In total, 120 Escherichia coli isolates positive for one of the gentamicin resistance (GEN(R)) genes aac(3)-II, aac(3)-IV or ant(2")-I were tested for gentamicin susceptibility by the agar dilution method. Isolates positive for aac(3)-IV or ant(2")-I had an MIC distribution of 8-64 mg/L, whereas isolates positive for aac(3)-II had MICs of 32 to > 512 mg/L, suggesting a relationship between the distribution of MICs and the specific GEN(R) mechanism. The MIC distribution, regardless of the GEN(R) mechanism, was 8 - > 512 mg/L, which supports the clinical breakpoint of MIC > 4 mg/L suggested by EUCAST and questions the breakpoint recommended by the CLSI (>= 16 mg/L).