5. CONCLUSIONS

In conclusion, we can confirm that the gene *blrA* is required for β-lactam resistance in the CA-MRSA strain MW2, but does not affect the expression of the gene *mecA* or the structure of the wall teichoic acids. At the same time, many auxiliary genes present in CA-MRSA strain MW2 may be involved in global cellular regulation, stress response and metabolic pathways.

6. CONFLICT OF INTEREST

The author declares no conflict of interest

7. LIMITATIONS OF LIABILITY

The author takes complete responsibility for the information presented in this original scientific article

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6. REFERENCES

1. Kwiecinski JM, Horswill AR. Staphylococcus aureus bloodstream infections: pathogenesis and regulatory mechanisms. Curr Opin Microbiol. 2020;53:51-60.

2. Oliveira K, Viegas C, Ribeiro E. MRSA Colonization in Workers from Different Occupational Environments—A One Health Approach Perspective. Atmosphere. 2022;13(5):658.

3. Dittmann K, Schmidt T, Müller G, Cuny C, Holtfreter S, Troitzsch D, et al. Susceptibility of livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) to chlorhexidine digluconate, octenidine dihydrochloride, polyhexanide, PVP-iodine and triclosan in comparison to hospital-acquired MRSA (HA-MRSA) and community-aquired MRSA (CA-MRSA): a standardized comparison. Antimicrob Resist Infect Control. 2019;8:122.

4. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nature Reviews Microbiology. 2019;17(4):203-18.

5. Okiki PA, Eromosele ES, Ade-Ojo P, Sobajo OA, Idris OO, Agbana RD. Occurrence of mecA and blaZ genes in methicillin-resistant Staphylococcus aureus associated with vaginitis among pregnant women in Ado-Ekiti, Nigeria. New Microbes New Infect. 2020;38:100772.

6. Mikkelsen K, Sirisarn W, Alharbi O, Alharbi M, Liu H, Nøhr-Meldgaard K, et al. The Novel Membrane-Associated Auxiliary Factors AuxA and AuxB Modulate β-lactam Resistance in MRSA by stabilizing Lipoteichoic Acids. International Journal of Antimicrobial Agents. 2021;57(3):106283.

7. Typas A, Banzhaf M, Gross CA, Vollmer W. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nature Reviews Microbiology. 2012;10(2):123-36.

8. Oliveira IA, Allonso D, Fernandes TVA, Lucena DMS, Ventura GT, Dias WB, et al. Enzymatic and structural properties of human glutamine:fructose-6-phosphate amidotransferase 2 (hGFAT2). J Biol Chem. 2021;296:100180.

9. Hummels KR, Berry SP, Li Z, Taguchi A, Min JK, Walker S, et al. Coordination of bacterial cell wall and outer membrane biosynthesis. Nature. 2023;615(7951):300-4.

10. Hrast M, Rožman K, Ogris I, Škedelj V, Patin D, Sova M, et al. Evaluation of the published kinase inhibitor set to identify multiple inhibitors of bacterial ATP-dependent mur ligases. J Enzyme Inhib Med Chem. 2019;34(1):1010-7.

11. Song Y, Lee JS, Shin J, Lee GM, Jin S, Kang S, et al. Functional cooperation of the glycine synthase-reductase and Wood–Ljungdahl pathways for autotrophic growth of Clostridium drakei. Proceedings of the National Academy of Sciences. 2020;117(13):7516-23.

12. Monteiro JM, Münch D, Filipe SR, Schneider T, Sahl H-G, Pinho MG. The pentaglycine bridges of Staphylococcus aureus peptidoglycan are essential for cell integrity. bioRxiv. 2018:479006.

13. Travis BA, Peck JV, Salinas R, Dopkins B, Lent N, Nguyen VD, et al. Molecular dissection of the glutamine synthetase-GlnR nitrogen regulatory circuitry in Gram-positive bacteria. Nature Communications. 2022;13(1):3793.

14. Nöldeke ER, Muckenfuss LM, Niemann V, Müller A, Störk E, Zocher G, et al. Structural basis of cell wall peptidoglycan amidation by the GatD/MurT complex of Staphylococcus aureus. Sci Rep. 2018;8(1):12953.

15. Kuk ACY, Hao A, Lee S-Y. Structure and Mechanism of the Lipid Flippase MurJ. Annual Review of Biochemistry. 2022;91(1):705-29.

16. Liu X, Meiresonne NY, Bouhss A, den Blaauwen T. FtsW activity and lipid II

synthesis are required for recruitment of MurJ to midcell during cell division in Escherichia coli. Molecular Microbiology. 2018;109(6):855-84.

17. Wacnik K, Rao VA, Chen X, Lafage L, Pazos M, Booth S, et al. Penicillin-Binding Protein 1 (PBP1) of Staphylococcus aureus Has Multiple Essential Functions in Cell Division. mBio. 2022;13(4):e0066922.

18. da Costa TM, de Oliveira CR, Chambers HF, Chatterjee SS. PBP4: A New Perspective on Staphylococcus aureus β-Lactam Resistance. Microorganisms. 2018;6(3).

19. Roch M, Lelong E, Panasenko OO, Sierra R, Renzoni A, Kelley WL. Thermosensitive PBP2a requires extracellular folding factors PrsA and HtrA1 for Staphylococcus aureus MRSA β-lactam resistance. Commun Biol. 2019;2:417.

20. Dalal V, Kumar P, Rakhaminov G, Qamar A, Fan X, Hunter H, et al. Repurposing an Ancient Protein Core Structure: Structural Studies on FmtA, a Novel Esterase of Staphylococcus aureus. Journal of Molecular Biology. 2019;431(17):3107-23.

21. G CB, Sahukhal GS, Elasri MO. Role of the msaABCR Operon in Cell Wall Biosynthesis, Autolysis, Integrity, and Antibiotic Resistance in Staphylococcus aureus. Antimicrob Agents Chemother. 2019;63(10).

22. Walter A, Unsleber S, Rismondo J, Jorge AM, Peschel A, Gründling A, et al. Phosphoglycerol-type wall and lipoteichoic acids are enantiomeric polymers differentiated by the stereospecific glycerophosphodiesterase GlpQ. J Biol Chem. 2020;295(12):4024-34.

23. Chee Wezen X, Chandran A, Eapen RS, Waters E, Bricio-Moreno L, Tosi T, et al. Structure-Based Discovery of Lipoteichoic Acid Synthase Inhibitors. Journal of Chemical Information and Modeling. 2022;62(10):2586-99.

24. Vickery CR, Wood BM, Morris HG, Losick R, Walker S. Reconstitution of Staphylococcus aureus Lipoteichoic Acid Synthase Activity Identifies Congo Red as a Selective Inhibitor. Journal of the American Chemical Society. 2018;140(3):876-9.

25. Zeng J, Platig J, Cheng TY, Ahmed S, Skaf Y, Potluri LP, et al. Protein kinases PknA and PknB independently and coordinately regulate essential Mycobacterium tuberculosis physiologies and antimicrobial susceptibility. PLoS Pathog. 2020;16(4):e1008452.

26. Jenul C, Horswill AR. Regulation of Staphylococcus aureus Virulence. Microbiol Spectr. 2019;7(2).

27. Bleul L, Francois P, Wolz C. Two-Component Systems of S. aureus: Signaling and Sensing Mechanisms. Genes (Basel). 2021;13(1).

28. Miragaia M. Factors Contributing to the Evolution of mecA-Mediated β-lactam Resistance in Staphylococci: Update and New Insights From Whole Genome Sequencing (WGS). Frontiers in Microbiology. 2018;9.

29. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J Mol Biol. 2019;431(18):3472-500.

30. Yadav AK, Espaillat A, Cava F. Bacterial Strategies to Preserve Cell Wall Integrity Against Environmental Threats. Front Microbiol. 2018;9:2064.

31. De Lencastre H, Wu SW, Pinho MG, Ludovice AM, Filipe S, Gardete S, et al. Antibiotic resistance as a stress response: complete sequencing of a large number of chromosomal loci in Staphylococcus aureus strain COL that impact on the expression of resistance to methicillin. Microb Drug Resist. 1999;5(3):163-75.

32. Xu J-Z, Ruan H-Z, Liu L-M, Wang L-P, Zhang W-G. Overexpression of thermostable meso-diaminopimelate dehydrogenase to redirect diaminopimelate pathway for increasing L-lysine production in Escherichia coli. Scientific Reports. 2019;9(1):2423.

33. Boyd SE, Livermore DM, Hooper DC, Hope WW. Metallo-β-Lactamases: Structure, Function, Epidemiology, Treatment Options, and the Development Pipeline. Antimicrobial Agents and Chemotherapy. 2020;64(10):e00397-20.

34. Aslanli A, Domnin M, Stepanov N, Efremenko E. "Universal" Antimicrobial Combination of Bacitracin and His(6)-OPH with Lactonase Activity, Acting against Various Bacterial and Yeast Cells. Int J Mol Sci. 2022;23(16).

35. Berger-Bächi B. Insertional inactivation of staphylococcal methicillin resistance by

Tn551. J Bacteriol. 1983;154(1):479-87.

36. Händel N, Schuurmans JM, Brul S, ter Kuile BH. Compensation of the metabolic costs of antibiotic resistance by physiological adaptation in Escherichia coli. Antimicrob Agents Chemother. 2013;57(8):3752-62.

37. Hernández SB, Dörr T, Waldor MK, Cava F. Modulation of Peptidoglycan Synthesis by Recycled Cell Wall Tetrapeptides. Cell Rep. 2020;31(4):107578.

38. Gaucher F, Rabah H, Kponouglo K, Bonnassie S, Pottier S, Dolivet A, et al. Intracellular osmoprotectant concentrations determine Propionibacterium freudenreichii survival during drying. Applied Microbiology and Biotechnology. 2020;104(7):3145-56.

39. Yang N, Ding R, Liu J. Synthesizing glycine betaine via choline oxidation pathway as an osmoprotectant strategy in Haloferacales. Gene. 2022;847:146886.

40. McNeil SD, Nuccio ML, Hanson AD. Betaines and Related Osmoprotectants. Targets for Metabolic Engineering of Stress Resistance1. Plant Physiology. 1999;120(4):945-9.

41. Salzer A, Keinhörster D, Kästle C, Kästle B, Wolz C. Small Alarmone Synthetases RelP and RelQ of Staphylococcus aureus Are Involved in Biofilm Formation and Maintenance Under Cell Wall Stress Conditions. Front Microbiol. 2020;11:575882.

42. Magnusson LU, Farewell A, Nyström T. ppGpp: a global regulator in Escherichia coli. Trends Microbiol. 2005;13(5):236-42.

43. Kudrin P, Dzhygyr I, Ishiguro K, Beljantseva J, Maksimova E, Oliveira SRA, et al. The ribosomal A-site finger is crucial for binding and activation of the stringent factor RelA. Nucleic Acids Res. 2018;46(4):1973-83.

44. Fritsch VN, Loi VV, Busche T, Tung QN, Lill R, Horvatek P, et al. The alarmone (p) ppGpp confers tolerance to oxidative stress during the stationary phase by maintenance of redox and iron homeostasis in Staphylococcus aureus. Free Radic Biol Med. 2020;161:351-64.

45. Madsen CD, Hein J, Workman CT. Systematic inference of indirect transcriptional regulation by protein kinases and phosphatases. PLoS Comput Biol. 2022;18(6):e1009414.