Abstract: A longitudinal study was conducted to determine the prevalence of Listeria spp. in a commercial fresh mushroom slicing and packaging environment. Samples were collected at three different sampling periods within a 13-month time interval. Of the 255 environmental samples collected, 18.8% tested positive for L. monocytogenes, 4.3% for L. innocua, and 2.0% for L. grayi. L. monocytogenes was most often found on wet floors within the washing and slicing and packaging areas. Each of the 171 L. monocytogenes isolates found in the environment could be placed into one of three different serotypes; 1/2c was predominant (93.6%), followed by 1/2b (3.5%) and 1/2a (2.9%). Of 58 isolates subtyped using multi-virulence-locus sequence typing, all 1/2c isolates were identified as virulence type (VT) 11 (VT11), all 1/2b isolates were VT105, and 1/2a isolates were either VT107 or VT56. VT11 was designated as the predominant and persistent clone in the environment because it was isolated repeatedly at numerous locations throughout the study. The overall predominance and persistence of VT11 indicates that it likely colonized the mushroom processing environment. Areas adjacent to the trench drain in the washing and slicing area and a floor crack in the packaging area may represent primary harborage sites (reservoirs) for VT11. Improvements made to sanitation procedures by company management after period 2 coincided with a significant (P ≤ 0.001) reduction in the prevalence of L. monocytogenes from 17.8% in period 1 and 30.7% in period 2 to 8.5% in period 3. This suggests that targeted cleaning and sanitizing procedures can be effective in minimizing the occurrence of L. monocytogenes contamination in processing facilities. Additional research is needed to understand why VT11 was predominant and persistent in the mushroom processing environment.