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Benchmarking cgMLST: The Impact of Allele Calling & ORF-based Post-Processing on Profile Similarity

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Core genome multilocus sequence typing (cgMLST) is a modern computational method widely used for bacterial genotyping and commonly utilized in bacterial outbreak investigations. By comparing allelic profiles, closely related isolates can be identified and infection chains reconstructed. As data is exchanged between laboratories, consistent allele assignments are essential for reliable cluster detection and epidemiological interpretation. However, different allele-calling tools produce slightly differing allele assignments from identical sequence data, affecting distance calculation and cluster definition.
For several integrated genomic surveillance (IGS) relevant pathogens we analyzed 100 samples each using multiple cgMLST allele-calling tools and investigated the impact of open reading frame validation. For each tool, three profile versions were generated and compared against the profile produced by MBioSEQ Ridom Typer with respect to missingness patterns and similarity: (1) consisted of the original allele calls, (2) only novel alleles reported by the tools were validated and filtered, and (3) both novel and known alleles were excluded based on additional validation of schema alleles.
Comparison of these profiles revealed differences in missingness patterns and concordance with SeqSphere across all versions. This demonstrates that allele-calling and post-processing strategies influence cgMLST profile comparability and affect downstream analyses strongly.

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`Bioinformatics, cgMLST, Clustering, Outbreak investigations`

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`Graduate Student`

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`No, I am not a Junior Scientist.`

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