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## Original Article

# Decoding the Complex Genetic Network of Antimicrobial Resistance in *Campylobacter jejuni* Using Advanced Gene Network Analysis

Prasanna Kumar Selvam[1], Santhosh Mudipalli Elavarasu[1], Hrituraj Dey[1], Karthick Vasudevan[1*] and George Priya Doss[2]

[1]*Department of Biotechnology, School of Applied Sciences, REVA University, Bengaluru, Karnataka, India;* [2]*Department of Integrative Biology, VIT University, Vellore, Tamil Nadu, India*

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## Abstract

**Background and objectives:** Antimicrobial resistance (AMR) poses a significant threat to public health in the 21st century, with bacteria such as *Campylobacter jejuni* (*C. jejuni)* exhibiting multidrug resistance due to the presence of AMR genes. Understanding the evolutionary patterns and functional relationships of these genes is crucial for addressing this issue effectively.

**Methods:** We conducted phylogenetic analysis to examine the evolution of AMR genes in *C. jejuni*. Additionally, we constructed and analyzed a gene interaction network comprising 39 functional relationships. Clustering analysis was employed to identify interconnected clusters associated with AMR processes. Functional enrichment analysis was performed to explore the involvement of cellular components, molecular functions, and biological processes.

**Results:** Our analysis revealed two interconnected clusters (C1 and C2) closely associated with AMR processes. Furthermore, genes encoding ribosomal proteins (*rplE*, *rplV*, *rplG*, *rplK*, *rplA*, *rplJ*, *rpsE*, *rplB*, *rpsL*, and *rpmA*) were identified as hub genes within the gene interaction network. These genes interact frequently with their functional counterparts, indicating their significance in AMR mechanisms. Enriched Kyoto Encyclopedia of Genes and Genomes pathway analysis highlighted the importance of the ribosome pathway in understanding antibiotic resistance mechanisms in *C. jejuni*.

**Conclusions:** The findings of this study enhance our understanding of the molecular mechanisms underlying AMR in *C. jejuni*. By elucidating the evolutionary patterns, gene interactions, and pathway enrichment, our study provides valuable insights that may contribute to the development of novel treatments for illnesses caused by this pathogen.

**Keywords:** Antimicrobial resistance; Gene interaction network; *Campylobacter jejuni*; AMR; *rpl*; Cytoscape; ClueGo.

**Abbreviations:** AMR, antimicrobial resistance; BPs, biological processes; *C. jejuni*, *Campylobacter jejuni*; CCs, cellular components; FA, Fusidic acid; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCODE, Molecular Complex Detection; MFs, molecular functions; STRING, Search Tool for the Retrieval of Interaction Genes/Proteins.

[*]**Correspondence to:** Karthick Vasudevan, Department of Biotechnology, School of Applied Sciences, REVA University, Bengaluru 560064, Karnataka, India. ORCID: https://orcid.org/0000-0001-7471-2527. Tel: +91-95971-56169, E-mail: karthick.vasudevan@reva.edu.in

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## Introduction

One of the greatest threats to public health in the 21st century is antimicrobial resistance (AMR) in bacteria. AMR occurs when bacteria undergo genetic changes that reduce the effectiveness of antibiotics used to treat infections. According to the UK Government-commissioned Study on Antimicrobial Resistance, AMR might result in the yearly death of 10 million people by 2050.[1] The World Health Organization, as well as numerous other organizations and researchers, concur that the development of AMR is a pressing issue that must be addressed through a global, coordinated action plan.[2,3]

Understanding the full cost of resistance is a significant obstacle in the fight against AMR, especially in areas with scant surveillance and limited available information.[1] Many studies have re-

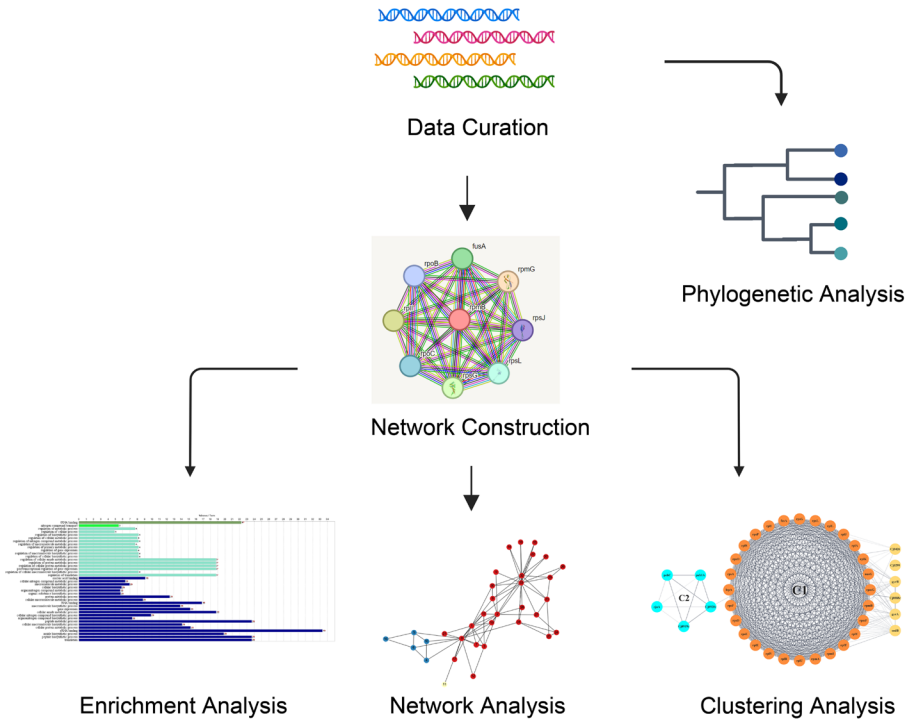


**Fig. 1. Visual representation of the methodology involved in the study.**

ported the impact of AMR for particular pathogen-drug combinations, particularly on incidence, fatalities, hospital length of stay, and healthcare costs.[4–7] The occurrence of AMR and the proliferation of antibiotic-resistant microorganisms encompass a range of significant human diseases. The spread of AMR from hospital environments, which are generally closed communities, is seen as a threat to public health.

*Campylobacter* species are gram-negative, spiral-shaped, and nonspore-forming bacteria that thrive best in microaerophilic environments. The first *Campylobacter* may have been discovered as early as 1913; however, it was not until 1963 that the genus *Campylobacter* was formally recognized, having been categorized as Vibrio spp. Currently, the family *Campylobacteriaceae* includes the genera *Campylobacter* and Arcobacter.[8] *Campylobacter* comprises 14 Campylobacter species, and *Campylobacter jejuni (C. jejuni)* is frequently linked to human gastroenteritis.

Most human infections caused by *Campylobacter* usually involve *C. jejuni*, one of several species within the *Campylobacter spp*.[9] In underdeveloped nations, *Campylobacter* infections are most commonly recorded in young people. Both children under one year of age and those under five in Southeast Asia have shown peak infection rates.[10] Between 2.9% and 15% of children in Southeast Asia were previously found to have *Campylobacter* spp.[11] According to the World Health Organization, AMR in *Campylobacter spp*. is a growing global concern and poses a significant public health threat.[12] The Centers for Disease Control and Prevention estimates that each year in the United States, *Campylobacter* causes 1.3 million cases of human illness.[13]

Understanding the molecular factors underlying the AMR pattern is crucial to address this challenge and propose effective solutions. To accomplish this, we developed a gene interaction network to identify highly interacting genes.[14] Our study highlights the critical importance of identifying hub genes within these networks to elucidate the mechanisms of AMR in *C. jejuni*. By focusing on these hub genes, researchers have gained valuable insights into the functional aspects of AMR, facilitating the identification of potential therapeutic targets. Furthermore, our approach highlights the significance of assessing biological pathways at the gene level, providing a dynamic framework for understanding AMR mechanisms comprehensively.[15,16] This study not only contributes to the scientific understanding of AMR but also provides essential insights necessary for the development of effective treatments for infections caused by *C. jejuni.*

## Materials and methods

### *NDARO and ABRicate*

NDARO (National Database of Antibiotic Resistance Organisms) is an online repository run by the National Center for Biotechnology Information that is used to store information on the AMR genes of disease-causing bacteria. It was developed to provide background information and host-specific data on bacterial AMR genes. Each of the AMR databases in ABRicate v. 0.8 (https://github.com/tseemann/abricate/), including NCBI AMRFinderPlus, CARD, and ResFinder, contains data on thousands of AMRs.[17] Figure 1 visually outlines the sequential steps and methodology employed in the study, offering a concise overview of the research process.

### *iTOL-a tool for phylogenetic tree development*

iTOL is a web-based tool used for displaying, manipulating, and annotating phylogenetic trees. It facilitates interactive rooting and pruning of trees. The tool also enables the mapping of various data types onto trees, including genome sizes and protein domain repertoires. Additionally, iTOL supports exporting images in a variety of bitmap and vector graphic formats.[18]

### *The STRING database for network analysis*

The STRING database (Search Tool for the Retrieval of Interaction Genes/Proteins) comprises several online platforms devoted to organism-wide protein interaction networks. The entire STRING database is precomputed, stored in a relational database, and independently accessible for download. Based on probabilistic confidence scores, the functional partners engaged in these interactions are described. STRING features a proprietary scoring method based on a reference set and several relationships.[19]

### *Cytoscape tool for network construction*

The open-source network analysis and visualization software Cytoscape were used to construct and analyze networks of social and biological interactions. Cytoscape includes essential tools for network analysis, data integration, and visualization.[20,21] By implementing Cytoscape plugins, additional functions can be introduced. Installing integrated database plugins provides access to data from other databases. In biological networks, each component is referred to as a node, and the connections between nodes are referred to as edges.[22]

### *MCODE tool for cluster analysis*

Molecular Complex Detection (MCODE) is a Cytoscape tool that locates cluster regions with many interconnections in a network. It is a clustering technique that is reasonably quick.[23,24] This approach is appropriate for academics who are focused on computation and biological research because of its simple interface. MCODE rates each cluster according to its size and density by giving it a score. Finally, the data are graphically represented as clusters.

### *NetworkAnalyzer*

NetworkAnalyzer is a flexible and intuitive tool for examining biological and other networks. This plugin seamlessly integrates with Cytoscape and utilizes effective graph algorithms to compute a comprehensive list of simple and sophisticated topology parameters.[25,26] It adds node properties for the outcomes and provides useful visualization settings to display and export the generated distributions.

### *ClueGO tool for gene ontology interpretation*

ClueGO offers preconfigured functional analysis settings that range from broad to highly detailed. Additionally, Gene Ontology (GO) is a standardized system for annotating genes and their products across different species. It provides a controlled vocabulary to describe gene and gene product attributes in any organism, consisting of three structured networks: Biological Process, Molecular Function, and Cellular Component. Users can adjust analysis parameters to focus on terms within a specific range of GO, enabling a more precise exploration of gene functions and biological processes. GO levels have a specific evidence code, or have a specific number and percentage of linked genes. ClueGO first generates a binary gene-term matrix consisting of the selected terms and their related genes. Using a term-term similarity matrix built on this matrix and chance-corrected kappa statistics, the association strength between the words is calculated. Similar to BiNGO, ClueGO may be used in conjunction with GOlorize for the functional analysis of a Cytoscape gene network.[27]

## Results

### *Data compilation from databases*

For this study, 25 AMR genes associated with *C. jejuni* were extracted from the ABRicate and NDARO databases. To ensure the exclusion of duplicate entries, a process of elimination was implemented. Consequently, a set of 25 distinct AMR genes was identified for analysis.

### *Phylogenetic tree construction*

The TREEFILE was aligned using MAFFT (Multiple Alignment using Fast Fourier Transform), a tool specifically designed for multiple sequence alignment. Following this alignment, iTOL, a program tailored for visualizing phylogenetic trees in a rooted format, was utilized to present the tree data (for details on network generation, refer to Appendix A in Supplementary file). Phylogenetic analysis was then carried out to examine the evolutionary connections among the *C. jejuni* strains.[28] Figure 2 displays the phylogenetic tree.

### *STRING analysis of gene interactions*

Seven specific genes (*bla, fusA, gyrA, lepA, cjaA, sodB, and peb1A*) associated with the 25 collected AMR genes were extracted from the STRING database. With a set minimum interaction score of 0.4 for medium confidence, our goal is to expand the network to include 39 nodes and 438 edges. This expansion aimed to maximize the representation of interactions among AMR genes, thereby enhancing our insight into their relationships. (For details on network generation, refer to Appendix B in supplementary file.)

### *Network analysis and hub gene identification*

NetworkAnalyzer, a tool in Cytoscape, was used to conduct the analysis. The results showed a total number of 39 nodes and 438 edges in the network. A strong association between genes was revealed by the data summary, which comprised the highest degree count, the clustering factor, and the shortest path length at the minimum (Table 1). Among the genes, *rplE, rplV, rpsG, rplK, rplA, rplJ, rpsE, rplB, rpsL, and rpmA* were identified with the greatest number of connections (Table 1).

### *Clustering analysis of gene networks*

The application of the MCODE clustering technique led to the identification of two distinct groups, In Figure 3, labelled as C1 and C2, there are densely interconnected regions. Out of the total 39 genes analyzed, 33 were organized into these clusters, while the remaining 6 genes did not form part of any specific cluster (refer to Appendix C in supplementary file for a detailed description of the network generation process).[29,30]

### *Functional enrichment analysis of gene clusters*

ClueGo was used to analyze overrepresented sequences and their functional components. The contributions of the numerous processes and pathways are clarified by this enrichment research. A moderate level of network specificity was used to evaluate the words and annotations from the Gene Ontology (GO) analysis. Moreover, pathway information was extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and UniProt databases using the STRING database, providing scientifically valuable pathway data for different genes. The analysis revealed that many pathways and activities, including biological processes (BPs), molecular functions (MFs), and cellular components (CCs), were associated with the AMR genes and their interactors. The network genes were shown to be enriched in 23 BPs, 21 CCs, and 16 MFs according to the enrichment analysis. The metabolic pathways involved in one KEGG pathway, 2 UniProt keywords along with gene expression (GO: 10467), cellular biosynthetic processes (GO: 44249), mac-

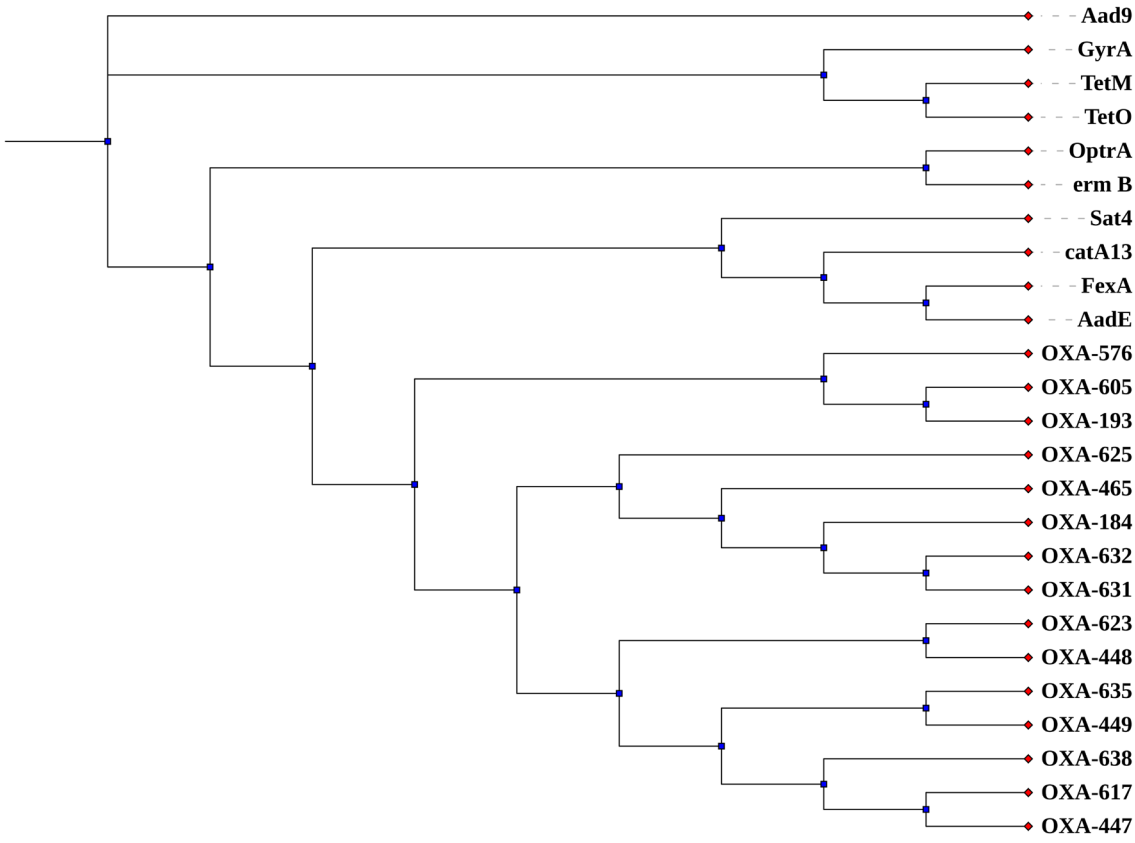


**Fig. 2. Schematic depiction of the rooted phylogenetic tree for the 25 genomic sequences for *C. jejuni* strains using the MAFFT (Multiple Alignment using Fast Fourier Transform).**

romolecule biosynthetic processes (GO: 9059), organic substance biosynthetic processes (GO:1901576), and translation were among the highly enriched BPs (GO:6412) (Table 2).[31] Figure 4 depicts the biosynthetic process facilitated by genes like *fusA*, *lepA*, *nusG*, *rplV*, *rplE*, among others.

## Discussion

Understanding the whole expense of resistance poses a significant challenge in the battle against AMR, particularly in regions with limited monitoring and information accessibility. To address this requirement and offer a workable remedy, one must comprehend the molecular mechanisms underlying AMR. Our analysis revealed two phylogenetic clusters for the *OXA* genes, with the second cluster further subdivided into two subclusters. Additionally, the phylogenetic clusters of *tetM and tetO* appeared to be closely related. Tetracyclines, a group of drugs that encompasses tigecycline, minocycline, doxycycline, and tetracycline, are utilized for the management and treatment of various bacterial infections. The evolution of tetracycline resistance in *Campylobacter*, a gram-negative bacterium, is supported by the similarities between *tetM* and *tetO*.[32] This finding reveals the evolution and family links of these gene variations. Genes within a network exhibiting the highest number of interactions are commonly known as hub genes. Due to their involvement in crucial BPs, these genes are considered essential.[33] Understanding these genes allows us to assess the molecular mechanisms and processes that underlie an organism's antibiotic resistance. In alignment with the results from the same study, the genes with the highest numbers of interactions included *rplE, rplV, rpsG, rplK, rplA, rplJ, rpsE, rplB, rpsL, and rpmA* (Table 1). The *rpl* gene is responsible for the assembly of the 60 s subunit of the eukaryotic ribosome, and plays a vital role in protein translation and cellular functions. Disruptions in ribosome assembly typically trigger a cellular stress response. Ribosomal proteins are essential for ribosome biogenesis and protein synthesis, and are crucial in diverse developmental processes. Furthermore, DNA replication, transcription, strand separation, repair, and DNA topoisomerase type II all require negative supercoiling, which is accomplished by the *gyrA* gene.[14,34]

Within the identified clusters, C1 emerged as the region with the highest connectivity, boasting 28 nodes and a notable score of 27.63. This suggests a robust interplay among the genes within C1, suggesting potential functional relationships. On the other hand, C2 comprised five nodes, each with a score of 5 (Table 3). This information provides insights into the structural organization of the network and highlights specific gene clusters that may play pivotal roles in antibiotic resistance.[35,36] BPs provide essential knowledge about cellular repair and interactions with cells, facilitating the interaction between molecular machinery and catalytic processes in MFs. CCs, such as cell structures and complexes, are essential for DNA storage and detoxification. Bacteria must rewire their cellular metabolic pathways to survive in the host and respond to antibiotic exposure, necessitating ATP synthesis.[37] Cellular metabolic processes are potential drug targets that are crucial for bacterial growth and survival.[38] Several genes, including *rpsL*, are respon-

**Table 1. List of the top 20 genes analyzed using NetworkAnalyzer considering various parameters, such as degree, average shortest path length, and betweenness centrality**

| Sl. | Gene | Degree | Average shortest path length | Betweenness centrality | Closeness centrality | Clustering coefficient |
|---|---|---|---|---|---|---|
| 1 | *rplE* | 32 | 1.243243243 | 0.021354984 | 0.804347826 | 0.796370968 |
| 2 | *rplV* | 31 | 1.27027027 | 0.012546176 | 0.787234043 | 0.838709677 |
| 3 | *rpsG* | 30 | 1.297297297 | 0.014254261 | 0.770833333 | 0.855172414 |
| 4 | *rplK* | 30 | 1.297297297 | 0.00695487 | 0.770833333 | 0.882758621 |
| 5 | *rplA* | 30 | 1.297297297 | 0.011056293 | 0.770833333 | 0.857471264 |
| 6 | *rplJ* | 30 | 1.189189189 | 0.24826994 | 0.840909091 | 0.816091954 |
| 7 | *rpsE* | 30 | 1.297297297 | 0.00695487 | 0.770833333 | 0.882758621 |
| 8 | *rplB* | 30 | 1.297297297 | 0.00695487 | 0.770833333 | 0.882758621 |
| 9 | *rpsL* | 29 | 1.324324324 | 0.005840363 | 0.755102041 | 0.903940887 |
| 10 | *rpmA* | 29 | 1.324324324 | 0.003670525 | 0.755102041 | 0.918719212 |
| 11 | *rpsA* | 29 | 1.324324324 | 0.013922199 | 0.755102041 | 0.849753695 |
| 12 | *rpmF* | 29 | 1.324324324 | 0.008913205 | 0.755102041 | 0.901477833 |
| 13 | *rpsF* | 29 | 1.324324324 | 0.008913205 | 0.755102041 | 0.901477833 |
| 14 | *rpsD* | 29 | 1.324324324 | 0.005015368 | 0.755102041 | 0.913793103 |
| 15 | *rpsP* | 28 | 1.351351351 | 0.001731023 | 0.74 | 0.955026455 |
| 16 | *rplS* | 28 | 1.351351351 | 0.001731023 | 0.74 | 0.955026455 |
| 17 | *rpsO* | 28 | 1.351351351 | 0.001731023 | 0.74 | 0.955026455 |
| 18 | *rpmB* | 27 | 1.378378378 | 0.001495463 | 0.725490196 | 0.96011396 |
| 19 | *rplI* | 27 | 1.378378378 | 0.000617 | 0.725490196 | 0.985754986 |
| 20 | *rplU* | 27 | 1.378378378 | 0.000617 | 0.725490196 | 0.985754986 |

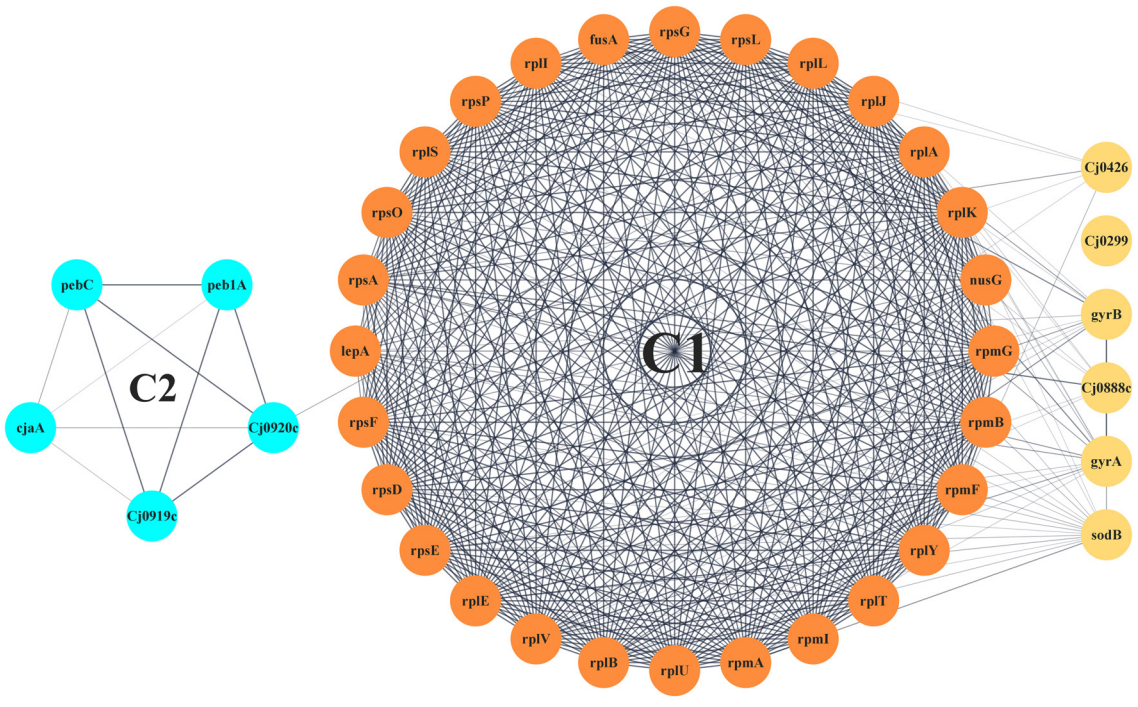


**Fig. 3. Clustering analysis of the gene interaction network using MCODE tool resulted in two clusters, C1 (orange) and C2 (blue) where C1 had the highest level of clustering.** Nodes highlighted in yellow represents the no zero degree of the clustering of the genes. MCODE, Molecular Complex Detection.

**Table 2. Gene Ontology terms significantly enriched in biological process's associated with genes**

| Sl | ID | Description | Genes |
|---|---|---|---|
| 1 | GO:10467 | gene expression | *fusA, lepA, nusG, rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 2 | GO:44249 | cellular biosynthetic process | *fusA, lepA, nusG, rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 3 | GO:9059 | macromolecule biosynthetic process | *fusA, lepA, nusG, rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 4 | GO:1901576 | organic substance biosynthetic process | *fusA, lepA, nusG, rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 5 | GO:6412 | translation | *fusA, lepA, rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |

GO, Gene Ontology.

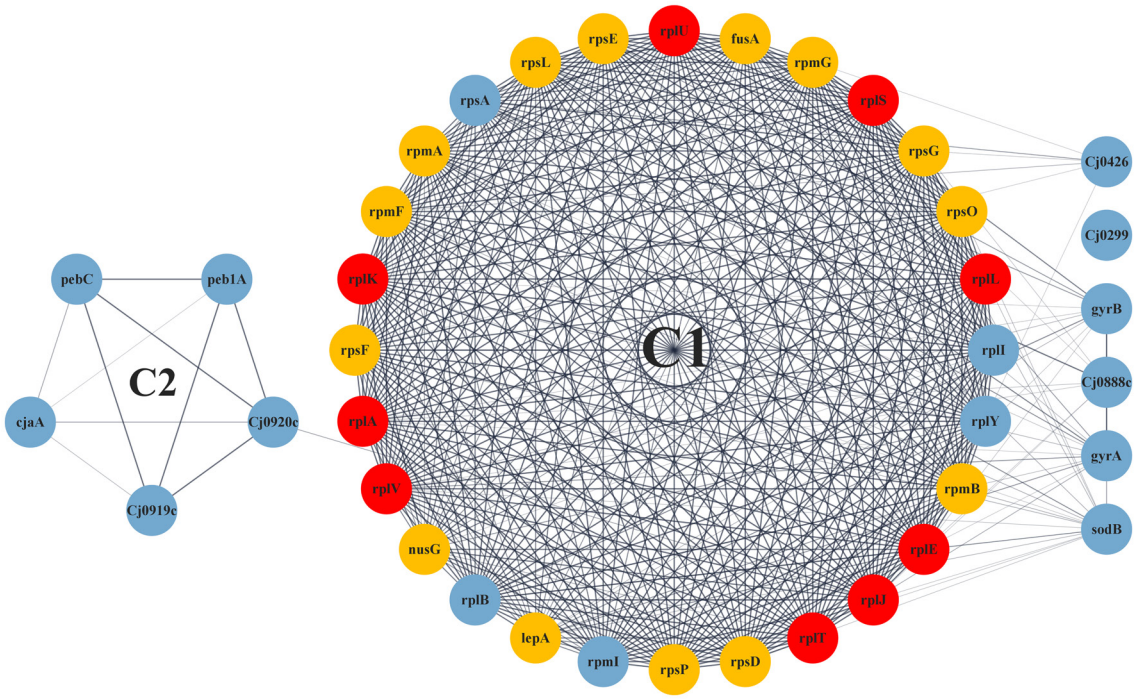


**Fig. 4. Enrichment of the AMR (antimicrobial resistance) genes using ClueGO involved in BPs.** The abundant genes in distinct BPs are highlighted in yellow, while the resistance-related *rpl* genes are highlighted in red. BPs, biological processes.

sible for resistance to aminoglycosides, likely due to efflux pump mechanisms or other unidentified resistance mechanisms.[39] The *CmeABC* multidrug resistance efflux pump is vital for *C. jejuni* colonization, regulating resistance to bile salts in the intestinal tract. The resistance-nodulation-division superfamily of bacterial transporters includes the tripartite efflux mechanism known as the multidrug resistance pump. The CmeABC complex allows *C. jejuni* to develop intrinsic resistance to a variety of antibiotics and other antimicrobial substances.[40,41]

The study also revealed that several ribosomal proteins are involved in the resistance mechanism. Clinical resistance to macrolides, lincosamides, streptogramins, and ketolides is related to

**Table 3. Clustering analysis of the gene interaction network**

| | Number of nodes | Number of edges | Cluster score = (density × no of nodes) | Gene |
|---|---|---|---|---|
| Cluster 1 | 28 | 373 | 27.63 | *rplJ, rpmI, rpsD, rplV, rpmB, rpsA, rpsG, rpsF, rplT, rplY, fusA, rpsL, rpsO, rpmA, rpsE, rpmG, rplA, rpll, lepA, rplL, rplE, rplb, rplK, rpmF, rpsP, rplU, nusG, rplS.* |
| Cluster 2 | 5 | 10 | 5 | *pebC, peb1A, Cj0919c, Cj0920c, cjaA.* |

**Table 4. Significantly enriched Gene Ontology terms in cellular component's with the associated genes**

| SI | ID | Description | Genes |
|---|---|---|---|
| 1 | GO:43229 | intracellular organelle | *rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsA, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 2 | GO:43228 | non-membrane-bounded organelle | *rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsA, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 3 | GO:43232 | intracellular non-membrane-bounded organelle | *rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsA, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |
| 4 | GO:5840 | ribosome | *rplA, rplE, rplJ, rplK, rplL, rplS, rplT, rplU, rplV, rpmA, rpmB, rpmF, rpmG, rpsA, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO, rpsP.* |

GO, Gene Ontology.

modifications in ribosomal proteins L4 and L22 in various bacteria.[42] Ribosomal proteins L4 and L22 interact with the CmeABC efflux pump to confer macrolide resistance. Understanding of the processes underlying *Campylobacter* resistance to macrolides is based on alterations in ribosomal proteins.[43] The research also indicated that a few GO terms were associated with mutations in *rpl* genes linked to azithromycin resistance. Fusidic acid (FA) resistance develops through either horizontal acquisition of the *fusA* gene, which encodes a binding protein shielding the translation apparatus from FA inhibition, or genetic anomalies in the EF-G (*fusA*) gene.[44] The antibiotic FA targets ribosome-bound EF-G in both translocation and ribosome recycling, thus inhibiting protein synthesis.[45] FA has been used to treat infections caused by gram-positive bacteria since it was first identified in the early 1960s. However, the increase in bacterial pathogen resistance has become a growing clinical concern, with FA resistance often resulting from mutations in the EF-G-encoding *fusA* gene or the reduction/loss of ribosomal protein L6. To control both intrinsic termination and global gene expression, *NusG* and *NusA* collaborate. Loss of *NusG* leads to an altered pattern in fla/che operon expression, causing a reduced motility phenotype.[46]

In this study, a few of the enriched CC terms were related to intracellular organelles (GO: 43229), nonmembrane-bound organelles (GO: 43228), intracellular nonmembrane-bound organelles (GO: 43232), and ribosomes (GO: 5840) (Table 4). These terms were associated with a set of genes, Genes such as *rplV*, *rplK*, *rplE*, and *rpsO* are shown in Figure 5. One of the mechanisms by which bacteria can resist the effects of antibiotics is through drug efflux pumps, primarily found in gram-negative bacteria, which maintain the internal environment. Among the significant genes identified, *rpsO* encodes one of the main rRNA binding proteins that directly binds to 16S rRNA, aiding in the assembly of the 30S subunit platform. The *rplV* gene encodes the ribosomal 50S subunit protein L22, which is essential for early ribosomal 50S subunit synthesis.[47,48] The nascent peptide exit tunnel of the growing ribosome needs to form.[49] *rplK* is a component of the ribosomal stalk,

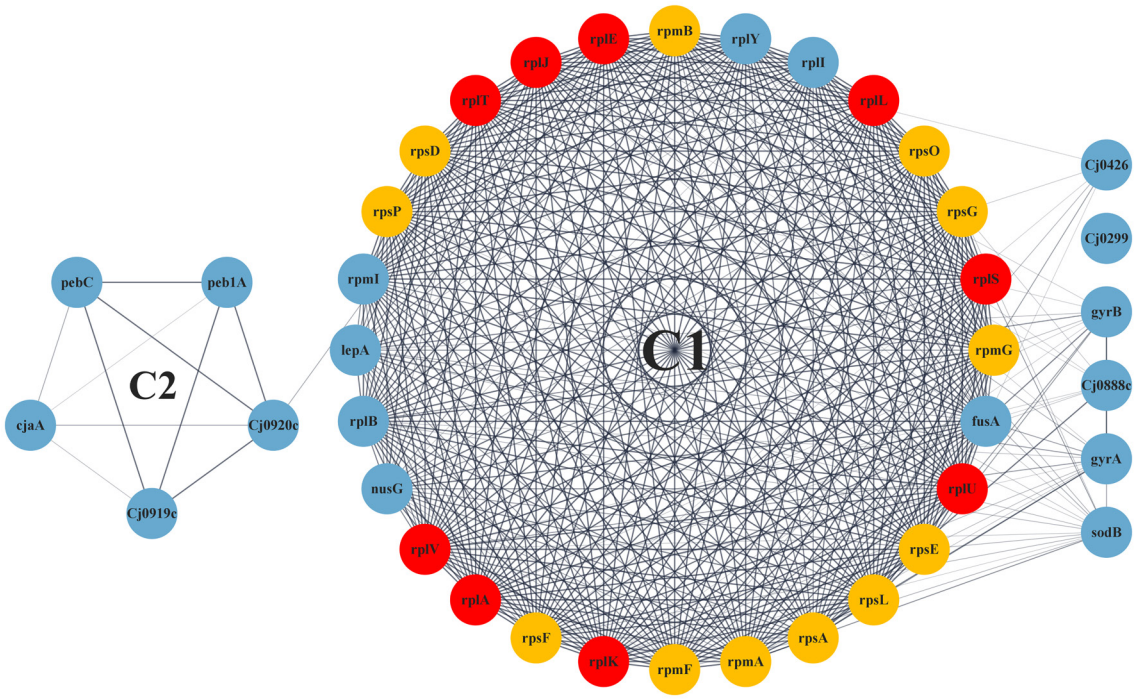


**Fig. 5. Enrichment of the AMR (antimicrobial resistance) genes using ClueGO involved in CCs.** The genes enriched in CCs are highlighted in yellow, whereas the top resistance genes are shown in red. CCs, cellular components.

**Table 5. Gene Ontology terms significantly enriched in molecular function's associated with genes**

| Sl | ID | Description | Genes |
|---|---|---|---|
| 1 | GO:3676 | nucleic acid binding | *fusA, lepA, rplA, rplE, rplJ, rplK, rplT, rplU, rplV, rpsA, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO.* |
| 2 | GO:3723 | RNA binding | *fusA, lepA, rplA, rplE, rplJ, rplK, rplT, rplU, rplV, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO.* |
| 3 | GO:19843 | rRNA binding | *rplA, rplE, rplJ, rplK, rplT, rplU, rplV, rpsD, rpsE, rpsF, rpsG, rpsL, rpsO.* |

GO, Gene Ontology.

facilitating the interaction of the ribosome with guanosine Triphosphate-bound translation factors. The enrichment of molecular activities, including ribosome structural elements and nucleic acid binding, was also a significant discovery from the study (GO:3676, GO:3723, and GO: 19843) (Table 5). These functions are linked to a cluster of genes, including *fusA*, *lepA*, *rplE*, *rplK*, *rplV*, and *rpsO*, represented in Figure 6. Erythromycin, spectinomycin, and streptomycin resistance have all been linked to mutations in ribosomal proteins L22, S5, and S12.[50] When bacteria are exposed to these medications, alterations in ribosomal proteins are integrated into the bacterium. In numerous bacterial species, including the intestinal pathogen *C. jejuni*, RNA-binding regulators have been shown to control posttranscriptional protein expression.[51,52]

Transformations in certain ribosomal proteins are known to be connected with antibiotic resistance, affecting the stability or translation of the mRNAs they bind to.[53] According to several studies, antibiotic exposure alters the metabolic state of bacteria. The activation of efflux pumps alters bacterial susceptibility to antibiotics, leading to the development of resistance mechanisms and influencing biofilm formation.[54,55] As a result, the enrichment of the ribosome pathway in the KEGG pathway is essential for understanding antibiotic resistance in bacteria.[56]

Through our studies, we have gained a better understanding of the intricate functional relationships between genes and their variations. Our results may have significant implications for the development of innovative therapies and diagnostic tools for *C. jejuni* infections because they illuminate the complex interplay of genetic variables in a range of BPs.

## Conclusions

*C. jejuni* stands out as a predominant pathogen in global foodborne outbreaks, notably amid increasing concerns about AMR. A recent study focused on tetracycline resistance genes *tetO* and *tetM*. By employing phylogenetic tree analysis, this research has provided valuable insights into the genetic landscape and variants associated with *C. jejuni*. The investigation highlighted the key hub genes such as *rplE, rplV, rplG,* and others, revealing their integral roles in AMR through GO keywords such as gene expression, cellular biosynthetic processes, and RNA binding. Crucially, this study highlighted the significance of the *rpl* gene in driving the AMR phase of *C. jejuni*. These hub genes, exhibiting a high degree of clustering with their functional partners, have emerged as potential drug targets. The study's findings raise hope that targeting these genes could pave the way for innovative treatments combating AMR in *C. jejuni* infections. This comprehensive ex-

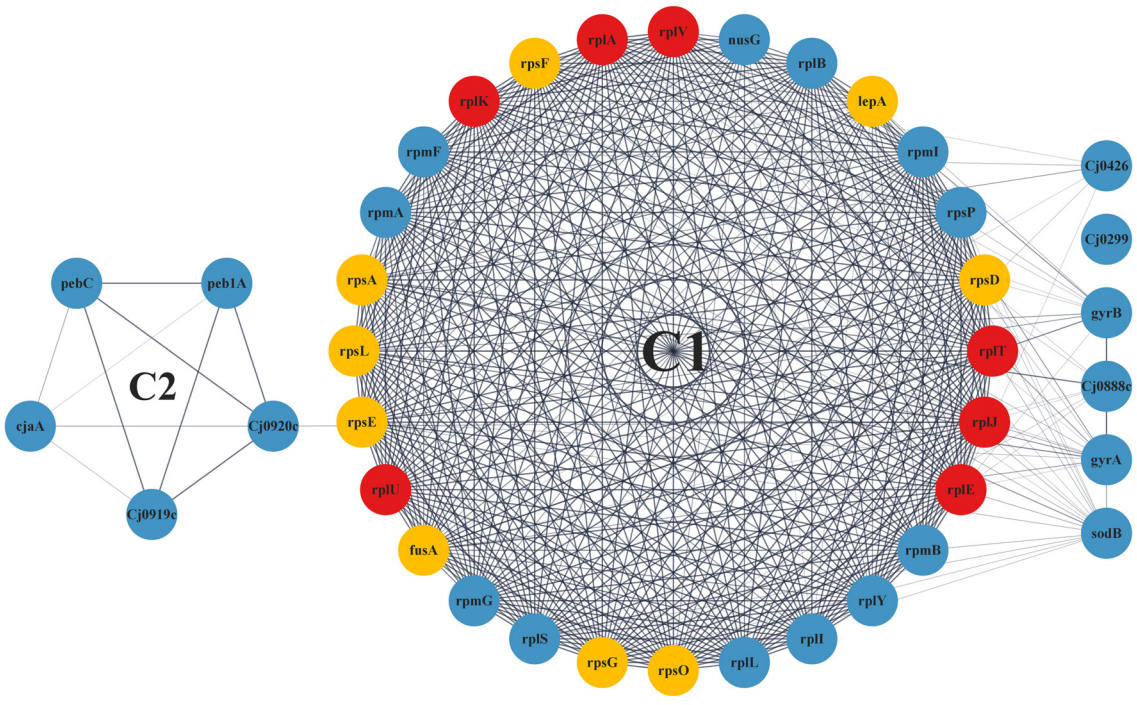


**Fig. 6. Enrichment of the AMR (antimicrobial resistance) genes using ClueGO involved in MFs.** The genes enriched in MFs were highlighted in yellow, whereas the top resistance genes are shown in red. MFs, molecular functions.

ploration of genetic and functional aspects offers valuable insights into the complex dynamics of AMR, providing a foundation for future therapeutic interventions and strategies in the ongoing battle against antibiotic resistance in *C. jejuni*.

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## Conflict of interest

The authors declare no conflict of interests.

## Author contributions

Writing-review & editing (PKS, SME, KV, GPDC), Writing-original draft (PKS), Visualization (PKS), Validation (PKS), Methodology (PKS), Formal analysis (PKS, SME, HD), Data curation (PKS), Conceptualization (PKS, SME, KV), Project administration (KV), Supervision (GPDC).

## Data sharing statement

The data will be made available upon request.

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