COMPARATIVE EVALUATION OF PATHOGENICITY, CYTOTOXICITY AT THE BLOOD-BRAIN BARRIER, AND ANTIBIOTIC SUSCEPTIBILITY PROFILES IN *LISTERIA MONOCYTOGENES* FROM FOOD AND CLINICAL SOURCES. Victoria A. Felton1, Hannah Keating2, & Rishi Drolia1,2, 1Old Dominion University, Dept. of Biological Sciences, 2 Eastern Kentucky University, Dept. of Biological Sciences. *Listeria monocytogenes* (*Lm*) is a model facultative intracellular and opportunistic foodborne pathogen. In high-risk populations, neonates, the aged, and immunocompromised individuals, *Lm* causes meningitis, encephalitis, liver abscessation, and abortion in pregnant women, with a case fatality rate of 20%–30%. *Lm* breaches the blood-brain barrier (BBB) to translocate into the brain parenchyma; however, the precise pathogenic mechanism of *Lm* translocation across the BBB is poorly understood. This study aimed to perform a comparative analysis of the pathogenicity of various food-origin-isolated and patient clinical-isolated *Lm* strains to invade the BBB (total 37 strains). A series of tests evaluated each strain's ability to adhere, invade, and translocate across the human brain endothelial cell (HBEC)-5i barrier via adhesion, invasion, and translocating screening efficiency assays. In addition, HBEC-5i cytotoxicity was measured via the quantification of host cell lactate dehydrogenase (LDH) release, and Kirby-Bauer antibiotic susceptibility was evaluated. There is no significant difference in adhesion % in food versus clinical-isolated strains. However, clinical strains 19115, 171, and CAP D-05 displayed the highest adhesion percentages, reporting ~26-27%. Similarly, the clinical *Lm* strains better invaded HBEC-5i strains F4264 and F4262, presenting ~0.50 and 0.19% invasion capacities, respectively. Clinical *Lm* strains also transmigrated through endothelial barriers better compared to food product-based isolates, with 171 and T543 reporting the highest translocation abilities (22 & 21%, respectively). The clinical *Lm* isolates19115 and CHLR1 induced the most cytotoxic effects on the HBEC-5i barrier (42 & 41%). Kirby-Bauer antibiotic susceptibility data showed that food and clinical *Lm* strains were most sensitive to Penicillin, Ampicillin, and vancomycin, whereas they were most resistant to Oxacillin and Cefotaxime. These data suggest that clinical *Lm* isolates possess hypervirulent traits that allow them to translocate across the BBB efficiently. Future studies will evaluate the expression levels of *Listeria*-associated virulence factors (InlA/B, LAP, P60) to understand the genetic differences between clinical hypervirulent and food hypovirulent isolates. This understanding is vital in combating the severe consequences of *Lm* brain infection, including meningitis, encephalitis, and brain abscess. Author contact: [Vfelt001@odu.edu](https://olddominion-my.sharepoint.com/personal/rdrolia_odu_edu/Documents/Vfelt001%40odu.edu). The research was supported by funds from the KY-INBRE (part of award # P20GM103436-22 from the National Institute of General Medical Sciences; R.D.), the start-up funds at EKU (R.D.) and ODU (R.D.), and USDA-NIFA (Award # 2023-67017-40051; R.D.).