Soil is the thin layer covering our planet earth, and is a very important resource for humans, animals, plants, and microorganisms. Many actions and initiatives to promote sustainable soil management and soil security have been undertaken by several institutions in many countries. Improper disposal of waste effluents from abattoirs have been documented to have effects on the soil properties and ultimately increasing the population of microflora, enteric and opportunistic pathogens. Soil-borne fungi are a major cause for different degrees of allergy and diseases in human and animals. This study therefore aimed at isolation and characterization of fungal microbes associated with abattoir soil samples. Five (5g) of soil samples were collected each from four different abattoirs, Eleya, Odo-ori, Kaara and Adeke in Iwo, Osun State. One (1) gramme of each soil sample was weighed, serially diluted using sterile water, and then 1mL aliquots of 10-³ and 10-⁵ dilutions were cultured on sterile Sabouraud dextrose agar medium impregnated with streptomycin (0.2g) using pour plate method. Incubation was carried out at $25-27^{\circ}C$ for 3days. Six (6) different fungal isolates were obtained and subjected to macroscopic and microscopic examinations. Antifungal susceptibility testing was also conducted using Kirby-Bauer disc diffusion technique. Molecular characterization of the fungal isolates by amplifying the internal spacer region (ITS) of ribosomal DNA using primers ITSI and ITS4, gel electrophoresis using mid-range ladder and an expected amplicon between (600-800bp), with sequence blasting using NCBI BLAST tool. Phylo genetic relationships among the six (6) fungal isolates were determined by neighbor-joining method, using MEGA II (Kimura 2- parameters) with 1000 bootstrap replication value. Result revealed that the isolates belong to five (5) different genera; Lasidioplodia, Geotrichum, Curvularia, Aspergillus, and Epicoccum, and percentage occurences were 23.08%, 23.08%, 15.38%, 30.77%, and 7.69% respectively. Antifungal sensitivity test showed that all the isolates were susceptible to Ketoconazole (100% susceptibility), five (5) to Clotrimazole (76.92% sensitivity), three (3) to Amphotericin B (46.15% susceptibility). But none was susceptible to nystatin (0% sensitivity). Molecular characterization and phylogenetic relationships among the six (6) fungal isolates results revealed species of the fungi as Lasiodiplodia theobromae, Geotrichum candidum, Curvularia aerial, Aspergillusallahabadii, Aspergillus flavus, and Epicoccum sorghum, with percentage identity between 98.36% to 100%. The presence of these opportunistic or potentially pathogenic microoranisms in abattoir soil, and their possibility of toxin production pose a significant threat to humans, pre-harvest and post-harvest crops. They are indicators of a potential health risk to abattoir workers, residents near abattoirs, meat consumers, and consumers of crops grown on contaminated soil. The use of conventional antifungal drugs against invasive fungal infection is of great importance in clinical treatments. Molecular technique has shown reliability in characterization of isolates to special level.