The aim of this project is to study the molecular characteristics of blaTEM-1 gene, and the associated ampicillin resistance mechanisms present in E. coli in north Jordan. In this study, 150 unrelated Escherichia coli bacterial samples were isolated from different clinical sources (urine, blood, pus and abscess) and tested for their susceptibility to 17 antimicrobial agents, including ampicillin. The isolates were typed by plasmid profiling, and were investigated by PCR for the presence of various resistance genes. Out of the 150 isolates, 14 strains were multi-resistant; they showed resistance to Cotrimoxazole (70%), Ampicillin (67%), nalidixic acid (51%), Cephalothin (27%), Augmentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%) and Gentamycin (12%). Plasmid analysis of clinical isolates showed the presence of 1 to 7 plasmids with size ranging from 1.9 to 21.1 Kb compared with the control E.coli ATCC 25922 (size range from 2 to 19.5 Kb). PCR results showed the presence of blaTEM-1 gene which was responsible for Ampicillin resistance in 5 of the 14 isolated E. coli strains; the gene was located on a plasmid having a size of 1190 bp. This is the first study describing the presence of the blaTEM-1 gene in bacterial isolates in Northern Jordan. The blaTEM-1 gene found in this plasmid showed strong correlation between genotype conferred resistance determined by PCR and antibiotic susceptibility patterns.