United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034591

Project Title

Antimicrobial Resistance Profiles of ESKAPE(E) Pathogens Isolated from Retail Fresh Produce in Athens, GA

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2026
Grant No. 2026-67011-46232	**Cumulative Award Amt.** $60,000.00
Proposal No. 2024-13108	**Multistate No.** (N/A)
Project Start Date May 01, 2026	**Project End Date** Apr 30, 2027
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Project Director

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Recipient Organization

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Performing Department

(N/A)

Non Technical Summary

The ESKAPE(E) group, which includes Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp., and Escherichia coli, primarily contains bacteria that don't tend to cause illness in people with healthy immune systems, but can cause severe illness in people who have weaker immune systems, such as people who are acutely ill or otherwise immunocompromised. These organisms are thus what we refer to as "opportunistic pathogens." ESKAPE(E) group opportunistic pathogens are naturally present in many environmental settings where humans and our food interface, including soil and water. Importantly, these bacteria are responsible for a large proportion of the burden of multidrug-resistant infections in clinical settings. Part of the reason for this is that ESKAPE(E) pathogens are particularly adept at evading the antibiotics meant to treat the infections they cause, and they are furthermore well-suited to survival in otherwise inhospitable environments, such as medical settings. While many members of the ESKAPE(E) group are found in

environmental reservoirs that human food sources regularly interface with, such as soil and water, comprehensive AMR characterization of these organisms along the human food system has been infrequently carried out. Due to growing recognition of the role of mobile genetic elements, that is, parts of a bacterium's genome that can move within the cell and/or between it and another cell, in transmission of AMR within complex microbial communities, it is crucial to more proactively monitor the AMR profiles of organisms along the food supply chain that are recognized to be particularly adept at evading clinical antibiotics. The isolates to be tested through this project have foodborne provenance, rather than clinical provenance, and as a consequence, we hypothesize that less than 25% of the 80 food-derived ESKAPE(E) isolates to be tested through this project will exhibit multidrug resistance, and furthermore, that isolate resistomes will primarily comprise genes encoding for intrinsic resistance determinants, such as outer membrane porins.We will test this central hypothesis by using antimicrobial susceptibility testing (AST) to assess if the isolates are able to resist exposure to increasing concentrations of first-line clinical antibiotic agents. We will additionally use next-generation sequencing of select isolates exhibiting more robust resistance phenotypes in the AST assays for whole genome sequencing, which will allow for the resolution of both chromosomally- and plasmid-encoded resistance determinants. The genomes of each isolate will be assembled and characterized using bioinformatic tools. Altogether, this approach will allow for reaching the first overarching goal of this project, which is to provision the AMR profiles of these foodborne ESKAPE(E) isolates to the scientific community and inform efforts to better characterize the scope of AMR along the U.S. food system. The project has a secondary overarching goal of supporting the professional development of the Project Director, which will involve technical training in bioinformatics, communication of project findings to technical and general audiences, and ultimately, the successful defense of the dissertation project that this project will be a component of. This project will allow for the provisioning of complete or near-complete genome sequences and comprehensive AMR profiles of the isolates tested to the scientific community, relevant and actionable findings to UGA Cooperative Extension agents, and cross-disciplinary training opportunities for the Project Director. We expect the characterization of the AMR profiles of the food-derived ESKAPE(E) isolates to be tested in this project to offer a more comprehensive understanding of AMR in the food system. Such information will be valuable to informing One Health efforts aimed at addressing the growing burden of AMR.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	100%
Applied	0%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
712	4010	1100	50%
712	4010	1040	50%

Knowledge Area

[712] Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

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Subject Of Investigation

[4010] Bacteria

Field Of Science

[1100] Bacteriology

[1040] Molecular biology

Keywords

antimicrobial resistance, food safety, genomics, interdisciplinary research, ESKAPE(E) group

Goals / Objectives

The overarching aim of this project is to characterize the resistomes and antimicrobial resistance (AMR) phenotypes of ESKAPE(E) group opportunistic pathogens isolated from retail fresh produce. This project has a second overarching aim of supporting the professional development of the Project Director so she may lead interdisciplinary food safety research projects in her future career. To meet these aims, this project has seven specific objectives:Objective 1 of this project is to characterize the resistomes of ESKAPE(E) isolates usingin silicoAMR gene prediction tools, specifically AMRFinderPlus, ResFinder, and RGIbwt. Here, we will extract genomic DNA from isolates using the QIAGEN DNeasy Blood and Tissue kit before fragment analysis with the Advanced Analytical Technologies Fragment AnalyzerTMAutomated CE System, DNA concentration analysis using a QubitTM4 fluorometer, and DNA quality assessment using a NanoDrop One spectrophotometer. Genomic DNA will be sequenced on using a MinION before filtering, assembly, and quality control of assemblies. Assemblies will be annotated using the Prokaryotic Genome Annotation Pipeline, and predicted AMR, virulence, and stress genes will be identified using AMRFinderPlus. The AMR gene prediction pipeline will also involve ResFinder and RGIbwt.Objective 2 of this project is to characterize the antimicrobial resistance phenotypes of the 80 ESKAPE(E) group isolates against first-line antibiotic agents using antimicrobial susceptibility testing. Here, isolates, alongside quality control strains, will be tested against first-line antibiotics using the Thermo ScientificTMSensititreTMGN7F broth microdilution plates, followed by disc elution using the BD BBLTMSensi-DiscTMantimicrobial susceptibility test discs. Susceptibility to agents will be assessed relative to the Clinical and Laboratory Standards Institute (CLSI) M100Enterobacterales breakpoints.Objective 3 of this project is to have two manuscripts pertaining to this project, one being a genome announcement, under peer review by the end of the project period.Objective 4 of this project is for the Project Director (PD) to complete the coursework required to earn the Graduate Certificate in Bioinformatics, offered through the Institute of Bioinformatics at the University of Georgia (UGA). This will directly support the PD's capacity to successfully address Objectives 1 and 3.Objective 5 of this project is for the PD to effectively communicate findings from this project to other scientists and food safety professionals at the annual International Association for Food Protection meeting in July of 2026, which will also serve to assess relevant future directions for such work within the broader food safety microbiology field.Objective 6 of this project is for the PD to communicate relevant findings from this project to UGA Cooperative Extension agents in the summer of 2026 and work with agents to determine feasibility of designing an educational resource based on the project findings that may be useful for the general public.Objective 7 of this project is for the PD to earn her PhD in Nutritional Sciences through having successfully defended an interdisciplinary dissertation project exploring neighborhood food environments, food safety microbiology, and antimicrobial resistance at the end of the project period.

Project Methods

This project builds upon earlier work wherein a total of 432 fresh produce samples (tomatoes, lettuce heads, and cucumbers) from retailers serving Athens, GA communities were tested for microbial hazards through standard culture-based methods. A total of 80 ESKAPE(E) group isolates were identified whose antimicrobial resistance (AMR) profiles

which will be characterized through this project. Two Klebsiella pneumoniae and 78 Enterobacter cloacae complex isolates were identified using matrix-assisted time-of-flight mass spectrometry. Efforts to be enacted to this end will first include assessing the AMR phenotypes of all 80 sample isolates using antimicrobial susceptibility testing. First, all isolates will be tested against first-line clinical antibiotics using the Thermo ScientificTM SensititreTM GN7F broth microdilution plates. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 will be used as quality control (QC) strains. Cryopreserved sample and QC isolates will be revived then inoculated into sterile purified water relative to a 0.5 McFarland standard prior to inoculation into cation-adjusted Mueller Hinton II broth, after which GN7F plates will be inoculated and incubated. Antibiotic susceptibility will be visually assessed relative to the Clinical and Laboratory Standards Institute (CLSI) M100 Enterobacterales breakpoints. Isolates with testing intermediate to any antibiotic agent on the GN7F plates will be further tested against the resistant breakpoint for Enterobacterales using disc elution with the BD BBLTM Sensi-DiscTM antimicrobial susceptibility test discs. Resistance to agents will be assessed relative to the CLSI M100 Enterobacterales breakpoints.Isolates with phenotypic resistance to two or more classes of antibiotic agents will subsequently undergo resistome characterization using in silico AMR gene prediction tools. Genomic DNA will be extracted from isolates using the QIAGEN DNeasy Blood & Tissue kit before fragment analysis with the Advanced Analytical Technologies Fragment AnalyzerTMAutomated CE System, DNA concentration analysis using a QubitTM4 fluorometer, and DNA quality assessment using a NanoDrop One spectrophotometer. Genomic DNA will be sequenced on using a MinION, with the super accuracy basecalling model. Reads will be quality filtered using Filtlong before genome assembly with Flye and quality control with BUSCO. Assemblies will be annotated using the Prokaryotic Genome Annotation Pipeline, and predicted AMR, virulence, and stress genes will be identified using AMRFinderPlus. The AMR gene prediction pipeline will also involve ResFinder and RGIbwt. Plasmids that may be present will be identified using PlasmidFinder.Evaluation of efforts put toward this project will be evaluated through generation of tangible outputs based on the project findings. The genome sequences derived from this project will be made publicly available through NCBI and formally published in a genome announcement. The AMR characterization findings and methodology will be published for use by the scientific community through presentation at the International Association for Food Protection 2026 Annual Meeting and a primary research manuscript that has made its way to the review stage by the close of the project period. Relevant findings will also be shared with University of Georgia (UGA) Family and Consumer Sciences (FACS) Cooperative Extension agents through one to four interactive presentations and discussions regarding the relevance of the findings to the greater public and how best to communicate such information. Furthermore, the professional development of the Project Director will be evaluated through successful completion of the coursework required to earn the UGA Graduate Certificate in Bioinformatics and successful defense of her dissertation project at the end of the project period.