**Interpretation of the Phylogenetic Tree for blaTEM Genes**

The phylogenetic tree constructed from the complete coding sequences (CDS) of the *blaTEM* gene across seven (7) multidrug-resistant bacterial strains reveals distinct evolutionary relationships and offers insights into potential horizontal gene transfer, allelic divergence, and the evolution of resistance.

**Closely Related Strains with Similar blaTEM Variants**

The upper clade of the tree includes the *blaTEM* genes from Escherichia coli (KJ923009.1), Pseudomonas aeruginosa (JN188364.1), Citrobacter freundii (JN188363.1), and Staphylococcus aureus (NG\_050162.1). These sequences cluster together with high bootstrap support (98%), indicating strong sequence similarity and suggesting they likely share a common ancestral variant of blaTEM. Given that *E. coli*, *P. aeruginosa*, and *C. freundii* are all Gram-negative bacteria and known to carry plasmids encoding beta-lactamase genes, the clustering is biologically plausible. The presence of *S. aureus*, a Gram-positive bacterium, in this group is notable and suggests the likely occurrence of horizontal gene transfer (HGT) between Gram-negative and Gram-positive bacteria, possibly through mobile genetic elements such as plasmids or transposons.

This cluster supports the hypothesis that conserved plasmid-borne TEM variants are being shared across diverse genera, emphasizing the role of plasmid-mediated resistance in the spread of *blaTEM* among clinically relevant pathogens.

**Unexpected Cluster: S.aureus Group with Gram-negative Bacteria**

The inclusion of *Staphylococcus aureus* within the same clade as *E. coli*, *C. freundii*, and *P. aeruginosa* is surprising due to its Gram-positive cell wall structure, which is quite different from the others in the cluster. This anomaly suggests a recent or uncommon acquisition of the *blaTEM* gene in *S. aureus*, likely through inter-species plasmid transfer in environments such as hospitals or wastewater, where bacteria from multiple genera coexist under antibiotic pressure. The S. aureus sequence may also represent a plasmid-encoded variant (e.g., TEM-116) that is nearly identical to variants found in Gram-negative hosts.

This grouping demonstrates that phylogenetic relationships of resistance genes do not always mirror organismal phylogeny, especially when mobile resistance elements are involved.

**Secondary Cluster: Salmonella and Acinetobacter**

A second, strongly supported cluster (bootstrap 100%) includes *Salmonella enterica* (NG\_050225.1) and *Acinetobacter baumannii* (NG\_052865.1). Although these organisms are from different genera and ecological niches, their *blaTEM* sequences show high similarity, suggesting the presence of a shared TEM-type allele, likely an extended-spectrum beta-lactamase (ESBL) variant. This could reflect a recent dissemination event of a specific TEM variant via a widely distributed conjugative plasmid, particularly in healthcare settings where these organisms often co-infect patients and are exposed to similar antibiotic regimens.

**Outlier: Klebsiella pneumoniae**

Interestingly, *Klebsiella pneumoniae* (MZ310395.1) forms a distinct outgroup, diverging significantly from all other sequences in the tree. This separation indicates that the *blaTEM* gene in this strain has accumulated unique mutations or undergone recombination, resulting in a divergent allelic form. It may also reflect the presence of a chimeric or hybrid TEM allele, or differences in flanking genetic elements that affect sequence context. The divergence of *K. pneumoniae* is significant because this species is known for its extensive resistance gene reservoirs, including carbapenemases (KPC) and multiple ESBL variants, suggesting that genetic diversification of blaTEM may be particularly active in this species due to strong selective pressure and genomic plasticity.

**Implications of the Tree**

* The high sequence conservation within major clusters points to functional constraint on the beta-lactamase gene, meaning the protein product is crucial for survival in antibiotic-rich environments.
* The cross-genus clustering (e.g., S. aureus with Gram-negatives) emphasizes the threat of inter-species gene transfer, especially in clinical settings.
* The divergence observed in *K. pneumoniae* may be an early indication of emerging allelic variants with possibly altered substrate profiles or resistance levels, this makes it a candidate for further functional and structural study.

**Conclusion**

This phylogenetic analysis demonstrates that although the blaTEM gene is widespread among clinically important MDR pathogens, the evolutionary path of each variant can differ significantly, likely shaped by plasmid dynamics, host biology, and selective antibiotic pressure. The high conservation among most sequences underscores the stability and success of TEM-type beta-lactamases in conferring resistance, while the divergence in *K. pneumoniae* highlights the potential for novel variants to evolve, possibly contributing to treatment failure and diagnostic challenges in the future.