Antibiotic resistance has been spreading rapidly throughout the world, faster than we can keep track of. Resistances of all kinds exist in gram-negative Enterobacteriaceae, ranging from colistin to carbapenems. Screening countries for the resistances present is important to understand the diffusion patterns or to acknowledge possible reservoirs around the globe. Our objective here was to establish whether resistance to last-resort antibiotics such as carbapenems and polymyxins may occur in a removed geographical area such São Tomé and Príncipe, an African island, where there is no access to those antibiotics. In this study, 50 patients were screened for strains resistant to carbapenems, aminoglycosides, and polymyxins. This was done using selective media to isolate the strains and then identify the resistance mechanisms using molecular methods. A total of 36 different strains were isolated from 23 separate patients, showing resistance to carbapenems, aminoglycosides, and polymyxins. 26 strains had the blaOXA-181 carbapenemase gene, 1 strain the rmtB RMTase gene conferring pan-resistance to aminoglycosides, 8 strains co-harbored both the blaOXA-181 and rmtB genes, and 1 strain housed the mcr-1 colistin resistance gene. A vast majority of the strains isolated were E. coli (31) with only a few K. pneumoniae (5). Additionally, many of the strains were clonal in nature and all strains that contained blaOXA-181 had the same plasmid backbone and size. These genetic features were also very similar to a previous study done in Angola that revealed carbapenemase-producing Enterobacteriaceae. It is possible that these strains did not evolve in São Tomé and Príncipe but instead were picked up in neighboring countries and brought in. More screenings need to be done worldwide so that we can gain a clearer picture of how resistance genes are diffusing out, and it can help us located possible reservoirs where these genes evolve and transfer.