James R. Brown, Daniel Gentry, Julie A. Becker, Karen Ingraham, David Holmes, Michael J. Stanhope. Horizontal transfer of antibiotic resistant aminoacyl-tRNA synthetase genes involving anthrax and Gram-positive pathogens.

Methods - PCR amplification primer sequences and conditions

Primers for the amplification of the loci *metS1* and *metS2* were designed to anneal to DNA sequences conserved between *S. pneumoniae* and *B. anthracis* while *ileS1* and *ileS2* primers were designed to nucleotide sequences conserved between *Staphylococcus aureus* and *B. anthracis*. For specific loci, the primer pairs and length of amplified products were: *metS1* (BCmetS1F 5' ATTACTACCCCAATTTATTATCCAAGTGG3' and BCmetS1R 5' TCCTCATCTTTCGCTAATACCCATGG3'; 1329bp), *metS2* (BCmetS2F 5' TGGCCGTATGCAAATGGTTCGTTACA 3' and BCmetS2R 5' CACTATTATGACTGTAAATAAATTCACGCCA3'; 1115 bp), *ileS1* (BCileS1F 5' GATACGCACGGTTTACCAATTGAACA 3' and BCileS1R 5' CCAGTCACCACGGTCACGAACCAT 3'; 1346 bp), and *ileS2* (BCileS2F 5' GTATTAAGAAAAGCGGGATGGGATAC 3' and BCileS2R 5' CACTATCAAACCAAACATCAATTACTTC 3'; 1331 bp).

Primer pairs were used in separate experiments. Colony isolates were grown on blood agar and transferred using a sterile needle to PCR reaction mixtures set-up according to vendor (Gibco-BRL PCR SuperMix High Fidelity). An initial cycle of 3 min 94oC was followed by 35 cycles of 30 sec 94°C, 45 sec 50°C and 1 min 72°C with a final cycle of 2 min 72°C. All experiments were performed in duplicate with positive and negative amplification controls. DNA fragments were visualized by electrophoresis on a 1.0 % agarose gel with a 1 kb molecular marker ladder. Amplification products were verified by sequencing randomly chosen DNA fragments in both directions using ABI3100 or 3700 automated sequencers.