Certificate of Analysis for NR-49455

Staphylococcus aureus, Strain LGA251

Catalog No. NR-49455

Product Description: *Staphylococcus aureus* (*S. aureus*), strain LGA251 was isolated in 2007 from a bulk milk sample taken from a cow dairy farm in Somerset, England, United Kingdom. *S. aureus*, strain LGA251 has a divergent *mecA* homologue, *mecA*$_{LGA251}$ (now referred to as *mecC*) and was deposited as resistant to penicillin, oxacillin and cefoxitin.

Lot[1]: 63950725 **Manufacturing Date: 06JAN2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[2]	Report results	Circular, convex, entire, smooth and gray (Figure 1)
Motility (wet mount)	Report results	Non-motile
Hemolysis[2]	Report results	β-hemolytic
Biochemical characterization		
Catalase	Positive	Positive
VITEK® 2 Compact (GP card)	≥ 90% probability of being *S. aureus*	*S. aureus* (99% probability)[3]
Antibiotic Susceptibility Profile		
Oxacillin resistance[4]	Resistant	Resistant
VITEK® (AST-GP71 card)[5]		
Beta-lactamase[6]	Report results	Positive
Cefoxitin screen	Report results	Negative
Benzylpenicillin	Report results	Resistant (= 0.25 µg/mL)
Gentamicin	Sensitive	Sensitive (≤ 0.5 µg/mL)
Ciprofloxacin	Sensitive	Sensitive (≤ 0.5 µg/mL)
Levofloxacin	Report results	Sensitive (≤ 0.12 µg/mL)
Moxifloxacin	Report results	Sensitive (≤ 0.25 µg/mL)
Clindamycin (inducible resistance)	Report results	Negative
Erythromycin	Sensitive	Sensitive (= 0.5 µg/mL)
Clindamycin	Sensitive	Sensitive (≤ 0.25 µg/mL)
Quinupristin/dalfopristin	Report results	Sensitive (≤ 0.25 µg/mL)
Linezolid	Sensitive	Sensitive (= 2 µg/mL)
Daptomycin	Report results	Non-susceptible (= 4 µg/mL)
Vancomycin	Report results	Sensitive (≤ 0.5 µg/mL)[7]
Minocycline	Report results	Sensitive (≤ 0.5 µg/mL)
Tetracycline	Sensitive	Sensitive (≤ 1 µg/mL)
Tigecycline	Report results	Sensitive (≤ 0.12 µg/mL)[8]
Nitrofurantoin	Report results	Sensitive (≤ 16 µg/mL)
Rifampicin	Sensitive	Sensitive (≤ 0.5 µg/mL)
Trimethoprim/sulfamethoxazole	Sensitive	Sensitive (≤ 10 µg/mL)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	≥ 99% sequence identity to *S. aureus*, strain LGA251 (GenBank: FR821779.1)	100% sequence identity to *S. aureus*, strain LGA251 (GenBank: FR821779.1)
PCR Assay of Extracted DNA[9]		
mecA	No amplicon	No amplicon
mecA$_{LGA251}$	~ 300 base pair amplicon	~ 300 base pair amplicon
Purity (post-freeze)[10]	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

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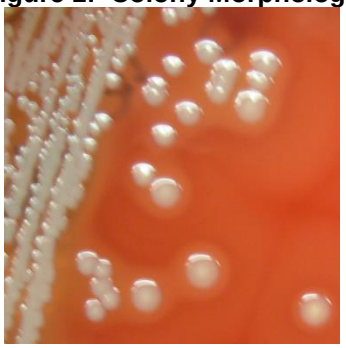
[1]NR-49455 was produced by inoculation of the deposited material into Tryptic Soy broth and grown 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles which were grown 1 day at 37°C in an aerobic atmosphere to produce this lot.
[2]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood
[3]Percent probabilities above 90% indicate a close match to the typical biochemical pattern for the given organism, with a percent probability of 99% being a perfect match between the test reaction pattern and the unique biochemical pattern of the given organism or organism group. For additional information, please refer to O'Hara, C.M. and J. M. Miller. "Evaluation of the VITEK 2 ID-GNB Assay for Identification of Members of the Family *Enterobacteriaceae* and Other Nonenteric Gram-Negative Bacilli and Comparison with the VITEK GNI+ Card." J. Clin. Microbiol. 41 (2003): 2096-2101. PubMed: 12734254.
[4]1 day at 30°C in an aerobic atmosphere on Mueller Hinton agar containing 6 µg/mL oxacillin and 4% NaCl (Oxacillin Screen agar BBL™ 221952)
[5]Minimum Inhibitory Concentration (MIC); MIC Interpretation Guideline: CLSI M100-S22 (2012)
[6]The production of beta-lactamase was detected using a Cefinase™ Paper Disc (BBL™ 231650).
[7]S. aureus, strain LGA251 is non-susceptible to daptomycin, which suggests that this strain may be a heterogeneous vancomycin-intermediate *S. aureus* (hVISA) strain in which subpopulations of cells of this strain are resistant to vancomycin (MIC ≥ 16 µg/mL). Antibiotic susceptibility testing using the VITEK® AST-GP71 card failed to detect vancomycin resistant subpopulations. For additional information, please refer to Cui, L., et al. "Correlation Between Reduced Daptomycin Susceptibility and Vancomycin Resistance in Vancomycin-Intermediate *Staphylococcus aureus*." J. Antimicrob. Chemother. 50 (2006): 1079-1082. PubMed: 16495273.
[8]MIC Interpretation Guideline: EUCAST Version 4.0 (2014)
[9]Cuny, C., et al. "Rare Occurrence of Methicillin-Resistant *Staphylococcus aureus* CC130 with a Novel *mecA* Homologue in Humans in Germany." PLoS One 6 (2011): e24360. PubMed: 21641281.
[10]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

Figure 1: Colony Morphology



Date: 01 MAR 2017 **Signature:**

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