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Whole Genome Sequence Analyses of Antibiotic Resistance and Virulence Genes of *S. argenteus* ST2250 from Bovine Mastitis

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Abstract
Staphylococcus argenteus (S. argenteus) is novel specie within the S. aureus complex (SAC) that is noticeably becoming emerging pathogen both in humans and domesticated animals. Staphylococcus aureus (S. aureus) is one of the most prevalent causative agents of contagious bovine mastitis and it can develop resistance easily to almost all antibacterial agents. In the present study, whole genome sequence approach was used to explore the virulence and resistance potentials of S. argenteus ST2250 isolate from a bovine mastitis clinical case. The sequence type of the isolate was determined using the Genomic multi-locus sequence typing database. Antimicrobial resistance genes and virulence genes of the isolate were predicted using the Abricate software and virulence factors database respectively. A number of multiple antibiotic resistance genes and virulence determinants were revealed. Resistance genes identified were APH(3')-IIIa, Blaz, arlR, arlS, dfrG, Mep A, Mep R, Sav 1866, norA, tet(34), and tet(L).The virulence genes detected were closely associated with those found in S. aureus and are known to play critical role in Biofilm formation, synthesis of capsular proteins, adhesion, secretion, and iron-regulation. The virulent and resistome potentials of this emerging pathogen were revealed. Horizontal gene transfer may be the possible source of these genes. This emerging pathogen is of increasing public health importance due to its adoptability and ability to cause disease both animals and humans.

Keywords: Genome, Staphylococcus argenteus, Resisitome, Resistance genes, Virulence genes

INTRODUCTION
Staphylococcus aureus (*S. aureus*) is one of the most prevalent causative agent of contagious bovine mastitis and it can develop resistance easily to almost all antibacterial agents (Wang *et al*., 2015). Many staphylococcal strains have shown to be resistant to a number of multiple antibiotics and possess reduced susceptibility to these antibiotics (Akpaka *et al*., 2017). This pathogen is transmitted among dairy cows through contact with contaminated surfaces and milk which leads to subclinical mastitis that is mostly not difficult to detect at the early phase. Consequently, this causes serious economic losses to the dairy industry (Feng *et al*., 2016).

Staphylococcus argenteus (*S. argenteus*) is novel specie within the *S. aureus* complex (SAC) (Tong *et al*., 2015), and a lineage that has been recently recovered from non-human primates (Schaumburg *et al*., 2015) and bats (Akobi *et al*., 2012). This genotypically divergent lineage received formal taxonomic classification in 2014. Based on routine diagnostic bacteriological

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procedures, *S. argenteus* cannot be distinguished from *S. aureus*. Additionally, the PCR identification of the nuc gene, which is the standard confirmatory marker for the *S. aureus*, may also be positive in *S. argenteus* (Feng *et al*., 2017;Wakabayashi *et a*l., 2018) It has also been observed that traditional MLST primers used for typing *S. aureus* cannot amplify some multilocus sequence typing genes in *S. argenteus*. This may result in *S. argenteus* being excluded or misidentified as *S. aureus* (Feng *et al*., 2017). The colony of *S. argenteus* is characteristically silver in colour in contrast to the usual golden colour of *S. aureus* colony, this is due to the absence of carotenoid pigment staphyloxanthin, that is encoded by crtOPQMN (Suzuki *et al*., 2017; Holt *et al*., 2011). Many reports have earlier suggested that *S. argenteus* was associated with rural and indigenous communities (Tong *et al*., 2013;Tong *et al*., 2010). However, it is currently considered to be worldwide in distribution and recovered from both humans and domestic animals as shown by many different studies (Wakabayashi *et al*., 2018; Li *et al*., 2018; Shi & Zhang, 2018; Ohnishi *et al*., 2018; Aung *et al*., 2017; Moradigaravand *et al*., 2017; Chantratita *et al*., 2016; Thaipadungpanit *et al*., 2015; Tong *et al*., 2015).

Various genes are significantly involved in antimicrobial resistance. Characterizing these genes are key to understanding resistance epidemiology, studying non-susceptible phenotypes and investigating those strains that are resistant, when genes are not strong enough to be expressed *in vitro* (Zankari *et al*., 2012). Typically, resistance genes are detected using PCR or microarray (Aarestrup *et al*., 2008). However, it is compulsory to perform additional sequencing of the PCR products in most cases (Hasman *et al*., 2005). As such, it can be costly and time demanding to carry out complete characterization of resistance genes available in a strain collection (Zankari *et al*., 2012). The recent technological development as well the cost reduction of whole genome sequencing allows the efficacious detection of antimicrobial drug resistance genes and determinants of virulence in bacterial pathogens (Vélez *et al*., 2017). However, to date there are limited or no molecular studies on *S. argenteus* associated with bovine mastitis in Malaysia.

In the present study, we conducted whole genome analysis of the *S. argenteus* ST2250 from bovine mastitis using next generation sequencing (NGS), in order to explore its virulence potentials and antibiotic resistance genes.

MATERIALS AND METHODS

S. argenteus ST2250 Isolation

Staphylococcus argenteus ST22500 was obtained from a clinical mastitis milk sample at a dairy herd in Banting, Selangor Malaysia. Samples were collected in a sterile universal sample bottle following the procedure previously described by the National Mastitis Council (NMC) (Oliver, 2004). Samples were cultured on blood agar (Oxoid, Hampshire, England) to amplify bacterial growth. Cultured plates were incubated aerobically for 24 hours at 37 ^{0}C. The plates were then examined for growth; morphological features such as colony size, shape color, and hemolytic characteristics. *Staphylococcus* presumptive colonies were selected and sub cultured on blood agar and incubated aerobically at 37 ^{0}C for 24 hours to obtain pure culture. Following isolation, colonies were characterized based on Gram reaction, catalase and coagulase tests. Finally, analytical profile index (API) system (Biomerieux, France) was used to identify the isolates following manufacturer's guidelines.

Genomic DNA Extraction

Genomic DNA extraction from the cultured cells was carried out using the Wizard Genomic DNA extraction kit (Promega) following manufacturer's guidelines. The quality of the DNA

samples was determined using the NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA integrity was further checked using 1% agarose gel electrophoresis. To prepare the sample for sequencing, it was re-suspended in a vial of 30 ul distilled water (pH: 7.0-8.5), the vial was sealed with para-film and sent for sequencing at Institute of Bioscience, Universiti Putra Malaysia.

Genome Sequencing, Assembly and Annotation

Genomic DNA was sequenced on Illumina Miseq 2000 platform using Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) following manufacturer's protocols. During preparation for sequencing, the bacterial genomic DNA fragments were attached to the NGS sequencing adapters, which contains the anchoring site of the sequencing primers. Because adapters are attached at both ends, if the sequencing length is longer than the bacterial genomic DNA fragment, the sequencing will precede onto the sequencing adapter, resulting in sequencing reads containing the bacterial genomic DNA and the NGS sequencing adapter sequences. Thus, it is recommended that all sequencing reads be scanned for presence of NGS sequencing adapters, and for the adapter sequences to be removed while retaining the portion containing the bacterial genomic DNA. In summary, the sequencing reads obtained were scanned for adapter sequences and low quality sequences (where parts of the reads containing these sequences were removed). Short sequencing reads were discarded after adapter and quality trimming by using BBDuk software (BBTools version 36). The sequenced reads were *de novo* assembled using the SPAdes to obtain Contigs (Bankevich *et al*., 2012). For genome annotation, the *rRNA* genes were predicted using RNAmmer (Lagesen *et al*., 2007) while the *tRNA* genes were predicted using ARAGORN (Laslett & Canback, 2004). The protein-coding genes were first predicted using Prodigal (Hyatt *et al*., 2010), and the predicted sequences were used to predict their function by using BLAST (Camacho *et al*., 2009) and HMMER (Eddy, 1998) to search against various sequence or domain databases.

Multi-locus Sequence Typing

Multi-locus sequence typing was performed as described previously (Larsen *et al*., 2012). Annotated fasta sequence format of isolate was used to obtain the sequence type (STs) using the multi-locus sequence typing database of total sequenced bacteria provided by Center for Genomic Epidemiology, MLST v2.0 (**https://cge.cbs.dtu.dk/services/mlst/**).

Antimicrobial Resistance Genes Analyses

Genome antibiotic resistance analyses was performed as previously described by Zankari *et al*. (2012). The sequence reads of the assembled genome was BLASted against all the genes from the ResFinder database (**https://cge.cbs.dtu.dk/services/ResFinder/**) using Abricate software version 0.8 (which enables mass screening of Contigs for acquired antimicrobial resistance). The best matching genes were given as output. A gene is reported resistant when it covers at least 2/5 of the length of the resistant gene in the database (Zankari *et al*., 2012). The best- matching genes were identified using identity threshold as 100%.

Virulence factors Analysis

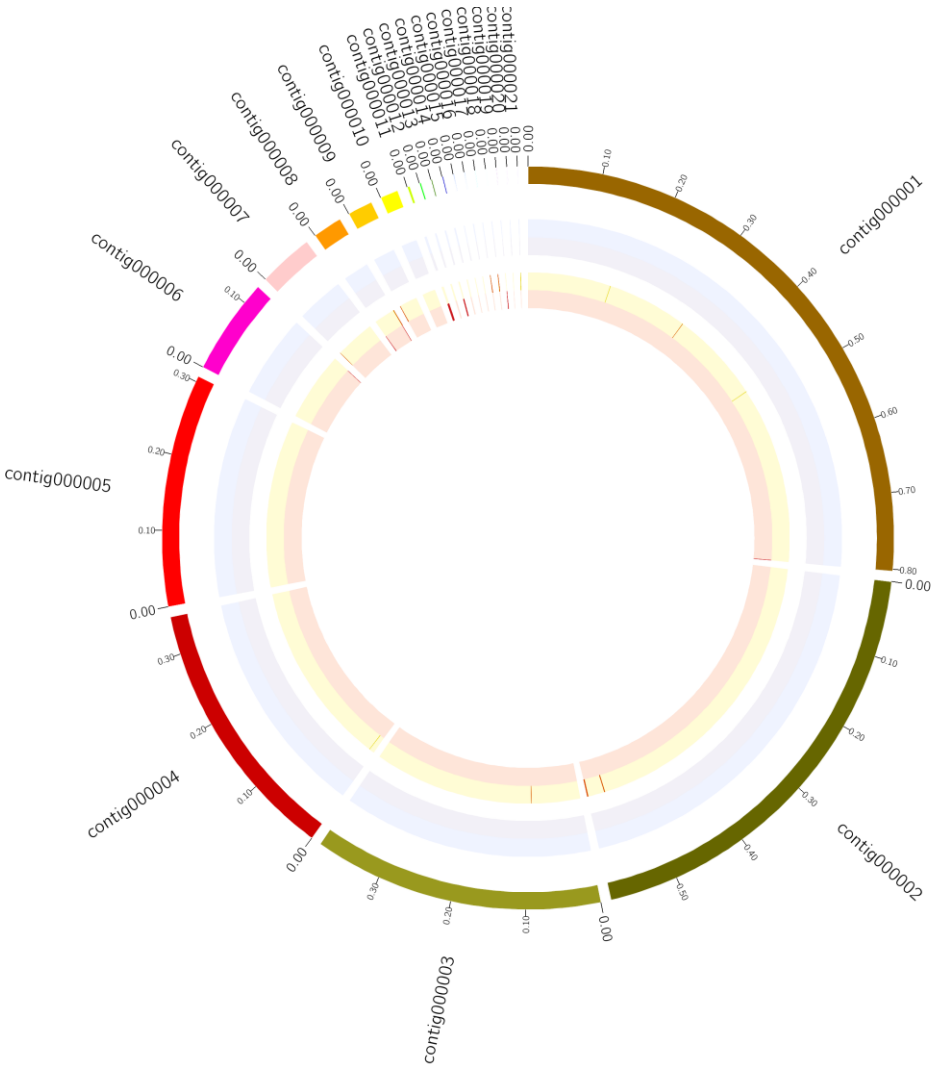
Analyses of Virulence factors was performed as previously described (Chen *et al*., 2005, Chen *et al*.,2016). Assembled sequence reads of *S. argenteus* genome were used for analyses of virulence factors using virulence factors database (VFdb) at **http://www.mgc.ac.cn/VFs.** The analysis was performed using BLASTN version 2.2.31+ at **ftp://ftp.ncbi.nlm.nih.gov/blast/.**

RESULTS

Genomic Features of *S. argenteus* ST2250

The *S. argenteus* ST2250 genome consists of a total of 1,322,858 bp (2x151) prior to quality control and trimming. The genome was assembled into 21 large Contigs, N50 of 591,279 bp, and a longest Contig size of 802,613 bp. The genome was approximately 2.7MB in size, with G+C content of 32.3%. The average coverage depth was 102.4X. For the genome annotation, the genome consists of 2520 protein-coding genes, 8 *rRNA* loci and 48 *tRNA*-coding genes.

The *S. argenteus* ST2250 genome was published in NCBI under the accession number **QLN00000000.1** at the following link: **https://www.ncbi.nlm.nih.gov/nuccore/QLNO00000000.1.** For the sequence reads, the data was deposited in the Sequence Reads Archive (SRA) in the following link **https://www.ncbi.nlm.nih.gov/sra/PRJNA476500.**



Antimicrobial resistance Genes in *S. argenteus* ST2250

Twelve antibiotic resistance genes were detected from the genome of *S. argenteus* ST2250. These include *APH(3')-IIIa, Blaz, arlR, arlS, dfrG, Mep A, Mep R, Sav 1866, norA, tet(34),* and *tet(L).*The description of antibiotics and mechanisms by which these genes act on are shown in Table 1.

Table 1. Detected antimicrobial resistance genes associated with *S. argenteus* ST2250

Resistance Gene	Description of antibiotics involved and mechanism of action
APH(3')-IIIa	Mediated resistance to Aminoglycosides resistance by antibiotic inactivation
Blaz	Coding for resistance to B-lactam antibiotics by antibiotic inactivation
arlR	Coding for multi-drug resistance by antibiotic efflux
arlS	Coding for multi-drug resistance by antibiotic efflux
dfrG	Coding for Trimethoprim resistance by antibiotic target replacement
Mep A	Coding for tetracycline antibiotics and Glycylcycline resistance by efflux mechanism
Mep R	Coding for tetracycline antibiotics and Glycylcycline resistance by efflux mechanism
Sav 1866	Coding for multiple antibiotic resistance by efflux mechanism
norA	Fluoroquinolone antibiotic and Acridine dye resistance by efflux pump mechanisms
mgrA	Peptide antibiotic, Penam, Cephalosporin, Acridindye, Fluoroquinolone antibiotic, Tetracycline antibiotic
tet(34)	Tetracycline antibiotic resistance by efflux pump
tet(L)	Tetracycline antibiotic resistance by antibiotic target protection

Virulence Genes Associated with *S. argenteus* ST2250

The various virulence genes harbored by this pathogen are shown in Table 2. They comprise of genes responsible for hemolysin factors, adhesion factors, Biofilm formation, synthesis of capsular proteins, iron- regulation proteins, Type VIII secretion system proteins and many others.

Table 2. List of predicted virulence factors associated with *S. argenteus* ST2250

Class of virulence factors	Virulence genes
Hemolysins	Gamma-hemolysin C *(hlgC)* Gamma-hemolysin B *(hlgB)* Delta-hemolysin *(hld)* Alpha-hemolysin *(hla)* Beta-hemolysin *(hlb)*
Adhesion factors	Clumping factor A *(clfA)* fibronectin-binding protein A *(fnbA)* Clumping factor B *(clfB)* Fibronectin- binding protein B *(fnbB)* Cell surface elastin binding protein *(ebp)* Ser-Asp rich fibrinogen- binding bone (*sdrC*) Ser-Asp rich fibrinogen- binding bone (*sdrD*) Ser-Asp rich fibrinogen- binding bone (*sdrE*)
Biofilm associated proteins	Intracellular adhesion protein C *(icaC)* Intracellular adhesion protein D *(icaD)* N-deacetylase (icaB)
Capsular biosynthesis proteins	*Cap8A, Cap8B, Cap8C, Cap8D, Cap8E, Cap8G, Cap8H, cap8I, Cap8J, Cap8K, Cap8L, Cap8M,Cap8N,Cap8O*
Iron-regulated proteins	*isdB,isdD,isdE,isdG*
Type VIII secretion system proteins	*essA,esxA,esaA,esaB,essB,essC*
Others	Leukocidin F *(lukF)* Pantom-valentine leukocidin chain F precursor (*lukF-PV)* Zinc metalloproteinase aureolysin (*aur*) Hyaluronate lyase precursor (*hysA*) Glycerol ester hydrolase (*geh*) Triacyleglycerol lipase precursor (*lip*) Adenosine synthase A (*adsA*) Staphylocoagulase precursor (*coa*) Stahpostatin B (*sspC*) Cestein proteinase (*sspB*) N-deacetylase (*icaB*) Secreted von Willebrand factor- bindind protein precursor(*vWbp*) ica operon transcriptional regulator IcaR (*IcaR*) N-aceyleglucosaminyltransferase (*icaA*)

DISCUSSION

Antibiotic resistance and virulence are of significant importance in clinical and public health settings. To the best of our knowledge, this is believed to be the first report on the resistance and virulence study on *S. argenteus* by whole genome sequencing in Malaysia. In this study, *S. argenteus* ST2250 demonstrated the possession of multiple antimicrobial resistance genes (Table 1). Antibiotic resistance genes in *S. aureus* Complex (SAC) have been reported elsewhere (Vélez *et al*., 2017; Yang *et a*l., 2016a; Liu *et al*., 2015; Gordon *et al*. 2014). This may be a clear indication that this pathogen may have acquired resistance genes as a result of indiscriminate use of antibiotics in the environment, and may be phenotypically resistant to most important antibiotics. Multiple drug resistance (MDR) defined as the tendency of pathogen to be insensitive to at least one antibiotic in three or more antibacterial groups (Ding *et al*., 2016).

Staphylococci harbor different virulence determinants that are responsible in causing diseases (Castro *et al*., 2018; Shi & Zhang, 2018). A particular virulent factor can play several functions in pathogenesis and many virulence determinants can play role in single function (Castro *et al*., 2018). The *S. argenteus* ST22500 in this study possess a large number of virulent factors similar to the ones traditionally known to be associated with *S. aureus* strains (Table 2). Studies have shown that *S. argenteus* has been known to contain homologs of all the virulence determinants coding for essential pathways in the pathogenesis of *S. aureus*, including icaA-D which encodes for intracellular adhesion protein D (important in Biofilm formation); *essA, esxA, esaA, esaB, essB, and essCI* coding for type VIII secretory system (Shi & Zhang, 2018).There were no genes responsible for both enterotoxins and exotoxins detected. This concurs with the fact that most of the virulence determinants not present in *S. argenteus* encodes for enterotoxins and exotoxins which are mostly located in mobile genetic elements and can easily be acquired or lost (Zhang *et al*., 2017). The *S. argenteus* ST22500 in this study was shown to harbour genes coding for Panton-Valentine Leukocidin (PVL). This agrees with the report that some of the *S. argenteus* isolates possess the PVL, justifying why this pathogen is able to cause clinical disease similar to that caused by *S. aureus*, including skin, soft tissue infection, abscess, bacterimia, pneumonia, sepsis, bone and joint infection (Thaipadungpanit *et al*., 2015; Tong *et al*., 2010). Hemolysins enhance the adherence of bacterial pathogens to host's epithelial tissues, other virulence enzymes (including hydrolase, lipase, protienase, deacetylase hyauluronate lyase) and non-enzyme like coagulase, help in promoting the bacterial escape from host immune response (Magro *et al*., 2017). The combinations of all these factors are central to the outcome of infection caused by *S. argenteus*.

CONCLUSION

This study provides the knowledge about the potential virulence and resistome associated with *S. argenteus* strains which are part of the *S. aureus* Complex (SAC). The likely source of these resistance genes may be from the horizontal gene transfer of mobile genetic elements. However, there is need to study the source of these resistance genes due to their potential threat to clinical and public health. Furthermore, the virulence potentials of this emerging pathogen should be of greater concern due to its easier adaptability to cause diseases in different animal species including humans.

REFERENCES

Aarestrup, F. M., Agerso, Y., Gerner-Smidt, P., Madsen, M., & Jensen, L. B. (2000). Comparison of antimicrobial resistance phenotypes and resistance genes in Enterococcus faecalis and Enterococcus faecium from humans in the community, broilers, and pigs in Denmark. *Diagnostic Microbiology and Infectious Disease*, *37*(2), 127–137. https://doi.org/10.1016/S0732-8893(00)00130-9

Akobi, B., Aboderin, O., Sasaki, T., & Shittu, A. (2012). Characterization of Staphylococcus aureus isolates from faecal samples of the Straw-Coloured Fruit Bat (Eidolon helvum) in Obafemi Awolowo University (OAU), Nigeria. *BMC Microbiology*, *12*(1), 279. https://doi.org/10.1186/1471-2180-12-279

Akpaka, P. E., Roberts, R., & Monecke, S. (2017). Molecular characterization of antimicrobial resistance genes against Staphylococcus aureus isolates from Trinidad and Tobago. *Journal of Infection and Public Health*, *10*(3), 316–323. https://doi.org/10.1016/j.jiph.2016.05.010

Aung, M. S., San, T., Aye, M. M., Mya, S., Maw, W. W., Zan, K. N., Htut, W. H. W., Kawaguchiya, M., Urushibara, N., & Kobayashi, N. (2017). Prevalence and genetic

characteristics of Staphylococcus aureus and Staphylococcus argenteus isolates harboring panton-valentine leukocidin, enterotoxins, and TSST-1 genes from food handlers in Myanmar. *Toxins*, *9*(8), 1–13. https://doi.org/10.3390/toxins9080241

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

Batchelor, M., Hopkins, K. L., Liebana, E., Slickers, P., Ehricht, R., Mafura, M., Aarestrup, F., Mevius, D., Clifton-Hadley, F. A., Woodward, M. J., Davies, R. H., Threlfall, E. J., & Anjum, M. F. (2008). Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. *International Journal of Antimicrobial Agents*, *31*(5), 440–451. https://doi.org/10.1016/j.ijantimicag.2007.11.017

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, *10*(1), 421. https://doi.org/10.1186/1471-2105-10-421

Castro, A., Silva, J., & Teixeira, P. (2018). Staphylococcus aureus, a Food Pathogen: Virulence Factors and Antibiotic Resistance. In *Foodborne Diseases* (Vol. 15). Elsevier Inc. https://doi.org/10.1016/B978-0-12-811444-5.00008-7

Chantratita, N., Wikraiphat, C., Tandhavanant, S., Wongsuvan, G., Ariyaprasert, P., Suntornsut, P., Thaipadungpanit, J., Teerawattanasook, N., Jutrakul, Y., Srisurat, N., Chaimanee, P., Anukunananchai, J., Phiphitaporn, S., Srisamang, P., Chetchotisakd, P., West, T. E., & Peacock, S. J. (2016). Comparison of community-onset Staphylococcus argenteus and Staphylococcus aureus sepsis in Thailand: A prospective multicentre observational study. *Clinical Microbiology and Infection*, *22*(5), 458.e11-458.e19. https://doi.org/10.1016/j.cmi.2016.01.008

Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., & Jin, Q. (2005). VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Research*, *33*(Database issue), D325-8. https://doi.org/10.1093/nar/gki008

Ding, Y., Zhao, J., He, X., Li, M., Guan, H., Zhang, Z., & Li, P. (2016). Pharmaceutical Biology Antimicrobial resistance and virulence-related genes of Streptococcus obtained from dairy cows with mastitis in Inner Mongolia, China Antimicrobial resistance and virulence-related genes of Streptococcus obtained from dairy cows wit. *Pezzuto Pharm Biol*, *54*(1), 162–167. https://doi.org/10.3109/13880209.2015.1025290

Eddy, S. R. (1998). Profile hidden Markov models. *Bioinformatics*, *14*(9), 755–763. https://doi.org/10.1093/bioinformatics/14.9.755

Feng, Y., Long-Hai, L., Ling, W., Xu-Rong, W., Li, X.-P., Jin-Yin, L., Zhe, Z., Zhang, S.-D., Zuo-Ting, Y., & Li, H.-S. (2017). ScienceDirect Penicillin-resistant characterization of Staphylococcus aureus isolated from bovine mastitis in Gansu, China. *Journal of Integrative Agriculture*, *16*(8), 1874–1878. https://doi.org/10.1016/S2095-3119(16)61531-9

Feng, Y., Qi, W., Xu-Rong, W., Ling, W., Li, X.-P., Jin-Yin, L., Zhang, S.-D., & Li, H.-S. (2016). ScienceDirect Genetic characterization of antimicrobial resistance in Staphylococcus aureus isolated from bovine mastitis cases in Northwest China. *Journal of Integrative Agriculture*, *15*(12), 2842–2847. https://doi.org/10.1016/S2095-3119(16)61368-0

Gordon, N. C., Price, J. R., Cole, K., Everitt, R., Morgan, M., Finney, J., Kearns, A. M., Pichon, B., Young, B., Wilson, D. J., Llewelyn, M. J., Paul, J., Peto, T. E. A., Crook, D. W., Walker, A. S., & Golubchik, T. (2014). Prediction of Staphylococcus aureus Antimicrobial Resistance by Whole-Genome Sequencing. *Journal of Clinical Microbiology*, *52*(4), 1182–

1191. https://doi.org/10.1128/JCM.03117-13

Hasman, H., Mevius, D., Veldman, K., Olesen, I., & Aarestrup, F. M. (2005). β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. *Journal of Antimicrobial Chemotherapy*, *56*(1), 115–121. https://doi.org/10.1093/jac/dki190

Holt, D. C., Holden, M. T. G., Tong, S. Y. C., Castillo-Ramirez, S., Clarke, L., Quail, M. A., Currie, B. J., Parkhill, J., Bentley, S. D., Feil, E. J., & Giffard, P. M. (2011). A very early-branching staphylococcus aureus lineage lacking the carotenoid pigment staphyloxanthin. *Genome Biology and Evolution*, *3*(1), 881–895. https://doi.org/10.1093/gbe/evr078

Hyatt, D., Chen, G.-L., LoCascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, *11*(1), 119. https://doi.org/10.1186/1471-2105-11-119

Lagesen, K., Hallin, P., Rødland, E. A., Stærfeldt, H.-H., Rognes, T., & Ussery, D. W. (2007). RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Research*, *35*(9), 3100–3108. https://doi.org/10.1093/nar/gkm160

Larsen, M. V, Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R. L., Jelsbak, L., Sicheritz-Pontén, T., Ussery, D. W., Aarestrup, F. M., & Lund, O. (2012). Multilocus sequence typing of total-genome-sequenced bacteria. *Journal of Clinical Microbiology*, *50*(4), 1355–1361. https://doi.org/10.1128/JCM.06094-11

Laslett, D., & Canback, B. (2004). ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Research*, *32*(1), 11–16. https://doi.org/10.1093/nar/gkh152

Li, Q., Li, Y., Tang, Y., Meng, C., Ingmer, H., & Jiao, X. (2018). Accepted Manuscript Prevalence and characterization of Staphylococcus aureus and Staphylococcus argenteus in chicken from retail markets in China. *Food Control*, *96*(August 2018), 158–164. https://doi.org/10.1016/j.foodcont.2018.08.030

Liu, C., Chen, Z.-J., Sun, Z., Feng, X., Zou, M., Cao, W., Wang, S., Zeng, J., Wang, Y., & Sun, M. (2015). Molecular characteristics and virulence factors in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate Staphylococcus aureus from central-southern China ScienceDirect. *Journal of Microbiology, Immunology and Infection*, *48*, 490–496. https://doi.org/10.1016/j.jmii.2014.03.003

Magro, G., Biffani, S., Minozzi, G., Ehricht, R., Monecke, S., Luini, M., & Piccinini, R. (2017). Virulence Genes of S. aureus from Dairy Cow Mastitis and Contagiousness Risk. *Toxins*, *9*(6), 195. https://doi.org/10.3390/toxins9060195

Moradigaravand, D., Jamrozy, D., Mostowy, R., Anderson, A., Nickerson, E. K., Thaipadungpanit, J., Wuthiekanun, V., Limmathurotsakul, D., Tandhavanant, S., Wikraiphat, C., Wongsuvan, G., Teerawattanasook, N., Jutrakul, Y., Srisurat, N., Chaimanee, P., Eoin West, T., Blane, B., Parkhill, J., Chantratita, N., & Peacock, S. J. (2017). Evolution of the Staphylococcus argenteus ST2250 Clone in Northeastern Thailand Is Linked with the Acquisition of Livestock-Associated Staphylococcal Genes. *MBio*, *8*(4), e00802-17. https://doi.org/10.1128/mBio.00802-17

Ohnishi, T., Shinjoh, M., Ohara, H., Kawai, T., Kamimaki, I., Mizushima, R., Kamada, K., Itakura, Y., Iguchi, S., Uzawa, Y., Yoshida, A., & Kikuchi, K. (2018). Purulent lymphadenitis caused by Staphylococcus argenteus, representing the first Japanese case of Staphylococcus argenteus (multilocus sequence type 2250) infection in a 12-year-old boy. *Journal of Infection and Chemotherapy*, *24*(11), 925–927. https://doi.org/10.1016/j.jiac.2018.03.018

Oliver, S. P. (2004). *Microbiological procedures for the diagnosis of bovine udder infection and determination of milk quality.* Verona, Wis. : NMC, 2004.

https://trove.nla.gov.au/work/35636994?q&versionId=44357531

Schaumburg, F., Pauly, M., Anoh, E., Mossoun, A., Wiersma, L., Schubert, G., Flammen, A., Alabi, A. S., Muyembe-Tamfum, J.-J., Grobusch, M. P., Karhemere, S., Akoua-Koffi, C., Couacy-Hymann, E., Kremsner, P. G., Mellmann, A., Becker, K., Leendertz, F. H., & Peters, G. (2015). Staphylococcus aureus complex from animals and humans in three remote African regions. *Clinical Microbiology and Infection*, *21*(4), 345.e1-345.e8. https://doi.org/10.1016/J.CMI.2014.12.001

Shi, X., & Zhang, D. F. (2018). Staphylococcus argenteus: an emerging foodborne pathogen? *Current Opinion in Food Science*, *20*, 76–81. https://doi.org/10.1016/j.cofs.2018.03.015

Thaipadungpanit, J., Amornchai, P., Nickerson, E. K., Wongsuvan, G., Wuthiekanun, V., Limmathurotsakul, D., & Peacock, S. J. (2015). Clinical and molecular epidemiology of Staphylococcus argenteus infections in Thailand. *Journal of Clinical Microbiology*, *53*(3), 1005–1008. https://doi.org/10.1128/JCM.03049-14

Tong, S. Y. C., Schaumburg, F., Ellington, M. J., Corander, J., Pichon, B., Leendertz, F., Bentley, S. D., Parkhill, J., Holt, D. C., Peters, G., & Giffard, P. M. (2015). Novel staphylococcal species that form part of a Staphylococcus aureus-related complex: the non-pigmented Staphylococcus argenteus sp. nov. and the non-human primate-associated Staphylococcus schweitzeri sp. nov. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *65*(Pt 1), 15–22. https://doi.org/10.1099/ijs.0.062752-0

Tong, Steven Y. C., Lilliebridge, R. A., Bishop, E. J., Cheng, A. C., Holt, D. C., McDonald, M. I., Giffard, P. M., Currie, B. J., & Boutlis, C. S. (2010). Clinical Correlates of Panton-Valentine Leukocidin (PVL), PVL Isoforms, and Clonal Complex in the *Staphylococcus aureus* Population of Northern Australia. *The Journal of Infectious Diseases*, *202*(5), 760–769. https://doi.org/10.1086/655396

Tong, Steven Y.C., Schaumburg, F., Ellington, M. J., Corander, J., Pichon, B., Leendertz, F., Bentley, S. D., Parkhill, J., Holt, D. C., Peters, G., & Giffard, P. M. (2015). Novel staphylococcal species that form part of a Staphylococcus aureus-related complex: the non-pigmented Staphylococcus argenteus sp. nov. and the non-human primate-associated Staphylococcus schweitzeri sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *65*(1), 15–22. https://doi.org/10.1099/ijs.0.062752-0

Vélez, J. R., Cameron, M., Rodríguez-Lecompte, J. C., Xia, F., Heider, L. C., Saab, M., McClure, J. T., & Sánchez, J. (2017). Whole-Genome Sequence Analysis of Antimicrobial Resistance Genes in Streptococcus uberis and Streptococcus dysgalactiae Isolates from Canadian Dairy Herds. *Frontiers in Veterinary Science*, *4*, 63. https://doi.org/10.3389/fvets.2017.00063

Wakabayashi, Y., Umeda, K., Yonogi, S., Nakamura, H., Yamamoto, K., Kumeda, Y., & Kawatsu, K. (2018). Staphylococcal food poisoning caused by Staphylococcus argenteus harboring staphylococcal enterotoxin genes. *International Journal of Food Microbiology*, *265*(October 2017), 23–29. https://doi.org/10.1016/j.ijfoodmicro.2017.10.022

Wang, D., Wang, Z., Yan, Z., Wu, J., Ali, T., Li, J., Lv, Y., & Han, B. (2015). Bovine mastitis Staphylococcus aureus: Antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant and methicillin-sensitive strains in China. *Infection, Genetics and Evolution*, *31*, 9–16. https://doi.org/10.1016/J.MEEGID.2014.12.039

Yang, F., Wang, Q., Wang, X. Rong, Wang, L., Li, X. Pu, Luo, J. Yin, Zhang, S. dong, & Li, H. sheng. (2016a). Genetic characterization of antimicrobial resistance in Staphylococcus aureus isolated from bovine mastitis cases in Northwest China. *Journal of Integrative Agriculture*, *15*(12), 2842. https://doi.org/10.1016/S2095-3119(16)61368-0

Yang, F., Wang, Q., Wang, X., Wang, L., Li, X., Luo, J., Zhang, S., & Li, H. (2016b). Genetic characterization of antimicrobial resistance in Staphylococcus aureus isolated from bovine mastitis cases in Northwest China. *Journal of Integrative Agriculture*, *15*(12), 2842–2847. https://doi.org/10.1016/S2095-3119(16)61368-0

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F. M., & Larsen, M. V. (2012). Identification of acquired antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy*, *67*(11), 2640–2644. https://doi.org/10.1093/jac/dks261

Zhang, D.-F., Zhi, X.-Y., Zhang, J., Paoli, G. C., Cui, Y., Shi, C., & Shi, X. (n.d.). *Preliminary comparative genomics revealed pathogenic potential and international spread of Staphylococcus argenteus*. https://doi.org/10.1186/s12864-017-4149-9