PHARMACON
Jurnal Farmasi Indonesia
Pharmaceutical Journal of Indonesia

Pharmacon: Jurnal Farmasi Indonesia
Vol. 22, No. 2, 2025, pp. 112–121
DOI:10.23917/pharmacon.v22i2.13565
p-ISSN: **1411-4283**, e-ISSN: **2685-5062**

Genomics and Network Analysis of Multidrug-Resistant Shigella flexneri from Raw Vegetables in Bangladesh for Risk Assessment and Targeted Therapeutic Intervention

K.M. Tanjida Islam[1*], Shahin Mahmud[2]

[1-2]Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh
**Corresponding author: tanjidaislam255@gmail.com*

ARTICLE HISTORY:
Submitted : 2025-10-30
Accepted : 2025-12-30
Published : 2025-12-31

KEYWORDS:
Bangladesh; Dissemination; Multidrug-resistant; Raw vegetables; *Shigella flexneri*

Citation:
Islam, K.M.T, Mahmud, S. (2025). Genomics and Network Analysis of Multidrug-Resistant Shigella flexneri from Raw Vegetables in Bangladesh for Risk Assessment and Targeted Therapeutic Intervention. Pharmacon: Jurnal Farmasi Indonesia, 22(2), 112-121. https://doi.org/10.23917/pharmacon.v22i2.13565

ABSTRACT

Shigella flexneri increasingly poses a threat to public health in developing nations through the dissemination of multidrug-resistant (MDR) strains via contaminated raw vegetables in urban agriculture systems. Despite extensive characterization of clinical isolates, the mechanistic insights and therapeutic vulnerabilities of food-borne *S. flexneri* strains from Bangladesh remain underexplored. Therefore, this study aimed to characterize MDR *S. flexneri* from Bangladeshi raw vegetables retrieved from the NCBI genome database and identify core and essential antibiotic-resistant genes as prioritized therapeutic targets. Whole-genome sequences of four *S. flexneri* strains isolated from tomatoes and green chilies across Gazipur and Dhaka were retrieved from NCBI GenBank and analyzed through comparative genomics to identify core genes and resistance determinants. Networks were constructed using the STRING database, followed by centrality-based topology analysis to identify hub genes that cross-referenced with the KEGG database and the Database of Essential Genes. Comparative analysis revealed 4273 core genes and 44 antibiotic resistance genes across all strains. Network topology analysis identified eight hub genes (*tolC*, *acrA*, *emrK*, *yegO*, *yjcP*, *emrB*, *yjcR*, *evgS*) based on degree, closeness, and betweenness centrality metrics. Five hub genes (*tolC*, *acrA*, *emrK*, *emrB*, *evgS*) were classified as essential for bacterial survival, representing critical nodes in efflux-mediated resistance and two-component regulatory systems. These essential hub genes constitute high-priority therapeutic targets whose disruption could compromise multidrug resistance mechanisms and bacterial viability in food-borne *S. flexneri*. Additionally, to mitigate their dissemination, raising public awareness on MDR pathogens from raw vegetables is recommended.

INTRODUCTION

Foodborne pathogens increasingly develop resistance to conventional antimicrobial therapies through complex mechanisms, presenting significant public health challenges that require novel therapeutic approaches (Berger & Loewy, 2024). *Shigella flexneri*, a Gram-negative enteropathogen responsible for endemic and epidemic bacillary dysentery, has emerged as a critical concern in low and middle-income countries where inadequate sanitation infrastructure and contaminated food sources facilitate its transmission (Gharpure et al., 2021; Nisa et al., 2020). Globally, *Shigella* species (spp.) cause approximately 270 million diarrheal disease cases annually, with significant mortality concentrated among children under five years old (Lu et al., 2024).

Although antibiotics have transformed modern medicine by successfully combating bacterial infections, the widespread overuse and misuse of these agents have driven the evolution of antibiotic resistance, enabling bacteria to develop survival mechanisms that significantly

© 2022 The Author(s). This work is licensed under a Creative Common Attribution 4.0 (CC-BY) International (https://creativecommons.org/licenses/by/4.0/)

diminish drug effectiveness (Ananna et al., 2024).

Shigella spp. in Bangladesh demonstrated substantial resistance to multiple first-line antibiotics, with the highest rates observed for fluoroquinolones (61.9%), trimethoprim–sulfamethoxazole (60.8%), azithromycin (38.8%), nalidixic acid (36.2%), ampicillin (34.5%), and ciprofloxacin (31.1%) (Ahmed et al., 2023).

Additionally, the environmental factors, including agricultural practices and contamination, contribute significantly to the emergence and dissemination of antimicrobial resistance (Sassi et al., 2025).

However, recent epidemiological studies have reported the identification and characterization of antimicrobial-resistant *S. flexneri* isolates from raw vegetables (Islam et al., 2024). Consequently, the consumption of these raw vegetables presents a substantial public health risk by serving as a potential vehicle for the dissemination of antibiotic resistance genes to humans (Kim & Ahn, 2022; Xiao et al., 2023) .

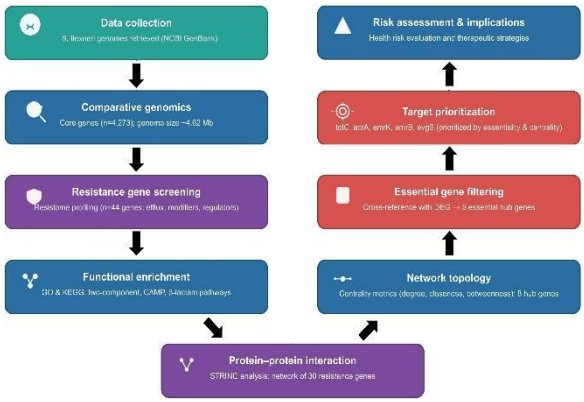

Figure 1. Systematic workflow for antimicrobial resistance gene identification and therapeutic target prioritization in *Shigella flexneri*.

Despite substantial advances in understanding the pathogenicity and MDR profiles of *S. flexneri* (Hossain et al., 2024), critical knowledge gaps persist regarding the foodborne MDR patterns, molecular mechanisms of resistance, and therapeutic strategies applicable to *S. flexneri* isolated from raw vegetables.

Therefore, this study aimed to characterize the MDR *S. flexneri* strains present in raw vegetables from Bangladesh and assess the potential risk of resistance gene dissemination, and identify essential hub genes within the resistance network that could serve as prioritized targets for therapeutic intervention. The flow diagram of this study is presented in **Figure 1**.

RESEARCH METHODS

Retrieval of Genomic Data of *S. flexneri*

To investigate the genome of *S. flexneri* strains contaminating raw vegetables in Bangladesh, sequences were retrieved from the NCBI Genome database (https://www.ncbi.nlm.nih.gov/datasets/genome/) (accessed September 13, 2025) (Pruitt et al., 2005). The NCBI genome database was selected as the primary data repository due to verified *S. flexneri* isolates from Bangladeshi raw vegetable sources and complete genome sequences with high-quality assembly metrics.

Screening of core genes from the genome

To identify core genes from these *S. flexneri* strains, we performed genomic annotation and pan-genome analysis. The genome sequences were annotated using Prokka (https://github.com/tseemann/prokka.git), a rapid prokaryotic genome annotation pipeline that identifies protein-coding sequences, tRNAs, rRNAs, and functional elements through integrated protein databases and sequence search tools, including UniProtKB, BLAST+, and HMMER3 (Seemann, 2014). The annotation was performed using default parameters with --kingdom Bacteria and --genus *Shigella* options. Pan-genome analysis was conducted using Roary (https://github.com/sanger-pathogens/Roary.git), selected for its computational efficiency in analyzing multiple bacterial genomes (Page et al., 2015). Roary was run with default parameters using a 95% sequence identity threshold (≥ 95) of homologous gene clustering for core gene definition.

Identification of Core Antimicrobial Resistance Genes

To identify the antimicrobial resistance (AMR) genetic determinants conferring multidrug resistance in S. flexneri strains and establish their conservation patterns, the previously screened core proteins that are

common to all S. flexneri strains from raw vegetables were interrogated against the Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca/) (Alcock et al., 2023). CARD was selected as the reference database for AMR gene identification due to its curated collection of experimentally validated resistance determinants and comprehensive coverage of known resistance genes. It also organized the associated molecular mechanisms and phenotypes through the Antibiotic Resistance Ontology framework, and regular updates that enable detection of both established and emerging resistance genes through robust sequence homology-based algorithms. AMR gene prediction was performed using the Resistance Gene Identifier (RGI) tool integrated within CARD, applying stringent filtering criteria of perfect and strict hit classifications with sequence identity ≥95% and high coverage parameters to ensure high-confidence gene annotations while minimizing false-positive predictions. Perfect hits represent exact matches to reference resistance sequences in CARD's curated dataset, whereas strict hits indicate close variants of known resistance genes based on detection models derived from homolog sequences, thereby balancing sensitivity and specificity in resistance gene identification.

Protein-Protein Interaction Network Construction

To elucidate the functional interconnections among AMR-associated proteins and identify critical nodes that may serve as potential therapeutic targets, protein-protein interaction (PPI) network analysis was conducted using the STRING database version 12.0 (https://string-db.org/) (Szklarczyk et al., 2023). STRING is a comprehensive resource that integrates experimentally validated interactions, curated pathway knowledge, and computationally predicted associations from co-expression, gene fusion, and phylogenetic co-occurrence data across numerous organisms. The core AMR genes were submitted to the STRING database with a minimum required interaction confidence score of 0.400 (medium confidence) to construct a balanced PPI network.

Hub Gene Identification

The balanced PPI network was subsequently exported and visualized using Cytoscape version 3.9.1, an open-source bioinformatics platform widely employed for network visualization, integration, and analysis of complex molecular interaction networks (Cline et al., 2007). Hub genes, defined as highly connected nodes that occupy central positions within the network topology and potentially play crucial regulatory or functional roles in maintaining multidrug resistance, were identified using the CytoHubba plugin integrated within Cytoscape. It employs multiple topological algorithms, including Degree, Closeness, and Betweenness, to rank nodes based on their network connectivity and centrality measures (Saito et al., 2012).

Gene Ontology and Pathway Enrichment Analysis

To systematically characterize the functional roles and biological pathways associated with the identified AMR genes and hub proteins, Gene Ontology (Biological Process) and KEGG pathway enrichment analyses were performed using the integrated analytical framework within the STRING database (https://string-db.org/) (Szklarczyk et al., 2023). STRING was employed for enrichment analysis due to its seamless integration with the PPI network data, its statistical framework that accounts for network topology in enrichment calculations, and its curated mappings to multiple functional classification systems, including GO terms (Biological Process). Subsequently, KEGG pathways were examined to represent molecular interaction and reaction networks (Kanehisa et al., 2025). Statistical significance of enrichment was assessed using hypergeometric tests with false discovery rate (FDR) correction to control for multiple hypothesis testing, with a significance threshold of FDR < 0.05.

Identification and Prioritization of Essential Genes as Therapeutic Targets

To identify clinically relevant therapeutic targets among the AMR genes that are indispensable for bacterial survival and virulence, essentiality analysis was conducted by cross-referencing the identified resistance genes and hub proteins against the Database of Essential Genes (DEG) (http://origin.tubic.org/deg), a manually

curated repository that catalogs experimentally validated essential genes from systematic gene knockout studies, transposon mutagenesis screens, and RNA interference experiments across diverse bacterial species under various growth conditions (Luo et al., 2021). This essentiality-guided target identification approach can reveal AMR-associated proteins whose inhibition would simultaneously compromise both antimicrobial resistance mechanisms and fundamental cellular processes critical for bacterial viability. Therefore, this approach could help reduce the likelihood of compensatory resistance evolution, facilitate the discovery of narrow-spectrum therapeutic interventions specific to *S. flexneri* while minimizing disruption to commensal microbiota, and provide a rational foundation for structure-based drug design efforts targeting essential resistance determinants.

RESULTS AND DISCUSSION

Retrieval and Genomic Information of MDR *S. flexneri* strains

The analysis of publicly available genomic data from foodborne pathogens is necessary to facilitate epidemiological surveillance and to identify potential therapeutic targets without the constraints of primary sample collection, particularly in regions where antimicrobial resistance poses a growing public health threat. In this study, four complete genome sequences of *S. flexneri* strains isolated from raw vegetables cultivated in rooftop gardens across Bangladesh were retrieved from the NCBI GenBank database (**Table 1**). Two strains were originally isolated from tomato samples (Tomato_1: GCA_032359965.1 from Gazipur; Tomato_2: GCA_032359825.1 from Dhaka), while the remaining two strains were recovered from green chilies (Green Chilies_1: GCA_032359905.1 from Gazipur; Green Chilies_2: GCA_032360065.1 from Dhaka), with all sequences submitted to GenBank on 04-10-2023. Genome size analysis revealed a narrow range from 4619435 bp to 4624521 bp, with the largest genome observed in the Green Chilies_1 strain (4624521 bp) and the smallest in the Green Chilies_2 strain (4619435 bp). The tomato-derived strains exhibited nearly identical genome sizes of 4623426 bp and 4623419 bp, differing by only 7 bp, which suggests high genomic conservation among strains from the same vegetable source and geographic proximity. These findings align with previous studies documenting the presence of MDR *S. flexneri* strains in Bangladesh (Azmi et al., 2014; Kar et al., 2025).

Table 1. *Shigella flexneri* strains from raw vegetables from Bangladesh

GenBank Accession	Sample	Source	Geographic Location	Genome Size (bp)	Submission Date to GenBank
GCA_032359965.1	Tomato_1	Rooftop of Garden	Gazipur	4623426	04-10-2023
GCA_032359825.1	Tomato_2	Rooftop of Garden	Dhaka	4623419	04-10-2023
GCA_032359905.1	Green Chilies_1	Rooftop of Garden	Gazipur	4624521	04-10-2023
GCA_032360065.1	Green Chilies_2	Rooftop of Garden	Dhaka	4619435	04-10-2023

Common genes and Core Antibiotic Resistance Determinants

The identification of core genomic features and antibiotic resistance genes across multiple strains is necessary to understand the conserved genetic architecture and resistance mechanisms that facilitate the persistence and spread of *S. flexneri* in contaminated food sources. Comparative genomic analysis of the four *S. flexneri* strains revealed a core genome comprising 4273 common genes shared across all isolates, which indicates substantial genetic homogeneity among strains from different vegetable sources and geographic locations. Subsequently, comprehensive screening for antibiotic resistance determinants identified 44 antibiotic resistance genes distributed across the genomes, encompassing multiple efflux pump systems, transcriptional regulators, and

modification enzymes. The resistance gene repertoire included critical multidrug efflux pump components such as *acrAB-tolC*, *emrAB*, *emrE*, *mdtABC*, *mdtEF*, *mdtGH*, *mdtMNOP*, *mdfA*, and *msbA*, as well as their associated regulatory elements, including *marA*, *emrR*, *evgAS*, *soxRS*, *leuO*, *CRP*, and *gadX*. Additional resistance genes identified were *acrD*, *acrEF*, *acrS*, *tolC*, *bacA*, *cpxA*, *baeR*, *kdpE*, *eptA*, *pmrF*, *H-NS*, *yojI*, and *EC-18*, which collectively confer resistance to a broad spectrum of antimicrobial agents through diverse mechanisms, including active drug extrusion, membrane modification, and stress response modulation. The presence of multiple overlapping efflux systems and their cognate regulators suggests that these strains possess highly redundant resistance mechanisms, which may contribute to their survival under selective antibiotic pressure in agricultural environments.

Our findings align with previous studies such as the efflux-associated genes *acrAB-tolC*, *mdfA*, and *marA,* which confer multidrug resistance in *Shigella* through reduced intracellular antimicrobial accumulation (Ayele et al., 2025; Ranjbar & Farahani, 2019). Thus, these prominent genes, *acrAB-tolC*, *mdfA*, and *marA,* validated our computational analysis and findings.

Therefore, the identification of MDR *S. flexneri* harboring 44 antibiotic resistance genes in raw vegetables from urban rooftop gardens in Gazipur and Dhaka highlights a substantial public health risk associated with the consumption of raw vegetable produce in Bangladesh. Hence, the consumption of uncooked vegetables could be a potential route for widespread MDR pathogen dissemination due to contaminated irrigation water, inadequate post-harvest handling, and insufficient sanitation infrastructure in densely populated urban centers is a major concern that necessary to be mitigated through rising public awareness in Bangladesh.

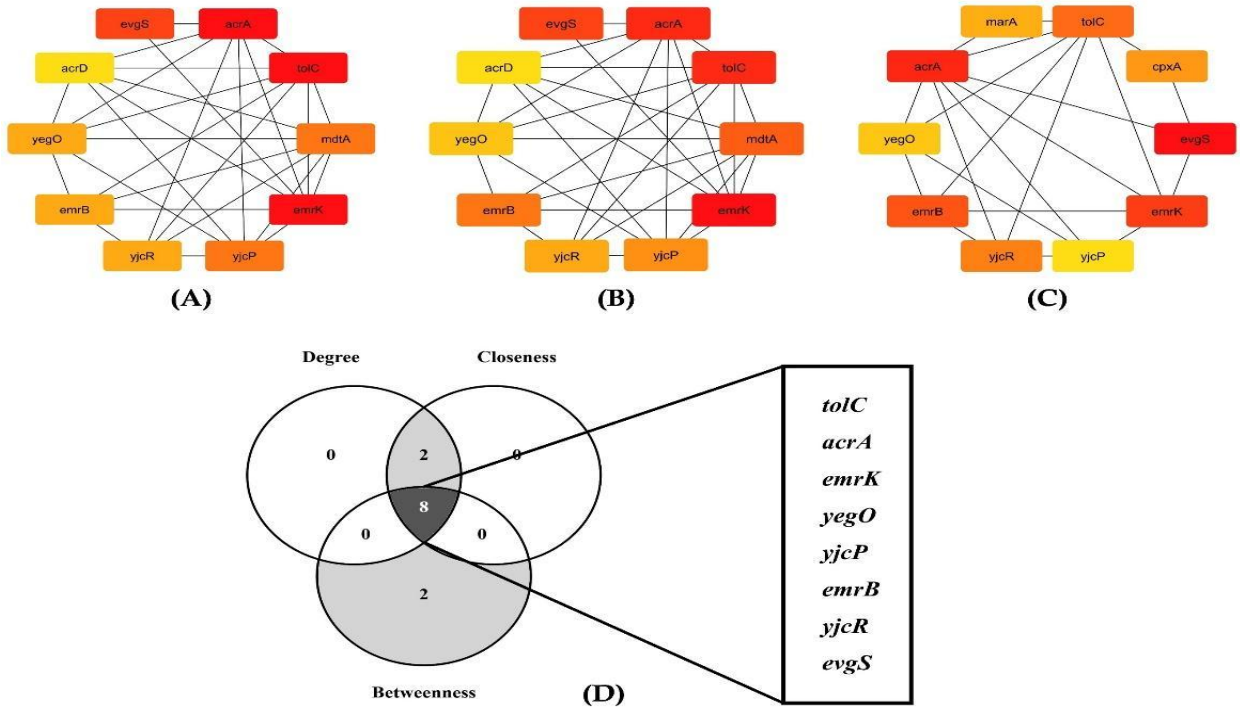

Figure 2. Hub gene networks. (A), (B), (C) Hub genes based on Degree, closeness, and betweenness centrality metrics, respectively. (D) Common hub genes from the three distinct centrality metrics

Protein-Protein Interaction Network and Hub Genes

The construction of PPI networks and the identification of hub genes are necessary to prioritize key nodes within the antibiotic resistance machinery that may serve as potential therapeutic targets for combating MDR *S. flexneri*. An interaction network was constructed using the STRING database, which incorporated 30 antibiotic resistance genes from the *S. flexneri* genomes, revealing complex interconnections

among efflux pump components, regulatory proteins, and stress response elements. Network topology analysis was performed using three distinct centrality metrics: degree, closeness, and betweenness, to identify hub genes that occupy critical positions within the resistance network architecture. The degree centrality analysis identified genes with the highest number of direct interactions, closeness centrality revealed genes with the shortest paths to all other nodes, and betweenness centrality highlighted genes that serve as critical bridges in network communication (**Figure 2A-C**). The intersection of these three complementary centrality measures identified 8 common hub genes: *tolC*, *acrA*, *emrK*, *yegO*, *yjcP*, *emrB*, *yjcR*, and *evgS* (**Figure 2D**), which represent the most interconnected and functionally critical components of the resistance network. Notably, *tolC* and *acrA*, which encode essential components of the *acrAB-tolC* multidrug efflux system, emerged as central nodes across all three metrics, suggesting their indispensable roles in coordinating multiple resistance pathways. The hub gene *acrAB-tolC* is also conserved in several *Shigella* species reported previously (Ranjbar & Farahani, 2019). The identification of these hub genes indicates that they function as critical control points in the resistance network and may represent high-priority targets for therapeutic intervention strategies aimed at disrupting the MDR phenotype in *S. flexneri*.

Functional Enrichment and Pathway Analysis

The functional characterization of antibiotic resistance genes through pathway enrichment analysis is necessary to elucidate the biological processes and molecular mechanisms underlying multidrug resistance in *S. flexneri* and to identify coordinated resistance strategies. Gene Ontology (GO) enrichment analysis of biological processes revealed two major functional categories: "Response to stimulus" and "Response to antibiotic" (**Figure 3A**). The "Response to stimulus" category was significantly enriched with 8 genes (*yjcF*, *yjcQ*, *yjcR*, *bacA*, *marA*, *evgA*, *evgS*, and *soxR*) at a false discovery rate (FDR) of 0.0055, while the "Response to antibiotic" category encompassed 5 genes (*yjcF*, *yjcQ*, *yjcR*, *bacA*, and *marA*) with an FDR value of 0.00053, indicating robust statistical significance. Furthermore, KEGG pathway enrichment analysis identified three critical resistance-associated pathways (**Figure 3B**): the "Two-component system" pathway was the most prominent with 11 genes (*acrD*, *tolC*, *baeR*, *cpxA*, *crp*, *emrY*, *evgA*, *evgS*, *mdtA*, and *yegO*) at FDR values of 1.81e-07, followed by "Cationic antimicrobial peptide (CAMP) resistance" with 5 genes (*acrA*, *acrB*, *tolC*, *marA*, and *cpxA*) at FDR of 0.00031, and "beta-Lactam resistance" with 3 genes (*acrA*, *acrB*, and *tolC*) at FDR of 0.0151. Notably, the hub genes *tolC*, *acrA*, *evgS*, and *yegO* were represented across multiple enriched pathways, confirming their central roles in coordinating diverse resistance mechanisms. The identified pathway-specific genes: *marA*, *tolC*, *acrA*, *acrB*, and *emrA* are also aligned with previous studies (Ayele et al., 2025; Jin et al., 2002; Ranjbar & Farahani, 2019). These findings demonstrate that the multidrug resistance phenotype in *S. flexneri* is mediated through interconnected pathways involving two-component regulatory systems, efflux-mediated drug extrusion, and adaptive responses to antimicrobial stress, which collectively enable bacterial survival under antibiotic pressure.

Identification of Essential Genes as Potential Therapeutic Targets

The identification of essential genes within the antibiotic resistance network is necessary to prioritize therapeutic targets whose disruption would compromise bacterial viability and overcome multidrug resistance in *S. flexneri*. Cross-referencing the hub genes with the Database of Essential Genes (DEG) revealed that 5 of the 8 hub genes were classified as essential for bacterial survival (**Table 2**).

These essential genes included *tolC* (DEG10260061), which encodes the outer membrane channel protein critical for multiple efflux systems; *acrA* (DEG10260004), encoding the acridine efflux pump periplasmic adaptor; *emrK* (DEG10560870), a multidrug efflux MFS transporter periplasmic adaptor subunit; *emrB* (DEG10050322), the multidrug resistance protein B component; and *evgS* (DEG10560871), an acid-sensing system histidine kinase involved in two-component regulatory networks. The resistance mechanisms associated with these

essential genes encompassed both structural components of efflux machinery (*tolC*, *acrA*, *emrK*, and *emrB*) and a regulatory element (*evgS*) that coordinates adaptive responses to environmental stress and antimicrobial agents. The dual criteria of high network centrality and essentiality for bacterial survival indicate that these genes represent high-priority targets for therapeutic intervention, as their inhibition would simultaneously disrupt multiple resistance pathways while compromising fundamental cellular processes required for *S. flexneri* viability. Among these genes, *acrA*, *emrK*, and *tolC were* identified as essential genes in previous studies (Freed et al., 2016; Pasqua et al., 2019). Thus, these previous observations confirm the validity of our computational findings.

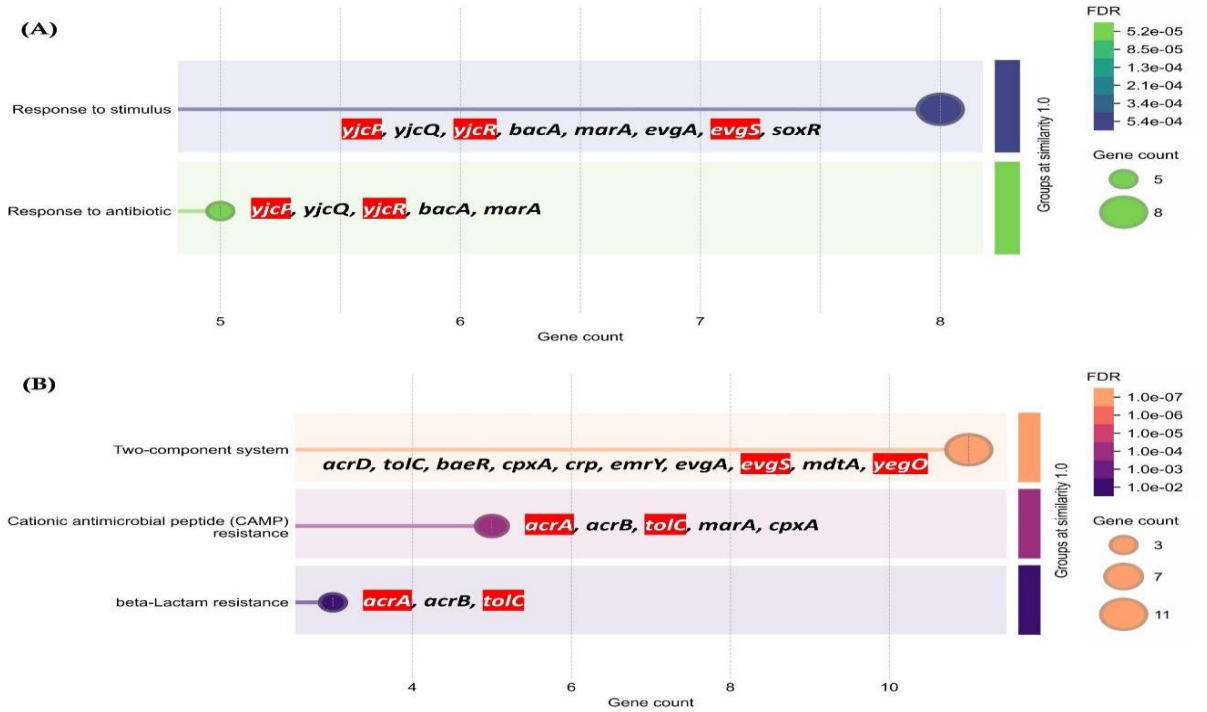

Figure 3. Pathway analysis of core antimicrobial resistant genes. (A) Biological process. (B) KEGG pathways. Red indicates core genes.

Table 2. Identification of essential genes that are responsible for bacterial survival from the core genes

Essential Gene ID	Genes	Resistance Mechanism
DEG10260061	*tolC*	Outer membrane channel protein
DEG10260004	*acrA*	Acridine efflux pump
DEG10560870	*emrK*	Multidrug efflux MFS transporter periplasmic adaptor subunit
DEG10050322	*emrB*	Multidrug resistance protein B
DEG10560871	*evgS*	Acid-sensing system histidine kinase

CONCLUSIONS

This study provides the first systematic integration of comparative genomics, network topology analysis, and essentiality screening to identify therapeutic targets in foodborne MDR *S. flexneri* from South Asian agriculture. Through comprehensive genomic characterization of MDR *S. flexneri* strains isolated from raw vegetables in Bangladesh, we identified five essential hub genes (tolC, acrA, emrK, emrB, and evgS) that occupy critical network positions coordinating efflux-mediated resistance and two-component regulatory systems. Disruption of these targets would simultaneously compromise multiple resistance pathways and bacterial viability, offering a strategic vulnerability for therapeutic exploitation. These

findings have immediate translational implications, such as the identified essential hub genes can guide rational design of novel antimicrobials, efflux pump inhibitors, or combination therapies targeting conserved vulnerabilities in MDR strains, potentially restoring efficacy of existing antibiotics while preventing further resistance evolution. However, validation through larger-scale studies across diverse geographic regions of South Asia, gene knockout experiments, PPI assays, and *in vitro* antimicrobial susceptibility testing remains essential. Future investigations should also examine whether similar essential hub genes represent universal targets across *Shigella* species and related Enterobacteriaceae, integrating genomic surveillance with phenotypic characterization, structural biology, and clinical trial frameworks to translate computational predictions into effective interventions. Therefore, these findings highlight the urgent need for expanded microbiological surveillance of raw vegetables and food matrices across Bangladesh, implementation of stringent food safety protocols throughout the agricultural supply chain, and targeted public health education campaigns to mitigate risks associated with consuming raw vegetables.

ACKNOWLEDGMENT

None

AUTHORS' CONTRIBUTIONS

K.M. Tanjida Islam: Conceptualization, Methodology, Data curation, Formal analysis, Validation, Writing- original draft. **Shahin Mahmud:** Supervision, Conceptualization, Methodology, Validation, Investigation, Writing-Review and editing.

CONFLICT OF INTERESTS

The author declared no conflicts of interest.

COPYRIGHT AND ETHICAL CONSIDERATION

Not applicable.

BIBLIOGRAPHY

Ahmed, S., Chowdhury, M. I. H., Sultana, S., Alam, S. S., Marzan, M., & Islam, M. A. (2023). Prevalence of Antibiotic-Resistant Shigella spp. in Bangladesh: A Systematic Review and Meta-Analysis of 44,519 Samples. *Antibiotics*, 12(5), 817. https://doi.org/10.3390/ANTIBIOTICS12050817/S1

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., Edalatmand, A., Petkau, A., Syed, S. A., & Tsang, K. K. (2023). CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Research*, 51(D1), D690–D699.

Ananna, N. F., Akter, A., Amin, Md. Al, Islam, K. M. T., & Mahmud, S. (2024). Ligand-based pharmacophore modeling targeting the fluoroquinolone antibiotics to identify potential antimicrobial compounds. *Computational and Structural Biotechnology Reports*, 1, 100021. https://doi.org/10.1016/J.CSBR.2024.100021

Ayele, B., Beyene, G., Mekonnen, Z., Esmael, A., Ayele, A., Alemayehu, D. H., & Beyene, G. T. (2025). Whole genome sequencing analysis of antibiotic resistant genes of Shigella species: A systematic review and meta-analysis. *PLOS ONE*, 20(10), e0334701. https://doi.org/10.1371/JOURNAL.PONE.0334701

Azmi, I. J., Khajanchi, B. K., Akter, F., Hasan, T. N., Shahnaij, M., Akter, M., Banik, A., Sultana, H., Hossain, M. A., Ahmed, M. K., Faruque, S. M., & Talukder, K. A. (2014). Fluoroquinolone Resistance Mechanisms of Shigella flexneri Isolated in Bangladesh. *PLOS ONE*, 9(7), e102533. https://doi.org/10.1371/JOURNAL.PONE.0102533

Berger, I., & Loewy, Z. G. (2024). Antimicrobial Resistance and Novel Alternative Approaches to Conventional Antibiotics. *Bacteria*, Vol. 3, Pages 171-182, 3(3), 171–182. https://doi.org/10.3390/BACTERIA3030012

Cline, M. S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., Christmas, R., Avila-Campilo, I., Creech, M., & Gross, B. (2007). Integration of biological networks and gene expression data using Cytoscape. *Nature Protocols*, 2(10), 2366–2382.

Freed, N. E., Bumann, D., & Silander, O. K. (2016). Combining Shigella Tn-seq data with gold-standard E. coli gene deletion data suggests rare transitions between essential and non-essential gene functionality. *BMC Microbiology*, 2016 16:1, 16(1), 203-. https://doi.org/10.1186/S12866-016-0818-0

Gharpure, R., Marsh, Z. A., Tack, D. M., Collier, S. A., Strysko, J., Ray, L., Payne, D. C., & Garcia-Williams, A. G. (2021). Disparities in Incidence and Severity of Shigella Infections Among Children—Foodborne Diseases Active Surveillance Network (FoodNet), 2009-2018. *Journal of the Pediatric Infectious Diseases Society*, 10(7), 782–788. https://doi.org/10.1093/JPIDS/PIAB045

Hossain, M. A., Al Amin, M., Khan, M. A., Refat, M. R. R., Sohel, M., Rahman, M. H., Islam, A., & Hoque, M. N. (2024). Genome-Wide Investigation Reveals Potential Therapeutic Targets in Shigella spp. *BioMed Research International*, 2024(1), 5554208. https://doi.org/10.1155/2024/5554208

Islam, Md. S., Pramanik, P. K., Rana, Md. L., Ullah, Md. A., Neloy, F. H., Ramasamy, S., Schreinemachers, P., Oliva, R., & Rahman, Md. T. (2024). Draft genome sequence of antibiotic-resistant Shigella flexneri MTR_GR_V146 strain isolated from a tomato (Solanum lycopersicum) sample collected from a peri-urban area of Bangladesh. *Microbiology Resource Announcements*, 13(4). https://doi.org/10.1128/MRA.00099-24

Jin, Q., Yuan, Z., Xu, J., Wang, Y., Shen, Y., Lu, W., Wang, J., Liu, H., Yang, J., Yang, F., Zhang, X., Zhang, J., Yang, G., Wu, H., Qu, D., Dong, J., Sun, L., Xue, Y., Zhao, A., … Yu, J. (2002). Genome sequence of Shigella flexneri 2a: insights into pathogenicity through comparison with genomes of Escherichia coli K12 and O157. *Nucleic Acids Research*, 30(20), 4432–4441. https://doi.org/10.1093/NAR/GKF566

Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y., & Ishiguro-Watanabe, M. (2025). KEGG: biological systems database as a model of the real world. *Nucleic Acids Research*, 53(D1), D672–D677. https://doi.org/10.1093/NAR/GKAE909

Kar, A., Ahmed, P., Sakib, N., Shibly, A. Z., Zohora, F. T., Dutta, A., Somadder, P. D., & Rahman, M. M. (2025). Prevalence, Antimicrobial Resistance Pattern of Shigella Spp. and Assessed Their Pathogenic Potential Using the Caenorhabditis elegans Infection Model Associated with Acute Diarrheal Patients in Tangail, Bangladesh. *Foodborne Pathogens and Disease*.

Kim, J., & Ahn, J. (2022). Emergence and spread of antibiotic-resistant foodborne pathogens from farm to table. *Food Science and Biotechnology*, 31(12), 1481–1499. https://doi.org/10.1007/S10068-022-01157-1/METRICS

Lu, T., Das, S., Howlader, D. R., Picking, W. D., & Picking, W. L. (2024). Shigella Vaccines: The Continuing Unmet Challenge. *International Journal of Molecular Sciences 2024*, Vol. 25, Page 4329, 25(8), 4329. https://doi.org/10.3390/IJMS25084329

Luo, H., Lin, Y., Liu, T., Lai, F.-L., Zhang, C.-T., Gao, F., & Zhang, R. (2021). DEG 15, an update of the Database of Essential Genes that includes built-in analysis tools. *Nucleic Acids Research*, 49(D1), D677–D686.

Nisa, I., Qasim, M., Yasin, N., Ullah, R., & Ali, A. (2020). Shigella flexneri: an emerging pathogen. Folia Microbiologica, 65(2), 275–291. https://doi.org/10.1007/S12223-020-00773-W/METRICS

Page, A. J., Cummins, C. A., Hunt, M., Wong, V. K., Reuter, S., Holden, M. T. G., Fookes, M., Falush, D., Keane, J. A., & Parkhill, J. (2015). Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics*, 31(22), 3691–3693. https://doi.org/10.1093/BIOINFORMATICS/BTV421

Pasqua, M., Grossi, M., Scinicariello, S., Aussel, L., Barras, F., Colonna, B., & Prosseda, G. (2019). The MFS efflux pump EmrKY contributes to the survival of Shigella within macrophages. *Scientific Reports 2019* 9:1, 9(1), 2906-. https://doi.org/10.1038/s41598-019-39749-3

Pruitt, K. D., Tatusova, T., & Maglott, D. R. (2005). NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Research*, 33(suppl_1), D501–D504. https://doi.org/10.1093/NAR/GKI025

Ranjbar, R., & Farahani, A. (2019). Shigella: Antibiotic-resistance mechanisms and new horizons for treatment. *Infection and Drug Resistance*, 12, 3137–3167. https://doi.org/10.2147/IDR.S219755;PAGE:STRING:ARTICLE/CHAPTER

Saito, R., Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P.-L., Lotia, S., Pico, A. R., Bader, G. D., & Ideker, T. (2012). A travel guide to Cytoscape plugins. *Nature Methods*, 9(11), 1069–1076.

Sassi, A., Basher, N. S., Kirat, H., Meradji, S., Ibrahim, N. A., Idres, T., & Touati, A. (2025). The Role of the Environment (Water, Air, Soil) in the Emergence and Dissemination of Antimicrobial Resistance: A One Health Perspective. *Antibiotics 2025*, Vol. 14, Page 764, 14(8), 764. https://doi.org/10.3390/ANTIBIOTICS14080764

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, 30(14), 2068–2069. https://doi.org/10.1093/BIOINFORMATICS/BTU153

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., & Pyysalo, S. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, 51(D1), D638–D646.

Xiao, R., Huang, D., Du, L., Song, B., Yin, L., Chen, Y., Gao, L., Li, R., Huang, H., & Zeng, G. (2023). Antibiotic resistance in soil-plant systems: A review of the source, dissemination, influence factors, and potential exposure risks. *Science of The Total Environment*, 869, 161855. https://doi.org/10.1016/J.SCITOTENV.2023.161855