Comparative genomic analysis and characterization of *Staphylococcus* sp. AOAB, isolated from a notoriously invasive *Mnemiopsis leidyi* gut revealed multiple antibiotic resistance determinants

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Abstract

Here, we describe the isolation and characterization of a coagulase-negative, vancomycin and oxacillin-susceptible novel bacterium of the genus *Staphylococcus*. *Staphylococcus* sp. strain AOAB was isolated from the stomodeum (gut) of the *Mnemiopsis leidyi* from Mobile Bay, Alabama USA. A polyphasic taxonomic approach comprised of phenotypic, chemotaxonomic and genotypic characteristics was used for analysis. The dominant respiratory quinone detected was MK-7 (100%). Major cellular fatty acids were anteiso-C₁₅:₀ (40.52%), anteiso-C₁₇:₀ (13.04 %), C-₁₈:₀ (11.53%) and C-₂₀:₀ (10.45%). The polar lipid profile consisted of glycolipid, phospholipid, phosphatidylglycerol and diphosphatidylglycerol. Although strain AOAB had a 16SrRNA gene sequence similarity of 99% with *S. warneri* SG1, *S. pasteuri*, *S. devriesei* KS-SP_60, *S. lugdunensis* HKU09-01, *S. epidermidis* RP62A, *S. haemolyticus* JCSC1435 and *S. hominis* DM 122, it was be distinguished from those species based on Multi-Locus Sequence Analysis (MLSA) using 6 marker genes (16S rRNA, *hsp60*, *rpoB*, *dnaJ*, *sodA* and *tuf*). MLSA revealed strain AOAB to be closely related to *S. warneri* SG1 and *S. pasteuri* SP1 but distinct from two hitherto known species. These results were confirmed by Average Nucleotide Identity (closest ANI of 84.93% and 84.58% identity against *S. warneri* SG1 and *S. pasteuri* SP1 respectively). *In-silico* DNA-DNA hybridization was <70% (33.1 % and 32.8% against *S. warneri* SG1 and *S. pasteuri* SP1 respectively), which further confirmed that the strain was a potential novel *Staphylococcus* species.
Abbreviations and key words: Average Nucleotide assembly (ANI), Multilocus Sequence Analysis (MLSA), DNA-DNA Hybridization (DDH)

Introduction

*Mnemiopsis leidyi* (Phylum Ctenophora) is a predatory gelatinous zooplankter endemic to the Western Atlantic, notorious for its invasion of all of the European enclosed and coastal seas, including the North Sea, the Baltic, the Black and Caspian Seas and the southern Adriatic and Mediterranean seas. *M. leidyi* is similarly notorious for its ability to alter the composition of native plankton communities (Delpy, Pagano, Blanchot, Carlotti, & Thibault-Botha, 2012; Jaspers et al., 2012; Lucic et al., 2012). *Mnemiopsis* blooms have been found to coincide with profound environmental perturbation (Purcell, 2012). Much like other zoonotic organisms in exotic habitats (DeLong, 2014), they also have been revealed to be vectors for microbial assemblages (Daniels & Breitbart, 2012; Hao, Gerdts, Peplies, & Wichels, 2015; Moss, Estes, Muellner, & Morgan, 2001). Although ctenophores are known to be parasitized by amoebae, dinoflagellates, sea anemones and bacteria (Daniels & Breitbart, 2012; Hammann, Moss, & Zimmer, 2015; Hao, Gerdts, Peplies, & Wichels, 2015; Moss, Estes, Muellner, & Morgan, 2001), there is no study that has isolated individual ctenophore gut bacteria for characterization.

This study used phenotypic, genotypic and phylogenetic analysis to characterize a *Staphylococcus* isolate AOAB. Previous studies have isolated novel staphylococci species from marine waters (Gunn & Colwell, 1983), fresh water (Hess & Gallert, 2015), marine crustaceans (Faghri, Pennington, Cronholm, & Atlas, 1984), domesticated animals (Yamashita et al., 2005) and human hosts (Trulzsch et al., 2007), among other sources.

Materials and Methods

Ctenophore gut (stomodeum) samples were collected from animals from Dauphin Island Marina, Mobile Bay, Alabama. Sterile toothpicks were inserted into the gut from the oral end and gently rotated, in order to collect a mucus-rich sample without damage to the stomodeal lining. Tips containing the mucoid gut samples were transferred into 1.5 mL tubes, stored on ice and transported to the laboratory for enrichment.

Enrichments were done using a modified protocol from Anacker and Ordal. Modifications were guided by Figueiredo et al. and Pilarski et al. (Anacker & Ordal, 1955; Figueiredo et al., 2005; Pilarski, Rossini, & Ceccarelli, 2008) (Supplementary Material 1). After an initial stage of
enrichment, cultures isolated and maintained using mannitol salt agar (Thavasi, Aparnadevi, Jayalakshmi, & Balasubramanian, 2007) and LB agar (Sigma Aldrich).

Phenotypic tests were carried out following minimum recommended standards to aid in discriminating species (Freney et al., 1999) using using carbohydrate fermentation with the API CH50 systems (bioMe`rieux). Optimal growth was obtained at 37°C after 24-48 hrs.

Chemotaxonomic analyses were also carried out to characterize phenotypes. To assign genus and confirm coagulase test of the isolate, occurrence of fatty acids ai-C15:0, i-C15:0, i-C17:0, ai-C17:0 and menaquinone (MK) in the cytoplasmic membrane was investigated as recommended (Freney et al., 1999; Heß & Gallert, 2015; B. J. Tindall, Rosselló-Móra, Busse, Ludwig, & Kämpfer, 2010). The Analysis for respiratory quinones was carried out by first separating them from other classes using thin layer chromatography on silica gel (Macherey-Nagel Art. No. 805 023), using hexane:tert-butylmethylether (9:1 v/v) as solvent. Menaquinones were then removed from the plate and analyzed using HPLC fitted with a reverse phase column (Macherey-Nagel, 2 mm x 125 mm, 3 µm, RP18) with methanol: heptane 9:1 (v/v) being used as the eluant. Polar lipids were extracted from 100 mg of freeze dried bacterial cells using chloroform: methanol: 0.3% aqueous NaCl mixture 1:2:0.8 (v/v/v), separated using two-dimensional silica gel TLC (Macherey-Nagel Art. No. 818 135) and detected using methods described by Tindall et al. (Brian J. Tindall, Sikorski, Smibert, & Krieg, 2007) (DSMZ Identification Services, Braunschweig, Germany). Cellular fatty acid composition for strain AOAB was determined using gas chromatography (chromatograph was fitted with a 5% phenyl-methyl silicone capillary column) using Sherlock MIS (MIDI Inc, Newark, USA) system. Using MIDI software package (MIDI Inc, Newark, USA), the fatty acid composition data was also used for clustering using Euclidean method for differentiation of isolate from closest species.

Antimicrobial susceptibility was performed using a standard disk diffusion method, following cutoff ranges as outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (Miller et al., 2003). Isolates were inoculated using spread plating with 100 µL of inoculum at log phase from LB broth onto LB agar before impregnating with antibiotic discs. Zones of inhibition (ZOI) were used to determine the ability of the antibiotics to inhibit the growth. The antibiotics used are shown in Table 1. Results were interpreted using commonly accepted zone breakpoints for Staphylococcus (Howe & Andrews, 2012). Control discs were sterile 6mm discs without antibiotics.
Genomic DNA was isolated using the CTAB protocol (Andreou, 2013) with minor modifications that included the use of 0.5 mm silica beads (Biospec Products, Inc. Cat. No. 110791052z), shaken using a specialized MO BIO Vortex-Genie² (MO BIO Laboratories). DNA purity was checked using a NanoDrop reader (ND-2000, NanoDrop Technologies, Wilmington, DE, USA) and precision-quantified using Qubit HS reagents (Life Technologies). The DNA template concentration was adjusted to 5 ng prior to use in touchdown PCR reactions. Amplification for most of the 16S rRNA gene was achieved using the universal primers 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and 1387r (5′-GGG CGG WGT GTA CAA GGC-3′) as described by Suriyachadkun et al. (Suriyachadkun et al., 2009).

Contents of a 25 μL PCR mixture included 12.5 μL of EconoTaq Plus Green 2X Master Mix (Lucigen), 0.5 μL of 20 μM of forward and reverse primers, 10.5 μL water and 1 μL of 5 ng μL⁻¹ genomic template DNA. PCR was carried out using ‘touchdown’ conditions: initial denaturation was at 95 °C for 5 min, followed by 20 cycles at 95 °C for 1 min 61°C for 45 sec, and extension at 72 °C for 90 sec. The touchdown method was followed by another 30 cycles at 95 °C (1 min) 51 °C (45 sec) 72 °C (90 sec). The final extension was done for 7 min at 72 °C.

The study also targeted a 370 bp tuf gene which is well established for Staphylococcus taxonomy (Martineau et al., 2001). Primer selection and melting temperature determination was done using an silico PCR simulator (http://insilico.ehu.es/PCR); (San Millan, Martinez-Ballesteros, Rementeria, Garaizar, & Bikandi, 2013). The PCR mixture included 0.4 μM of each Staphylococcus-specific primers (tuf-F (TStaG422) 5′-GCC CGT GTT GAA CGT GTG CAA ATC A-3 and tuf-R (TStag76) 5′TIA CCA TTT CAG TAC CTT CTG GTA A-3′) (Tm 59.3°C). The PCR reagent ratios were similar to those used in the 16S rRNA gene amplification. Touchdown PCR conditions were as previously described (Martineau et al., 2001) with modifications that included 5 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 55°C and 45 s at 72°C, and final extension for 7 min at 72°C. Amplified PCR products were purified using QIAquick (Qiagen, Maryland, USA) purification kit following manufacturer’s instructions prior to sequencing using ABI 3100 DNA Genetic Analyzer at Auburn University’s Genomics & Sequencing Laboratory (GSL).

Genomic DNA of the isolate was used for library construction. We used the Agilent 2100 BioAnalyzer (Agilent Technologies, USA) to perform size fractionation and quantification of DNA. DNA was then fragmented and libraries prepared using Nextera XT (Illumina) according
to manufacturer’s protocol before being run on an Illumina Miseq (for 2 x 250 bp paired-end reads) sequencing platform at the Auburn University’s Biological Sciences Department. Quantification of final library before loading on MiSeq sequencer was performed using the Kapa quantification kit (RT-PCR) for next generation sequencing with the Illumina platform (Kapa Biosystems, Wilmington, MA USA). Sequence reads were quality filtered and assembled using CLC Genomics Workbench 8.0.1 (CLCbio, Aarhus, Denmark) (K. U. Kim et al., 2013), SPAdes 3.6 (Bankevich et al., 2012) and Velvet 1.2 (Zerbino & Birney, 2008). QUAST β (http://quast.bioinf.spbau.ru/) (Gurevich, Saveliev, Vyahhi, & Tesler, 2013a, 2013b) was used to check for the quality of the assemblies and determine % G+C content of genomes. FASTA-formatted genomic sequences of closely related Staphylococcus genomes were obtained from the RefSeq database on GenBank and uploaded to PATRIC for annotation.

Protein coding genes in genomes were identified using GeneMarkS (Borodovsky & Lomsadze, 2014). Non-coding RNA prediction was achieved using RNAMMER 1.2 online server (Lagesen et al., 2007). Predictions for tRNA and tmRNA genes were done with the ARAG ORN tRNA and tmRNA prediction program (Laslett & Canback, 2004). Functional annotation of the protein gene models was achieved using multiple bioinformatic softwares including the RAST server (Overbeek et al., 2014), PATRIC (Wattam et al., 2014) and IMG/M (Markowitz et al., 2012). Annotation for antibiotic resistance genes was achieved using PATRIC via BLASTP sequence homology search from the Antibiotic Resistance Genes Database (ARDB) (Liu & Pop, 2009) and the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al., 2013) databases. To remove database-based redundancy, replicates were removed.

Analysis for occurrence of metal resistance genes (MRG) was performed using BLASTX against the Antibacterial Biocide and Metal Resistance Genes Database (MRDB) database with an e-value cutoff of 0.01 (Altschul et al., 1997). To achieve this, the BaCMet experimentally confirmed database of MRDB was used (Pal, Bengtsson-Palme, Rensing, Kristiansson, & Larsson, 2014).

Using the 16S rRNA gene sequences of strain AOAB and closely related *Staphylococcus* representatives were structurally aligned using SSU-ALIGN v0.1.1 (Nawrocki, 2009) and used for reconstruction of neighbor-joining tree. Separate sequence alignments was done using ClustalW algorithm in MEGA7 (Kumar, Stecher, & Tamura, 2016) for unrooted neighbor-joining tree (Supplementary Figure S3).
FASTA sequences of housekeeping genes from the closest species were obtained from GenBank. Concatenated DNA sequences from six marker genes (16S rRNA, tuf, sodA, dnaJ, hsp60 and rpoB) were used for MLSA. Sequences were aligned using MUSCLE (Edgar, 2004). The evolutionary history of strain AOAB was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model (Jukes & Cantor, 1969). Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

To infer genomic distance between strain AOAB and the closest species, pairwise average nucleotide identity (ANI) was computed using IMG/M system (Varghese et al., 2015). With a species cut off set at 96%, this method has been found to be robust in delineating bacteria based on genome sequence data (M. Kim, Oh, Park, & Chun, 2014).

*In-silico* DNA-DNA hybridization (DDH) was achieved using GBDP (Genome Blast Distance Phylogeny), which reliably infers genome-to-genome distances by utilizing Genome Blast Distance Phylogeny using logistic regression (Meier-Kolthoff, Auch, Klenk, & Goker, 2013).

**Results and Discussion**

*Staphylococcus* sp. strain AOAB as determined by scanning electron microscopy (SEM) (Hitachi) depicted numerous clumps of coagulase-negative, vancomycin and oxacillin-susceptible bacterium (Figure 1). *Staphylococcus* sp. strain AOAB grown on LB and Mannitol agar at 30 °C produced yellow, medium (2-3mm), round, smooth colonies after 3 days (Supplementary Material 2) after t periods of growth. The isolate is catalase-positive and coagulase-negative. The polar lipid profile consisted of glycolipid, phospholipid, phosphatidylglycerol and diphosphatidylglycerol (Supplementary Figure S5), typical of *Staphylococcus* species (Nahae, Goodfellow, Minnikin, & Hajek, 1984). But unlike *S. warneri* and other *Staphylococcus* species (Nahae et al., 1984), strain AOAB did not have detectable β-gentiobiosyl diacylglycerol (Supplementary Figure S5) as part of its polar lipid profile.

The presence of fatty acids, ai-C15:0, i-C15:0, i-C17:0, ai-C17:0, confirmed Genus of isolates as *Staphylococcus* (Supplementary Figure S4). Cluster analysis of the fatty composition of our isolate based on Euclidean distance revealed that our isolate does not belong to any known species (Figure 4) as MIDI dendrogram software places same species link at about 10 Euclidian
Distance (http://www.midilabs.com/fatty-acid-analysis). The presence of menaquinone (MK-7) in the cytoplasmic membrane helped confirm the isolated as coagulase negative (Heß & Gallert, 2015).

Disk diffusion confirmed resistance of S. mneniopsis AOAB against the penicillins (Penicillin and Ampicillin), fluoroquinolones (Ciprofloxacin, Nalidixic acid), a polypeptide (Bacitracin) and an aminoglycoside (Kanamycin) (Table 2). Also, strain AOAB was revealed to be susceptible to vancomycin, oxacillin, tetracycline, Amoxicillin/clavulanic acid and chloramphenicol (Table 1). S. pasteuri strains have mixed results against tetracycline (Chesneau et al., 1993).

Phylogenetic analysis using the 16S rRNA gene using neighbor-joining method indicated that the isolate was closely related to S. pasteuri and S. warneri (Figure 2). Further phylogenetic analysis using MLSA (Figure 3) clustered the isolate as novel bacteria. Both ANI and DDH using genome sequence data confirmed delineation of the isolate as a novel species. None of the closest species met the ANI cut off of 96% or the DDH cut off of 70% (Table 3).

Whole genome sequencing yielded a total of 905,410 paired reads for strain AOAB. The PATRIC annotated genome size was 2,617,061 bp. A number of genomic features differentiated it from the two closest relatives. Unlike S. mnemiopsis, S. pasteuri was experimentally confirmed to be susceptible to kanamycin (Chesneau et al., 1993), which helped in discrimination between the two species (Table 4).

Genomic characterization of strain AOAB revealed that the isolate harbors antibiotic resistance determinants (virulence factors, antibiotic resistance and drug targets and heavy metal resistance genes) (Table 5). In addition to antibiotic resistance genes, MRG-like sequences were detected in the genome of strain AOAB. The most dominant ones were copper (22), arsenic (11) and zinc (4) metal resistance genes. S. warneri had copper (12), zinc (7) cadmium (7) and arsenic (5) as the dominant metal resistance genes and for S. pasteuri SP1, it was copper (12), zinc (7), cadmium (6) and arsenic (5), thus aiding in further discrimination. This study forms the first report of Staphylococcus isolation from the stomodeum of M. leidyi. The results also suggest that M. leidyi, a notoriously invasive zooplankton harbors culturable but previously uncharacterized Staphylococcus species, some of which harbor antibiotic resistance genes. Future efforts will involve investigation of host-microbe interaction.
Data Availability

The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession LZFL00000000. The version described in this paper is version LZFL01000000. The SRA accession number is SRP076995, 16S rRNA gene (KU497670.1), tuf (LC158075), sodA (LC158854), rpoB (LC158855), hsp60 (LC158856) and dnaJ (LC158857). RAST server IDs are: Strain AOAB (6666666.127652), S. pasteurii SP1 (6666666.123700), S. warneri SG1 (6666666.123634). PATRIC Genome IDs: Strain AOAB (1279.166), S. pasteurii SP1 (1276282.3), S. warneri SG1 (1194526.3). Supplementary can be accessed via:

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Figure Legends

Figure 1: *Staphylococcus* sp. strain AOAB as determined by scanning electron microscopy (SEM) (Hitachi) depicted numerous clumps.

Figure 2: Neighbor-joining tree based on SSU-ALIGN for 16S rRNA gene sequences of strain AOAB with closely related *Staphylococcus* species. Numbers at nodes indicate the percentage of bootstrap support based on 1500 replications. Rooting was done using *Macrococcus caseolyticus* for discriminatory purposes.

Figure 3: Evolutionary history of strain AOAB inferred by using Maximum Likelihood method based on Jukes-Cantor model. Evolutionary tree was constructed using MEGA7. The tree is drawn to scale. Numbers on the nodes indicate bootstrap values as a percentage based on 1000 replications with branch lengths measured in the number of substitutions per site. The tree was based on concatenated MLSA of 16S rRNA, *tuf*, *sodA*, *dnaJ*, *hsp60* and *rpoB* gene sequences.

Figure 4: A dendrogram showing GC fatty acid profile similarities with 19 other *Staphylococcus* species.
Figure 1
Figure 2
Figure 3

![Phylogenetic tree diagram showing relationships between various Staphylococcus species. The tree includes labels for S. capitis CR01, S. caprae C87, S. epidermidis ATCC12228, S. muenchen ATCC12228, S. pasteuri SP1, S. warneri SG1, S. aureus MRSA252, S. haemolyticus JCSC1435, S. lugdunensis HKU09-01, S. pettenkoferi VCU012, S. saprophyticus ATCC15305, S. pseudintermedius HKU10-03, and M. caseolyticus JCSC5402. The scale bar indicates 0.020 evolutionary changes.]
Figure 4

Staphylococcus-dolphini
Staphylococcus-chromogenes
Staphylococcus-schleiferi
Staphylococcus-hyicus
Staphylococcus-felis
Staphylococcus-cnnii-urbaniticus
Staphylococcus-intermedius
Staphylococcus-hominis-hominis
K-11 (01-2018-22)
Staphylococcus-haemolyticus
Staphylococcus-midas-GC subgroup B
Staphylococcus-saprophyticus
Staphylococcus-cnnii-cnnii
Staphylococcus-pisciferoceanthane
Staphylococcus-kloosii
Staphylococcus-wernerii
Staphylococcus-pasteuri
Staphylococcus-capitis-capitis
Staphylococcus-aureus-GC subgroup A
Staphylococcus-muscae

Euclidean distance
Table Legends

**Table 1:** Antibiotic susceptibility: ZOI (Zone of inhibition) as measured (in mm) from the edge of disc to edge of inhibition zone (Kaushik, Kessel, Ratnayeke, & Gordon, 2015). Resistant (R) and susceptible (S) assigned based on previously defined breakpoints (Howe & Andrews, 2012). Below the breakpoints, a given isolate is resistant. Antibiotics with unrevised break points are indicated as dash (-). Experiments done in triplicate.

**Table 2:** Phenotypic characterization investigation using carbohydrate fermentation with the API CH50 systems (bioMe´rieux)

**Table 3:** Pairwise (ANI) and DDH between *S. mnemiopsis* AOAB (ANI2) and closely related species. Entries represent per cent values.

**Table 4:** Genome characteristics of *S. mnemiopsis* AOAB and close relatives.

**Table 5:** Antibiotic resistance genes from *S. mnemiopsis* AOAB (PATRIC Genome ID: 1279.166). Entries represent number of genes of the identified type.
Table 1

| Antibiotic                                      | ZOI 1 | ZOI 2 | ZOI 3 | Avg. | R/S | Breakpoint (mm) |
|------------------------------------------------|-------|-------|-------|------|-----|-----------------|
| Bacitracin 0.04 iu (A)                          | 6     | 6     | 6     | 6    | R   | >14             |
| Nalidixic acid 30 µg (NA30)                     | 8     | 6     | 7     | 7    | R   | >19             |
| Ciprofloxacin 5 µg (Cip5)                       | 13    | 15    | 14    | 14   | R   | >21             |
| Kanamycin 30 µg (K30)                           | 15    | 14    | 14    | 14.3 | R   | >18             |
| Penicillin (P10 iu)                             | 15    | 15    | 15    | 15   | R   | >25             |
| Ampicillin 10 µg (AM10)                         | 15    | 17    | 17    | 16.3 | R   | >26             |
| Vancomycin (VA 30 ug)                           | 19    | 18    | 18    | 18.3 | S   | >12             |
| Tetracycline 30 µg (TE30)                       | 24    | 23    | 23    | 23.4 | S   | >20             |
| Amoxicillin/Clavulanic acid 20/10 µg (AMC30)    | 24    | 23    | 28    | 25   | S   | >25             |
| Chloramphenicol 30 µg (C30)                     | 30    | 27    | 28    | 28.3 | S   | >15             |
| Novobiocin 30 µg (NB30)                         | 35    | 35    | 35    | 35   | S   | >16             |
| Oxacillin 1µg (OX1)                             | 20    | 19    | 18    | 19   | S   | >15             |
| Control: Polymyxin B ( PB300 iu)                | 6     | 6     | 6     | 6    | R   | NA              |

R=resistant; S=susceptible
Table 2

| Test/Characteristic | S. mnemiopsis | S. pasteur | S. warneri | S. hominis | S. lugdunensis | S. haemolyticus |
|---------------------|---------------|------------|------------|------------|----------------|----------------|
| GAL                 | ?             | +          | -          | +          | +              | +              |
| GLU                 | +             | +          | +          | +          | +              | +              |
| FRU                 | +             | +          | +          | +          | +              | -              |
| MNE                 | -             | -          | -          | -          | +              | -              |
| MAN                 | +             | +          | +          | -          | -              | -              |
| SOR                 | -             | -          | -          | -          | -              | -              |
| NAG                 | -             | -          | -          | +          | +              | -              |
| MAL                 | +             | +          | +          | +          | +              | +              |
| LAC                 | -             | -          | -          | +          | +              | ?              |
| SAC                 | +             | +          | +          | +          | +              | +              |
| TRE                 | +             | +          | +          | +          | +              | +              |
| DXYL                | ++            | -          | -          | ND         | ND             | ND             |

Only the tests with a positive result were included. GAL, D-galactose; GLU, D-glucose; FRU, D-fructose; MNE, D-mannose; MAN, D-mannitol; SOR, Sorbitol; NAG, N-acetylglucosamine; MAL, D-maltose; LAC, D-lactose SAC, Sucrose; TRE, D-trehalose; DXYL, D-Xylose.
Table 3

| Species                        | ANI1>2 | ANI2>1 | DDH  |
|-------------------------------|--------|--------|------|
| S. warneri SG1                | 85.02  | 84.93  | 27.80|
| S. pasteuri SP1               | 84.79  | 84.58  | 27.60|
| S. hominis C80                | 79.62  | 79.59  | 22.40|
| S. haemolyticus JCSC1435      | 79.52  | 79.31  | 22.60|
| S. saprophyticus ATCC 15305   | 78.03  | 77.66  | 21.90|
| S. aureus MRSA252             | 78.85  | 78.73  | 22.70|
| S. epidermidis ATCC 12228     | 79.4   | 79.36  | 19.00|
| S. lugdunensis HKU09-01       | 78.21  | 78.04  | 22.0 |
| S. hominis VCU122             | 79.62  | 79.59  | 22.40|
Table 4

| Feature                        | S. mnemiopsis | S. warneri SG1 | S. pasteuri SP1 |
|--------------------------------|---------------|----------------|-----------------|
|                                | **AOAB**      |                |                 |
| Size (bp)                      | 2,617,061     | 2,560,716      | 2,559,946       |
| % G+C                         | 32.13         | 32.73          | 32.71           |
| CDS                            | 2,695         | 2,435          | 2,457           |
| rRNA                           | 9             | 16             | 16              |
| tRNA                           | 59            | 60             | 48              |
| tmRNA                          | 1             | 1              | 1               |
| Hypothetical proteins          | 595           | 539            | 559             |
| Proteins functional assignments| 2,100         | 1,896          | 1,898           |
| Proteins with EC number assignments | 770      | 739            | 719             |
| Proteins with GO assignments   | 695           | 737            | 728             |
| Proteins with Pathway assignments | 630        | 579            | 559             |
| Proteins with FIGfam assignments | 2,294     | 2,214          | 2,194           |
| Kanamycin                      | resistant     | ND             | susceptible     |
| Novobiocin                     | susceptible   | susceptible    | susceptible     |
Table 5

| Gene                                           | Source       | PATRIC ID          | GO assignments | Highest sequence similarity | Organism                  |
|------------------------------------------------|--------------|--------------------|----------------|----------------------------|---------------------------|
| Multi antimicrobial extrusion protein, MATE family of MDR efflux pumps | ARDB         | fig|1279.166.peg.1539  | GO:0015559         | 93                        | *S. warneri* L37603        |
| Quinolone resistance protein norA               | ARDB / CARD  | fig|1279.166.peg.2125  | -              | 94                        | *S. warneri* L37603        |
| Undecaprenyl-diphosphatase                      | ARDB         | fig|1279.166.peg.858   | GO:0050380      | 96                        | *S. warneri* L37603        |
| Beta-lactamase                                  | ARDB / CARD  | fig|1279.166.peg.218   | GO:0008800      | 100                       | *S. aureus*                |
| Topoisomerase IV subunit A                      | CARD         | fig|1279.166.peg.1344  | GO:0003916      | 84                        | *S. aureus USA300_TCH1516 |
| Topoisomerase IV subunit B                      | CARD         | fig|1279.166.peg.1345  | GO:0003916      | 91                        | *S. aureus USA300_FPR3757 |
| Lipid A export ATP-binding / permease protein MsbA | CARD       | fig|1279.166.peg.2634  | -              | 90                        | *S. aureus* Mu50            |
| Two component system histidine kinase ArlS      | CARD         | fig|1279.166.peg.1493  | GO:0000155      | 82                        | *S. epidermidis* ATCC 12228 |
| Transcriptional regulator, MarR family | CARD | fig|1279.166.peg.350 | - | 92 | S. aureus JH1 |
| Translation elongation factor Tu | CARD | fig|1279.166.peg.2336 | - | 97 | S. aureus ED98 |
| DNA gyrase subunit A | CARD | fig|1279.166.peg.417 | GO:0003918 | 89 | S. epidermidis ATCC 12228 |
| Alkaline phosphatase synthesis transcriptional regulatory protein PhoP | CARD | fig|1279.166.peg.1439 | - | 86 | S. aureus MW2 |
| Putative response regulator ArlR | CARD | fig|1279.166.peg.1494 | - | 82 | S. aureus ED98 |
| Transcriptional regulator MgrA (Regulator of autolytic activity) | CARD | fig|1279.166.peg.861 | - | 93 | S. aureus Mu3 |
| DNA-directed RNA polymerase beta subunit | CARD | fig|1279.166.peg.594 | GO:0003899 | 97 | S. aureus USA300_TCH1516 |
| Mn-dependent transcriptional regulator MntR | CARD | fig|1279.166.peg.1106 | - | 83 | S. aureus MRSA252 |
| Beta-lactamase regulatory sensor-transducer BlaR1 | CARD | fig|1279.166.peg.217 | - | 99 | S. aureus |
| Beta-lactamase repressor BlaI | CARD | fig|1279.166.peg.216 | - | 100 | S. aureus USA300_TCH959 |