Sakarya Üniversitesi Fen Bilimleri Dergisi
Sakarya University Journal of Science

e-ISSN : 2147-835X
Publisher : Sakarya University

Vol. 29, No. 6, 699-716, 2025
DOI: https://doi.org/10.16984/saufenbilder.1710007

Research Article

Systems-Level Identification of Resistance-Associated Metabolites in Foodborne Pathogens

Fatma Gizem Avci[1,2]

[1] Department of Bioengineering, Faculty of Engineering and Natural Sciences, Üsküdar University, Istanbul, Türkiye, fatmagizem.avci@uskudar.edu.tr, ror.org/02dzjmc73
[2] Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany, ror.org/02hpadn98

ARTICLE INFO

Keywords:
Foodborne pathogens
Antimicrobial resistance
Reporter metabolites
Stress response
Drug target

Article History:
Received: 30.05.2025
Revised: 09.10.2025
Accepted: 20.10.2025
Online Available: 14.12.2025

ABSTRACT

Foodborne pathogens remain a pressing global health concern and account for serious infections and even deaths. Among the leading bacterial pathogens contributing to these infections are *Campylobacter jejuni*, *Listeria monocytogenes*, and *Salmonella enterica*, all of which are associated with serious health complications and pose significant challenges for public health systems and food safety. The improper uses of antimicrobials in both human and veterinary settings compound this issue, as the growing threat of antimicrobial resistance is exacerbated by these practices. In the current study, the stress responses in foodborne pathogens *C. jejuni* subsp. *jejuni* 81-176, *L. monocytogenes* EGD-e (serotype 1/2a), and *S. enterica* serovar Typhimurium were investigated through reporter metabolite analysis. By integrating systems-level insights, it is aimed to discover novel targets and pathways involved in survival and resistance under several stress conditions. The findings will provide a basis for more effective treatment strategies to combat foodborne infections and mitigate the spread of antimicrobial resistance.

1. Introduction

Foodborne diseases resulting from the contamination of food products by bacterial pathogens pose a serious health threat and contribute significantly to rising mortality rates. It is estimated that unsafe food causes illnesses in 600 million people and results in 420,000 deaths annually [1]. *Salmonella, Campylobacter, Vibrio cholerae, Listeria,* and enterohaemorrhagic *Escherichia coli* are the leading foodborne pathogens [2, 3].

Campylobacter jejuni is among the most prevalent foodborne zoonotic pathogens globally. It is responsible for over 80% of campylobacteriosis cases and is linked to serious complications such as reactive arthritis, bacteremia, and Guillain-Barré syndrome. The bacterium establishes colonization in its host by surviving the acidic environment of the stomach, attaching to intestinal epithelial cells through surface structures, and producing virulence factors that enable it to infect and multiply within the gastrointestinal tract. *C. jejuni* infections in humans are mostly transmitted through contaminated poultry products but can also result from contaminated water or milk [4-6].

Listeria monocytogenes is a widespread intracellular bacterium found in diverse environments, including soil, water, raw and ready-to-eat (RTE) meat and dairy products, as well as food processing environments. This microorganism causes listeriosis. In humans, listeriosis can range from mild, self-limiting gastrointestinal symptoms to severe invasive disease. Although infections are relatively rare due to their opportunistic nature, their high mortality rate and ability to survive for extended periods make *L. monocytogenes* a major challenge in the food industry [7-9].

Owing to its efficient transmission through contaminated food (found in meat, poultry, or

Cite as: F. G. Avci, "Systems-Level Identification of Resistance-Associated Metabolites in Foodborne Pathogens," Sakarya University Journal of Science, vol. 29, no. 6, pp. 699-716, 2025. https://doi.org/10.16984/saufenbilder.1710007

eggs) and water, *Salmonella enterica* is associated with widespread outbreaks. Infections caused by *S. enterica* span a clinical spectrum from self-limiting gastroenteritis to severe systemic diseases such as typhoid fever. Disease manifestations vary by serotype, ranging from diarrhea to septicemia. Among its serovars, *S. enterica* serovar Typhimurium is a major cause of human salmonellosis worldwide and illnesses linked to the food industry. Due to the extensive health impact of *S. enterica*, it remains a focal point of microbiological and epidemiological research [10-12].

Antimicrobials have a crucial role in managing bacterial infections, including those caused by foodborne pathogens. However, the misuse of these pharmaceuticals in both human and veterinary medicine has led to the emergence and spread of resistant strains, which make the treatment of infections difficult [2]. All major foodborne pathogens have demonstrated resistance to multiple antimicrobials, presenting a significant risk to public health [13].

This study investigates the molecular basis of stress responses in the foodborne pathogens *C. jejuni* subsp. *jejuni* 81-176*, L. monocytogenes* EGD-e (serotype 1/2a), and *S. enterica* subsp. enterica serovar Typhimurium. Using reporter metabolite analysis, it is aimed to uncover novel molecular targets to develop more effective treatment strategies. This comprehensive understanding at the systems level holds promise for combating antimicrobial resistance and enhancing food safety interventions.

2. General Methods

2.1. Determination of antimicrobial resistance and stress genotypes

The data belonging to the isolates, *C. jejuni*, *L. monocytogenes*, and *S. enterica*, were retrieved from the Pathogen Detection Web Browser of the National Biotechnology Information Center (NCBI) (accessed on 28 December 2024). For each organism, prevalent genotypes associated with antimicrobial resistance and stress response mechanisms were identified based on the information provided within the database.

2.2. Gene expression datasets

Transcriptome datasets of three foodborne pathogens, *C. jejuni* subsp. *jejuni* 81-176, *L. monocytogenes* EGD-e, and *S. enterica* subsp. enterica serovar Typhimurium, were used for the study [14]. The datasets are accessible at GEO with the accession number GSE152295.

2.3. Draft genome-scale metabolic model reconstruction

Using the RAVEN Toolbox v2.10.3 [15] within MATLAB R2024b, the draft genome-scale metabolic models (GEMs) of the microorganisms were reconstructed based on the protein sequence FASTA files retrieved from NCBI.

De novo model reconstruction was carried out by the function *getKEGGModelForOrganism*, using the KEGG database [16, 17] as the primary annotation source. For each microorganism, two preliminary draft models were independently generated. The first model was based on the organism's KEGG identifier to obtain annotated pathway and reaction data. The second model was built by aligning the microorganism's proteome against Hidden Markov Models derived from prokaryotic protein families sharing at least 90% sequence identity. The draft models were then integrated into a single reconstruction using the *mergeModels* function, and the resulting GEMs were utilized for reporter metabolite analyses.

2.4. Reporter metabolite analysis

Reporter metabolites were identified using the *Reporter Features* algorithm, which maps transcriptomic perturbations onto the topological structure of the metabolic network [18, 19]. The metabolites and enzymes are represented as nodes and their biochemical interactions as edges. Within this framework, metabolite nodes receive scores according to the transcriptional significance (p-values) of the neighboring enzyme-coding genes. These scores reflect the extent of transcriptional perturbation around each metabolite. Metabolites adjacent to enzymes exhibiting the most statistically significant differential expression are identified as reporter metabolites. Only those with p-values below 0.05 were deemed statistically significant and included in the subsequent metabolic enrichment analyses [20].

2.5. Pathway enrichment analysis based on reporter metabolites

To investigate the functional roles of the significant reporter metabolites, pathway enrichment analysis was conducted using the Metabolites Biological Role (MBRole) database, version 3.0 [21]. Reporter metabolites were assigned to KEGG compound identifiers and subjected to enrichment analysis. Pathways enriched with a false discovery rate (FDR) below 0.05 were considered statistically significant and used for the interpretation of condition and pathogen-specific metabolic responses.

3. Results

3.1. Determination of antimicrobial resistance and stress genotypes

According to the data obtained from the Pathogen Detection Web Browser, microorganisms were mainly isolated from humans, animals, food, animal feed, or environment. The number of isolates based on their isolation types is provided in Table 1.

Table 1. The number of isolates according to their isolation types

	C. jejuni	*L. monocytogenes*	*S. enterica*
Clinical	50,936	23,552	510,330
Environmental	60,916	36,293	151,164
Not indicated	20,339	10,508	44,498
Total	132,191	70,353	705,992

The information on antimicrobial resistance genotypes for each pathogen is presented in Table 2.

Table 2. Antimicrobial resistance genotypes of the foodborne pathogens

Pathogen	Antimicrobial Resistance Genotypes
C. jejuni	23S_A2074C, 23S_A2074G, 23S_A2074T, 23S_A2075G, 50S_L22_A103V, *aac(6')*, *aac(6')*-E449, *aac(6')*-E64, *aac(6')-Ie/aph(2'')-Ia*, *aac(6')-Ie/aph(2'')-If2*,*aac(6')-Im*, *aad9*, *aadA1*, *aadA2*, *aadD1*, *aadE*, *aadE-Cc*, *ant(2'')-Ia*, *ant(6)*, *ant(6)-Ia*, *ant(6)-Ib*, *ant(9)-Ia*, *aph(2'')-I*, *aph(2'')-If*, *aph(2'')-Ig*, *aph(2'')-Ih*, *aph(2'')-IIa*, *aph(2'')-IIIa*, *aph(2'')-II*, *aph(2'')-IVa*, *aph(3')*, *aph(3')*-H510, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(3')-IIIa*, *aph(3')-VIIa*, *aph(6)-Id*, *apmA*, *blaOXA*, *blaOXA-184*, *blaOXA-185*, *blaOXA-193*, *blaOXA-446*, *blaOXA-447*, *blaOXA-448*, *blaOXA-449*, *blaOXA-450*, *blaOXA-451*, *blaOXA-452*, *blaOXA-453*, *blaOXA-460*, *blaOXA-461*, *blaOXA-465*, *blaOXA-466*, *blaOXA-489*, *blaOXA-493*, *blaOXA-518*, *blaOXA-576*, *blaOXA-577*, *blaOXA-578*, *blaOXA-579*, *blaOXA-580*, *blaOXA-581*, *blaOXA-582*, *blaOXA-583*, *blaOXA-584*, *blaOXA-585*, *blaOXA-586*, *blaOXA-587*, *blaOXA-588*, *blaOXA-589*, *blaOXA-590*, *blaOXA-591*, *blaOXA-592*, *blaOXA-593*, *blaOXA-594*, *blaOXA-595*, *blaOXA-596*, *blaOXA-597*, *blaOXA-599*, *blaOXA-185*, *blaOXA-193*, *blaOXA-446*, *blaOXA-447*, *blaOXA-448*, *blaOXA-449*, *blaOXA-450*, *blaOXA-451*, *blaOXA-452*, *blaOXA-453*, *blaOXA-460*, *blaOXA-461*, *blaOXA-465*, *blaOXA-466*, *blaOXA-489*, *blaOXA-493*, *blaOXA-518*, *blaOXA-576*, *blaOXA-577*, *blaOXA-578*, *laOXA-579*, *blaOXA-580*, *blaOXA-581*, *blaOXA-582*, *blaOXA-583*, *blaOXA-584*, *blaOXA-585*, *blaOXA-586*, *blaOXA-587*, *blaOXA-588*, *blaOXA-589*, *blaOXA-590*, *blaOXA-591*, *blaOXA-592*, *blaOXA-593*, *blaOXA-594*, *blaOXA-595*, *blaOXA-596*, *blaOXA-597*, *blaOXA-599*, *blaOXA-601*, *blaOXA-602*, *blaOXA-603*, *blaOXA-604*, *blaOXA-605*, *blaOXA-608*, *blaOXA-609*, *blaOXA-61*, *blaOXA-61*_G-57T, *blaOXA-610*, *blaOXA-611*, *blaOXA-614*, *blaOXA-615*, *blaOXA-616*, *blaOXA-617*, *blaOXA-618*, *blaOXA-619*, *blaOXA-620*, *blaOXA-621*, *blaOXA-622*, *blaOXA-623*, *blaOXA-624*, *blaOXA-625*, *blaOXA-626*, *blaOXA-627*, *blaOXA-628*, *blaOXA-629*, *blaOXA-630*, *blaOXA-631*, *blaOXA-632*, *blaOXA-633*, *blaOXA-634*, *blaOXA-635*, *blaOXA-636*, *blaOXA-637*, *blaOXA-638*, *blaOXA-639*, *blaOXA-640*, *blaOXA-658*, *blaOXA-659*, *blaOXA-783*, *blaOXA-784*, *blaOXA-785*, *blaOXA-786*, *blaOXA-85*, *blaROB-1*, *blaTEM*, *blaTEM-1*, *blaTEM-116*, *blaTEM-57*, *catA*, *catA13*, *cat-TC*, *cblA*, *cfr(C)*, *cfxA*, *cfxA3*, *cmeB*, *dfrA1*, *dfrG*,, *erm*(53), *erm*(A), *erm*(B), *erm*(C), *erm*(F), *estT*, *fexA*, *fosXCC*, *gyrA*_D90N, *gyrA*_D90Y, *gyrA*_P104S, *gyrA*_T86A, *gyrA*_T86I, *gyrA*_T86K, *gyrA*_T86V, *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(P), *lsa*(A), *lsa*(E), *mef*(En2), *narA*, *narB*, *optrA*, *qnrB19*, *qnrD1*, *rplD*_G74D, *rplV*_A103V, *rplV*_A88E, *rplV*_G86E, *rpsL*_K43R, *rpsL*_K88Q, *rpsL*_K88R, *sat2*, *sat4*, *satA*, *spw*, *sul1*, *sul2*, *tet*, *tet*(32), *tet*(44), *tet*(A), *tet*(L), *tet*(M), *tet*(O), *tet*(O/32/O), *tet*(O/M/O), *tet*(O/W/32/O), *tet*(Q), *tet*(W), *tet*(X2), *vanR-A*, *vanS-A*
L. monocytogenes	*aac(6')*, *aac(6')-I*, *aac(6')-Ib-cr5*, *aac(6')-Ie/aph(2'')-Ia*, *aadA5*, *aadD1*, *abc-f*, *ant(6)-Ia*, *ant(9)-Ia*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(3')-IIa*, *aph(3')-IIIa*, *aph(4)-Ia*, *aph(6)-Id*, *arr*, *arr-3*, *bcrA*, *blaI*, *blaNDM-1*, *blaOXA*, *blaR1*, *blaTEM*, *blaTEM-1*, *blaTEM-116*, *catA1*, *catB3*, *cat-TC*, *cfr*(D), *dfrA17*, *dfrD*, *dfrG*, *emr*C, *erm*(A), *erm*(B), *erm*(C), *erm*(G), *erm*(T), *erm*(Y), *erm*, *fexA*, *floR*, *fosA*, *fosB*, *fosD*, *fosX*, *fusB*, *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(D), *lnu*(G), *lsa*(A), *lsa*(E), *lsa*, *mecA*, *mef*(A), *mef*(F), *blaTEM-181*, *blaTEM-57*, *blaZ*, *bleO*, *ble-Sh*, *catA*, *mph*(A), *mph*(B), *mph*(C), *msr*(A), *msr*(C), *msr*(D), *msr*(G), *narA*, *narB*, *optrA*, *qnrB*, *qnrB19*, *qnrD*, *sat4*, *spd*, *spw*, *str*, *sul1*, *sul2*, *tet*(A), *tet*(C), *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(S), *tetA*(46), *tetB*(46), *vanC*, *vanR*, *vanR-C*, *vanS-C*, *vanT-C*, *vanXY-C*, *vat*(B), *vat*, *vga*(A), *vga*(G)

Table 2. Antimicrobial resistance genotypes of the foodborne pathogens (Continued)

S. enterica	aac(2')-IIa, aac(3), aac(3)-C322, aac(3)-I, aac(3)-Ia, aac(3)-Ic, aac(3)-Id,aac(3)-II, aac(3)-IId, aac(3)-IIe, aac(3)-IIg, aac(3)-IV, aac(3)-IVa, aac(3)-VIa, aac(6'), aac(6')-33, , aac(6')-F309, aac(6')-Ian, aac(6')-Ib, aac(6')-Ib', aac(6')-Ib11, aac(6')-Ib3, aac(6')-Ib4, aac(6')-Ib-cr, aac(6')-Ib-cr4, aac(6')-Ib-cr5, aac(6')-Ie, aac(6')-Ie/aph(2'')-Ia, aac(6')-IIa, aac(6')-IIc, aac(6')-Il, aac(6')-Im, aacA34, aacA38, aacA56, aacA57-2, aad9, aadA1, aadA11, aadA12, aadA13, aadA15, aadA16, aadA2, aadA21, aadA22, aadA25, aadA3, aadA31, aadA4, aadA5, aadA6, aadA7, aadA8, aadA9, aadD1, aadE, aadS, acrB_R717L, acrB_R717Q, acrF, ampC, ant(2'')-Ia, ant(3''), ant(3'')-Ia, ant(6)-Ia, ant(9)-Ib, aph(2'')-I, aph(2'')-Ia, aph(2'')-IIa, aph(3''), aph(3')-I, aph(3')-I(H957), aph(3')-Ia, aph(3')-Ib, aph(3'')-Ib, aph(3')-Id, aph(3')-II, aph(3')-IIa, aph(3')-IIc, aph(3')-IIIa, aph(3')-VI, aph(3')-VIa, aph(3')-XV, aph(4)-Ia, aph(6)-Ic, aph(6)-Id, aphA16, armA, arr, arr-2, arr-3, bla, blaACC, blaACC-1, blaACT-84, blaADC-30, blaADC-73, blaBPU, blaC, blaCARB-2, blaCARB-4, blaCARB-50, blaCARB-58, blaCMY, blaCMY-111, blaCMY-16, blaCMY-2, blaCMY-27, blaCMY-30, blaCMY-4, blaCMY-42, blaCMY-43, blaCMY-55, blaCMY-59, blaCMY-6, blaCTX-M, blaCTX-M-1, blaCTX-M-10, blaCTX-M-101, blaCTX-M-104, blaCTX-M-115, blaCTX-M-12, blaCTX-M-123, blaCTX-M-125, blaCTX-M-130, blaCTX-M-137, blaCTX-M-14, blaCTX-M-15, blaCTX-M-153, blaCTX-M-165, blaCTX-M-169, blaCTX-M-190, blaCTX-M-199, blaCTX-M-2, blaCTX-M-206, blaCTX-M-231, blaCTX-M-24, blaCTX-M-261, blaCTX-M-27, blaCTX-M-3, blaCTX-M-32, blaCTX-M-40, blaCTX-M-5, blaCTX-M-55, blaCTX-M-56, blaCTX-M-64, blaCTX-M-65, blaCTX-M-8, blaCTX-M-9, blaCTX-M-90, blaCTX-M-96, blaCTX-M-98, blaDHA, blaDHA-1, blaDHA-15, blaDHA-22, blaDHA-27, blaDHA-4, blaDHA-6, blaDHA-9, blaEC, blaFONA, blaFOX, blaFOX-5, blaHER, blaHER-3, blaI, blaIMP-27, blaKLUC-2, blaKPC-2, blaKPC-3, blaKPC-4, blaL2, blaLAP, blaLAP-1, blaLAP-2, blaMOR-2, blaNDM, blaNDM-1, blaNDM-13, blaNDM-5, blaNDM-9, blaOXA, blaOXA-1, blaOXA-10, blaOXA-129, blaOXA-17, blaOXA-181, blaOXA-2, blaOXA-23, blaOXA-232, blaOXA-392, blaOXA-4, blaOXA-48, blaOXA-66, blaOXA-9, blaOXY-5-3, blaP, blaPC1, blaPEN-E, blaPSE, blaR1, blaROB, blaROB-11, blaSCO, blaSCO-1, blaSHV, blaSHV-1, blaSHV-11, blaSHV-12, blaSHV-189, blaSHV-2, blaSHV-2A, blaSHV-30, blaSHV-5, blaSHV-7, blaTEM, blaTEM-1, blaTEM-10, blaTEM-103, blaTEM-104, blaTEM-105, blaTEM-116, blaTEM-12, blaTEM-128, blaTEM-131, blaTEM-135, blaTEM-144, blaTEM-15, blaTEM-166, blaTEM-169, blaTEM-171, blaTEM-176, blaTEM-181, blaTEM-19, blaTEM-190, blaTEM-191, blaTEM-2, blaTEM-20, blaTEM-207, blaTEM-210, blaTEM-215, blaTEM-219, blaTEM-238, blaTEM-251, blaTEM-29, blaTEM-30, blaTEM-31, blaTEM-32, blaTEM-33, blaTEM-34, blaTEM-35, blaTEM-36, blaTEM-40, blaTEM-52, blaTEM-54, blaTEM-57, blaTEM-63, blaTEM-70, blaTEM-71, blaTEM-84, blaVEB, blaVEB-1, blaVEB-17, blaVEB-8, blaVIM-1, blaVIM-2, blaZ, ble, bleO, catA, catA1, catA13, catA2, catA3, catB, catB11, catB2, catB3, catB8, cfr, cfxA, cmlA, cmlA1, cmlA4, cmlA5, cmlA6, cmx, crpP, dfrA, dfrA1, dfrA10, dfrA12, dfrA14, dfrA15, dfrA16, dfrA17, dfrA19, dfrA21, dfrA22, dfrA23, dfrA25, dfrA27, dfrA29, dfrA31, dfrA32, dfrA34, dfrA36, dfrA42, dfrA48, dfrA5, dfrA51, dfrA7, dfrA8, dfrB1, dfrB3, dfrB4, dfrB5, dfrE, dfrF, dfrG, dfrI, emrA, emrB, emrC, emrD, ere(A), ere(D), erm, erm(42), erm(49), erm(52), erm(53), erm(B), erm(C), erm(F), erm(G), erm(T), estT, farB, fexA, floR, floR2, folP_F28L, fos, fosA, fosA10, fosA3, fosA4, fosA7, fosA7.2, fosA7.3, fosA7.4, fosA7.5, fosA7.6, fosA7.7, fosA7.8, fosA8, fosB, fosL, fosL1, fosL2, fosX, gar, grdA, gyrA_A119E, gyrA_D72G, gyrA_D82G, gyrA_D82N, gyrA_D87G, gyrA_D87N, gyrA_D87Y, gyrA_G81C, gyrA_G81D, gyrA_S83A, gyrA_S83F, gyrA_S83I, gyrA_S83Y, gyrB_E466D, gyrB_S464F, gyrB_S464T, gyrB_S464Y, lnu(A), lnu(C), lnu(F), lnu(G), mcr-1, mcr-1.1, mcr-1.19, mcr-1.2, mcr-1.26, mcr-1.5, mcr-1.6, mcr-2.1, mcr-3, mcr-3.1, mcr-3.2, mcr-3.20, mcr-3.21, mcr-3.24, mcr-3.39, mcr-3.40, mcr-3.5, mcr-4, mcr-4.1, mcr-4.2, mcr-4.3, mcr-4.6, mcr-5, mcr-5.1, mcr-9, mcr-9.1, mcr-9.2, mdsA, mdsB, mdtM, mecA, mef(A), mef(B), mef(C), mef(F), mph, mph(A), mph(B), mph(E), mph(F), mph(G), msr, msr(D), msr(E), msr(G), nimE, norm, oqxA, oqxA2, oqxB, oqxB2, parC_E84G, parC_E84K, parC_G78D, parC_S80I, parC_S80R, parC_W106G, parE_A499T, parE_H462Y, parE_S458P, parE_V461G, pmrA_15R, pmrA_G53E, pmrA_R81C, pmrA_R81H, pmrB_E166K, pmrB_G206R, pmrB_G206W, pmrB_M186I, pmrB_R89P, pmrB_RQRL11del, pmrB_S124P, pmrB_S305R, pmrB_T147P, pmrB_T156M, pmrB_V161G, pmrB_V161L, pmrB_V161M, qepA, qepA1, qepA2, qepA4, qepA8, qnrA, qnrA1, qnrA3, qnrA6, qnrB, qnrB1, qnrB17, qnrB19, qnrB2, qnrB38, qnrB4, qnrB6, qnrB7, qnrB77, qnrB9, qnrB91, qnrD, qnrD1, qnrD2, qnrE, qnrE1, qnrE2, qnrE3, qnrS, qnrS1, qnrS11, qnrS13, qnrS2, qnrS4, qnrVC, qnrVC1, qnrVC4, qnrVC5, ramR_E160D, ramR_G25A, ramR_M84I, ramR_R46P, ramR_T18P, ramR_T50P, ramR_Y59H, rmtB, rmtB1, rmtC, rmtE, rmtE1, rmtF, rmtF1, rmtG, sat2, sat4, smeF, soxR_G121D, soxR_R20H, str, sul1, sul2, sul3, sul4, tet, tet(31), tet(32), tet(40), tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), tet(H), tet(H-J), tet(J), tet(K), tet(L), tet(M), tet(O), tet(Q), tet(W), tet(X2), tet(X4), tet(X5), tet(Y), tetA(46), tetB(46), tmexC, tmexC2, tmexC3, tmexD, toprJ, toprJ1, vanA, vanB, vanC, vanH-A, vanH-B, vanR-A, vanR-B, vanR-C, vanS-A, vanS-B, vanW-B, vanX-A, vanX-B, vanY-A, vanY-B, vanZ-A, vat, vga(G)

Since the reporter metabolite analyses were carried out under several stress conditions, the details about the stress genotypes of the isolates are given in Table 3.

It is worth noting that each microorganism has at least one of the genotypes with one or more resistance profiles.

Table 3. Stress genotypes of the isolated foodborne pathogens. The number of isolates for each genotype was given in parentheses

Pathogen	Stress Genotypes
C. jejuni	*acr3* (47,286), *arsP* (37,862)
L. monocytogenes	*arsA* (6,619), *arsB* (7,372), *arsD* (10,770), *arsD2* (5,754), *arsR* (7,367), *bcrB* (11,580), *bcrC* (11,575), *cadC* (24,822), *clpL* (14,185), *gadD1* (31,129), *merA* (1,045), *merB* (1,031), *merR1* (1,106), *merR2* (1,107), *merT* (1,030), *qacH* (4,691)
S. enterica	*arsA* (33,069), *arsB* (34,558), *arsC* (49,096), *arsD* (201,567), *arsR* (79,426), *asr* (552,701), *golS* (669,965), *golT* (667,997), *merA* (57,239), *merB* (7,019), *merC* (58,118), *merD* (53,336), *merE* (53,068), *merP* (69,930), *merR* (73,093), *merT* (66,270), *pcoB* (32,543), *pcoC* (61,015), *pcoD* (59,478), *pcoE* (29,334), *pcoS* (62,965), *qac* (65,790), *qacE* (60,278), *qacEdelta1* (57,940), *qacL* (8,593), *silA* (65,080), *silB* (64,882), *silC* (64,839), *silE* (65,048), *silF* (64,905), *silP* (64,537), *silR* (64,888), *terD* (16,600), *terW* (16,151), *terZ* (16,062)

3.2. Reconstruction of GEMs and metabolic graphs

RAVEN toolbox v2.10.3 [15] was used to reconstruct draft GEMs for the target microorganisms (Table 4). The resulting draft models, derived using KEGG annotations, were utilized for further analyses.

Table 4. Summary of the draft GEMs

	# Genes	# Reactions	# Metabolites
C. jejuni	452	934	1,162
L. monocytogenes	783	1,281	1,454
S. enterica	1,193	1,913	1,771

All spontaneous reactions were systematically excluded from the draft GEMs to refine the predictive accuracy. Metabolic graphs were subsequently constructed in accordance with the guidelines outlined in *Reporter Features* [18, 19], enabling detailed annotation and functional categorization of reactions based on KEGG pathway classifications.

3.3. Identification of reporter metabolites and enriched pathways

RNA-Seq data were mapped onto the metabolic networks by incorporating gene expression p-values, allowing the identification of reporter metabolites. Metabolites with a p-value below 0.05 under any of the eleven different stress conditions applied to the foodborne pathogens were considered statistically significant. Table 5 presents the counts of significant and non-significant reporter metabolites for each microorganism.

Table 5. The number of significant/non-significant reporter metabolites for each foodborne pathogen under different stress conditions

	C. jejuni	*L. monocytogenes*	*S. enterica*
Acidic stress	97/832	125/1,104	150/1,534
Bile stress	68/861	78/1,151	106/1,578
Hypoxia	77/852	75/1,154	112/1,572
Low iron	58/871	82/1,147	88/1,596
Nitrosative stress	78/851	109/1,120	124/1,560
Nutritional downshift	88/841	68/1,161	168/1,516
Osmotic stress	71/858	111/1,118	100/1,584
Oxidative stress	69/860	129/1,100	119/1,565
Stationary phase	74/855	102/1,127	158/1,526
Temperature	56/873	92/1,137	128/1,556
Virulence inducing	55/874	87/1,142	110/1,574

Among the significant reporter metabolites, those that were common to at least four stress conditions were determined and provided in Table 6. These metabolites were subsequently assigned to pathways using KEGGMapper to identify potential targets.

To further elucidate the functional implications of the identified reporter metabolites, enrichment analysis was conducted using the MBROLE v3.0 server. Pathways exhibiting an FDR value less than 0.05 were regarded as statistically enriched. Figure 1 displays the top enriched pathways, those with the lowest FDR values, for each bacterial strain across the different stress conditions.

To assess if bacteria respond similarly to different stress conditions, enriched pathways were thoroughly examined. Figure 2 illustrates the number of shared enriched pathways for each bacterium under various stress conditions. The most commonly enriched pathways included amino acid biosynthesis, cofactor biosynthesis, carbon metabolism, and methane metabolism.

4. Discussion

The advancement of antimicrobial agents has played a crucial role in infection prevention; however, the growing resistance among bacteria has made many treatments less effective. The rapid spread of multidrug-resistant bacteria complicates and raises the cost of developing new drugs. As antimicrobial-resistant microorganisms can spread through various routes, including the food supply, they present a significant risk to both human health and food safety, highlighting the urgent need to discover new antimicrobial agents with new mechanisms of action or innovative structural designs. Current research efforts focus on discovering new drugs, identifying novel targets, exploring synergistic drug combinations, and repurposing existing medications [22, 23].

Pathogens must withstand several challenges to survive harsh conditions and grow rapidly before causing infection in humans. For instance, they need to defend themselves against the natural barriers of the body, such as gastric acid (pH stress), fever (thermal stress), and nutrient limitation (starvation stress) [24]. These stress conditions can alter bacterial sensitivity to various antimicrobial agents. This modulation is mediated by the induction of stress response pathways, which can activate resistance mechanisms or induce physiological adaptations that diminish antimicrobial efficacy [25]. Pathogens that manage to survive these treatments can contribute to the development and spread of antimicrobial resistance [26]. Therefore, understanding the mechanisms underlying microbial stress responses and how pathogens detect and swiftly adapt to environmental changes is crucial [27]. Owing to their pivotal role in antimicrobial and multidrug resistance, these stress responses present promising targets for novel therapeutic interventions.

NCBI Pathogens Isolates Browser (https://www.ncbi.nlm.nih.gov/pathogens/) provides data on stress and antimicrobial resistance genotypes collected from both environmental and clinical pathogen samples. Therefore, in this study, antimicrobial resistance and stress genotypes were first determined for *C. jejuni*, *L. monocytogenes*, and *S. enterica* by retrieving the data from the NCBI Pathogen Detection Web Browser (Table 2 and Table 3).

Table 6. Common reporter metabolites for each foodborne pathogen under different stress conditions

Pathogen	Common Reporter Metabolites
C. jejuni	L-Alanine, Phosphoenolpyruvate, L-Tryptophan, Acetaldehyde, Ferricytochrome c, Ferrocytochrome c, Reduced ferredoxin, Oxidized ferredoxin, Palmitoyl-CoA, 2-Oxo acid, Diacylglycerol, D-Xylulose 5-phosphate, 3-Phospho-D-glyceroyl phosphate, Taurine, Hexadecanoic acid, Nicotinate, Isocitrate, Indole, 3-Carboxy-cis,cis-muconate, UDP-N-acetylmuramoyl-L-alanine, 2-Carboxy-2,5-dihydro-5-oxofuran-2-acetate, Hydrogen selenide, Leukotriene C4, R-S-Glutathione, (S)-2-Aminobutanoate, 3-Cyano-L-alanine, (5-L-Glutamyl)-peptide, 5-L-Glutamyl amino acid, (5-L-Glutamyl)-L-amino acid, UDP-2-acetamido-4-dehydro-2,6-dideoxyglucose, 2-Oxo-5-methylthiopentanoic acid, Homomethionine, 2-Oxo-6-methylthiohexanoic acid, Dihomomethionine, D-glycero-alpha-D-manno-Heptose 7-phosphate, GDP-D-glycero-alpha-D-manno-heptose, UDP-2-acetamido-3-amino-2,3-dideoxy-alpha-D-glucuronate, UDP-2-acetamido-2-deoxy-alpha-D-ribo-hex-3-uluronate, KDO2-lipid A 1-(2-aminoethyl diphosphate), KDO2-lipid A 4'-(2-aminoethyl diphosphate), KDO2-lipid A 1,4'-bis(2-aminoethyl diphosphate), 1-Dephospho-KDO2-lipid A, 1-PEtN-KDO2-lipid A
L. monocytogenes	CoA, Heme, Succinate, Phosphoenolpyruvate, L-Cysteine, Propanoyl-CoA, IMP, Nicotinamide, Adenosine, Thymidine, Guanine, Hydrogen sulfide, Xanthine, N-Carbamoyl-L-aspartate, O-Acetyl-L-serine, D-Alanyl-D-alanine, Hydrogen selenide, L-Cystathionine, beta-D-Fructose, Protein glutamine, tRNA containing 6-isopentenyladenosine, 5'-Deoxyadenosine, beta-D-Fructose 6-phosphate, L-Selenocysteine, Selenohomocysteine, L-Selenocystathionine, Methaneselenol, UDP-N-acetylmuramoyl-L-alanyl-gamma-D-glutamyl-L-lysine, 5-Fluorodeoxyuridine, Fe2+, Enzyme N6-(lipoyl)lysine, Enzyme N6-(dihydrolipoyl)lysine, 3-Methyl-1-hydroxybutyl-ThPP, [Dihydrolipoyllysine-residue (2-methylpropanoyl)transferase] S-(3-methylbutanoyl)dihydrolipoyllysine, 2-Methyl-1-hydroxypropyl-ThPP, [Dihydrolipoyllysine-residue (2-methylpropanoyl)transferase] S-(2-methylpropanoyl)dihydrolipoyllysine, 2-Methyl-1-hydroxybutyl-ThPP, [Dihydrolipoyllysine-residue (2-methylpropanoyl)transferase] S-(2-methylbutanoyl)dihydrolipoyllysine, [Dihydrolipoyllysine-residue acetyltransferase] S-acetyldihydrolipoyllysine, Protein N5-methyl-L-glutamine, 2-(alpha-Hydroxypropyl)thiamine diphosphate, Enzyme N6-(S-propyldihydrolipoyl)lysine

Table 6. Common reporter metabolites for each foodborne pathogen under different stress conditions (Continued)

S. enterica	Diphosphate, AMP, Phosphoenolpyruvate, H+, dATP, Guanine, Hypoxanthine, alpha-D-Ribose 1-phosphate, alpha-D-Glucose 6-phosphate, 2-Deoxy-D-ribose 1-phosphate, trans-2,3-Dehydroacyl-[acyl-carrier protein], Reduced coenzyme F420, 4-Hydroxy-2-oxoglutarate, S-Adenosylmethioninamine, tRNA(Met), L-Methionyl-tRNA, 2-Succinylbenzoyl-CoA, (R)-3-Hydroxybutanoyl-CoA, Coenzyme M, N-Acetyl-alpha-D-glucosamine 1-phosphate, Coenzyme B, Coenzyme M 7-mercaptoheptanoylthreonine-phosphate heterodisulfide, Dodecanoyl-[acyl-carrier protein], Selenomethionyl-tRNA(Met), Butyryl-[acp], Hexanoyl-[acp], Octanoyl-[acp], trans-Dec-2-enoyl-[acp], Decanoyl-[acp], Hexadecanoyl-[acp], Cobyrinate, L-4-Hydroxyglutamate semialdehyde, alpha-D-Glucosamine 1-phosphate, [Dihydrolipoyllysine-residue succinyltransferase] S-glutaryldihydrolipoyllysine, Hydrogenobyrinate, Adenosyl cobyrinate hexaamide, Cobalt-precorrin 8, [Dihydrolipoyllysine-residue succinyltransferase] S-succinyldihydrolipoyllysine, [Dihydrolipoyllysine-residue acetyltransferase] S-acetyldihydrolipoyllysine, Trinitrotoluene, 4-Hydroxylamino-2,6-dinitrotoluene, 2-Hydroxylamino-4,6-dinitrotoluene, Aminopropylcadaverine, 6-Thioxanthine 5'-monophosphate, (8S)-3',8-Cyclo-7,8-dihydroguanosine 5'-triphosphate

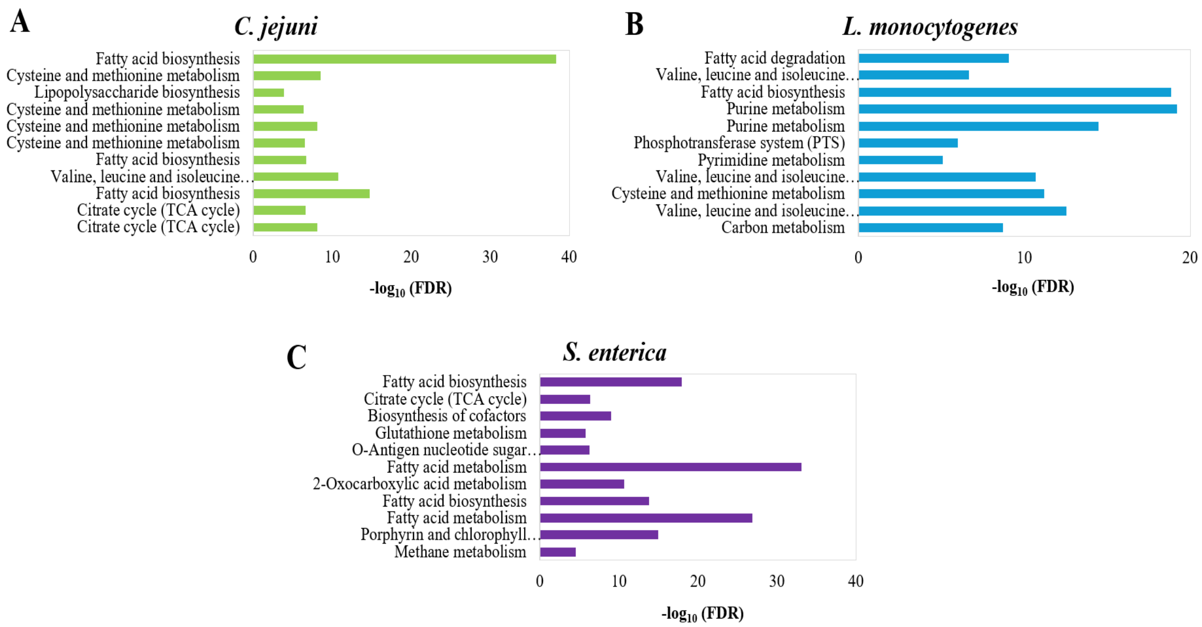

Figure 1. The top enriched metabolic pathways for each foodborne pathogen, ordered from the top according to different stress conditions: acidic stress, bile stress, hypoxia, low iron, nitrosative stress, nutritional downshift, osmotic stress, oxidative stress, stationary phase, temperature, and virulence-inducing conditions for A) *C. jejuni*, B) *L. monocytogenes*, and C) *S. enterica*

The proteins encoded by stress genotypes and nonhomologous to their hosts can be potential targets for the new antimicrobials. However, these genotypes reflect stress conditions specific to the isolation environment of the pathogens. To capture a broader response, gene expression data obtained under diverse stress conditions were analyzed more comprehensively using a systems biology approach to identify potential antimicrobial targets.

A pathway-based computational algorithm [19] was utilized to investigate reporter metabolites associated with resistance-inducing stress. The metabolites obtained were narrowed by focusing on those with p-values below 0.05. Subsequently, pathway enrichment analysis was conducted to associate these key reporter metabolites with organism-specific KEGG pathways. The top enriched pathways are given in Figure 1. Although most of these pathways represent the individual responses of the pathogens, they also share common pathways. Figure 2 shows the pathways that are common for at least four different stress conditions. As shown in the figure, carbon metabolism, amino acid biosynthesis, cofactor biosynthesis, and methane metabolism are common among the three microorganisms. These metabolisms have already been demonstrated as antimicrobial targets in previous studies [28-30]. Other pathways are common for two out of three or individual responses.

The pathways provide mechanistic insights into the bacterial stress response, but their potential as antimicrobial targets is limited. This is primarily due to the presence of conserved metabolic reactions shared with the host (human), which poses a risk of off-target effects and host toxicity. Therefore, it is important to discover the targets that are nonhomologous to humans [31].

By considering individual enriched pathways and common reporter metabolites, potential targets for each microorganism were determined and are detailed below.

4.1. *Campylobacter jejuni*

Campylobacter is a genus of Gram-negative bacteria and a leading cause of foodborne illnesses globally, with *C. jejuni* being the most frequently identified species linked to human infections [32]. As a significant etiological agent of acute gastroenteritis, *C. jejuni* is responsible for approximately 500 million cases of diarrhea annually [33].

Analysis of the antimicrobial resistance genotypes (Table 2) reveals that *C. jejuni* exhibits resistance to a broad spectrum of advanced antibiotics, including aminoglycosides, macrolides, oxazolidinones, pleuromutilins, streptogramins, lincosamides, phenicol antibiotics, beta-lactams (penicillin and cephalosporin), fusidanes, fluoroquinolones, diaminopyrimidines, nucleoside antibiotics, sulfonamides, glycopeptide antibiotics, tetracyclines, and phosphonic acids.

Integrated analysis of the common reporter metabolites and the significantly enriched pathways under various stress conditions revealed multiple metabolic pathways that are markedly perturbed in response to stress. 2-oxocarboxylic acid metabolism, carbon metabolism, biosynthesis of cofactors, carbohydrate metabolism, energy metabolism, amino acid metabolism, metabolism of other amino acids, glycan biosynthesis and metabolism, and metabolism of cofactors and vitamins are the most common responsive pathways in *C. jejuni*. Although lipid metabolism pathways were less commonly enriched (< 4 conditions), fatty acid biosynthesis was the most significantly perturbed pathway under acidic stress, osmotic stress, and stationary phase.

Shawan et al. employed a subtractive genomics approach to discover potential drug targets in *C. jejuni* ATCC 700819 by analyzing human non-homologous essential metabolic proteins. Their study identified 30 such proteins, of which 16 were further categorized into 8 metabolic pathway classes, including methane metabolism, C5-branched dibasic acid metabolism, and lipopolysaccharide (LPS) biosynthesis, pathways also enriched in the current study. Notably, they identified *ccoN*, the gene encoding the cbb3-type cytochrome c oxidase subunit I, as a target. This enzyme facilitates the oxidation of ferrocytochrome c to ferricytochrome c while reducing oxygen to water [34]. Given that both ferrocytochrome c and ferricytochrome c were among the common reporter metabolites in this study, CcoN may serve as a promising drug target.

Similarly, Mehla and Ramana employed a subtractive genomics and comparative pathway analysis approach to identify novel drug targets in *C. jejuni* NCTC11168 [35]. They discovered 16 metabolic pathways involving 326 proteins unique to *Campylobacter*. Of these, 115 were classified as essential according to the Database of Essential Genes, and 66 exhibited no similarity to the human proteome. Screening via DrugBank identified 34 candidates with drug development potential, leading to the selection of 14 top-priority targets, including eight matching approved drug targets involved in critical processes such as cell wall formation and energy metabolism. Pathways such as methane metabolism, C5-branched dibasic acid metabolism, and LPS biosynthesis again overlapped with those found in this study. Among the reported target candidates, *murA* to *murF* genes are essential and highly conserved across various bacterial species, making them strong candidates for the development of future therapeutic drugs. Additionally, the CcoP subunit of cbb3-type cytochrome c oxidase, which mediates electron transfer via heme groups, and *trpC*, involved in tryptophan biosynthesis, emerged as potential therapeutic candidates.

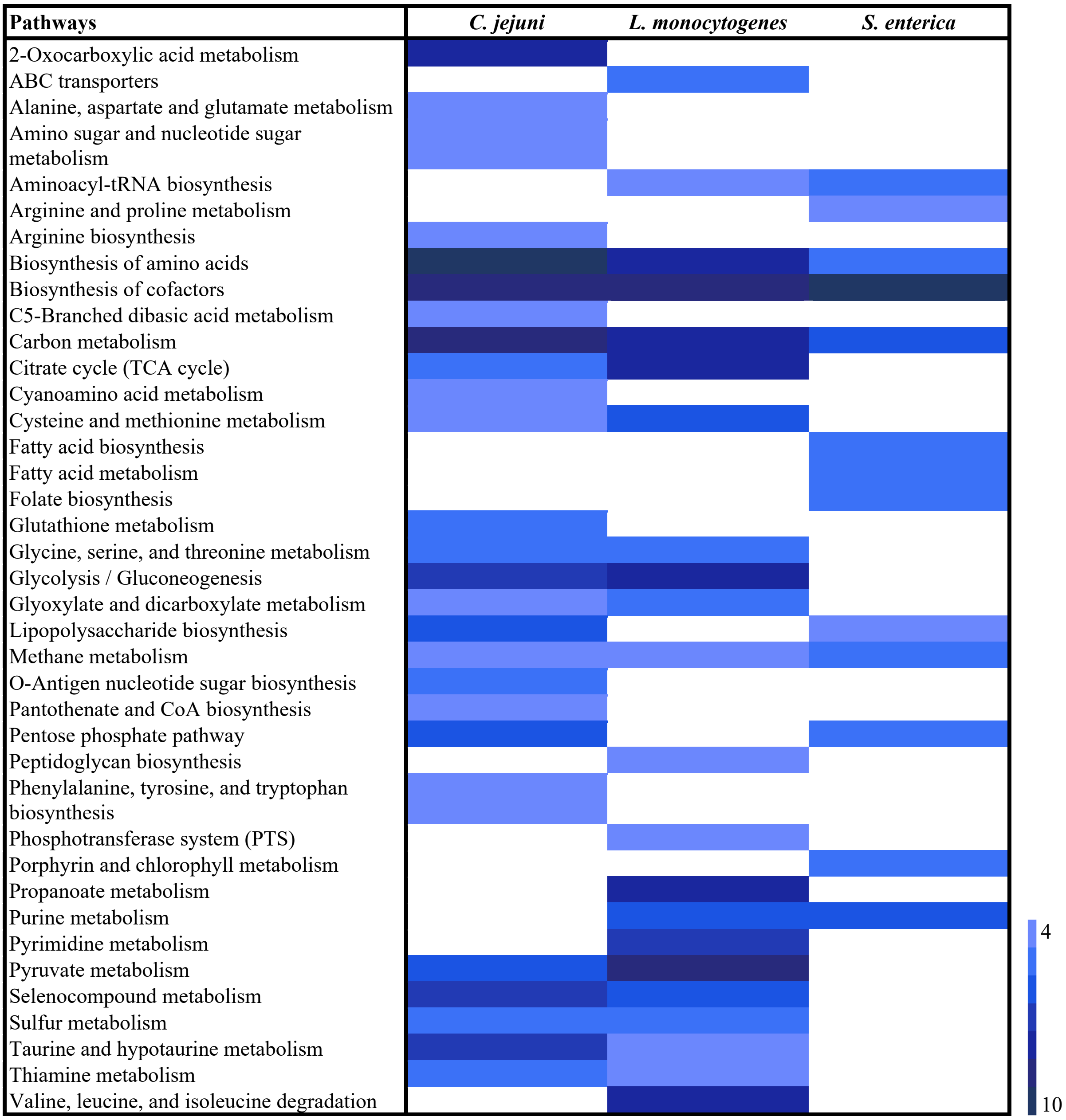

Figure 2. Common enriched pathways for *C. jejuni*, *L. monocytogenes*, and *S. enterica*. The pathways that are not common to at least four stress conditions are not provided. The color bar represents the number of common pathways. As the color gets darker, the number of common pathways increases

Tryptophan biosynthesis enzymes, particularly TrpA and TrpB, are also promising targets. The critical role of this pathway in pathogenicity has been demonstrated. For instance, *Mycobacterium tuberculosis* mutants deficient in tryptophan biosynthesis show impaired survival within macrophages and attenuated virulence in animal models [36]. The *trpB*/*trpA* double mutant, along with the individual gene mutants, showed impaired growth of *S. enterica* serovar Typhimurium *in vitro*; however, only the double mutant was unable to sustain infection *in vivo*. This highlights earlier findings that tryptophan is crucial for the infection [37]. Recent efforts to develop inhibitors targeting this pathway further validate its therapeutic potential [38].

The protein GmhB (D-glycero-alpha-D-manno-heptose 1,7-bisphosphate 7-phosphatase) represents a potential target due to its role in LPS biosynthesis. In *Helicobacter pylori*, disruption of the homologous *HP0860* gene (encoding GmhB) led to increased novobiocin sensitivity and impaired adherence [39], supporting its significance in virulence and its potential as a target in *C. jejuni*.

As key components of the Gram-negative bacterial outer membrane, lipid A and its biosynthetic intermediates are considered attractive drug targets. Compounds disrupting LPS production have been proposed as a strategy to combat antibiotic resistance [40]. Lipid A derivatives (KDO2-lipid A 1-(2-aminoethyl diphosphate), KDO2-lipid A 4'-(2-aminoethyl diphosphate), KDO2-lipid A 1,4'-bis(2-aminoethyl diphosphate), 1-dephospho-KDO2-lipid A, 1-PEtN-KDO2-lipid A) gain attention as the common reporter metabolites in this study. These lipid A derivatives are associated with the protein EptA (lipid A ethanolaminephosphotransferase), which has been suggested as a future therapeutic target to overcome polymyxin resistance [41, 42].

Overall, the convergence of metabolic pathway enrichment data and drug target predictions from previous studies reinforces the potential of several key metabolic enzymes and pathways, including LPS biosynthesis, tryptophan biosynthesis, and cytochrome c oxidase, as promising targets for antimicrobial development against *C. jejuni*.

4.2. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive bacterium known for causing listeriosis, a foodborne disease. It demonstrates a high level of adaptability, enabling it to survive under a wide range of adverse environmental conditions, including extreme pH levels, low temperatures, and high salt concentrations [43, 44].

Genotypic data on antimicrobial resistance (Table 2) show that *L. monocytogenes* isolates possess resistance genes against a broad spectrum of antibiotic classes. These include aminoglycosides, rifamycins, peptide antibiotics, beta-lactams (penicillins, cephalosporins, carbapenems), phenicol antibiotics, oxazolidinones, lincosamides, glycopeptide antibiotics, diaminopyrimidines, fluoroquinolones, macrolides, phosphonic acids, fusidanes, streptogramins, pleuromutilins, nucleoside antibiotics, sulfonamides, and tetracyclines.

Metabolic pathway analysis under various stress conditions revealed that *L. monocytogenes* primarily activates stress response mechanisms through pathways associated with carbon metabolism, energy metabolism, biosynthesis of cofactors, carbohydrate metabolism, nucleotide metabolism, amino acid metabolism, metabolism of other amino acids, and membrane transport. The most enriched pathways also include some of these metabolisms, in addition to fatty acid degradation and fatty acid biosynthesis.

Chandra et al. applied a comparative metabolic pathway analysis to identify potential drug targets against *L. monocytogenes* EGD-e. By utilizing the KEGG database, they identified 28 metabolic pathways unique to *L. monocytogenes* EGD-e, in contrast to 71 pathways that were shared with humans. Although some reporter metabolites involved in lipoic acid metabolism, valine, leucine, and isoleucine degradation, and selenocompound metabolism identified in this study fall within these shared pathways, they were not recommended as therapeutic targets due to potential off-target effects in the human host. Among the 28 unique pathways identified, methane metabolism, peptidoglycan biosynthesis, and the phosphotransferase system overlapped with the enriched pathways observed in the current study, reinforcing their relevance in pathogen-specific metabolism. Chandra et al. also identified 15 potential drug target enzymes that are essential for bacterial survival and lack human homologs. Among these, four enzymes were prioritized as particularly promising candidates for vaccine or drug development: UDP-N-acetylglucosamine 1-carboxyvinyltransferase (*lmo2526-murA*), acetate kinase (*lmo1581-ackA*), phosphate acetyltransferase (*lmo2103-eutD*), and aspartate kinase (*lmo1235*) [45]. Notably, two of these, *lmo2103* and *lmo1235*, also emerged as relevant findings in the current study, further validating their potential as selective antimicrobial targets against *L. monocytogenes*.

In a comprehensive pan-genomic study, Zhang et al. analyzed 355 genome sequences, including 343 *L. monocytogenes* strains and 12 from other *Listeria* species and non-Listerial genomes, identifying 357 potential target genes, which were narrowed down to 20 key pathways via KEGG enrichment. Common pathways between their study and the current analysis include peptidoglycan biosynthesis, glycine/serine/threonine metabolism, phosphotransferase system, and ABC transporters. The potential target genes were subsequently filtered by a hub gene selection approach to discover novel targets. The identified key hub genes related to pathogenicity [46] also align with common reporter metabolites and targets identified in this study (*davD-lmo0913*, *tilS-lmo0219*, and *gshA-lmo2770*), supporting their relevance as therapeutic targets.

During peptidoglycan synthesis, L-alanine is converted into D-alanine by alanine racemase (Dal or Alr), and subsequently, two D-alanine molecules are linked by D-Ala:D-Ala ligase (Ddl) to form a dipeptide [47]. Inhibitors of Ddl have been identified across various organisms [48-52], suggesting this enzyme as a viable therapeutic target for *L. monocytogenes* as well.

Another potential target is CysK, cysteine synthase, which has a vital role in the major metabolic activities of Gram-positives [53]. The deletion of *cysK* gene in *Staphylococcus aureus* resulted in reduced cell viability, diminished stress tolerance, and impaired survival [54]. CysK has already been suggested as a promising therapeutic target due to its essential role in bacterial physiology [55].

Together, the findings underscore the importance of integrating genomic, metabolic, and functional analyses to uncover novel drug targets. The overlap of metabolic stress responses and essential pathways in multiple independent studies provides robust evidence supporting specific genes/enzymes such as *davD*, *tilS*, *murA*, *ddl*, *cysK*, and others as promising candidates for future antimicrobial development against *L. monocytogenes*.

4.3. *Salmonella enterica*

S. enterica serovar Typhimurium is a well-established model organism for studying Gram-negative intracellular pathogens. As a non-typhoidal strain, it is a common cause of acute gastroenteritis and food poisoning [56]. Due to its increasing resistance to fluoroquinolones, it is listed in the Bacterial Priority Pathogens by WHO [57]. In addition to fluoroquinolones, *S. enterica* exhibits resistance to aminoglycosides (gentamycin, streptomycin), tetracyclines (glycylcycline, tigecycline), beta-lactams (penicillin, cephalosporin, carbapenem, monobactam, cephamycin), phenicol antibiotics, rifamycins, fluoroquinolones, oxazolidinones, lincosamides, streptogramins, diaminopyrimidines, macrolides, phosphonic acids, peptide antibiotics, nucleoside antibiotics, nitroimidazoles, nitrofurans, sulfonamides, glycopeptide antibiotics, and pleuromutilins (Table 2).

The analysis of common reporter metabolites and common enriched pathways highlighted porphyrin metabolism as a prominent enriched pathway, with particular attention to cobalamin biosynthesis. CbiA (cobyrinic acid a,c-diamide synthase) and CbiC (precorrin-8X/cobalt-precorrin-8 methylmutase) proteins were identified as potential targets. Supporting these findings, Gu et al. investigated the prevalence of antimicrobial resistance and virulence genes in *Salmonella* isolates from livestock in Central China. Pathway analysis associated with multidrug resistance and pathogenicity resulted in several overlapped metabolisms/pathways with the current study, including carbon metabolism, biosynthesis of amino acids, pentose phosphate pathway, methane metabolism, arginine and proline metabolism, and porphyrin and chlorophyll metabolism. Bioinformatics analysis highlighted 10 hub genes (including *cbiA* and *cbiC*) as key players in antimicrobial resistance and virulence [58]. Similarly, Liu et al. identified 9 hub proteins of *S.* Typhimurium DT104 by performing a protein-protein interaction network analysis. CbiQ, CbiC, and CbiT, which are part of the cobalamin biosynthesis pathway, were implicated in adaptive responses and suggested as potential targets for growth inhibition [59].

Another enzyme of interest is MenE (O-succinylbenzoic acid-CoA ligase), which functions in menaquinone biosynthesis, a process essential to bacterial metabolism but not synthesized endogenously in humans. Therefore, enzymes involved in the menaquinone biosynthesis pathway represent promising therapeutic targets [60]. Previously, several inhibitors of MenE were discovered in different organisms, among which are *M. tuberculosis*, *S.*

aureus [61, 62], and *Bacillus anthracis* [63]. The results suggest MenE may also serve as a novel target in *S. enterica.*

The fatty acid synthesis (FAS) pathway is crucial for producing key components of bacterial cell structures, such as LPSs, lipoproteins, and phospholipids. There are two types of FAS: FAS-I in eukaryotes, which uses a single multifunctional enzyme complex, and FAS-II in prokaryotes, which relies on separate enzymes for each step of fatty acid chain elongation. The FAS-II pathway, unique to bacteria, presents a promising target for developing new antimicrobials. A key enzyme in this pathway is enoyl-ACP reductase (ENR), which performs the final step in each elongation cycle. Among the ENR isoforms, FabI has gained particular attention as a significant antibacterial target due to its essential role in bacterial fatty acid synthesis [64-66]. Although several studies focused on the FabI inhibition of *S. aureus* [67], *Plasmodium falciparum* [68], and *S. enterica* by different molecules, further exploration of FabI inhibitors in *Salmonella* is warranted.

Finally, N-acetyl-glucosamine-1-phosphate uridyltransferase (GlmU), a bifunctional enzyme (acetylation and uridylation) catalyzing the final steps in UDP-N-acetylglucosamine (UDP-GlcNAc) biosynthesis [69, 70], represents a potential drug target. Being involved in the synthesis of both LPS and peptidoglycan in bacteria makes GlmU an attractive target for developing antimicrobials. Indeed, both the acetyl- and/or uridyltransferase activities of *Haemophilus influenzae* [71], *M. tuberculosis* [72-74], *Xanthomonas oryzae* [75], *E. coli* [76], and *Streptococcus pneumoniae* [77] have been targeted as a novel antibiotic strategy, and their inhibition could similarly be effective against *S. enterica*.

The findings emphasize the therapeutic potential of targeting essential metabolic proteins involved in cobalamin, menaquinone, fatty acid, and cell wall biosynthesis in *S. enterica*, especially given its extensive antimicrobial resistance.

5. Conclusion

To combat the growing threat of foodborne pathogens, there is a pressing demand for new antimicrobial agents since they are resistant to most of the existing antibiotics. Targeting unconventional biological processes specifically active during infection and suitable for high-throughput screening holds great promise for revolutionizing antimicrobial development.

The current study identified several targets for common foodborne pathogens *C. jejuni*, *L. monocytogenes*, and *S. enterica,* by employing a systems biology approach. Identification of reporter metabolites across several stress conditions pointed out several key pathways and proteins that could be important for the stress response. Although it is possible to find several stress genotypes using several databases, the responses are limited to the isolation environment. Through a comprehensive analysis, this study characterized bacterial stress responses under several stress conditions. The off-target effects were considered during the identification of the potential targets. The findings highlighted that the use of systems biology approaches in antimicrobial discovery can enhance the accuracy of target identification and lower the screening costs, thereby contributing to early-stage drug development.

In summary, this integrative approach combining genomic data with stress-induced metabolic responses has identified several promising, non-human-homologous antimicrobial targets across major foodborne pathogens. These findings lay the groundwork for targeted therapeutic development, especially against multidrug-resistant strains.

Article Information Form

Acknowledgments
The author would like to thank Kemal Avican for sharing large-scale differential expression analysis data and read count tables.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the author.

Artificial Intelligence Statement
No artificial intelligence tool has been used while writing this article.

Copyright Statement

References

[1] World Health Organization. (2022). WHO global strategy for food safety 2022–2030: towards stronger food safety systems and global cooperation: executive summary [Online]. Available: https://iris.who.int/handle/10665/364638

[2] D. G. White, S. Zhao, S. Simjee, D. D. Wagner, P. F. McDermott, "Antimicrobial resistance of foodborne pathogens," Microbes and Infection, vol. 4, no. 4, pp. 405–412, 2002.

[3] A. H. Havelaar, M. D. Kirk, P. R. Torgerson, H. J. Gibb, T. Hald, R. J. Lake, et al., "World health organization global estimates and regional comparisons of the burden of foodborne disease in 2010," PLoS Medicine, vol. 12, no. 12, e1001923, 2015.

[4] Z. Bukari, T. Emmanuel, J. Woodward, R. Ferguson, M. Ezughara, N. Darga, et al, "The global challenge of *Campylobacter*: antimicrobial resistance and emerging intervention strategies," Tropical Medicine and Infectious Disease, vol. 10, no. 1, p. 25, 2025.

[5] Y. El Dessouky, S. W. Elsayed, N. A. Abdelsalam, N. A. Saif, A. Álvarez-Ordóñez, M. Elhadidy, "Genomic insights into zoonotic transmission and antimicrobial resistance in *Campylobacter jejuni* from farm to fork: A one health perspective," Gut Pathogens, vol. 14, p. 44, 2022.

[6] J. Lu, D. Linz, I. Struewing, S. P. Keely, M. Jahne, T. M. Gruber, E. N. Villegas, "Virulence and infectious assessment of a *Campylobacter jejuni* strain isolated from California gull," Microbiology Spectrum, vol. 13, no. 5, p. e01705-24, 2025.

[7] H. Jamali, M. Paydar, S. Ismail, C. Y. Looi, W. F. Wong, B. Radmehr, et al., "Prevalence, antimicrobial susceptibility and virulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fish markets," BMC Microbiology, vol. 15, p. 144, 2015.

[8] K. Linke, I. Rückerl, K. Brugger, R. Karpiskova, J. Walland, S. Muri-Klinger, et al., "Reservoirs of *Listeria* species in three environmental ecosystems," Applied and Environmental Microbiology, vol. 80, no. 18, pp. 5583–5592, 2014.

[9] L. Hafner, M. Pichon, C. Burucoa, S. H. A. Nusser, A. Moura, M. Garcia-Garcera, et al., "*Listeria monocytogenes* faecal carriage is common and depends on the gut microbiota," Nature Communications, vol. 12, p. 6826, 2021.

[10] V. Yadav, A. K. Poonia, D. Pal, "*Salmonella* Typhimurium biofilm," in: *Salmonella* Biofilms, Infection, and Therapeutic Management, A. Kumar, A. Upadhyay, Academic Press, 2025, pp. 63–74.

[11] C. Vinueza-Burgos, J. Medina-Santana, M. Ishida, B. Sauders, G. Deiulio, A. Dickey, et al., "*Salmonella* isolated from street foods and environment of an urban park: A whole genome sequencing approach," PLoS One, vol. 20, no. 4, p. e0320735, 2025.

[12] Y. Xiang, K. Zhu, K. Min, Y. Zhang, J. Liu, K. Liu, et al., "Characterization of a *Salmonella enterica* serovar Typhimurium lineage with rough colony morphology and multidrug resistance," Nature Communications, vol. 15, p. 6123, 2024.

[13] M. Farrukh, A. Munawar, Z. Nawaz, N. Hussain, A. Bin Hafeez, P. Szweda, "Antibiotic resistance and preventive strategies in foodborne pathogenic

bacteria: A comprehensive review," Food Science and Biotechnology, vol. 34, pp. 2101–2129, 2025.

[14] K. Avican, J. Aldahdooh, M. Togninalli, A. K. M. F. Mahmud, J. Tang, K. M. Borgwardt, et al., "RNA atlas of human bacterial pathogens uncovers stress dynamics linked to infection," Nature Communications, vol. 12, p. 3282, 2021.

[15] H. Wang, S. Marcišauskas, B. J. Sánchez, I. Domenzain, D. Hermansson, R. Agren, et al., "RAVEN 2.0: A versatile toolbox for metabolic network reconstruction and a case study on *Streptomyces coelicolor*," PLoS Computational Biology, vol. 14, p. e1006541, 2018.

[16] M. Kanehisa, S. Goto, "KEGG: Kyoto Encyclopedia of Genes and Genomes," Nucleic Acids Research, vol. 28, pp. 27–30, 2000.

[17] M. Kanehisa, S. Goto, M. Hattori, K. F. Aoki-Kinoshita, M. Itoh, S. Kawashima, et al., "From genomics to chemical genomics: New developments in KEGG," Nucleic Acids Research, vol. 34, pp. D354–D357. 2006.

[18] A. P. Oliveira, K. R. Patil, J. Nielsen, "Architecture of transcriptional regulatory circuits is knitted over the topology of bio-molecular interaction networks," BMC Systems Biology, vol. 2, p. 17, 2008.

[19] K. R. Patil, J. Nielsen, "Uncovering transcriptional regulation of metabolism by using metabolic network topology," Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 8, pp. 2685–2689, 2005.

[20] F. G. Avci, "Unraveling bacterial stress responses: Implications for next-generation antimicrobial solutions," World Journal of Microbiology and Biotechnology, vol. 40, p. 285, 2024.

[21] J. Lopez-Ibanez, F. Pazos, M. Chagoyen, "MBROLE3: improved functional enrichment of chemical compounds for metabolomics data analysis," Nucleic Acids Research, vol. 51, pp. W305–W309, 2023.

[22] G. Cox, A. Sieron, A. M. King, G. De Pascale, A. C. Pawlowski, K. Koteva, et al., "A Common platform for antibiotic dereplication and adjuvant discovery," Cell Chemical Biology, vol. 24, no. 1, pp. 98–109, 2017.

[23] G. Kiskó, B. Bajramović, F. Elzhraa, P. Erdei-Tombor, V. Dobó, C. Mohácsi-Farkas, et al., "The invisible threat of antibiotic resistance in food," Antibiotics, vol. 14, p. 250, 2025.

[24] S. Dühring, S. Germerodt, C. Skerka, P. F. Zipfel, T. Dandekar, S. Schuster, "Host-pathogen interactions between the human innate immune system and *Candida albicans*-understanding and modeling defense and evasion strategies," Frontiers in Microbiology, vol. 6, p. 144328, 2015.

[25] G. Jorge, D. Silva, J. Dawan, J. Ahn, "Bacterial Stress Responses as Potential Targets in Overcoming Antibiotic Resistance," Microorganisms, vol. 10, no. 7, p. 1385, 2022.

[26] R. Pei, L. Zhang, C. Duan, M. Gao, R. Feng, Q. Jia, et al., "Investigation of stress response genes in antimicrobial resistant pathogens sampled from five countries," Processes, vol. 9, no. 6, p. 927, 2021.

[27] K. J. Boor, "Bacterial stress responses: What doesn't kill them can make them stronger," PLOS Biology, vol. 4, no. 1, p. e23, 2006.

[28] T. M. Amorim Franco, J. S. Blanchard, "Bacterial branched-chain amino acid biosynthesis: Structures, mechanisms, and drugability," Biochemistry, vol. 56, pp. 5849–5865, 2017.

[29] A. Wróbel, K. Arciszewska, D. Maliszewski, D. Drozdowska, “Trimethoprim and other nonclassical antifolates an excellent template for searching modifications of dihydrofolate reductase enzyme inhibitors,” The Journal of Antibiotics, vol. 73, pp. 5–27, 2019.

[30] M. Tong, E. D. Brown, “Food for thought: Opportunities to target carbon metabolism in antibacterial drug discovery,” Annals of the New York Academy of Sciences, vol. 1524, no. 1, pp. 51–64, 2023.

[31] F. M. Mobegi, S. A. F. T. van Hijum, P. Burghout, H. J. Bootsma, S. P. W. de Vries, C. E. van der Gaast-de Jongh, E. Simonetti, J. D. Langereis, P. W. M. Hermans, M. I. de Jonge, A. Zomer, “From microbial gene essentiality to novel antimicrobial drug targets,” BMC Genomics, vol. 15, p. 958, 2014.

[32] N. O. Kaakoush, N. Castaño-Rodríguez, H. M. Mitchell, S. M. Man, “Global epidemiology of *Campylobacter* infection,” Clinical Microbiology Reviews, vol. 28, no. 3, pp. 687–720, 2015.

[33] D. A. Rossi, C. F. Dumont, A. C. de S. Santos, M. E. de Lourdes Vaz, R. R. Prado, G. P. Monteiro, et al., “Antibiotic Resistance in the Alternative Lifestyles of *Campylobacter jejuni*,” Frontiers in Cellular and Infection Microbiology, vol. 11, p. 535757, 2021.

[34] M. M. A. K. Shawan, H. Al Mahmud, P. S. Gope, N. M. Salauddin, H. Rahman, M. A. Ahmed, et al., “*Campylobacter jejuni* ATCC 700819: An *in silico* approach to identify and categorize probable drug targets by subtractive genome analysis,” Journal of Pure and Applied Microbiology, vol. 10, no. 1, pp. 229–241, 2016.

[35] K. Mehla, J. Ramana, “Novel Drug Targets for food-borne pathogen *Campylobacter jejuni*: An integrated subtractive genomics and comparative metabolic pathway study,” OMICS: A Journal of Integrative Biology, vol. 19, no. 7, pp. 369–434, 2015.

[36] D. A. Smith, T. Parish, N. G. Stoker, G. J. Bancroft, “Characterization of auxotrophic mutants of *Mycobacterium tuberculosis* and their potential as vaccine candidates,” Infection and Immunity, vol. 69, no. 2, pp. 1142–1150, 2001.

[37] L. Jelsbak, H. Hartman, C. Schroll, J. T. Rosenkrantz, S. Lemire, I. Wallrodt, et al., “Identification of Metabolic pathways essential for fitness of *Salmonella* typhimurium *In Vivo*,” PLoS One, vol. 9, no. 7, p. e101869, 2014.

[38] S. Consalvi, C. Scarpecci, M. Biava, G. Poce, “Mycobacterial tryptophan biosynthesis: A promising target for tuberculosis drug development?” Bioorganic and Medicinal Chemistry Letters, vol. 29, no. 23, p. 126731, 2019.

[39] S. F. Chiu, K. W. Teng, P. C. Wang, H. Y. Chung, C. J. Wang, H. C. Cheng, et al., “*Helicobacter pylori* GmhB enzyme involved in ADP-heptose biosynthesis pathway is essential for lipopolysaccharide biosynthesis and bacterial virulence,” Virulence, vol. 12, no. 1, pp. 1610–1628, 2021.

[40] S. H. Joo, “Lipid a as a drug target and therapeutic molecule,” Biomolecules and Therapeutics (Seoul), vol. 23, no. 6, pp. 510–516, 2015.

[41] A. Samantha, A. Vrielink, “Lipid a phosphoethanolamine transferase: Regulation, structure and immune response,” Journal of Molecular Biology, vol. 432, no. 18, pp. 5184–5196, 2020.

[42] V. C. Thai, K. A. Stubbs, M. Sarkar-Tyson, C. M. Kahler, “Phosphoethanolamine transferases as drug discovery targets for therapeutic treatment of multi-drug resistant pathogenic gram-negative bacteria,” Antibiotics, vol. 12, no. 9, p. 1382, 2023.

[43] M. Kumaraswamy, C. Do, G. Sakoulas, P. Nonejuie, G. W. Tseng, H. King, et al., "*Listeria monocytogenes* endocarditis: Case report, review of the literature, and laboratory evaluation of potential novel antibiotic synergies," International Journal of Antimicrobial Agents, vol. 51, no. 3, pp. 468–478, 2018.

[44] H. Zhang, J. Wang, Z. Chang, X. Liu, W. Chen, Y. Yu, et al., "*Listeria monocytogenes* contamination characteristics in two ready-to-eat meat plants from 2019 to 2020 in Shanghai," Frontiers in Microbiology, vol. 12, p. 729114, 2021.

[45] N. Chandra, T. Qidwai, S. Singh, "Drug Target Identification for *Listeria monocytogenes* by Subtractive Genomics Approach," Journal of Pure and Applied Microbiology, vol. 17, no. 3, pp. 1893–1906, 2023.

[46] B. Zhang, H. Ren, X. Wang, C. Han, Y. Jin, X. Hu, et al., "Comparative genomics analysis to explore the biodiversity and mining novel target genes of *Listeria monocytogenes* strains from different regions," Frontiers in Microbiology, vol. 15, p. 1424868, 2024.

[47] G. A. Prosser, L. P. S. De Carvalho, "Kinetic mechanism and inhibition of *Mycobacterium tuberculosis* D-alanine: D-alanine ligase by the antibiotic D-cycloserine," FEBS Journal, vol. 280, no. 4, pp. 1150–1166, 2013.

[48] V. Škedelj, E. Arsovska, T. Tomašić, A. Kroflič, V. Hodnik, M. Hrast, et al., "6-Arylpyrido[2,3-d]pyrimidines as Novel ATP-Competitive Inhibitors of Bacterial D-Alanine:D-Alanine Ligase," PLoS One, vol. 7, no. 8, p. e39922, 2012.

[49] R. Murakami, Y. Muramatsu, E. Minami, K. Masuda, Y. Sakaida, S. Endo, et al., "A novel assay of bacterial peptidoglycan synthesis for natural product screening," Journal of Antibiotics, vol. 62, pp. 153–158, 2009.

[50] M. Hrast, B. Vehar, S. Turk, J. Konc, S. Gobec, D. Janežič, "Function of the D-alanine: D-alanine ligase lid loop: A molecular modeling and bioactivity study," Journal of Medicinal Chemistry, vol. 55, no. 15, pp. 6849–6856, 2012.

[51] Y. Nakatani, H. K. Opel-Reading, M. Merker, D. Machado, S. Andres, S. S. Kumar, et al., "Role of alanine racemase mutations in *Mycobacterium tuberculosis* D-cycloserine resistance," Antimicrobial Agents and Chemotherapy, vol. 61, no. 12, p. e01575-17, 2017.

[52] S. Rahman, A. Bhattacharya, P. Jana, M. Ganguly, A. K. Das, D. Hazra, et al., "Subtractive proteomics unravel the potency of D-alanine-D-alanine ligase as the drug target for *Burkholderia pseudomallei*," International Journal of Biological Macromolecules, vol. 314, p. 144106, 2025.

[53] M. A. Mraheil, R. Frantz, L. Teubner, H. Wendt, U. Linne, J. Wingerath, et al., "Requirement of the RNA-binding protein SmpB during intracellular growth of *Listeria monocytogenes*," International Journal of Medical Microbiology, vol. 307, no. 3, pp. 166–173, 2017.

[54] J. K. Lithgow, E. J. Hayhurst, G. Cohen, Y. Aharonowitz, S. J. Foster, "Role of a Cysteine Synthase in *Staphylococcus aureus*," Journal of Bacteriology, vol. 186, no. 6, pp. 1579–1590, 2004.

[55] P. Joshi, A. Gupta, V. Gupta, "Insights into multifaceted activities of CysK for therapeutic interventions," 3 Biotech, vol. 9, p. 44, 2019.

[56] K. Nishino, E. Nikaido, A. Yamaguchi, "Regulation and physiological function of multidrug efflux pumps in *Escherichia coli* and *Salmonella*, Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, vol. 1794, vol. 5, pp. 834–843, 2009.

[57] World Health Organization. (2024). WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance, 2024 [Online]. Available: https://www.who.int/publications/i/item/9789240093461

[58] Y. Gu, X. Kuang, A. Sajid, Y. Wang, Z. Zhang, Z. Xu, et al., "Prevalence and mechanism of antimicrobial resistance and pathogenicity of *Salmonella* isolated from foodborne animal in China," LWT Food Science and Technology, vol. 184, p. 114906, 2023.

[59] Y. Liu, F. Zhang, J. L. Hawkins, J. R. Elder, G. M. Baranzoni, Z. Huang, et al., "Comparative Gene Expression Analysis of *Salmonella* Typhimurium DT104 in Ground Chicken Extract and Brain Heart Infusion Broth," Microorganisms, vol. 12, p. 1461, 2024.

[60] J. S. Matarlo, C. E. Evans, I. Sharma, L. J. Lavaud, S. C. Ngo, R. Shek, et al., "Mechanism of MenE inhibition by acyl-adenylate analogues and discovery of novel antibacterial agents," Biochemistry, vol. 54, no. 42, pp. 6514–6524, 2015.

[61] X. Lu, R. Zhou, I. Sharma, X. Li, G. Kumar, S. Swaminathan, et al., "Stable analogues of OSB-AMP: Potent inhibitors of MenE, the o-Succinylbenzoate-CoA synthetase from bacterial menaquinone biosynthesis," ChemBioChem, vol. 13, no.1, pp. 129–136, 2012.

[62] C. E. Evans, J. S. Matarlo, P. J. Tonge, D. S. Tan, "Stereoselective synthesis, docking, and biological evaluation of difluoroindanediol-based MenE inhibitors as antibiotics," Organic Letters, vol. 18, no. 24, pp. 6384–6387, 2016.

[63] Y. Tian, D. H. Suk, F. Cai, D. Crich, A. D. Mesecar, "*Bacillus anthracis* o-succinylbenzoyl-CoA synthetase: Reaction kinetics and a novel inhibitor mimicking its reaction intermediate," Biochemistry, vol. 47, pp. 12434–12447, 2008.

[64] C. O. Rock, J. E. Cronan, "*Escherichia coli* as a model for the regulation of dissociable (type II) fatty acid biosynthesis," Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, vol. 1302, no. 1, pp. 1–16, 1996.

[65] S. Smith, A. Witkowski, A.K. Joshi, "Structural and functional organization of the animal fatty acid synthase," Progress in Lipid Research, vol. 42, no. 4, pp. 289–317, 2003.

[66] J. W. Campbell, J. Cronan, "Bacterial fatty acid biosynthesis: Targets for antibacterial drug discovery," Annual Review of Microbiology, vol. 55, pp. 305–332, 2001.

[67] C. J. Zheng, M. J. Sohn, W. G. Kim, "Vinaxanthone, a new FabI inhibitor from *Penicillium* sp.," Journal of Antimicrobial Chemotherapy, vol. 63, no. 5, pp. 949–953, 2009.

[68] T. Banerjee, S. K. Sharma, N. Surolia, A. Surolia, "Epigallocatechin gallate is a slow-tight binding inhibitor of enoyl-ACP reductase from *Plasmodium falciparum*," Biochemical and Biophysical Research Communications, vol. 377, no. 4, pp. 1238–1242, 2008.

[69] V. Soni, E. H. Rosenn, R. Venkataraman, "Insights into the central role of N-acetyl-glucosamine-1-phosphate uridyltransferase (GlmU) in peptidoglycan metabolism and its potential as a therapeutic target," Biochemical Journal, vol. 480, no. 15, pp. 1147–1164, 2023.

[70] J. A. Wyllie, M. V. McKay, A. S. Barrow, T. P. Soares da Costa, "Biosynthesis of uridine diphosphate N-Acetylglucosamine: An underexploited pathway in the search for novel antibiotics?" IUBMB Life, vol. 74, no. 12, pp. 1232–1252, 2022.

[71] I. Mochalkin, S. Lightle, Y. Zhu, J. F. Ohren, C. Spessard, N. Y. Chirgadze, et al., "Characterization of substrate binding and catalysis in the potential antibacterial target N-acetylglucosamine-1-phosphate uridyltransferase (GlmU)," Protein Science, vol. 16, no. 12, pp. 2657–2666, 2007.

[72] H. M. Patel, M. Palkar, R. Karpoormath, "Exploring MDR-TB Inhibitory Potential of 4-Aminoquinazolines as *Mycobacterium tuberculosis* N-Acetylglucosamine-1-Phosphate Uridyltransferase (GlmUMTB) Inhibitors," Chemistry and Biodiversity, vol. 17, no. 8, p. e2000237, 2020.

[73] Y. Li, Y. Zhou, Y. Mac, X. Li, "Design and synthesis of novel cell wall inhibitors of *Mycobacterium tuberculosis* GlmM and GlmU," Carbohydrate Research, vol. 346, no. 13, pp. 1714–1720, 2011.

[74] R. Sharma, I. A. Khan, "Mechanism and Potential Inhibitors of GlmU: A Novel Target for Antimicrobial Drug Discovery," Current Drug Targets, vol. 18, no. 14, pp. 1587–1597, 2017.

[75] J. Min, D. Lin, Q. Zhang, J. Zhang, Z. Yu, "Structure-based virtual screening of novel inhibitors of the uridyltransferase activity of *Xanthomonas oryzae* pv. *oryzae* GlmU," European Journal of Medicinal Chemistry, vol. 53, pp. 150–158, 2012.

[76] R. Sharma, C. Rani, R. Mehra, A. Nargotra, R. Chib, V. S. Rajput, et al., "Identification and characterization of novel small molecule inhibitors of the acetyltransferase activity of *Escherichia coli* N-acetylglucosamine-1-phosphate-uridyltransferase/glucosamine-1-phosphate-acetyltransferase (GlmU)," Applied Microbiology and Biotechnology, vol. 100, no. 7, pp. 3071–3085, 2016.

[77] E. B. Brazel, A. Tan, S. L. Neville, A. R. Iverson, S. R. Udagedara, B. A. Cunningham, et al., "Dysregulation of *Streptococcus pneumoniae* zinc homeostasis breaks ampicillin resistance in a pneumonia infection model," Cell Reports, vol. 38, no. 2, p. 110202, 2022.