PROTEOMIC AND MOLECULAR ANALYSIS OF METHICILLIN RESISTANCE AND SELECTED TOXIGENIC GENES IN COAGULASE-NEGATIVE STAPHYLOCOCCUS SPP FROM FOOD AND ANIMAL SOURCES

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Racheal Aye

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Great Plains Institute of Food Safety

April 2010

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Proteomic and Molecular Analysis of Methicillin Resistance and Selected Toxigenic Genes

in Coagulase-Negative Staphylococcus spp. from Food and Animal Sources.

By

RACHEAL AYE

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

ABSTRACT

Aye, Racheal; M.S.; Great Plains Institute of Food Safety; College of Agriculture, Food Systems, and Natural Resources; North Dakota State University; April 2010. Proteomic and Molecular Analysis of Methicillin Resistance and Selected Toxigenic genes in Coagulase-negative *Staphylococcus* spp From Food and Animal Sources. Major Professor: Dr. Robert Barigye.

While most coagulase-negative *Staphylococcus* (CNS) are apathogenic, recent evidence suggests some food and animal derived CNS isolates may carry and express virulence factors including classical enterotoxins, toxic shock syndrome, and methicillin resistance genes. The present study was designed to assess the potential role of CNS in the epidemiology of foodborne illnesses and to determine the likelihood of food and domestic animals as transmission vehicles of methicillin resistance. Of the animal-derived food samples tested, 27.3% (39/143) were *Staphylococcus*-positive compared to only 9.5% (23/242) of the plant foods. A total of 92 *Staphylococcus* spp cultured from 385 food (62/92) and 30 diagnostic animal (30/92) samples were tested by the polymerase chain reaction (PCR) for classical enterotoxin (*sea, seb, sec, sed,* and *see*), toxic shock syndrome toxin-1 (*tsst-1*), and *mecA* genes. All PCR-positive isolates were further tested by immunoblotting for production of the corresponding toxin. Susceptibility patterns of both CNS and coagulase-positive *Staphylococcus* (CPS) were assessed for β-lactam antimicrobial agents and the isolates analyzed for presence of the *mecA* gene. Of all study isolates, 20/92 (21.7%) were CPS and 72/92 (78.3%) CNS. Of the 20 CPS isolates, 15.4% (2/13) *S. aureus* cultured from steak were *sec* positive

while only 1/7 (14.3%) CPS (*S. aureus*) from a diagnostic feline sample was positive for both *sec* and *tsst-1* genes. Both toxigenic *S. aureus* isolates from steak and a diagnostic feline sample also expressed detectable amounts of SEC and TSST-1 toxins. On the other hand, 1.4% (1/49) of the CNS (*S. lugdunensis*) from strawberries was positive for the *sec* gene but negative for SEC toxin and 2/49 (2.8%) of the CNS (*S. hominis*) gave unexpected base pair PCR products with *see* primers. All CNS isolates were generally susceptible to test β-lactam antimicrobial agents; 80% of the CPS isolates were resistant to penicillin and amoxicillin of which 1/20 (5%) were positive for the *mecA* gene. Based on this data, 3/20 (15%) of CPS and 3/72 (4.2%) of CNS isolates were positive for toxigenic genes thus underscoring the potential role food-derived CNS isolates may have in the epidemiology of foodborne illnesses. Although only 5% of the CPS and none of the study CNS isolates expressed *mecA* gene, 80% of the CPS were resistant to penicillin, suggesting other mechanisms of drug resistance. The presence of *mecA*-positive CPS and toxin producing *Staphylococcus* isolates underscore the public health significance of organisms from this genus.

ACKNOWLEDGMENTS

I thank God the Almighty for His grace and mercy. I am grateful to Dr. Robert Barigye, my major advisor, for his patience and wonderful mentorship. I enjoyed working with you. To the other graduate committee members, Dr. Charlene Hall, Dr. Penelope Gibbs and Dr. Glen Dorsam, thank you for walking this journey with me. To Heather Vinson and Arshi Reyaz, thank you for the help and support in the laboratory.

Last but not least, I thank all my friends for the friendship and laughter. Funding for this research was provided through the USDA/APHIS Biosurveillance Grant #FARG014465.

DEDICATION

To my parents for their love and support, I have come this far because you have always encouraged me to aim higher.

To my son Emmanuel, I am so blessed to have you in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Foodborne diseases have been estimated to cause about 9,000 deaths and between 6 to 81 million illnesses in the United States (US) annually (*5, 6, 17, 164*). Of all the foodborne illnesses caused by known pathogens, conservative estimates indicate that *Staphylococcus aureus* is responsible for about 185,000 cases in the US annually (*125*). In France, toxigenic *S. aureus* is the second most important cause of food poisoning after *Salmonella* (*98, 107*). Staphylococcal food poisoning (SFP) occurs following ingestion of at least 1.0 µg of preformed staphylococcal enterotoxin in food (*62*). Such a level of contamination is reached when bacterial populations exceed 100,000 staphylococci per gram of food (*62*). Foods frequently incriminated in SFP include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products such as cream-filled pastries; sandwich fillings; and milk and dairy products (*62*). Foods that require considerable handling during preparation, and/or those kept at slightly elevated temperatures after preparation are frequently involved in SFP (*62*).

Members of the genus *Staphylococcus* are ubiquitous. They are found in air, dust, sewage, water, milk and food in addition to food handling equipment, environmental surfaces. However, it is humans and animals that serve as primary reservoirs of this organism since *Staphylococcus* spp are widely found as normal flora on hair and skin, and in the nasal passages and throat of more than 50% of healthy individuals (*62, 127, 176*). In fact, the FDA report, 2009 (*62*) cites food handlers as the main source of food contamination in most SFP outbreaks. To a

lesser extent, equipment and environmental surfaces can also be significant sources of contamination (*62*).

Staphylococcus spp are non-motile Gram-positive cocci that are broadly divided into coagulase-positive *Staphylococcus* (CPS) and coagulase-negative *Staphylococcus* (CNS) sub-groups. *S. aureus,* the prototype under the CPS sub-group, is responsible for a number of human diseases including but not limited to food poisoning, skin infections, toxic shock syndrome and sepsis (*89, 155*). Although previously presumed non-pathogenic, evidence now suggests some CNS are important nosocomial pathogens (*3, 63, 97, 128*). In fact, a number of CNS strains have been recognized for their ability to elaborate toxins like: staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1 (TSST-1), hemolysins, coagulase (*85, 145, 152, 175*) and exfoliative toxins (*85, 108*) among others. More recently, a number of workers have reported enterotoxin and other toxigenic genes in CNS isolates from foods (*50, 186*). These findings underscore the potential the CNS have to cause food poisoning (*93*) and possibly other human diseases. Among the CNS with documented clinical significance, *S. epidermidis* is the most commonly isolated species (*63*). In fact, enterotoxin genes have been demonstrated in *S. epidermidis* plus several other CNS like *S. cohnii*, *S. xylosus* and *S. haemolyticus* (*13*). Waites et al. (1984) (*177*) isolated *S. warneri,* a CNS, along with other organisms from amniotic fluids of a 34-year-old pregnant woman that aborted in the mid-trimester of pregnancy (Waites et al. 1984) (*177*). Kamath et al (1992) (*97*) have also associated *S. warneri* with severe bacteremia and

endocarditis in immunocompromised patients (*97*). Recently, *S. warneri* was also isolated from a case of bovine abortion in which histological and bacteriologic evidence suggested potential virulence (*12*). Among other domestic animal species, *S. warneri* has been isolated from cerebrospinal fluids of a dog with a severe thrombotic meningoencephalitis (*59*). All these incidents suggest the potential virulence that may be expressed by various CNS.

Staphylococcus aureus (*9, 79, 185*) is increasingly becoming important due to methicillin resistance (*60, 61, 72*). Whereas majority of the methicillin resistant *S. aureus* (MRSA) cases have been associated with nosocomial and community acquired infections (*180*), current evidence suggests that food may be an important transmission vehicle for MRSA (*53, 114, 143*). Interestingly, a few reports of methicillin resistant CNS strains have also been cited (*1, 77, 93*). This justifies multidisplinary studies to determine the exact role CNS have in the epidemiology of troublesome foodborne staphylococcal diseases.

1.1. Hypothesis

i) Coagulase-Negative *Staphylococcus* from foods and domestic animals express virulence factors that include classical enterotoxins and toxic shock syndrome toxin-1 (TSST-1),

ii) Coagulase-Negative *Staphylococcus* from foods and domestic animal samples express the methicillin resistance gene.

1.2. Objectives

i) Use polymerase chain reaction (PCR) to screen study coagulase-negative *Staphylococcus* spp from food, and animal sources for genes that encode the five classical enterotoxins [*entA* (*sea*), *entB (seb), entC (sec), entD (sed),* and *entE* (*see*)], and toxic shock syndrome toxin-1 (*tsst-1*);

ii) Use immunoblotting analysis to screen study coagulase-negative *Staphylococcus* spp for classical enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1);

iii) Describe antimicrobial susceptibility patterns of the study *Staphylococcus* spp, and use PCR analysis to elucidate the incidence of methicillin resistance by determining the frequency of the *mecA* gene among coagulase negative *Staphylococcus* spp from food and domestic animal sources.

2. LITERATURE REVIEW

2.1. The Genus *Staphylococcus*

The genus *Staphylococcus* is composed of 41 species (*55*) primarily found on skin and mucous membranes of humans and animals. Member species are facultative anaerobes, catalase positive and oxidase negative Gram-positive cocci that frequently form grape-like clusters. *Staphylococcus* spp grow in the presence of bile salts, 6.5% salt solution and are resistant to Bacitracin. Generally, the members of the genus are classified into two major groups based on their ability to clot blood; the coagulase-positive *Staphylococcus* spp (CPS) produce the enzyme coagulase that can clot blood, while coagulase-negative *Staphylococcus* spp (CNS) do not produce coagulase and therefore fail to clot blood.

2.2. Staphylococcal Diseases

2.2.1. Staphylococcal Food Poisoning

Staphylococcal food poisoning (SFP) is caused by ingestion of preformed *Staphylococcus*-derived heat stable toxins in food (*18*). Clinical signs include abdominal cramps, nausea, and vomiting, which is sometimes followed by diarrhea (*107*). The onset of the symptoms is rapid but spontaneous recovery is observed within 24-48 hours. Production of enterotoxins by CPS is widely reported (*11, 16, 88, 153*). On the other hand, the few studies that have investigated enterotoxin production by CNS have given conflicting results (*48, 68, 77, 137, 149*). Some researchers have documented classical enterotoxins in CNS (*1, 77, 93*) while

others report unsuccessful efforts to detect such toxins (*91, 92, 103, 137, 141, 149*).

2.2.2. Other Staphylococcal Diseases in Humans

Staphylococcus spp affect all age groups but newborns and individuals with underlying diseases are most susceptible. *Staphylococcus* spp cause disease by two major mechanisms: direct tissue invasion and toxin production (*126*). The toxin-mediated diseases are caused by a number of exotoxins which may produce either localized or generalized lesions. Among the important staphylococcal diseases of humans are: *toxic shock syndrome* a serious disease reported in association with the use of high absorbent tampons during menstruation (*23*); *scalded skin syndrome* characterized by large cutaneous bullae and peeling of the epidermis in newborne babies; and superficial or deep skin infections resulting from direct tissue invasion (*126*). Cellulitis or focal and deep cutaneous abscesses are also commonly reported, and *Staphylooccus* spp are also implicated in burn-wound infections and in mastitis of nursing mothers. In the immunocompromised, *Staphylococcus* spp may cause bacteremia, meningitis and pneumonia (*126*). Because of great resistance to most antibiotics, *Staphylococcus* spp are greatly recognized for their role in hospital and community acquired MRSA infections.

2.2.3. Coagulase-Negative *Staphylococcus* in Human Disease

Although formerly regarded apathogenic, a number of virulent CNS have been reported (*25, 76*). In the US alone, CNS are estimated to cause about 50,000-120,000 nosocomial infections annually (*144*). Majority of the nosocomial

CNS infections in humans are associated with indwelling medical devices like catheters and artificial heart valves (*7, 39, 117*). According to a study reported by Archer, 1995 (*7*), *S. epidermidis* was the most frequently isolated CNS species from patients with indwelling medical devices. Even though CNS-associated bacteremias are seldom life threatening, frank sepsis may occur especially in immunocompromised patients (*74*). *S. epidermidis*, *S. warneri*, and *S. lugdunensis* have been associated with valvular endocarditis (*87, 156, 183*). *S. saprophyticus* often causes urinary tract infections in young females and women aged 16-35 years (*67*). Clinical signs include hematuria, pyuria, flank pain and pyelonephritis; sepsis and rarely endocarditis. Coagulase negative staphylococci are the second most common cause of postoperative infections especially in patients with implanted foreign material (*35*). Most problems are caused by the patients' own normal skin flora but infections contracted from operating room personnel have also been reported (*26*). For example, *S. epidermidis* from patient's own flora causes 15% of endophthalmitis cases following penetrating eye injury (*56*). Different studies have shown that CNS can express toxins similar to those elaborated by *S. aureus* (*50, 186*).

2.2.4. Staphylococcal Diseases in Animals

Staphylococcus aureus causes 25-30% of the mastitis cases in lactating domestic ruminants (*71*). In addition, *S. aureus* and other *Staphylococcus* spp have been reported in sporadic livestock abortions (*12, 45, 58*). In poultry, this agent causes important pathological conditions like dermatitis, osteomyelitis,

arthritis, synovitis and "Bamboo foot" (*29*). The latter is a localized foot lesion in domestic fowls and other birds that is almost exclusively caused by *S. aureus*. The latter is one of the major causes of leg weakness in broiler flocks (*71*). In pigs, exfoliative toxin producing *S. hyicus* strains are the cause of exudative epidermitis - also called *"greasy pig disease"* (*179*). Leukocidal toxin producing *S. intermedius* and staphylococcal enterotoxin have been associated with pyoderma in dogs (*49, 71*).

2.3. Staphylococcal Superantigens

2.3.1. Staphylococcal Enterotoxins

Staphylococcus aureus as well as many other enterotoxin-producing *Staphylococci* have been widely reported in SFP outbreaks (*9, 18, 85, 89, 107, 119, 155*). Strains of enterotoxigenic *Staphylococcus* are responsible for 185,000 cases of food-borne illness in the US each year (*125*). Five classical enterotoxins namely SEA, SEB, SEC, SED, and SEE have been reported (*54, 80, 90*). The enterotoxins are small peptides of about 23 to 29 kDa in size characterized by significant amino acid sequence similarities (*9*). The SEs are heat stable (*75, 80*) and are remarkably resistant to gastrointestinal proteases (*9*). Studies have shown that SEs may retain some biological activity after exposure to 121°C heat for 28 min (*4*). Staphylococcal enterotoxins belong to a family of pyrogenic or superantigenic toxins (*9*) that cause nonspecific T-cell activation and proliferation ("superantigen activity"). The latter is a pathophysiological mechanism that is associated with emesis fever, and immunosuppression (*9*). The superantigenic and

emetic activities are two separate biological functions that are localized on different domains of the SE molecule (*54*). The linkage between the two activities remains unclear (*107*).

2.3.1.1. *Staphylococcus* Enterotoxin A (SEA). The SEA toxin is a 27.1 kDa protein (*22*) that causes about 77.8% of all the SFP outbreaks (*32, 78*). Carried on a temperate bacteriophage, the *sea* gene (*21, 24*) is composed of 771 base pairs and encodes a 257 amino acid precursor protein (*84*). The *sea* gene is not regulated by the "accessory regulator" (*agr*), and is expressed during the mid-exponential phase of growth (*165*). Once expressed, the nascent toxin molecule undergoes posttranslational processing to yield the monomeric and biologically functional proteinaceous toxin. *Staphylococcus* enterotoxin A has a zinc ion coordination site involved in MHC class II binding (*9*). Three SEA isoforms with different isoelectric points have been described (*151*).

2.3.1.2. *Staphylococcus* Enterotoxin B (SEB). The SEB toxin is a 28 kDa protein that is encoded by the chromosomal *seb* gene (*69*). In other *Staphylococcus* strains, however, the *seb* gene is carried by a 750 kb plasmid (*154*). The toxin is initially translated as a 267 amino acid precursor that requires posttranslational modification to enhance its potency (*69*). The incidence of SEB intoxication is unknown because in most cases the symptoms are mild and patients do not seek treatment (*69*). In addition to food poisoning, SEB has also been associated with a non-menstrual toxic shock syndrome (*33*). SEB has also been studied as a potential biological warfare agent because it is stable, can easily

be aerosolized and can cause widespread systemic damage and death when inhaled at very high dosages (*169*).

2.3.1.3. *Staphylococcus* Enterotoxin C (SEC). There are 3 sub-types of the SEC toxin: SEC1, SEC2 and SEC3 (*20*). These are highly conserved proteins that show significant immunological cross-reactivity (*20*). SEC3 differs from SEC1 and SEC2 by four and nine amino acids, respectively (*9*). The *sec3* gene has 801 nucleotides and encodes a 267 amino acid precursor protein (*81*). Ninety percent of the *sec3* nucleotide sequences are similar to those of *sec1* (*46*). The similarities between the genes that encode the three toxin subtypes suggest that an ancestral *sec1*-like gene was formed by recombination between the *sec3* and *seb* genes (*46*). Different animal species have unique and host-specific *sec* receptors (*118*) implying that heterogeneity is due to selection for modified *sec* sequences that facilitate the survival of *S. aureus* in their respective hosts (*9*).

2.3.1.4. *Staphylococccs* Enterotoxin D (SED). Enterotoxin D is the second most common SE that is associated with SFP outbreaks (*14*). The *sed* gene is located on a 27.6 kb penicillinase plasmid (*14*) and encodes a 258 amino acid protein (SED). The 258 amino acids include a 30-amino acid signal peptide and a 228 amino- acid mature polypeptide (*14*) with a high degree of sequence similarity to other SEs (*161*). The SED toxin demonstrates high affinity for zinc ions for its interaction with MHC class II molecules (*161*).

2.3.1.5. *Staphylococcus* Enterotoxin E (SEE). The SEE is a 26-kDa protein that is encoded by the *see* gene (*47*). The toxin shares about 81% sequence homology with SEA (*171*).

2.3.2. Toxic Shock Syndrome Toxin-1 (TSST-1).

The TSST-1 is a superantigenic toxin that causes non-specific T-cell activation and proliferation (*124*). Subsequently, the TSST-1-induced T-cell activation leads to clinical toxic shock syndrome (TSS), a systemic syndrome characterized by fever, hypotension, myalgia, rash, and multiple-organ failure (*124*). Toxic shock syndrome is associated with staphylococcal colonization of the vagina, and other sites like the respiratory tract or following surgical procedures (*23, 54*). *In vitro* studies have shown that TSST-1 production is dependent on environmental factors such as a low partial pressure of oxygen (ppO_2), and high partial pressure of carbon dioxide ($ppCO_2$). Other risk factors include concentration of iron, pH, and temperature (*23*). The incidence of TSS is low, approximately 1.05 per 100,000 menstruating women (*37*). Unlike most of the superantigen genes discussed above, the *tsst-1* gene is chromosomal in location (*18, 85*).

2.3.3. Non-Classical Superantigens

A number of new superantigens have been described (*64, 90, 105, 110, 135, 138, 139, 140, 184*). Of the new toxins, only those that induce emesis in a simian model, like SEG, SEH and SEI, have been classified as enterotoxins (*129, 160*). Those that lack emetic activity are classified as "*staphylococcal enterotoxin-*

like (SEI) superantigens" and include: SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER and SEU (*139*). All SE and SEI have superantigen activity (*123, 142*).

2.4. Methicillin Resistance

2.4.1. Epidemiology

Methicillin resistant *S. aureus* (MRSA) is resistant to most if not all β-lactam antibiotics (*42, 43, 166*). About 80-90% of penicillin-resistant CPS are typically *S. aureus* (*36*). However, some reports indicate that about 65% of CNS isolated from bacteremias are resistant to oxacillin (*10*). From epidemiological viewpoint, MRSA cause two major forms of disease: "hospital" (*41*) and "community" (*40*) acquired MRSA infection. Hospital-acquired –MRSA (HA-MRSA) has been recognized for decades (*41*) and primarily affects people in healthcare settings, such as patients that have recently had surgery, or those with surgically implanted medical devices (*41*). Infections caused by MRSA also tend to affect the elderly, immunocompromised, and patients undergoing kidney dialysis (*8*). In 2005, a total of 94,360 people in the US developed invasive MRSA (*39*) and about 18,650 of them died during a hospital stay (*39*). A recent study demonstrated that 1% of all in-patient stays per year (292,045 cases) were associated with staphylococcal infection. Sadly, 14,000 of these patients died during the same period. Infection with staphylococci increased the length of hospital stay, cost of treatment by about 3 times, and 5 times the risk of in-hospital death (*104*). Community-associated MRSA (CA-MRSA) has an elusive origin because it affects people who do not have an apparent association with a healthcare setting. Skin infections

caused by CA-MRSA are spread in crowded settings (*39, 100*). Symptoms of cutaneous MRSA infection include red, swollen, and painful skin lesions that may drain pus; more severe form of infection may lead to chest pain, cough, malaise, fever, bradycardia, muscle aches, fever and headache (*157*).

2.4.2. Food as a Transmission Vehicle of Methicillin Resistance

In 2002, an outbreak of SFP reported in Tennessee State was traced to a MRSA strain originating from a food handler that had apparently contaminated shredded pork and colestraw sold at a convenience store (*95*). During investigations done shortly after the outbreak, *sed*-positive MRSA was cultured from the food handler in question and from the ill patrons that had consumed the contaminated food (*95*). In another report, 5% of the meat samples tested during a study contained MRSA (*143*). Although the MRSA isolated from the meat samples was of human origin, other studies have demonstrated MRSA can be zoonotic (*52, 113, 159, 178, 181*). The few reports reviewed above underscore the potential threat posed by foodborne MRSA infections (*102*), and therefore warrant investigations on the role of food-derived CNS in the epidemiology of methicillin resistance.

2.4.3. The *mecA* Gene

Resistance to methicillin is encoded by the *mecA* gene which is located on the chromosome and is regulated by two other genes, namely *mec1* and *mecR1* (*42*). Methicillin and other β-lactam antibiotics have a structure similar to that of "penicillin-binding protein" (PBP). The latter is necessary in the transpeptidation of

the bacterial cell wall (*121*). Normally, β-lactam antibiotics irreversibly bind to PBP thereby disrupting cell wall synthesis. This results in cell death due to lack of cell wall integrity. In *S. aureus,* three PBPs (PBP 1, PBP 2 and PBP 3) are usually used in the synthesis of the cell wall. Methicillin resistant staphylococci possess a supplementary PBP termed PBP2' or PBP2a. Coupled with the presence of *mecA* gene, these species are able to grow in the presence of methicillin and other β-lactam antibiotics (*101, 162*). Although *mecA* resistance is found in all cells of an intrinsically resistant population, the gene may only be expressed in a small percentage of cells a phenomenon called "heterogeneous resistance" (*121*). Research done by van Griethuysen et al. (2005) (*172*) has shown that the *mecA* gene can be lost with time following storage at -80 °C.

3. MATERIALS AND METHODS

3.1. Food and Diagnostic Animal Samples

Sample collection for the study was done from April to October 2009. The sample size was determined using the *"Method of Proportions"* (*66*): where "*n*" is the sample size, "*Z*" is the *Z* value for the corresponding confidence level (1.96 for 95% confidence; "*e*" is the margin of error (.05 = ± 5%); "*p*" is the estimated value for the proportion of *Staphylococcus* spp that express the toxins of interest (50%). Substituting these figures in the formula below, a sample size of 385 food samples was derived.

$$n = \frac{Z^2 \, p \, (1 - p)}{e^2}$$

Food samples were bought two times a week from two selected stores in Fargo, North Dakota, U.S.A. The stores were chosen on the basis of proximity to the research laboratory and because they provided a steady supply of study food samples. Food samples bought were categorized into foods of animal origin including chicken, beef, pork, sausage, cheese and mushrooms; and food of plant origin including grapes, strawberries, salsa, salad and carrots. Samples were kept at 4° C and analyzed within 12 hours of purchase.

Diagnostic animal samples were obtained from the North Dakota State University Veterinary Diagnostic Laboratory (NDSU-VDL), Fargo, North Dakota, U.S.A. The sampling for these samples was somewhat biased since most of the

study *Staphylococcus* isolates were cultured from diagnostic samples taken from dead animals.

3.2. Study and Experimental Control *Staphylococcus* spp

A total of 62 *Staphylococcus* spp from food samples were analyzed during the study. The other 30 study *Staphylococcus* isolates came from samples of companion and food animal tissues submitted to the NDSU-VDL. Seven reference ATCC *S. aureus* strains carrying the *sea* (ATCC # 13565), *seb* (ATCC # 14450), *sec-1 (ATCC #19095), sed-1* (ATTC # 23235), *see* (ATCC # 27664), *tsst-1* (ATCC # 51650) and *mecA* (ATCC # 43300) genes were used as positive experimental controls.

3.3. Food Sample Preparation

Food samples were washed with 250 mL sterile phosphate buffered saline, pH 7.4 (PBS) for 10 min. and 25 mL of the PBS washings added to 10 mL of Giolitti - Cantoni broth, an enrichment media for the isolation of *Staphylococcus* spp from food. The tubes were incubated at 37 °C for 18-24 hours with shaking at 200 × rpm. Two loopfulls of 10 µl inoculating loop were streaked on Baird Parker Agar; a *Staphylococcus* selective medium. The plates were incubated at 37 °C for 18-24 hours after which single colonies were streaked onto blood agar plates and further incubated at 37 °C for 12-18 hrs.

3.4. Identification of *Staphylococcus* spp

Suspect *Staphylococcus* colonies were identified based on colony morphology (white-yellow, smooth, and small colonies) and Gram staining

characteristics. Briefly, for the Gram's staining technique, the inoculating loop was sterilized by flaming, cooled in a drop of sterile water held on a clean glass slide, and then used to pick a suspect colony from the blood plate. A smear was made, air dried and heat fixed, after which it was flooded with crystal violet and allowed to stand for 1 min. The smears were then gently rinsed with distilled water, flooded with Gram's iodine solution and allowed to stand for 1 min. The glass slides were then rinsed with distilled water, decolorized for 10 sec in the decolorizer solution after which the smears were washed thoroughly with distilled water, followed by flooding with safranin stain. After 1 min, the slides were rinsed gently with distilled water, blot dried with absorbent paper, and examined under the oil immersion objective lens (100x) of the light microscope: confirmation of the *Staphylococcus* species was done using the sensititre system. The identified species were cryo-preserved in 600µl of 50% glycerol at -70 °C.

3. 5. Coagulase Test

Two mL of rabbit serum were inoculated with a colony of the test *Staphylococcus* spp and the tube incubated at 37 °C for 24 hours. During this time, the tubes were examined for coagulation every 1 hour for the first 6 hours. The final results were read after 24 hours.

3.6. DNA Extraction

Single bacterial colonies were inoculated in 40 µL of 99% 1×TE and 1% Proteinase K. DNA was extracted using Single Cell Lysis Buffer (SCLB) protocol (55 °C and 80 °C for 10 min, and 20 °C until retrieved for testing) on the

thermocycler. Following DNA extraction, each sample was diluted with 80 µL of ddH_2O.

3.7. Polymerase Chain Reaction

The PCR assay used primers (see below) designed at Trilink Biologicals Inc. San Diego CA based on sequences of the 5 classical enterotoxin genes (*sea, seb, sec-1, sed-1,* and *see*), and toxic shock syndrome toxin (*tsst-1*) genes initially reported by Johnson et al. ,1991 (*94*). Twenty five µL PCR reactions were set up each containing 5 µL of 5× buffer, 0.2 µL dNTP, 0.25 µL each forward and reverse primers, 0.125 µL DNA polymerase, 17.175 µL water and 2 µL DNA. The PCR was run on a thermocycler program: 94 °C for 5 min; 30 cycles of 94 °C for 2 min, 55 °C for 1 min, and 72 °C for 1 min; 72 °C for 7 min and 20 °C forever. Primers to the methicillin resistance gene *mecA* were also designed at Trilink Biologicals Inc. San Diego CA, and the PCR assay done based on the protocol described by Thomas *et al.* (2006) (*163*). Twenty-five µL tubes of PCR mixture were made as the enterotoxins above and run in the thermocycler program 95 °C for 2 min; 30 cycles of 95 °C for 30s, 50 °C for 30 sec, and 72 °C for 30 sec; 72°C for 3 min; and 20 °C until retrieved for testing. PCR products were visualized in 1.5% agarose gels stained with ethidium bromide. Briefly, gels were stained in diluted ethidium bromide (500 µL EBr: 500 mL of water) for 30 min and then destained in distilled water for 15 min.

sea gene:

- Forward primer: 5' TTGGAAACGGTTAAAACGAA 3'
- Reverse primer: 5' GAACCTTCCCATCAAAAACA 3'

- ***seb*** gene:
  - Forward primer: 5' TCGCATCAAACTGACAAACG 3'
  - Reverse primer: 5' GCAGGTACTCTATAAGTGCC 3'
- ***sec-1*** gene:
  - Forward primer: 5' GACATAAAAGCTAGGAATTT 3'
  - Reverse primer: 5' AAATCGGATTAACATTATCC 3'
- ***sed-1*** **gene:**
  - Forward primer: 5' CTAGTTTGGTAATATCTCCT 3'
  - Reverse primer: 5' TAATGCTATATCTTATAGGG 3'
- ***see*** **gene:**
  - Forward primer: 5' TAGATAAAGTTAAAACAAGC 3'
  - Reverse primer: 5' TAACTTACCGTGGACCCTTC 3'
- ***tsst-1*** **gene:**
  - Forward primer: 5' ATGGCAGCATCAGCTTGATA 3'
  - Reverse primer: 5' TTTCCAATAACCACCCGTTT 3'
- ***mecA*** **gene:**
  - Forward primer: AAAATCGATGGTAAAGGTTGGC
  - Reverse primer: AGTTCTGCAGTACCGGATTTGC

3.8. Proteomic Analysis of Study *Staphylococcus* spp

3.8.1. Reactivation of the *Staphylococcus* Isolates

Individual study *Staphylococcus* isolates were removed from cryopreservation, inoculated on blood agar plates and incubated for 18 h at 37 °C. The next day, 3 colonies from each isolate were reinoculated in15 mL tubes containing 3 mL of LB broth. The cultures were grown for 18 h at 37 °C with continuous shaking at 200 × rpm.

3.8.2. Extraction of *Staphylococcus* Protein

Bacterial cells were harvested from *Staphylococcus* cultures by centrifugation at 10,000 ×g for 10 min at 4 °C., the supernatants discarded and the pellet subjected to two washings in sterile phosphate buffer saline (SIGMA), pH 7.2 (PBS) containing 1mM of the protease inhibitor phenylmethylsulphonyl fluoride (PMSF, Amresco, Solon, OH). For each wash, the pellet was resuspended in 2 mL of PBS/1mM PMSF, the mixture vortexed and centrifuged at 10,000 ×g for 20 min at 4 °C. After the second wash, the pellet was resuspended in 150 µL of SoluLyse™ Bacterial Protein Extraction Reagent (Genlantis, SanDiego, California), followed by a 10 min incubation on a rocking platform at room temperature (rT). This was followed by centrifugation at 14,000 ×g for 5 min at rT, after which the supernatant containing the soluble protein fraction was transferred into a clean tube. The pellet constituting the insoluble protein fraction was resuspended in 300 µL SoluLyse™ Reagent and added to the soluble protein fraction. Protein concentration was determined by the NanoDrop ND-1000 Spectrometer.

3.8.3. Determination of Protein Concentration

Using the software Protein A280, protein concentration was determined with NanoDrop ND-1000 Spectrometer. The instrument was initialized with 1 µL of SoluLyse™ Bacterial Protein Extraction Reagent. The protein concentration was determined by loading 1 µL of sample.

3.9. Sodium Dodecyl Polyacrylamide Gel Electrophoresis

Proteins extracted from *Staphylococcus* spp were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Twenty µg of extracted protein from each of the study isolates were diluted 1:2 in sample buffer (625 Mm Tris-HCl, pH 6.8 [vol/vol]; 10% glycerine [vol/vol]; 5% SDS [wt/vol]; 0.002% bromophenol blue [wt/vol] plus 10% β-mercaptoethanol [vol/vol]. Samples were boiled for 4 min at 95 °C, cooled and loaded onto a 12% Tris-HCl precast polyacrylamide gel (Bio-Rad Laboratories, Inc., Hercules, CA). Two µL of Precision Plus Protein Standards, dual colored molecular weight marker (Bio-Rad Laboratories, Inc., Hercules, CA) were loaded in a separate lane to permit determination of the apparent molecular weight of interest protein. To fractionate the bacterial proteins, SDS-PAGE was carried out in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) at 150 V at constant voltage for 2 hours or until the bromophenol blue dye front reached the bottom of the separating gel. Following fractionation, the separated proteins were visualized by staining the gels with Silver Stain Plus (Bio-Rad Laboratories, Inc.. Hercules, CA). This was performed as described in the manufacturer's protocol. The gel was incubated in silver staining

kit 'fixative enhancer solution' [50% methanol (vol/vol), 10% acetic acid (vol/vol), 10% fixative enhancer concentrate (vol/vol), 30% ddH_2O (vol/vol)] for 20 min at rT with gentle agitation. After fixing the gels, they were washed twice with 400 mL ddH_2O for 10 min. The gels were then reacted with fresh 'staining solution' [5% silver complex solution (vol/vol), 5% reduction moderator solution (vol/vol), 5% image development reagent (vol/vol), 50% development accelerator solution (vol/vol), 35% ddH_2O]. After the desired staining intensity was attained, the reaction was stopped by adding 100 mL of 'stop solution' [5% acetic acid (vol/vol)] at rT for 5 min with gentle agitation. Finally, the gels were washed twice with ddH_2O for 5 min and scanned (HP Scanjet G3010, CA, USA).

3.10. Immunobloting

Following SDS-PAGE, the *Staphylococcus* proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane. This was done in a wet transfer cell (Bio-Rad Laboratories, Inc., Hercules, CA) using transfer buffer (25 Mm Tris, 192 mM gylcine, 20% [vol/vol] methanol, pH 8.3) at 100 V for 1 hr, at rT. The PVDF was incubated overnight at 4 °C in blocking solution (5% Nonfat dry milk, 10% TBS with 0.2 M Tris, 1.37 M NaCl, pH 7.6) with gentle agitation. The following day, the membrane was washed in 10% TBS (0.2 M Tris, 1.37 M NaCl, pH 7.6) plus 0.2% Tween 20 (TBS-Tween), and then reacted with 20 mL diluted primary antibody (anti-*Staphylococcus* enterotoxin mouse monoclonal antibody diluted 1:80,000 Antibody buffer; anti-*Staphylococcus* tsst-1 mouse monoclonal antibody diluted 1:1600 Antibody buffer) for 2 hr at rT. The membrane was then washed

with TBS-Tween and reacted for 1 hr with diluted secondary antibody (goat anti-mouse IgG ALP 1; 10,000 [Abcam, Inc., Cambridge, MA]) at rT with gentle agitation. After incubation with secondary antibody, the membrane was washed three times with TBS-Tween, and then developed with premixed BCIP/NBT substrate solution (Bio-Rad Laboratories, Inc., Hercules, CA) according to the manufacturer's instructions. After obtaining the desired background, the reaction was stopped by washing 2 times with 5% acetic acid for 5-10 min and then air dried at rT.

3.11. Statistical Analysis

The chi square test was done at a p-value of 0.0001 using EpiInfor 4.0

4. RESULTS

4.1. *Staphylococcus* Isolates

The plant-derived foods tested during the study include: salads (n=34), grapes (n=60), carrots (n=28), mushrooms (n=29), strawberries (n=29) and salsa (n=62), while foods of animal origin are: cheese (n=28), chicken (n=29), beef (n=29), pork (n=27), and sausages (n=30). Overall, 18.0% (n=62) of all the 385 study food samples were *Staphylococcus*-positive, and 82.0% (n=323) were *Staphylococcus*-negative (Table 1). In summary, of the 385 food samples tested, 62.9% (n=242) were of plant origin and 37.1% (n=143) were of animal origin (Table 1).

Food samples	***Staphylococcus* negative samples**	***Staphylococcus* positive samples**
Plant origin	219	23
Animal origin	104	39
	n= 323 (82%)	**n= 62 (18%)**

Table 1. Total number of food samples tested during the study (n=385).

A statistically significant difference was found with (x^2=21.01, p=<0.0001) in the proportion of *Staphylococcus* positive samples in foods of animal origin 26.3 %(39/143) and plant origin 9.5 % (23/242).

Of the foods of plant origin, mushrooms yielded the highest number of *Staphylococcus* isolates (n=13). Two isolates came from strawberries, 4 from grapes, 2 from salads, 1 from carrots and 1 from salsa (Table 2).

Food type	*Staphylococcus* negative	*Staphylococcus* positive	*Staphylococcus* isolates
Salad	32 (94.1%)	2 (5.9%)	*S. heamolyticus* (1); *S. hyicus* (1)
Grapes	56 (93.3%)	4 (6.6%)	**S. aureus* (2); *S. warneri* (1); *S. xylosus* (1)
Carrots	27 (96.4%)	1 (3.6%)	*S. hyicus* (1)
Mushrooms	16 (55.2%)	13 (44.8%)	*S. epidermidis* (1); *S. hyicus* (2); *S. sciuri* (5); *S. xylosus* (5)
Strawberries	27 (93.1%)	2 (6.9%)	*S. lugdunensis* (1); *S. saprophyticus* (1)
Salsa	61 (98.4%)	1 (1.6%)	*S. warneri* (1)

Table 2. The different *Staphylococcus* spp cultured from foods of plant origin (n= 242). *- Coagulase-positive *Staphylococcus*.

A statistically significant difference was found between the proportion of *Staphylococcus* positive mushrooms and the other food of plant origin, however, there was no significant statistical difference among the other foods of plant origin (Table 3).

Of the foods of animal origin, on the other hand, 40.7% (n=11) of the pork, 37.9% (n=11) of the beef, 37.9% (n=11) of the chicken, 10.7% (n=3) of the cheese, and 10% (n=3) of the sausage samples were *Staphylococcus*-positive (Table 4).

Food Type	*Staphylococcus* positive isolates	No. of samples	95% Confidence Interval (CI)
Salad	5.9% (2)	34	0.72-19.68
Grapes	6.6% (4)	60	1.85-16-20
Carrots	3.6% (1)	28	0.90-18.35
Mushrooms	**44.8% (13)**	**29**	**26.45-64.31**
Strawberries	6.9% (2)	29	0.85-22.77
Salsa	1.6% (1)	62	0.04-8.66

Table 3. Comparison of the proportion of *Staphylococcus* positive isolates among food of plant origin.

Food type	*Staphylococcus* negative samples	*Staphylococcus* positive samples	*Staphylococcus* isolates
Cheese	25 (89.3%)	3 (10-7%)	*S. saprophyticus* (3)
Chicken	18 (62.1%)	11 (37.9%)	**S. aureus* (4); *S. hominis* (3); *S. xylosus* (1); *S. hyicus* (3).
Beef	18 (62.1%)	11 (37.9%)	**S. aureus* (4); *S. warneri* (3); *S. ureolyticus* (1); *S. epidermidis* (3)
Pork	16 (59.3%)	11 (40.7%)	**S. aureus* (3); *S. warneri* (4); *S. mitis* (2); *S. hominis* (1); *S. scheleferi* (1)
Sausage	27 (90%)	3 (10%)	*S. warneri* (1); *S. hyicus* (1); *S. saprophyticus* (1)

Table 4. *Staphylococcus* spp cultured from foods of animal origin (n= 143).

*- Coagulase-positive *Staphylococcus.*

A statistically significant difference was found between the proportion of *Staphylococcus* positive cheese and the proportion of *Staphylococcus* positive beef, chicken and pork. Similarly, a statistically significant difference was found between the proportion of *Staphylococcus* positive sausage and the proportion of *Staphylococcus* positive beef, chicken and pork. However, there was no significant statistical difference between pork, beef and chicken (Table 5).

Of the 62 *Staphylococcus* spp identified in the study, 13 were CPS and 49 were CNS.

	Cheese	Chicken	Beef	Pork	Sausage
Cheese		**6.91**	**6.91**	**6.53**	0.12
		(0.009)*	**(0.009)***	**(0.011)***	(0.732)
Chicken			0.00	0.05	**6.36**
			(1.000)	(0.830)	**(0.012)***
Beef				**0.05(0.830)**	**6.36(0.012)***
Pork					**7.25**
					(0.007)*
Sausage					

Table 5. Comparison of the proportion of *Staphylococcus* positive isolates among foods of animal origin. An asterisk denoted a p-value that has a statistically significant difference.

4.2. *Staphylococcus* spp from Diagnostic Animal Samples

A total of 30 *Staphylococcus spp* isolated were cultured from diagnostic animal samples submitted to the NDSU-VDL. The animal species studied were bovine (n=7), pig (n=10), dog (n=4), cat (n=7), goose (n=1) and deer (n=1) (Table 6). Of the 30 *Staphylococcus* isolates studied, 7 isolates were CPS and the other 23 were CNS.

Animal species	No. of isolates	*Staphylococcus* isolates
Bovine	7	*S. intermedius* (3); *S, hyicus* (1); *S. xylosus* (2); *S. warneri* (1).
Pig	10	*S. hyicus* (5); *S. saprophyticus* (1); *S. heamolyticus* (1); *S. xylosus* (1); *S. warneri* (1); **S. aureus* (1)
Dog	4	**S. intermedius* (2); *S. warneri* (1); *S. intermedius* (1)
Cat	7	**S. lentus* (1); **S. aureus* (2); *S. felis* (1); *S. xylosus* (1); *S. hyicus* (1); *S. intermedius* (1)
Goose	1	**S. gallinarum* (1)
Deer	1	*S. hyicus* (1)

Table 6. The different *Staphylococcus* spp cultured from diagnostic animal samples (n=30). *- Coagulase-positive *Staphylococcus.*

4.3. Coagulase Test Results

Of the 72 CNS isolates, 18 were *S. hyicus*, 13 were *S. warneri*, 11 were *S. xylosus*, 6 were *S. saprophyticus*, 5 were *S. sciuri*, 3 were *S. intermedius*, *S. hominis* and *S. epidermidis*, 2 were S. heamolyticus, 1 S. scheleferi, S. lentus, *S. gallinarum*, *S. ludgnensis*, *S. mitis*, *S. ureolyticus*, and *S. felis* respectively. Overall,

Staphylococcus hyicus was the most frequently isolated *Staphylococcus* (19.6%) from all the samples. Other species isolated include: *S. aureus* (17.4%), *S. warneri* (15.2%), *S. xylosus* (12.0%), *S. saprophyticus* (6.5%), *S. sciuri* (5.4%), *S. intermedius* (5.4%), *S. epidermidis* (4.3%), *S. hominis* (4.3%), *S. heamolyticus* (2.2%), *S. scheleferi (1.1%), S. mitis (1.1%), S. ludgnensis (1.1%), S. ureolyticus* (1.1%), *S. lentus (1.1%), S. gallinarum (1.1%)* and *S. felis* (1.1%) (Figure 1).

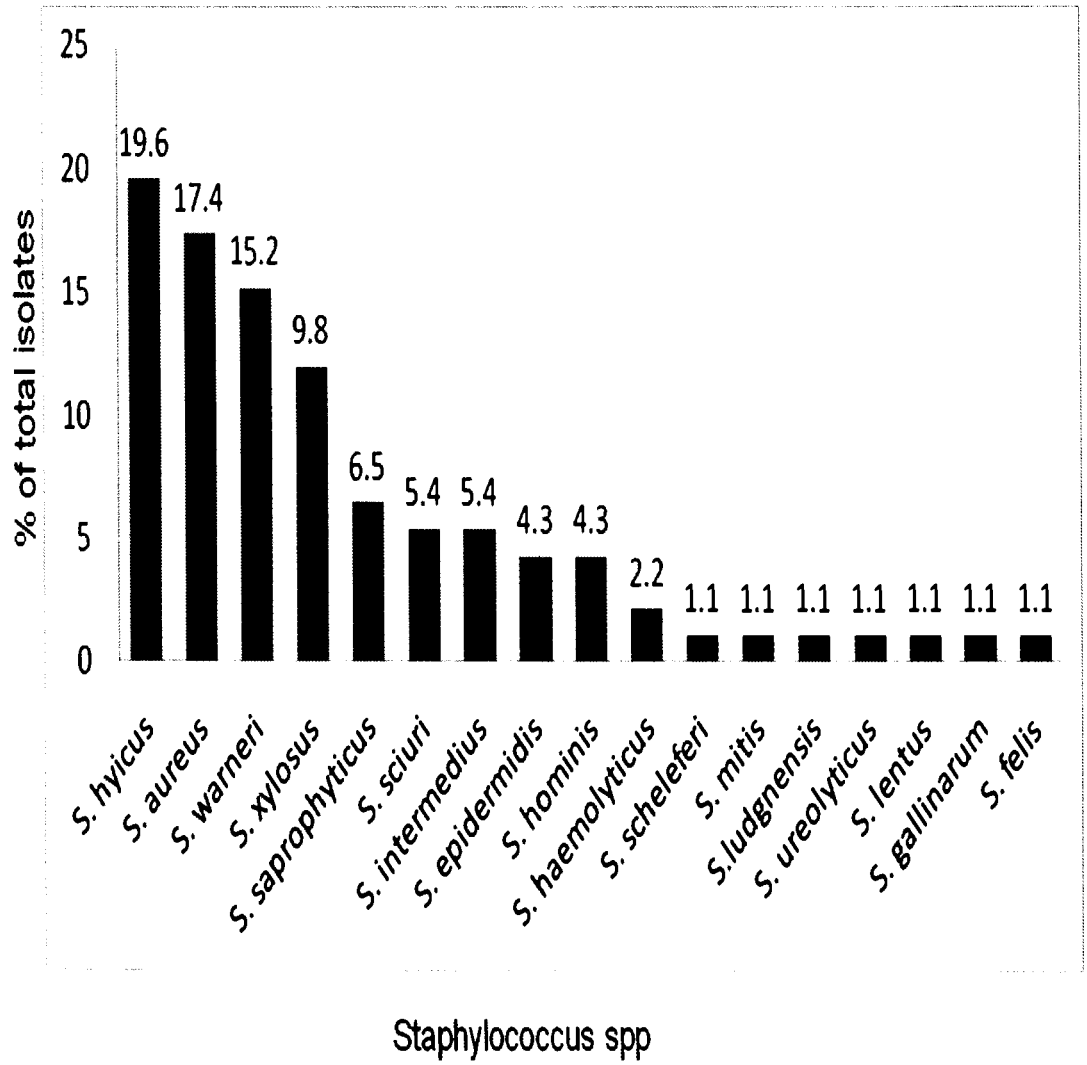


Figure 1. Study *Staphylococcus* isolates (n=92)

Of the 92 *Staphylococcus* isolates, 21.7% (n=20) were CPS while 78.3% (n=72) were CNS. Of the 20 CPS isolates, 16 were *S. aureus* (2 from diagnostic cat samples, 1 from pig, 4 from pork, 4 from chicken, 3 from beef, and 2 from grapes). The other CPS were 2 isolates of *S. intermedius* from diagnostic dog samples, 1 *S. gallinarum* from a diagnostic goose sample and 1 *S. lentus* from a diagnostic cat sample (Tables 2, 4 and 6).

4.4. PCR Analysis of *Staphylococcus* Toxin Genes and Immunoblot Analysis

Staphylococcus spp were separated into two groups based on coagulase test results after which they were divided into batches of 13 and analyzed by the PCR assay. Isolates that were positive for enterotoxin genes by PCR were further analyzed by immunoblotting for the expression of the corresponding toxigenic protein. For clarity, the study isolates from food samples (n=62) were analyzed separately from the diagnostic samples (n=30). Of the 13 CPS isolates from food samples, 15.4% (n=2) were positive for the *sec* gene (Figure 2a) and SEC toxin (Figure 2b). The two *sec*-positive isolates were both *S. aureus* cultured from steak procured from a grocery store in Fargo, ND.

One CPS isolate from a cat diagnostic sample also tested positive for *sec* gene (Figure 3a) and SEC toxin (Figure 3b). The same diagnostic sample was also positive for the *tsst-1* gene (Figure 4a) and TSST-1 toxin (Figure 4b).

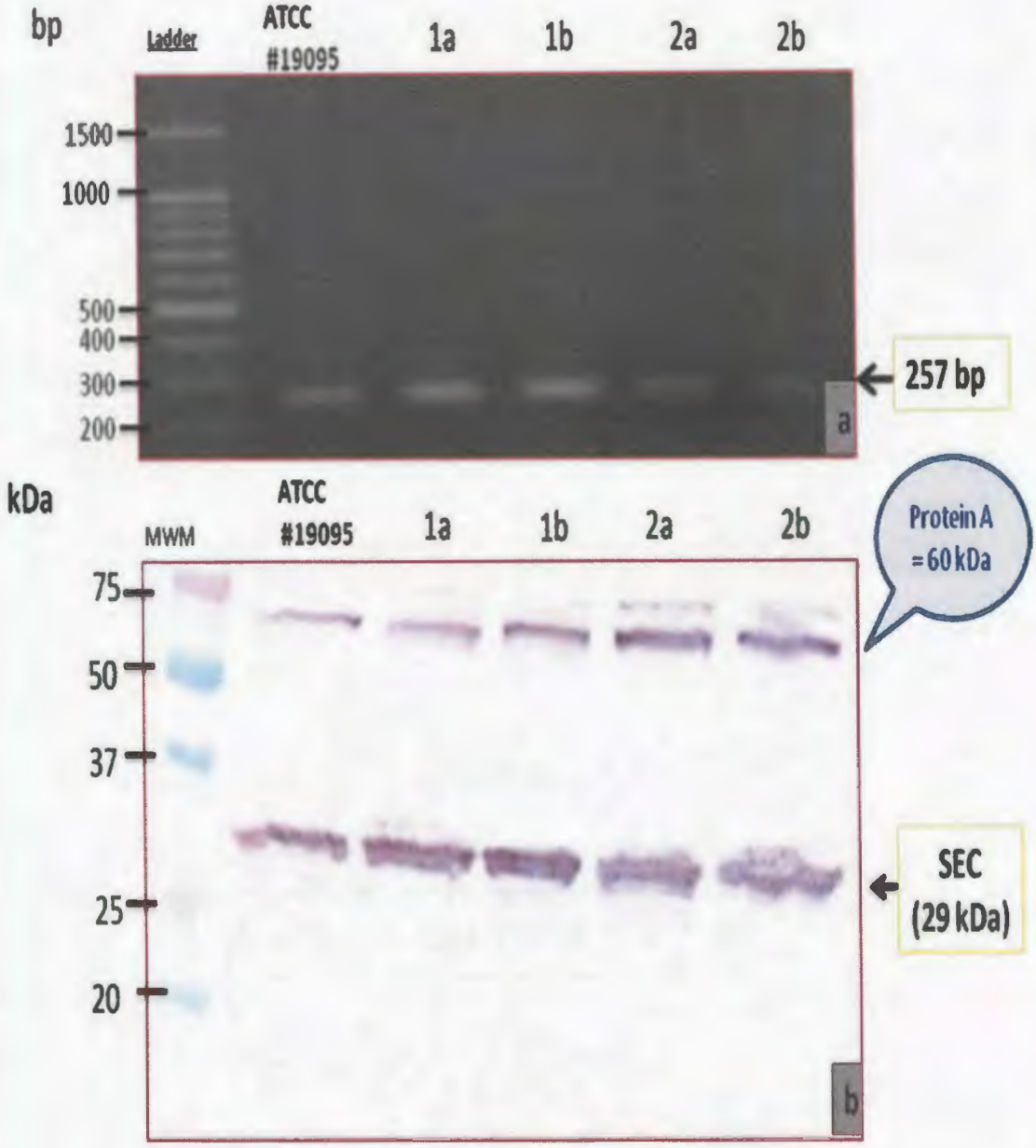


Figure 2. Two coagulase-positive *Staphylococcus* isolates of food origin were positive for the *sec* gene (Fig. 2a). Both isolates also expressed the corresponding protein toxin (Fig. 2b). In addition, all *S. aureus* isolates included in the immunoblot above expressed a prominent 60 kDa band interpreted on the basis of molecular weight and immunoreactivity to be Protein A.

***Legend**: Ladder- Promega DNA ladders #G210A; MWM- Bio-rad # 161-0374 (Precision plus protein standard dual color); S. aureus ATCC #19095- (sec positive control); 1a and 1b- S. aureus (steak); 2a and 2b- S.aureus (steak).*

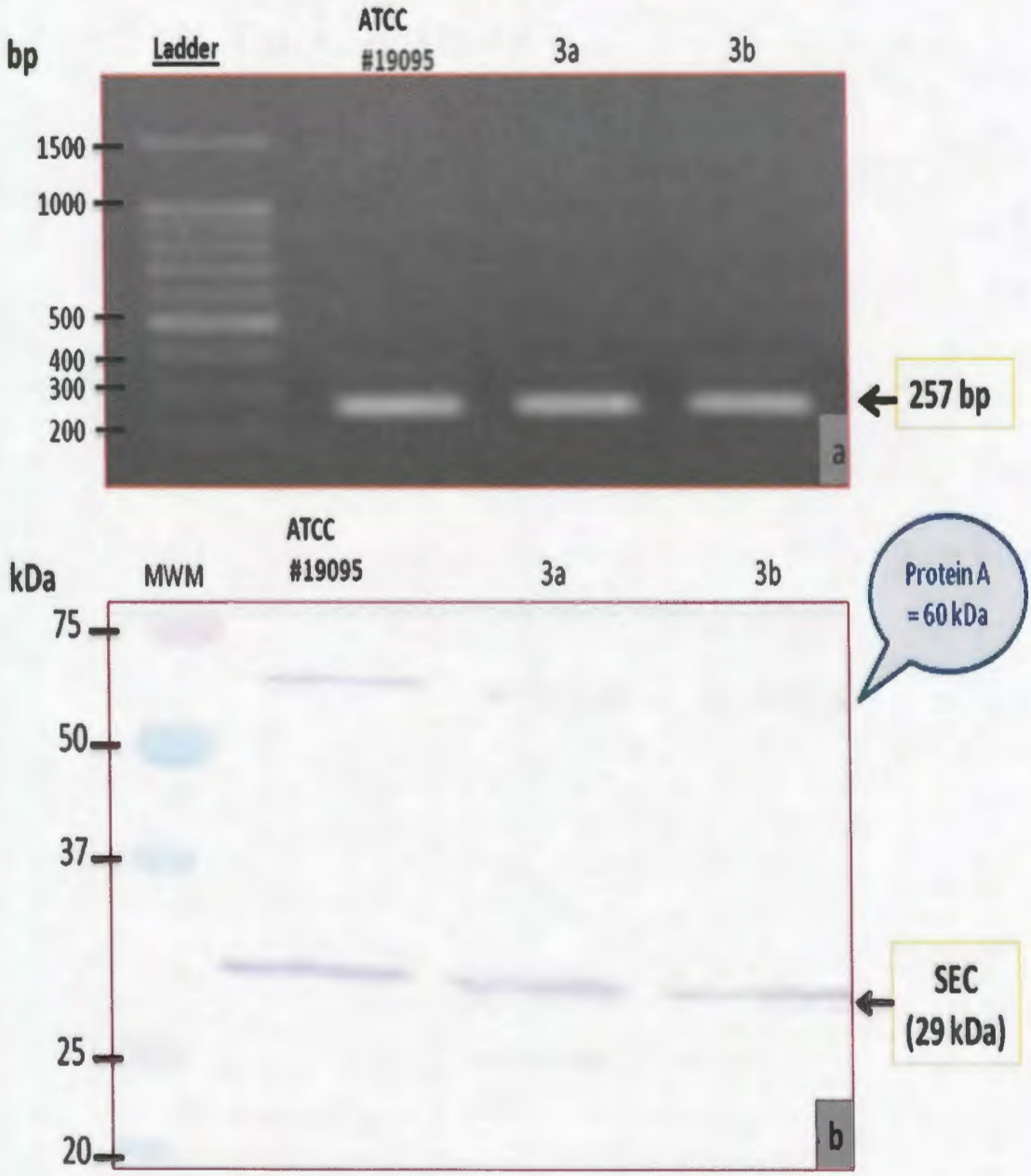


Figure 3. A coagulase-positive *Staphylococcus* isolate cultured from a diagnostic cat sample was positive for the *sec* gene (Fig. 3a) and the corresponding SEC toxin (Fig. 3b).

Legend: *Ladder- Promega DNA ladders #G210A; MWM- Bio-rad # 161-0374 (Precision plus protein standard dual color); S. aureus ATCC # 19095- sec; 3a and 3b- S. aureus (feline diagnostic sample).*

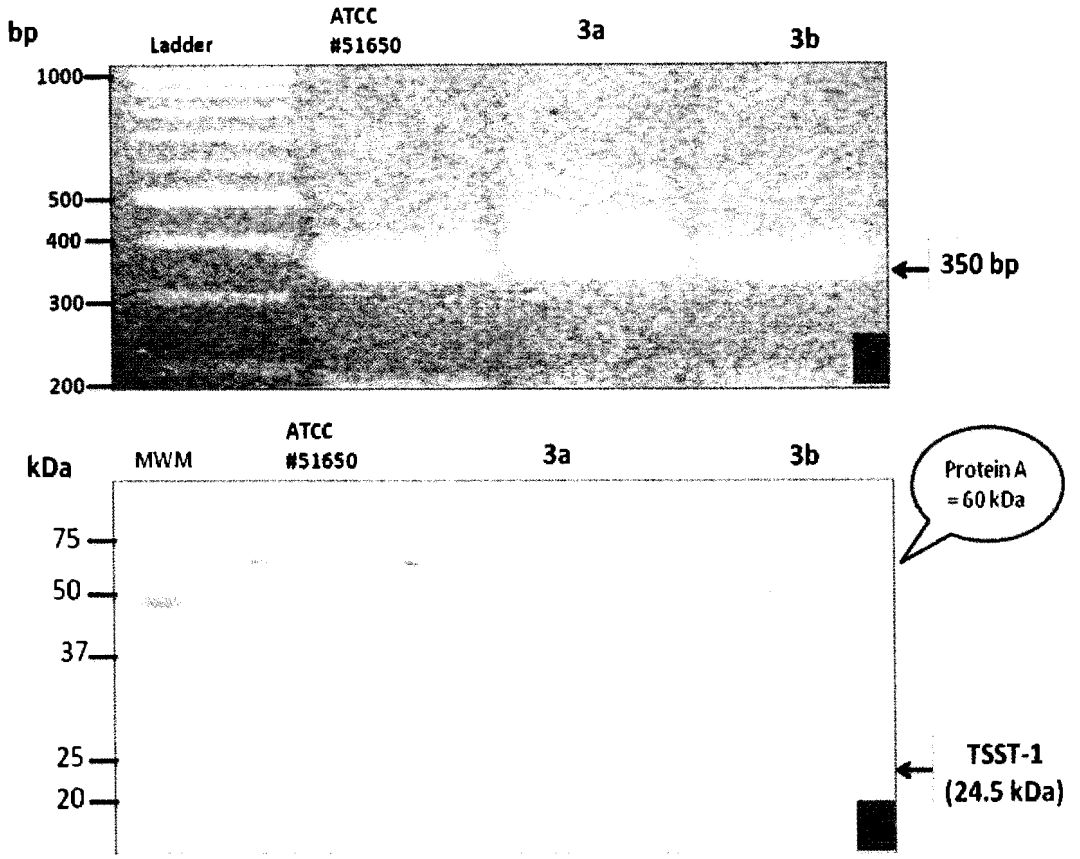


Figure 4: A coagulase-positive *Staphylococcus* isolate cultured from a diagnostic cat sample was positive for the tsst-1 gene (Fig. 4a) and the corresponding SEC toxin (Fig. 4b).

Legend: *Ladder- Promega DNA ladders #G210A; MWM- Bio-rad # 161-0374 (Precision plus protein standard dual color); S. aureus ATCC # 51650- tsst-1; 3a and 3b- S. aureus (diagnostic cat sample).*

Of the 72 CNS isolates, 1 *S. lugdunensis* isolate cultured from strawberries tested positive for the *sec* gene (Figure 5a) but was negative for the SEC toxin (Figure 5b), while 2 *S. hominis* isolates showed unexpected base pair products with primers designed against the *see* gene (Figure 6a) and were likewise negative for the corresponding SEE toxin (Figure 6b).

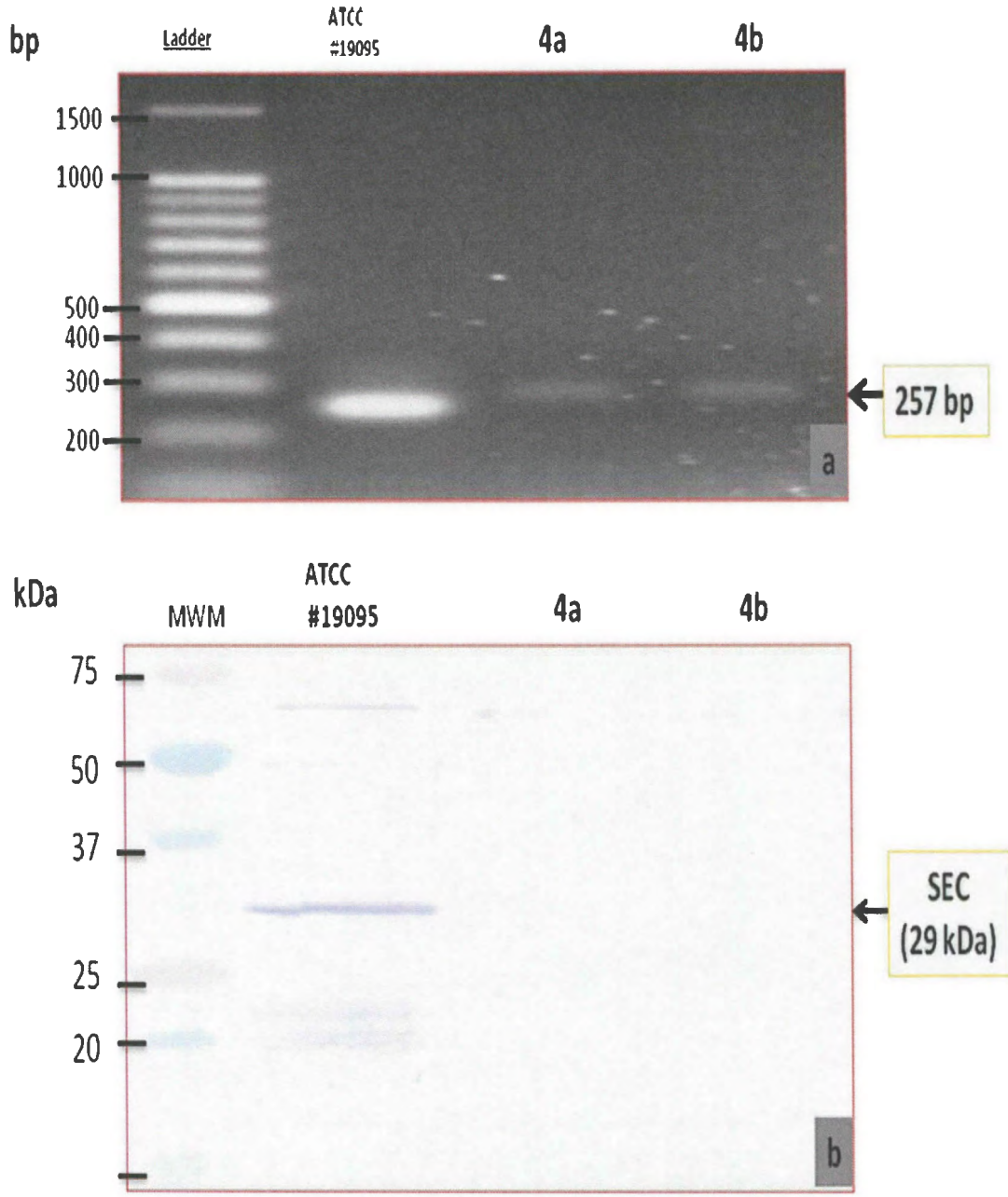


Figure 5. A coagulase-negative *Staphylococcus* isolate cultured from strawberries was positive for the *sec* gene (Fig. 5a) but negative for the SEC toxin (Fig. 5b).

Legend: *Ladder- Promega DNA ladders #G210A; MWM- Bio-rad # 161-0374 (Precision plus protein standard dual color); S. aureus ATCC # 19095- entC; 4a and 4b- S. lugdunensis (strawberries).*

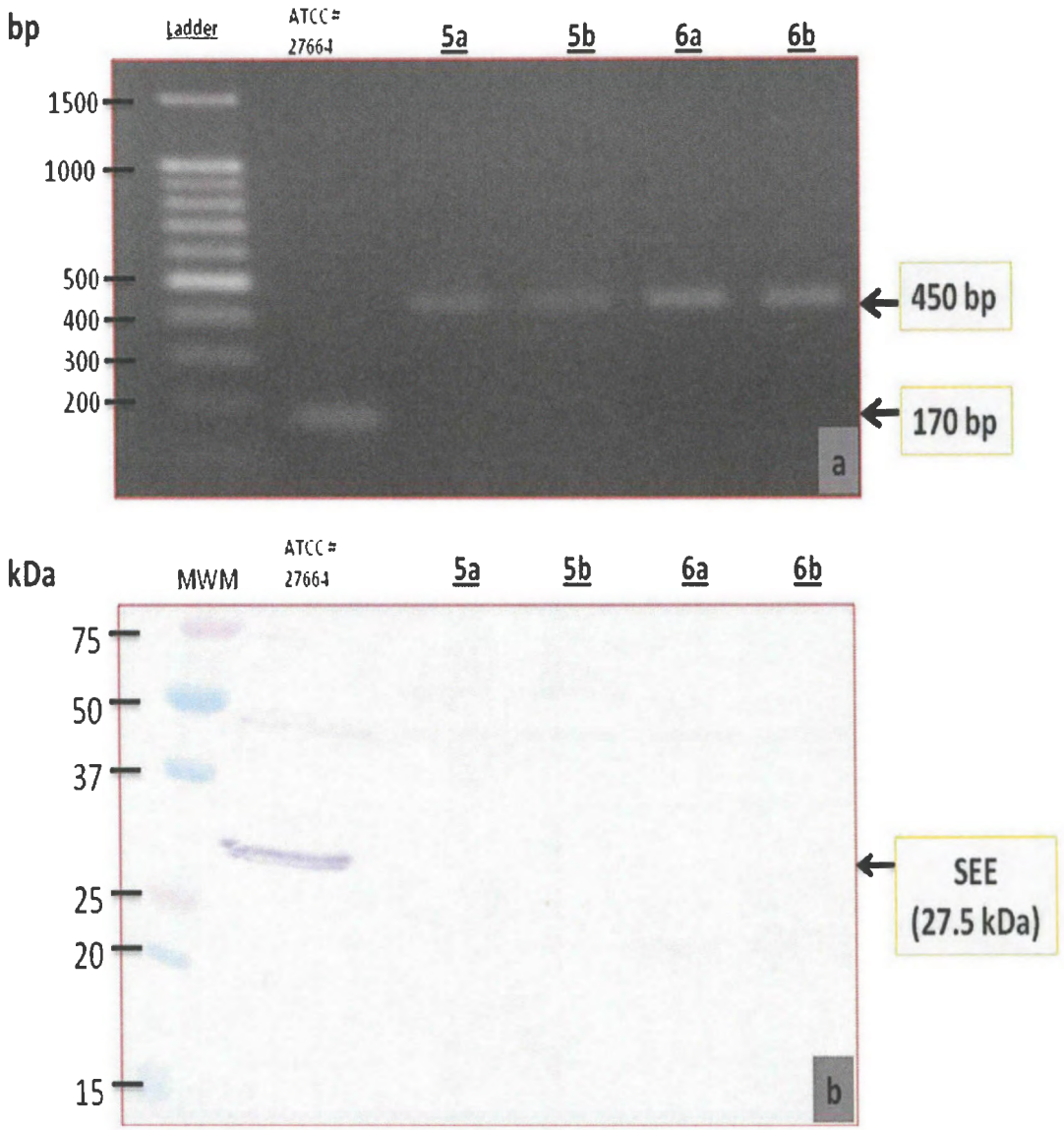


Figure 6. Two coagulase-negative *Staphylococcus* spp cultured from chicken were tested by PCR and gave unexpected base pair products of a different size with *see* primers (Fig. 6a). Both isolates were negative for the SEE toxin by immunoblotting (Fig. 6b).

Legend: *Ladder- Promega DNA ladders #G210A; MWM- Bio-rad # 161-0374 (Precision plus protein standard dual color); S. aureus ATCC # 27664- entE; 5a and 5b- S. hominis (chicken) and S. hominis (chicken).*

All the other study *Staphylococcus* isolates tested negative for the classical enterotoxin and *tsst-1* genes (Figure 7).

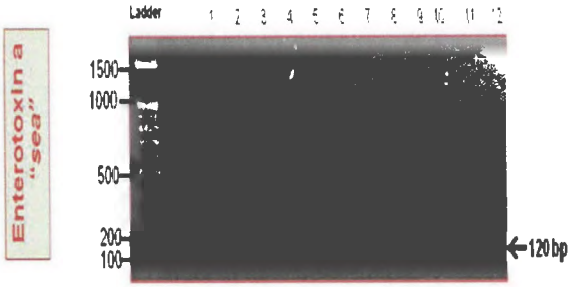


Fig. 7a. *Legend*: Ladder - Promega # G210A; *S. aureus* ATCC # 13565- *sea.*

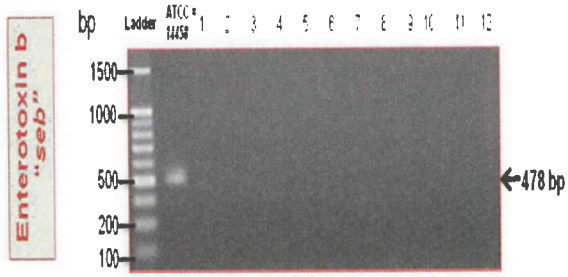


Fig. 7b. *Legend*: Ladder -Promega #G210A; *S. aureus* ATCC # 14458- *seb.*

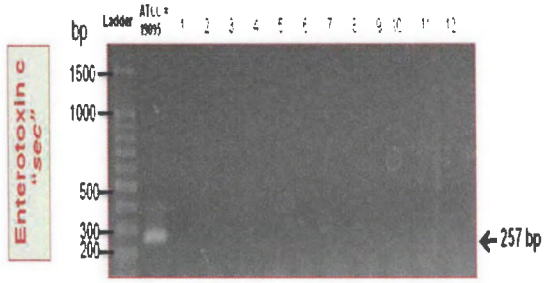


Fig. 7c. *Legend*: Ladder - Promega # G210A; *S. aureus* ATCC # 19095- *sec.*

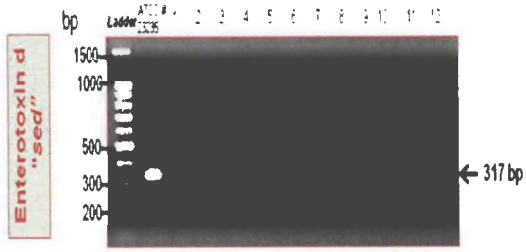


Fig. 7d. *Legend*: Ladder - Promega #G210A; *S. aureus* ATCC # 23235- *sed.*

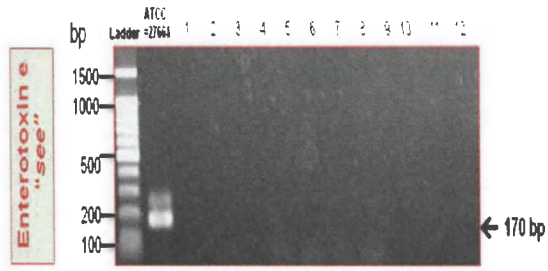


Fig. 7e. *Legend*: Ladder - Promega # G210A; *S. aureus* ATCC # 27664- *see.*

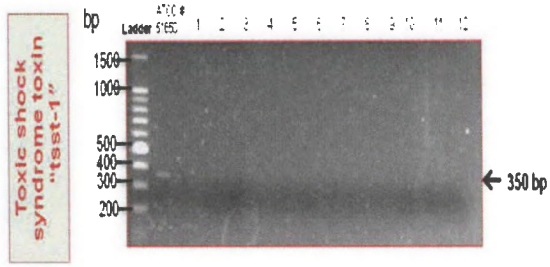


Fig. 7f. *Legend*: Ladder – Promega # G210A; *S. aureus* ATCC #- 51650- *tsst-1.*

Figures 7a-f: A total of 86 *Staphylococcus* isolates tested negative for classical enterotoxin and *tsst-1* genes.

4.5. Antimicrobial Susceptibility Patterns

All the study 92 *Staphylococcus* isolates were tested for susceptibility patterns of 20 antimicrobial agents. For this analysis, the isolates were grouped into CNS and CPS, and the antibacterial agents grouped into β-lactams and non-β lactam antibacterial agents. Overall, most of the isolates were sensitive to the 20 antibacterial agents tested (results not included). On the other hand, resistance to old generation β-lactam antibacterial agents like penicillin and ampicillin was as high as 80.0% within the CPS sub-group, and 50.0% among the new generation β-lactam antibacterial agents (Table 7).

Drug	Resistant (%)	Sensitive (%)	No interpretation possible (%)	Intermediate (%)
Amoxicillin/ Clavulanic acid	50	50		
Ampicillin	70	10	20	
Cefazolin	50	50		
Cefoxitin	50	50		
Cefpodoxime	50	50		
Imipenem	40	45		5
Oxacillin + 2% Nacl	50	50		
Penicillin	80	5	15	
Ticarcillin	50	30	30	
Ticarcillin/ Clavulanic acid	50	45	5	

Table 7. Antimicrobial agent susceptibility patterns of coagulase positive *Staphylococcus* (n=20) to β-lactam agents.

When compared to non-β-lactam antibacterial agents, 85.0% of the CPS isolates were sensitive (results not included). Resistance to β-lactam antibiotics by CNS was generally lower (>50.0%) compared to the CPS sub-group (Table 8).

Drug	Resistant (%)	Sensitive (%)	No interpretation possible (%)	Intermediate (%)
Amoxicillin/ Clavulanic acid	36	64		
Ampicillin	35	11	54	
Cefazolin	36	64		
Cefoxitin	29	71		
Cefpodoxime	35	65		
Imipenem	33	67		10
Oxacillin + 2% Nacl	33	77		
Penicillin	42	58		
Ticarcillin	39	61		
Ticarcillin/ Clavulanic acid	39	61		

Table 8. Antimicrobial agent susceptibility patterns of coagulase-negative *Staphylococcus* spp (n=72) to β-lactam antibacterial agents.

4.6. Incidence of the *mecA* gene

All 92 study *Staphylococcus* spp isolates were tested for the carriage of *mecA* gene. Five percent (1/20) of the CPS isolates was positive for *mecA* gene (Figure 8). The *S. gallinarum* was isolated from a diagnostic goose sample.

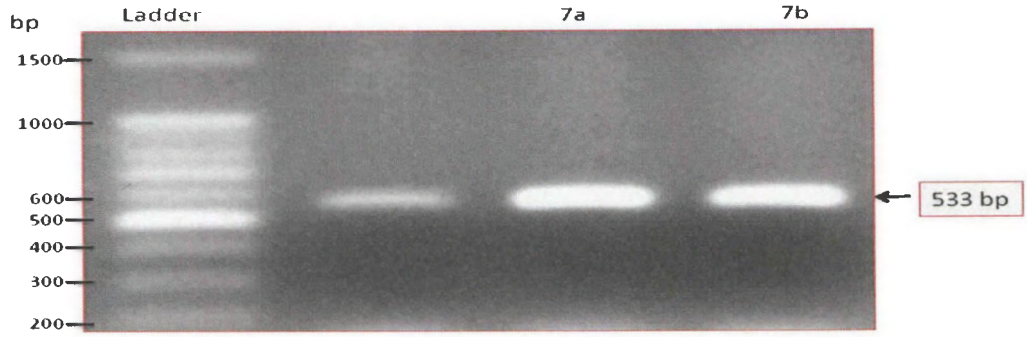
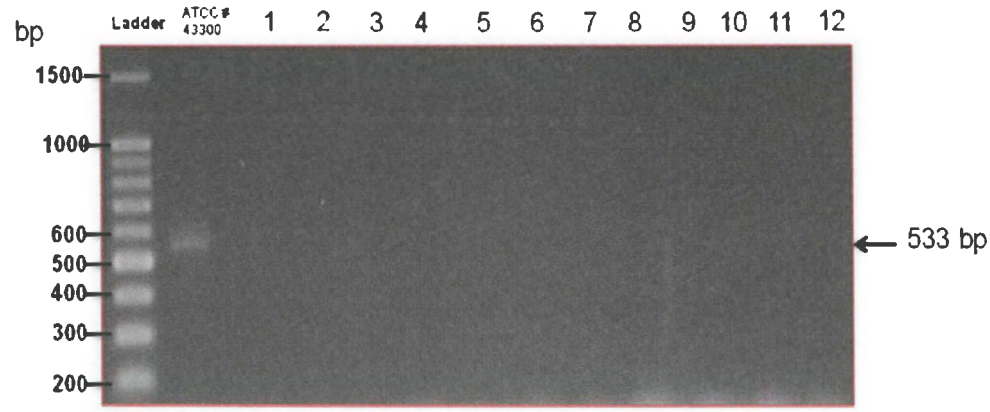


Figure 8. A coagulase-positive *Staphylococcus* spp cultured from a diagnostic goose was positive for the *mecA* gene.

***Legend**: Ladder- Promega DNA ladders #G210A; S. aureus ATCC #43300-(mecA positive control); 7a and 7b- S. gallinarum (goose).*

All CNS (62/62) and 95.0% (19/20) CPS were negative for *mecA* gene (Figure 9).



Figures 9: A total of 91 *Staphylococcus* isolates tested negative for the *mecA* gene.

***Legend:** Ladder- Promega DNA ladders #G210A; S. aureus ATCC #43300- (mecA positive control).*

5. DISCUSSION AND CONCLUSIONS

Of all the 143 animal-derived food samples tested, 27.3% (39/143) were *Staphylococcus*-positive compared to only 9.5% (23/242) of the plant foods (Table 1). Overall, 75.0% (69/92) of the *Staphylococcus* isolates came from animal sources; of the 69 isolates, 56.5% (39/69) were cultured from foods of animal origin (Table 1), and 43.5% (30/69) from diagnostic animal samples (Table 6). As would have been expected, this data indicates that animal-derived foods have a higher *Staphylococcus* contamination rate compared to foods of plant origin (Chi square; $p<0.0001$). It is widely documented in literature that animals and humans are the main *Staphylococcus* reservoir with ≥50% of healthy individuals being colonized by the organism (*62, 127, 176*). Supported by literature, therefore, the present data would appear to suggest that foods of animal origin are contaminated by the animal itself. It is also widely believed that food handlers are a major source of contamination of table foods (*62*) including those that were tested during the present study. However, testing of humans that came into contact with the study food and animal samples was not attempted. Contamination of food by humans in food processing plants is minimized by wearing protective gear as well as maintaining unidirectional flow of food along the processing chain. Future studies should attempt to evaluate the efficiency of such systems as they may have significant implications in the epidemiology of staphylococcal food poisoning (SFP).

Among the foods of plant origin, mushrooms yielded the highest number of *Staphylococcus* spp isolates (Table 2). Previously, other researchers have also reported a surprisingly high incidence of *Staphylococcus* in mushrooms (*28, 34, 111, 120, 158*). Based on the reports by these researchers, the finding in the current study appears not to be a random event but rather underscores the potential that mushrooms may have in the epidemiology of SFP. Interestingly, none of the previous studies have given a satisfactory explanation for this observation. Subject to further investigations, the anatomy, and probably other biological features unique to mushrooms, may facilitate growth and survival of this agent.

Quite remarkably, 19.6% (18/92) of the *Staphylococcus* spp isolated from both food and diagnostic samples were *S. hyicus* (Figure 1). The latter is the most frequently isolated pathogenic *Staphylococcus* spp in pigs in which it causes a devastating disease called exudative dermatitis (*73*). However of all the 18 *S. hyicus* isolates, only 5 were from pig samples (Table 6). This finding appears to imply that although routinely considered a primary pathogen of pigs, *S. hyicus* may be more widely distributed in nature.

In this study, *Staphylococcus* isolates were either grouped as CPS or CNS and their ability to produce toxins analyzed. Coagulase is an enzyme produced by some *Staphylococcus* spp that causes clot formation and *Staphylococcus* spp are grouped into CPS and CNS based on this characteristic. The present experimental data shows that 21.7% (20/92) of the study *Staphylococci* were coagulase-positive

and 78.3% (72/92) coagulase-negative. Of the CPS isolates, 65% (13/20) were from food samples and 35% (7/20) from diagnostic samples. Thirty one point nine percent (23/72) of the CNS isolates were from diagnostic samples and 68.1% (49/72) from food samples (Tables 2, 4 and 6). Despite the fact that *S. aureus* is the prototype of the CPS subgroup, there is evidence that indicates that some of *S. aureus* strains may be coagulase-negative (*122*). Likewise, a number of coagulase-positive *S. intermedius* (*148*), *S. delphini* (*173*), *S. hyicus* (*148*), *S. lutrae* (*65*) and *S. schleiferi* subspp *coagulans* (*86*) strains have been reported. Based on the above literature, therefore, demonstration of 2 coagulase-positive *S. intermedius* isolates is not surprising. However, the isolation of coagulase-positive *S. gallinarum* and *S. lentus* is quite interesting since no previous literature exists that documents such strains of *Staphylococcus*. *S. aureus*, the prototype of CPS group, has been associated with SFP since the early 1970s (*83*). Several studies have since demonstrated toxigenic *S. aureus* in food stuffs (*2, 31, 106, 149, 150*). At the beginning of the last decade, coagulase positive *S. intermedius* was shown to express SEs (*15*). Whereas CNS were initially thought to be avirulent, research has shown over time that members of the subgroup can also express SEs (*13, 93, 168*) and *S. intermedius* has been linked to food poisoning (*99*).

Using the PCR assay, all study *Staphylococcus* isolates were screened for genes that encode five classical enterotoxins and toxic shock syndrome toxin-1. The results were further corroborated by immunoblotting analysis to establish if the PCR-positive isolates expressed the corresponding toxins. As presented in

results under 4.4 above, 15.4% (3/20) of the CPS isolates were positive for both the *sec* gene and SEC toxin but not any other classical enterotoxins. These results are in agreement with the reported range of toxigenic *S. aureus* isolates (*48*). The toxigenicity of CPS is well documented with 5-20% of *S. aureus* reported to be toxigenic (*48*). The percentage of toxigenic CPS reported here is most likely an underestimate of potentially enterotoxigenic coagulase-positive *Staphylococci* since only the five classical enterotoxins, and none of the newly described enterotoxin-like genes, were screened for in the present study. Therefore, it is reasonable to speculate that if the study *Staphylococcus* spp were screened for all the staphylococcal enterotoxin genes, the newly described ones included, the prevalence of enterotoxigenic staphylococci might be much higher than currently reported (*132*). It is important to note that 2 of the *sec*/SEC positive isolates were cultured from steak. Staphylococcal food poisoning in humans normally follows ingestion of food containing about 100,000 bacterial cells/g of food (*62*). Since SEs are heat stable, it is plausible to conclude that the *sec*/SEC-positive-*S. aureus* from the steak could have potentially caused food poisoning to the consumer since SEs are heat stable. Huang et al (2005) (*82*), reported an incident in Louisiana State in which SFP occurred in persons that ate contaminated ham. The other *sec*/SEC-positive *Staphylococcus* isolate was from a diagnostic cat sample. Interestingly the same isolate was also *tsst-1*/TSST-1-positive. Isolation of a *sec*/SEC positive *Staphylococcus* strain from a diagnostic cat sample underscores the risk of potential zoonotic transmission of toxigenic *Staphylococcus* spp from companion

animals to humans. It is also possible that humans getting infected from their cats, and/or the infected cat itself may then contaminate food supplies. *Staphylococcus* spreads by direct contact often via the hands of an infected food handler (*38, 109*). As a matter of fact, food handlers have been implicated in SFP outbreaks (*44, 95, 115*). Zonootic transmission by direct contact is possible but its clinical significance is still controversial (*57*).

In the present study, the enterotoxigenicity of CNS isolates was evaluated using PCR and immunoblotting. Of the various CNS isolates tested, a *S. lugdunensis* isolate cultured from strawberries was positive for the *sec* gene but not the corresponding SEC toxin. The pathogenicity of CNS has been reported by a number of researchers (*13, 15, 93, 99*) but also questioned by others (*51, 174, 186*). To definitively determine toxin producing ability in the *sec* gene-positive *S. lugdunensis* isolate would need to be subjected to growth conditions that favor SE production. As a number of workers have shown, the production of SE is dependent on a number of factors including but not limited to the expression of *agr* (*131*); various amino acids (*136*); and neutral pH (*146*). Likewise, the production of SE is inhibited by glucose (*19*), low pH (*131, 147*) and salt concentrations above 12% (*130*). A number of researchers have previously reported enterotoxigenic CNS including: *S. cohnii*, *S. epidermidis*, *S. xylosus*, *S. heamolyticus*, *S. hyicus*, *S. lentus*, *S. capitis*, *S. lentus*, *S. warneri*, *S. hominis*, *S. saprophyticus*, *S. schleferi* and *S. equorum* (*1, 9, 13, 27, 48, 80, 134, 168, 174*). However, literature indicates that *S. intermedius* is the only CNS species that has

been definitively demonstrated in SFP outbreaks (*99*). Several workers have also reported that CNS may produce *tsst-1* alone or in combination with other toxins (*48, 96, 168*). Based on data obtained in the present study, none of the CNS tested positive to *tsst-1.* A study by Valle et al, 1991 (*170*) reported up to 16% of CNS as *tsst-1* positive.

Two *S. hominis* isolates from chicken gave unexpected base pair products with primers designed against the *see* gene. Classical SEs are encoded by a cluster of polymorphic genes that show a high degree of homology in their base sequences. Base sequence similarities in the range of 15.0 to 90.0% are not surprising and have in fact been reported for the *sea* and *see* genes (*142, 171*) suggesting that primers designed for the *see* gene may inappropriately bind the *sea* or other enterotoxin genes.

Based on the immunoblotting data, all the *S. aureus* isolates included in the Western blots expressed Protein A, a 60 kDa protein which was not expressed by the other *Staphylococcus* isolates (Figure 2-6). Protein A binds the Fc portion of the immunoglobulin G (IgG) molecule and because the Protein- IgG binding is in the wrong direction, the resulting steric interference disrupts opsonisation and phagocytosis. This phenomenon increases the virulence of isolates that express the protein by acting as an immunologic disguise and preventing phagocytosis (*70*).

As part of the research objectives, the antimicrobial sensitivity profiles for all the study *Staphylococcus* isolates were tested against 20 antibacterial agents.

Generally, most of the isolates were sensitive to most of the antibacterial agents used in the study (results not included). However, up to 80% of the CPS isolates were resistant to the old generation β-lactam antimicrobial agents and 50% were resistant to the new generation antibiotics (Table 5). Generally, on the other hand, less than 50% of the CNS were resistant to β-lactam antibiotics (Table 6). Notably, 80% of the CPS were resistant to penicillin similar to data reported by the CDC, (CDC, 2005); but less than 50% of CNS were resistant to penicillin. When penicillin was introduced in 1944, 94% of *Staphylococcus* spp were susceptible to the drug (*112*). However, at present, 80-90% of *Staphylococci* are resistant to penicillin (*36, 133*). In the presnet study, 5% (1/20) of the CPS and none of the CNS isolates were positive for *mecA* gene. The *mecA*-positive isolate was also resistant to penicillin and oxacillin +2% NaCl confirming that the methicillin resistance mechanism was most likely responsible for the poor antimicrobial sensitivity observed. Research by Meas, 2002 (*116*) showed *S. cohnii* and *S. lugdunesis* that were resistant to oxacillin MICs did not amplify *mecA* with PCR suggesting that the resistance was mediated by a mechanism other than one involving *mecA*. In another study, 11 *Staphylococcus* isolates that were susceptible to oxacillin tested positive for the *mecA* gene and of the 11 isolates, 4 were CNS (*43*). Methicilin resistant *Staphylococcus* aureus (MRSA) are a public health problem worldwide. Research carried out in different parts of the world has shown predominance of MRSA over susceptible isolates (*182*). Recently, a *Staphylococcus* strain associated with a food poisoning outbreak was also resistant to methicilin (*95*). This

documents the potential of MRS to cause food poison in a community setting. Phenotypic and genotypic methods are used to detect resistance in *Staphylococcus* spp with genotypic methods having higher sensitivity and specificity compared to phenotypic methods; however, genotypic methods are not always available in all laboratories (*167*).

The major goal of this study was to assess the potential role played by CNS in the epidemiology of foodborne illnesses, and to determine the likelihood of food and domestic animals as transmission vehicles of mecithillin resistance. A single CNS isolate (*S. lugdunensis*) from strawberries tested positive for the *sec* gene but not the SEC toxin while 2 isolates of *S. hominis* from chicken gave unexpected base pair products with *see* primers. These findings underscore the potential role played by food-derived CNS in the epidemiology of SFP, and warrant more extensive studies involving a bigger sample size to address the research question. The 2 *sec*/SEC positive *S. aureus* isolates from steak confirm the undisputed role CPS have in the epidemiology of SFP. Also, the *sec*/SEC and *tsst-1*/TSST-1 positive *S. aureus* from a diagnostic cat sample further highlights the importance of companion animal as potential reservoirs for *Staphylococci* of zoonotic and public health importance. Together with the *mecA*-positive *S. gallinarum* isolate from a diagnostic goose sample suggest that food may serve as a vehicle of foodborne disease caused mecithillin resistant *Staphylococci*. A much broader study involving more samples should exhaustively investigate the role of CNS in the epidemiology of food poisoning.

6. REFERENCES

1. **Adesiyun, A. A., S. R. Tatini,** and **D. G. Hoover.** 1984. Production of enterotoxin(s) by *Staphylococcus hyicus*. *Vet Microbiol* 9: 487–495.

2. **Akineden, O., C. Annemüller, A. A. Hassan, C. Lämmler, W. Wolter,** and **M. Zschöck.** 2001. Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clin. Diagn. Lab. Immunol. 8*: 959-964.

3. **Anday, E.K.** and **G.H. Talbot**. 1985. Coagulase-negative *Staphylococcus* bacteremia–a rising threat in the newborn infant. *Ann Clin Lab Sci* 15:246–251.

4. **Anderson, J.E., R.R. Beelman,** and **S. Doores.** 1996. Persistence of serological and biological activities of staphylococcal enterotoxin A in canned mushrooms. *J. Food Prot.* 59:M1292–1299.

5. **Anonymous.** 1994. Foodborne pathogens: risks and consequences. Ames, IA: Council of Agricultural Science and Technology.

6. **Archer, D.L.,** and **J. E. Kvenberg.** 1985. Incidence and cost of foodborne diarrheal disease in the United States. *J Food Protect* 48:887-94.

7. **Archer, G. L.** 1995. *Staphylococcus epidermidis* and other coagulase-negative staphylococci. In *Principles and Practice of Infectious Diseases,* ed. GL Mandell, JE Bennett, R Dolin, 4:1777.84. New York: Churchill Livingston.

8. **Archer, G. L.** 2007. Staphylococcal infections. In: Goldman L, Ausiello D, eds. *Cecil Medicine*. 23rd ed. Philadelphia, Pa: Saunders Elsevier. ch 310.

9. **Balaban, N.** and **A. Rasooly.** 2000. Staphylococcal enterotoxins. *Int. J. Food Microbiol.* 61: 1-10.

10. **Baquero, F.** 1997. Gram-positive resistance: challenge for development of new antibiotics. *J. Antimicrob. Chemother.* 39 (Suppl A): 1-6.

11. **Barg, N. L.,** and **T. Harris.** 1997. Toxin-mediated syndromes. In: Crossley KB, Archer GL (eds): The staphylococci in human disease. Churchill Livingstone, New York pp 527–543.

12. **Barigye, R., L. Schann, S.G. Gibbs, E. Schamber,** and **N.W. Dyer.** 2007. Diagnostic evidence of *Staphylococcus warneri* as a possible cause of bovine abortion. *J. Vet. Diag. Invest.* 19: 694-696.

13. **Bautista, L., P. Gaya, M. Medina,** and **M. Nunez.** 1988. A quantitative study of enterotoxin production by sheep milk staphylococci. *Appl. Environ. Microbiol.* 54: 566-569.

14. **Bayles, K.W.** and **J.J. Iandolo.** 1989. Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. *J. Bacteriol.* 171: 4799–4806.

15. **Becker, K., B. Keller, C. Von Eiff, M. Brück, G. Lubritz, J. Etienne,** and **G. Peters.** 2001. Enterotoxigenic potential of *Staphylococcus intermedius*. *Appl. Environ. Microbiol. 67*: 5551-5557.

16. **Bendahou, A., M. Abid, B. Nadine, D. Catelejine,** and **M. Lebbadi.** 2009. Enterotoxigenic coagulase positive Staphylococcus in milk and milk products, lben and jben, in northern Morocco. *J Infect Developing Countries* 3(3):169-176.

17. **Bennett, J., S. Holmberg, M. Rogers,** and **S. Solomon.** 1987. Infectious and parasitic diseases. In: Amler R, Dull H, editors. Closing the gap: the burden of unnecessary illness. New York: Oxford Univ Press. 102-14.

18. **Bergdoll, M.S.** 1983. Enterotoxins. In: *Staphylococci and Staphylococcal Infections* (Easman, C.S.F. and Adlam, C., eds.). Academic Press, London, UK, pp. 559-598.

19. **Bergdoll, M.S.** 1989. *Staphylococcus aureus*. In: *Foodborne Bacterial Pathogens* (Doyle, M.P., ed.). Marcel Dekker, Inc., New York, NY, USA, pp. 463-523.

20. **Bergdoll, M.S., C.R. Borja,** and **R.M. Avena.** 1965. Identification of a new enterotoxin as enterotoxin C. *J. Bacteriol.* 90: 1481–1485.

21. **Betley, M.J.** and J.**J. Mekalanos.** 1985. Staphylococcal enterotoxin A is encoded by phage. *Science* 229: 185–187.

22. **Betley, M.J.** and **J.J. Mekalanos.** 1988. Nucleotide sequence of the type A staphylococcal enterotoxin gene. *J. Bacteriol.* 170: 34–41.

23. **Bohach, G. A., L. Jablonski, M. Roggiani, I. SadleI, P. M. Schlievert, D. Mitchell,** and **D. Ohlendorf.** 1998. Biological activity of pyrogenic toxins

delivered at the mucosal surface in J. Arbuthnott, B. Fuman (Eds). European conference on Toxic shock syndrome. International congress and symposium series 229. (Royal society of Medicine press Ltd., NY). 170-172.

24. **Borst, D.W.** and **M.J. Betley.** 1994. Phage-associated differences in staphylococcal enterotoxin A gene (sea) expression correlate with sea allele class. *Infect. Immun.* 62: 113–118.

25. **Boyce, J. M.** 1997. Epidemiology and prevention of nosocomial infections. In *The Staphylococci in Human Disease*, ed. KB Crossley, GL Archer, 1:309.29. New York: Churchill Livingston.

26. **Boyce, J. M., G. Potter-Bynoe, S. M. Opal et al.** 1990. A common source outbreak of *Staphylococcus epidermidis* infections among patients undergoing cardiac surgery. *J. Infect. Dis.* 161:493.99.

27. **Breckinridge, J. C.** and **M.S. Bergdoll.** 1971. Outbreak of food-borne gastroenteritis due to a coagulase-negative enterotoxin-producing *Staphylococcus*. *NE J Med.* 284: 541–543.

28. **Brunner, K. E. G.** and **A. M. C. L. Wong.** 2006. Staphylococcus aureus Growth and Enterotoxin Production in Mushrooms. *J Food Sci 57*(3): 700-703.

29. **Butterworth A.** 1999. Infectious components of broiler lameness: a review. *World Poult. Sci. J.* 55: 327- 352.

30. **Caputo, G.M., G. L. Archer, S. B. Calderwood, et al.** 1987. Native valve endocarditis due to coagulase-negative staphylococci. Clinical and microbiological features. *Am. J. Med.* 83:619.25.

31. **Cardoso, H.F., N. Silva, M. J. Sena,** and **L. S. Carmo.** 1999. Production of enterotoxins and toxic shock syndrome toxin by *Staphylococcus aureus* isolated from bovine mastitis in Brazil. *Lett. Appl. Microbiol. 29*: 347-349.

32. **Casman, E.P.** 1965. Staphylococcal enterotoxin. *Ann. NY Acad. Sci.* 128: 124–131.

33. **CDC.** 1982. Toxic-shock syndrome, United States, 1970-1982. *MMWR Morb Mortal Wkly Rep.* 31(16):201-4.

34. **CDC.** 1989. Epidemiologic Notes and Reports Multiple Outbreaks of Staphylococcal Food Poisoning Caused by Canned Mushrooms. Center for Disease Control. MMWR 38(24); 417-418.

35. **CDC.** 1997. CDC National Nosocomial Infections Surveillance (NNIS) Report, Data Summary from October 1986. *Am. J. Infect. Control* 25:477.87.

36. **CDC.** 2005. Laboratory detection of: oxacillin/methicillin resistant *Staphylococcus aureus*. Depatment of human services, Center for disease control.

37. **CDC.** 2005. Toxic shock syndrome. Department of health and human services, Center for disease control.

38. **CDC.** 2006. Staphylococcal food poisoning. Department of health and human services, Center for disease control.

39. **CDC.** 2007. Fact sheet: Invase MRSA. Department of human services, Center for disease control. Available at: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_Invasive_FS.html.

40. **CDC.** 2009. Community-associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA). Department of human services, Center for disease control. Available at: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca.html.

41. **CDC.** 2009. Healthcare-associated Methicillin Resistant *Staphylococcus aureus* (HA-MRSA). Department of human services, Center for disease control. Available at: http://www.cdc.gov/ncidod/dhqp/ar_mrsa.html.

42. **Chambers, H. F.** 1997. Methicillin resistance in staphylococci: molecular and biomedical basis and clinical implications. *Clin. Microbiol. Rev.* 10:781-791.

43. **CLSI.** 2005. Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100-S15. Clinical and Laboratory Standards Institute, Wayne, Pa, U.S.A.

44. **Colombari, V., M. D. B. Mayer, Z. M. Laicini, E. Mamizuka, Franco, D. G. M. Bernadette, M. T. Destro,** and **M. Landgraf.** 2007. Foodborne Outbreak Caused by *Staphylococcus aureus*: Phenotypic and Genotypic Characterization of Strains of Food and Human Sources. *J. of Food Protection*. 70 (2): 489-493 (5).

45. **Corbellini, L. G., C. A. Pescador, F. J. Frantz, M. Cardoso,** and **D. Driemeier.** 2006. *Staphylococcus* spp. Abortion: Skin Lesions Caused by *Staphylococcus aureus* Infection in an Aborted Bovine-Fetus. *Vet. Res. Comm.* 30 (7): 717-721.

46. **Couch, J.L.** and **M.J. Betley.** 1989. Nucleotide sequence of the type C3 staphylococcal enterotoxin gene suggests that intergenic recombination causes antigenic variation. *J. Bacteriol.* 171: 4507–4510.

47. **Couch, J.L., M.T. Soltis,** and **M.J. Betley.** 1988. Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. *J. Bacteriol.* 170: 2954–2960.

48. **Crass, B. A.,** and **M. S. Bergdoll.** 1986 Involvement of staphylococcal enterotoxins in nonmenstrual toxic shock syndrome. *Journal of Clinical Microbiology* 23: 1138–1139.

49. **Cree, R.G.A.** and **W. C. Noble.** 1995. In vitro indices of tissue adherence in *Staphylococcus intermedius*. *Lett. Appl. Microbiol* 20: 168-170.

50. **Cunha, M. L. R. S.,** and **R. A. O. Calsolari.** 2007. Toxigenicity in *Staphylococcus* with emphasis on coagulasenegative staphylococci Communicating Current Research and Educational Topics and Trends in Applied Microbiology.

51. **Cunha, M. L. R. S., E. Peresi, R. A. O. Calsolari,** and **J. P. A. Junor.** 2006. Detection of enterotoxins genes in coagulase-negative staphylococci isolated from food. *Braz. J Microbiol* 37: 70-74.

52. **Cuny, C., J. Kuemmerle, C. Stanek, B. Willey, B. Strommenger,** and **W. Witte.** 2006. Emergence of MRSA infections in horses in a veterinary hospital: strain characterization and comparison with MRSA from humans. *Euro Surveill* 11:44–7.

53. **de Boer, E., J. T. Zwartkruis-Nahuis, B. Wit, et. al.** 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Int J Food Microbiol.* 134(1-2):52-56.

54. **Dinges, M.M., P.M. Orwin,** and **P.M. Schlievert.** 2000. Exotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev. 13*: 16-34.

55. **DSMZ.** 2004. German Collection of Microorganisms and Cell Cultures. DSMZ. Available at: http://www.dsmz.de/dsmz/?menu_id=2.

56. **Duch-Samper, A. M., J. L. Menezo,** and **M. Hurtado-Sarrio.** 1997. Endophthalmitis following penetrating eye injury. *Acta Ophthalmol. Scand.* 75:104.6.

57. **Duquette, R. A.,** and **T. J. Nuttall.** 2006. Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *Journal of Small Animal Practice* 45(12): 591-597.

58. **Edwards, J. F., A. L. Lassala,** and **T. E. Spencer.** 2008. *Staphylococcus*-associated abortions in ewes with long-term central venous catheterization. *Vet pathol.* 45(6):881-8.

59. **Espino, L., R. Bermudez, L.E. Figaldo, et al.**2006. Meningoencephalitis associated with *Staphylococcus warneri* in a dog. *J Small Anim Pract* 47:598–602.

60. **Eveillard, M., F. Eb, B. Tramier, J.L. Schmit, F.X. Lescure, M. Biendo, B. Canarelli, F. Daoudi, G. Laurans, F. Rousseau,** and **D. Thomas.** 2001. Evaluation of the contribution of isolation precautions in prevention and control of multi-resistant bacteria in a teaching hospital. *J Hosp Infect.* 47(2):116-24.

61. **Faibis, F., C. Laporte, A. Fiacre, C. Delisse, G. Lina, M.C. Demachy,** and **F. Botterel**. 2005. An outbreak of methicillin-resistant *Staphylococcus aureus* surgical-site infections initiated by a healthcare worker with chronic sinusitis. *Infect Control Hosp Epidemiol.* 26(2):213-5.

62. **FDA.** 2009. *Staphylococcus aureus*. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. Food and Drug Administration. US Department of Health and Human services.

63. **Fidalgo, S., F. Vazquez, M.C. Mendoza et al.** 1990. Bacteremia due to *Staphylococcus epidermidis*: microbiologic, epidemiologic, clinical, and prognostic features. *Rev Infect Dis* 12:520–528.

64. **Fitzgerald, J.R., S.R. Monday, T.J. Foster, G.A. Bohach, P.J. Hartigan, W.J. Meaney** and **C.J. Smyth.** 2001. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens, *J. Bacteriol.* 183: 63–70.

65. **Foster, G., H. M. Ross, R. A. Hutson,** and **M. D. Collins.** 1997. Staphylococcus lutrae sp. nov., a New Coagulase-Positive Species Isolated from Otters. *Int J Syst Bacteriol.* 47 (3): 724-726.

66. **FSRRN.** 2007. Sample size calculations. Food Safety Research and Response Network. Available at: http://www.fsrrn.net/modules/content/index.php?id=13.

67. **Gatermann, S. G.,** and **K. B. Crossley.** 1997. Urinary tract infections. In *The Staphylococci in Human Disease*, ed. KB Crossley, GL Archer, 1:493.508. New York: Churchill Livingston.

68. **Gemmell, C. G.,** and **F. Schumacher-Perdreau.** 1986. Extracellular toxins and enzymes elaborated by coagulase-negative staphylococci. In: Mardh PA, Schleifer KH (eds): Coagulase-negative staphylococci. Almqvist and Wiksell, Stockholm pp 809–827.

69. **Gleason, B. A.,** and **K. D. Huebner.** 2009. CBRNE - Staphylococcal Enterotoxin B, 2009. Available at: http://emedicine.medscape.com/article/830715-overview.

70. **Goodyear, C. S.** and **G. J. Silverman.** 2003. Death by a B-cell superantigen: In vivo VH targeted apoptotic supra-clonal B-cell deletion by a staphylococcal toxin. *J. Exp. Med.* 197: 1125-1139.

71. **Gyles, C. L., J. F. Prescott, J. G. Songer,** and **C. O. Thoen.** 2004. Pathogenesis of Bacterial Infections in Animals, 3rd Edition. Blackwell Publishing Professional. Ch 4: 48-51.

72. **Haley, R.W., N.B. Cushion, F.C. Tenover, T.L. Bannerman, D. Dryer, J. Ross, P.J. Sanchez,** and **J.D. Siegel.** 1995. Eradication of endemic methicillin-resistant Staphylococcus aureus infections from a neonatal intensive care unit. *J Infect Dis.* 171(3):614-24.

73. **Hassler, C., S. Nitzsche, C. Iversen, C. Zweifel** and **R. Stephan.** 2008. Characteristics of *Staphylococcus hyicus* strains isolated from pig carcasses in two different slaughterhouses. *Meat Science* 80(2): 505-510.

74. **Henderson D. K.** 1988. Intravascular device associated infection: current concepts and controveries. *Infect. Med.* 5: 459:64.

75. **Hernandez, F.J., J. Goyache, J.A. Orden, J.L. Blanco, A. Domenech, G. Suarez *et al.*** 1993. Repair and enterotoxin synthesis by *Staphylococcus aureus* after thermal shock. *Appl. Environ. Microbiol.* 59: 1515–1519.

76. **Heubner, J.,** and **D. A.Goldman.** 1999. Coagulase-Negative staphylococci: Role as Pathogens. *Annual Reviews of Medicine* 50: 223-236.

77. **Hirooka, E. Y., E. E. Muller, J. C. Freitas, E. Vicente, Y. Yoshimoto,** and **M. S. Bergdoll.** 1988. Enterotoxigenicity of *Staphylococcus intermedius* of canine origin. *International Journal of Food Microbiology* 7:185–191.

78. **Holmberg, S.D.** and **P.A. Blake.** 1984. Staphylococcal food poisoning in the United States. New facts and old misconceptions. *J. Am. Med. Assoc.* 251: 487–489.

79. **Honeyman, A.L., H. Friedman,** and **M. Bendinelli.**2001. *Staphylococcus aureus* Infection and Disease, Kluwer Academic/Plenum Publishers, New York; USA.

80. **Hoover, D.G., S.R. Tatini,** and **J.B. Maltais.** 1983. Characterization of staphylococci. *Appl. Environ. Microbiol.* 46: 649–660.

81. **Hovde, C.J., S.P. Hackett,** and **G.A. Bohach.** 1990. Nucleotide sequence of the staphylococcal enterotoxin C3 gene: sequence comparison of all three type C staphylococcal enterotoxins. *Mol. Gen. Genet.* 220: 329–333.

82. **Huang, A., C. B. Ocfemia,** and **D. C. Hunt.** 2005. Staphylococcal food poisoning in southwest Kansas- December 2005. Kansas Department of Health and Environment. Bureau of Epidemiology and Disease prevention. Available at: http://www.kdheks.gov/epi/download/foodborne_illness_outbreak_report_dec_2005.pdf.

83. **Huang, I.Y.** and **M. S. Bergdoll.** 1970. The primary structure of staphylococcal enterotoxin B. II. Isolation, composition, and sequence of chymotryptic peptides. *J. Biol. Chem. 245*: 3511-3517.

84. **Huang, I.Y., J.L. Hughes, M.S. Bergdoll,** and **E.J. Schantz.** 1987. Complete amino acid sequence of staphylococcal enterotoxin A. *J. Biol. Chem.* 262: 7006–7013.

85. **Iandolo, J. J.** 1989. Genetic analysis of extracellular toxins of *Staphylococcus aureus. Annu. Rev. Microbiol.* 43:375-402.

86. **Igimi, S., E. Takahashi,** and **T. Mitsuoka.** 1990. *Staphylococcus schleiferi* subsp. *coagulans* subsp. nov. Isolated from the External Auditory Meatus of Dogs with External Ear Otitis. *Int J Syst Bacteriol.* 40 (4): 409-411.

87. **Ing, M. B., L. M. Baddour,** and **A. S. Bayer.** 1997. Bacteremia and infective endocarditis: pathogenesis, diagnosis, and complications. In *The Staphylococci in Human Disease*, ed. KB Crossley, GL Archer, 1:331.54. New York: Churchill Livingston.

88. **Islam, M. J., M. S. Uddin, M. S. Nasrin, K. H. M. N. Nazir, M. T. Rahman,** and **M. M. Alam.** 2007. Prevalence of enterotoxigenic and toxic

shock syndrome toxin-1 producing coagulase positive *Staphylococcus aureus* in human and their characterization. *Bangladesh Journal of Veterinary Medicine.* 5: (1&2).

89. **Jablonski, L.M.** and **G.A. Bohach.** 2001. *Staphylococcus aureus.* In: M.P. Doyle, L.R. Beuchat and T.J. Montville, Editors, *Food Microbiology: Fundamentals and Frontiers* (2nd ed.), ASM Press, Washington, D.C., USA, pp. 411–434.

90. **Jarraud, S., M.A. Peyrat, A. Lim, A. Tristan, M. Bes, C. Mougel, J. Etienne, F. Vandenesch, M. Bonneville** and **G. Lina.** 2001. egc, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*, *J. Immunol.* 166: 669–677.

91. **Jaulhac, B., M. Bes, N. Bornstein, Y. Piemont, Y. Brun,** and **J. Fleurette.** 1992. Synthetic DNA probes for detection of genes for enterotoxins A, B, C, D, E and for TSST-1 in staphylococcal strains. *J App Bacteriol.* 72:386–392.

92. **Jaulhac, B., M. L. De Buyser, F. Dilasser, G. Prevost,** and **Y. Piedmont.** 1991. Screening of staphylococci for the toxic shock syndrome toxin-1 (TSST-1) gene. *Letters in Applied Microbiology* 13: 90–92.

93. **Jay, J.M.** 1992. Staphylococcal gastroenteritis. In: *Modern Food Microbiology* (Nostrand, V., ed.). 4th edn. Van Norstrand Reinhold, New York, NY, USA, pp. 455-478.

94. **Johnson, W.M., S.D. Tyler, E.P. Ewan, F.E. Ashton, D.R. Pollard** and **K.R. Rozee.** 1991. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction, *J. Clin. Microbiol.* 29: 426–430.

95. **Jones, T. F., M. E. Kellum, S. S. Porter, M. Bell,** and **W. Schaffner.** 2002. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus. Emerg. Infect. Dis.* 8:82-84.

96. **Kahler, R. C., J.M. Boyce, M.S. Bergdoll, W.R. Lockwood** and **M.R. Taylor.** 1986. Toxic shock syndrome associated with TSST-1 producing coagulase-negative staphylococci, *A. J. Med. Sci.* 292: 310–312.

97. **Kamath, U., C. Singer,** and **H.D. Isenberg.** 1992. Clinical significance of *Staphylococcus warneri* bacteremia. *J Clin Microbiol* 30:261–264.

98. **Kérouanton, A., J.A. Hennekinne, C. Letertre, L. Petit, O. Chesneau, A. Brisabois,** and **M.L.D. Buyser.** 2007. Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France, *Int. J. Food Microbiol.* 115: 369–375.

99. **Khambaty, F.M., R.W. Bennet,** and **D.B. Shah.** 1994. Application of pulse-field gel electrophoresis to the epidemiological characterization of *Staphylococcus intermedius* implicated in a food-related outbreak. *Epidemiol. Infect. 113*: 75-80.

100. **Klevens , R. M.,M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray,L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey,** and **S. K. Fridkin.** 2007. Invasive Methicillin-Resistant *Staphylococcus aureus* Infections in the United States. *JAMA* 298(15): 1763-1771.

101. **Kloos, W. E.,** and **T. L. Bannaerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282-298. In Murray, P. R., Baron, E. J.Pfaller, M. A., Tenover, F. C., and Yolken R. H. (eds), Manual of clinical microbiology, ASM Press, Washington, D.C.

102. **Kluytmans, J., W. van Leeuwen, W. Goessens, R. Hollis, S. Messer, L. Herwaldt, H. Bruining, M. Heck, J. Rost, N. van Leeuwen, A. van Belkum,** and **H. Verbrugh.** 1995. Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping. *J. Clin. Microbiol.* 33:1121-1128.

103. **Kreiswirth, B.N., P. M. Schlievert,** and **R. P. Novick.** 1987. Evaluation of coagulase-negative staphylococci for ability to produce toxic shock syndrome toxin 1. *Journal of Clinical Microbiology* 25: 2028–2029.

104. **Kuehnert, M. J., H. A. Hill, B. A. Kupronis, J. I. Tokars, S. L. Solomon,** and **D. B. Jernigan.** 2005. Methicillin-resistant *Staphylococcus aureus* hospitalizations, United States. *Emerg Infect Dis.* 11:868–72.

105. **Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N.K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi** and **K. Hiramatsu.** 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*, *Lancet* 357: 1225–1240.

106. **Larsen, H.D., A. Huda, N. H. R. Eriksen,** and **N. E. Jensen.** 2000. Differences between Danish bovine and human *Staphylococcus aureus* isolates in possession of superantigens. *Vet. Microbiol. 76*: 153-162.

107. **Le Loir, Y., F. Baron,** and **M. Gautier.** 2003. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* 31: 63–76.

108. **Lee, C. Y., J. J. Schmidt, A. D. Johnson-Winegar, L. Spero,** and **J. J. landolo.** 1987. Sequence determination and comparison of the exfoliative toxin A and toxin B genes from *Staphylococcus* aureus. *J. Bacteriol.* 169:3904-3909.

109. **Lee, J. H.** 2003. Methicillin (Oxacillin)-Resistant *Staphylococcus aureus* Strains Isolated from Major Food Animals and Their Potential Transmission to Humans. *Appl Environ Microbiol.* 69(11): 6489–6494.

110. **Letertre, C., S. Perelle, F. Dilasser,** and **P. Fach.** 2003. Identification of a new putative enterotoxin SEU encoded by the egc cluster of *Staphylococcus aureus, J. Appl. Microbiol.* 95: 38–43.

111. **Levine, W. C., W. B. Reginald, C. Youngsook, K. J. Henning, J. R. Rager, K. A. Hendricks, D. P. Hopkins, R. A. Gunn,** and **P. M. Griffin.** 1996. Staphylococcal Food Poisoning Caused by Imported Canned Mushrooms. *J. Inf. Dis.* 173(5): 1263-1267.

112. **Livernore, D. M.** 2000. Antibiotic resistance in staphylococci. *Int. J. Antimicrob. Agents* 16: S3-10.

113. **Loeffler, A., A. K. Boag, J. Sung, J. A. Lindsay, L. Guardabassi, A. Dalsgaard, et al.** 2005. Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *J Antimicrob Chemother* 56:692–7.

114. **Lozano, C., M. Lopez, E. Gomez-Sanz, F. Ruiz-Larrea, C. Torres** and **M. Zarazaga.** 2009. Detection of methicillin-resistant Staphylococcus aureus ST398 in food samples of animal origin in Spain. *J Antimicrob Chemother* 64(6):1325-6.

115. **Luiz, S. C., L. S., R. S. Dias, V. R. Linardi, M. J. de Sena.,** and **D. A. dos Santos.** 2003. An outbreak of staphylococcal food poisoning in the Municipality of Passos, MG, Brazil. *Braz. arch. biol. technol.* 46: 4.

116. **Maes, N., J. Magdalena, S. Rottiers, Y. D. Gheldre,** and **M. J. Struelens.** 2002. Evaluation of a triplex PCR assay to discriminate Staphylococcus aureus from coagulase-negative Staphylococci and determine methicillin resistance from blood cultures. *J Clin Microbiol* 40(4):1514-7.

117. **Malanoski, G. J., M. H. Samore, A. Pefanis,** and **A. W. Krachmer.** 1995. *Staphylococcus aureus* catheter-associated bacteremia. Minimal effective therapy and unusual infectious complications associated with arterial sheath catheters. *Arch. Intern. Med.* 155:1161.66.

118. **Marr, J.C., J. D. Lyon, J. R. Roberson, M. Lupher, W. C. Davis, G. A. Bohach, *et al.*** 1993. Characterization of novel type C staphylococcal enterotoxins: biological and evolutionary implications. *Infect. Immun.* 61: 4254–4262.

119. **Marrack, P.** and **J. Kappler.** 1990. The staphylococcal enterotoxins and their relatives. *Science* 248:705-711.

120. **Martin, S. T.,** and **R. B. Beelman.** 1996. Growth and Enterotoxin Production of *Staphylococcus aureus* in Fresh Packaged Mushrooms (*Agaricus bisporus*). *J Food Protection.* 59(8): 819-826.

121. **Martins, A.,** and **L. M. Cunha.** 2007. Methicillin resistance in Staphylococcus aureus and coagulase-negative staphylococci: epidemiological and molecular aspects. *Microbiol Immunol* 51(9):787-95.

122. **Matthews, K. R., J. Roberson, B. E. Gillespie, D. A. Lvther,** and **S. P. Oliver.** 1997. Identification and Differentiation of Coagulase-Negative *Staphylococcus aureus* by Polymerase Chain Reaction. *J Food Protection* 60(6): 686-688.

123. **McCormick, J. K., J.M. Yarwood,** and **P.M. Schlievert.** 2001. Toxic shock syndrome and bacterial superantigens: an update, *Annu. Rev. Microbiol.* 55: 77–104.

124. **McKenna, U. G.,** and **J. A. Meadows.** 1980. 3rd, Brewer NS, Wilson WR, Perrault J. Toxic shock syndrome, a newly recognized disease entity. Report of 11 cases. *Mayo Clin Proc* 55:663-72.

125. **Mead, P.S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin,** and **R. V. Tauxe.** 1999. Food-Related Illness and Death in the United States, Emerging infectious diseases, Center for disease control and prevention.

126. **Merck Manuals.** 2005. Infectious Disease: Staphylococcal Infections. Merck and Co., Inc, Whitehouse Station, NJ, USA.

127. **Mertz, D., R. Frei, N. Periat, M. Zimmerli, M. Battegay**, and **U. Flückiger.** 2009. Exclusive *Staphylococcus aureus* throat carriage: at-risk populations. *Arch Intern Med.* 169(2):172-8.

128. **Moyer, M.A., L. D. Edwards,** and **L. Farley.** 1983. Comparative culture methods on 101 intravenous catheters. Routine, semiquantitative, and blood cultures. *Arch Intern Med 143*:66–69.

129. **Munson, S. H., M.T. Tremaine, M.J. Betley,** and **R.A. Welch.** 1998. Identification and characterization of staphylococcal enterotoxin types G and I from *Staphylococcus aureus. Infect. Immun.* 66: 3337–3348.

130. **Notermans, S.** and **C. J. Heuvelman.** 1983. Combined effects of water activity, pH, and suboptimal temperature on growth and enterotoxin production. *J. Food Sci. 48*: 1832-1835.

131. **Novick, R.P.** 2000. Pathogenicity factors and their regulation. In: *Gram Positive Pathogens* (Fischetti, V.A., Novick, R.P., Feretti, J.J., Portnoy, D.A. and Rood, J.I., eds.). ASM Press, Washington, D.C., USA, pp. 392-407.

132. **Oh, S. K., N. Lee, Y.S. Cho, D.B. Shin, S.Y. Choi,** and **M. Koo.** 2007. Occurrence of toxigenic *Staphylococcus aureus* in ready-to-eat food in Korea. *Journal of Food Protection* 70: 1153–1158.

133. **Oliveira, G. A., J. B. Faria, C. E. Levy,** and **E. M. Mamizuka.** 2001. Characterization of the Brazillian endemic clone of methicillin-resistant *Staphylococcus aureus* (MRSA) from hospitals throughout Brazil. *Braz. J. Infect. D* 5: 163-170.

134. **Olsvik, O., K. Fossum** and **B.P. Berdal.** 1982. Staphylococcal enterotoxin A, B, and C produced by coagulase-negative strains within the family *Micrococcaceae, Acta pathologica, microbiologica, et immunologica Scandinavica. Section B, Microbiol* 90: 441–444.

135. **Omoe, K., D.L. Hu, H. Takahashi-Omoe, A. Nakane,** and **K. Shinagawa.** 2003. Identification and characterization of a new staphylococcal enterotoxin-related putative toxin encoded by two kinds of plasmids, *Infect. Immun.* 71: 6088–6094.

136. **Onoue, Y.** and **M. Mori.** 1997. Amino acid requirement for growth and enterotoxin production by *Staphylococcus aureus* in chemically defined media. *Int. J. Food Microbiol. 36*: 77-82.

137. **Orden, J. A., D. Cid, M. E. Blanco, J. A. Ruiz Santa Quiteria, E. Gomez Lucía,** and **R. de la Fuente.** 1992. Enterotoxin and toxic shock syndrome toxin-one production by staphylococci isolated from mastitis in sheep. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* 100:132–134.

138. **Orwin, P. M., D.Y. Leung, H.L. Donahue, R.P. Novick,** and **P.M. Schlievert.** 2001. Biochemical and biological properties of Staphylococcal enterotoxin K. *Infect. Immun.* 69: 360–366.

139. **Orwin, P.M., D.Y. Leung, T.J. Tripp, G.A. Bohach, C.A. Earhart, D.H. Ohlendorf** and **P.M. Schlievert.** 2003. Characterization of a novel staphylococcal enterotoxin-like superantigen, a member of the group V subfamily of pyrogenic toxins. *Biochem.* 41: 14033–14040.

140. **Orwin, P.M., J.R. Fitzgerald, D.Y. Leung, J.A. Gutierrez, G.A. Bohach,** and **P.M. Schlievert.** 2003. Characterization of *Staphylococcus aureus* enterotoxin L. *Infect. Immun.* 71: 2916–2919.

141. **Parsonnet, J., A. E. Harrison, S. E. Spencer, A. Reading, K.C. Parsonnet,** and **E. H. Kass.** 1987. Nonproduction of toxic shock syndrome toxin 1 by coagulase-negative staphylococci. *J. Clin. Microbiol.* 25:1370–1372.

142. **Proft T.** and **J.D. Fraser.** 2003. Bacterial superantigens, *Clin. Exp. Immunol.* 133: 299–306.

143. **Pu, S., F. Han,** and **B. Ge.** 2009. Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* Strains from Louisiana Retail Meats. *Appl Environ Microbiol.*75 (1): 265–267.

144. **Raad,II** and **Bodey GP.** 1992. Infectious complications of indwelling vascular catheters. *Clin. Infect. Dis.* 15:197.210.

145. **Rainard P., J.C. Corrales, M.B. Barrio, T. Cochard,** and **B. Poutrel.** 2003. Leucotoxic activities of *Staphylococcus aureus* strains isolated from cows, ewes and goats with mastitis: importance of LukM/LukF'-PV leukotoxin, *Clin. Diagn. Lab. Immunol.* 10: 272–277.

146. **Regassa, L.B.** and **M. J. Betley.** 1992. Alkaline pH decreases expression of the accessory gene regulator (*agr*) in *Staphylococcus aureus*. *J. Bacteriol. 174*: 5095-5100.

147. **Regassa, L.B., R. P. Novick,** and **M. J. Betley.** 1992. Glucose and nonmaintained pH decrease expression of the accessory gene regulator (*agr*) in *Staphylococcus aureus*. *Infect. Immun. 60*: 3381-3388.

148. **Roberson, J. R., L. K. Fox, D. D. Hancock, J. M. Gay,** and **T. E. Besser.** 1996. Prevalence of coagulase-positive staphylococci, other than *Staphylococcus aureus*, in bovine mastitis. *Am J Vet Res.* 57(1):54-8.

149. **Rosec, J. P., J. P. Guiraud, C. Dalet,** and **N. Richard.** 1997. Enterotoxin production by staphylococci isolated from foods in France. *International Journal of Food Microbiology* 35:213–221.

150. **Rosec, J.P.** and **O. Gigaud.** 2002. Staphylococcal enterotoxin genes of classical and new types detected by PCR in France. *Int. J. Food Microbiol. 77*: 61-70.

151. **Schad, E.M., I. Zaitseva, V. N. Zaitsev, M. Dohlsten, T. Kalland, P. M. Schlievert, *et al.*** 1995. Crystal structure of the superantigen staphylococcal enterotoxin type A. *EMBO J.* 14: 3292–3301.

152. **Schuberth, H.J, C. Krueger, H. Zerbe, E. Bleckmann,** and **W. Leibold.** 2001. Characterization of leukocytotoxic and superantigen-like factors produced by *Staphylococcus aureus* isolates from milk of cows with mastitis, *Vet. Microbiol.* 82: 187–199.

153. **Serhat, K.,** and **O. Kuplulu.** 2009. Detection the enterotoxin producing capacity of coagulase positive *Staphylococcus* by EIA (Enzyme Immuno Assay) isolated from turkey meat. *Ankara Üniv Vet Fak Derg* 56: 183-186.

154. **Shalita, Z., I. Hertman,** and **S. Sand.** 1977. Isolation and characterization of a plasmid involved with enterotoxin B production in *Staphylococcus aureus*. *J. Bacteriol.* 129: 317–325.

155. **Shimizu A., M. Fujita, H. Igarashi, M. Takagi, N. Nagase, A. Sasaki,** and **J. Kawano.** 2000. Characterisation of *Staphylococcus aureus* coagulase Type VII isolates from staphylococcal food poisoning outbreaks (1980–1995) in Tokyo, Japan, by pulsed-field gel electrophoresis, *J. Clin. Microbiol.* 38: 3746–3749.

156. **Shuttleworth, R.,** and **W. D. Colby.** 1992. *Staphylococcus lugdunesis* endocarditis. *J. Clin. Microbiol.* 30:1948.52.

157. **Siegel, J. D., E. Rhinehart, M. Jackson,** and **L. Chiarello.** 2006 Healthcare Infection Control Practices Advisory Committee. Management of multi-drug resistant organisms in healthcare settings, 2006. US Centers for Disease Control and Prevention.

158. **Stefan, M. T.,** and **R. B. Beelman.** 1996. Growth and Enterotoxin Production of *Staphylococcus aureus* in Fresh Packaged Mushrooms (*Agaricus bisporus). J. Food Protection.* 59(8): 819-826.

159. **Strommenger, B., C. Kehrenberg, C. Kettlitz, C. Cuny, J. Verspohl, W. Witte, et al.** 2006. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *J Antimicrob Chemother* 57:461–5.

160. **Su, Y. C.** and **A.C. Wong.** 1995. Identification and purification of a new staphylococcal enterotoxin, *H. Appl. Environ. Microbiol.* 61: 1438–1443.

161. **Sundstrom, M., L. Abrahmsen, P. Antonsson, K. Mehindate, W. Mourad,** and **M. Dohlsten.** 1996. The crystal structure of staphylococcal enterotoxin type D reveals Zn^{2+}-mediated homodimerization. *EMBO J.* 15: 6832–6840.

162. **Swenson, J. M.** 2002. New tests for detection of oxacillin resistant *Staphylococcus aureus. Clin. Microbial. Newslet.* 24: 159-162.

163. **Thomas L. C., H. F. Giddin, A. N. Gin, T. Olma,** and **Iredell.** 2006, Development of a real-time *Staphylococcus aureus* and MRSA (SAM-) PCR for routine blood cultutre. *Journal of Microbiological Methods* 68: 296-302.

164. **Todd, E. C. D.** 1989. Preliminary estimates of costs of foodborne disease in the United States. *J Food Protect* 52:595-601.

165. **Tremaine, M.T., D. K. Brockman,** and **M. J. Betley.** 1993. Staphylococcal enterotoxin A gene (sea) expression is not affected by the accessory gene regulator (agr). *Infect. Immun.* 61: 356–359.

166. **Turnridge, J.,** and **M. L. Grayson.** 1993. Optimum treatment of staphylococcal infections. *Drugs* 45: 353-366.

167. **Tveten, Y., A. Jenkins A. Digranes, K. K. Melby, A. G. Allum,** and **B. E. Kristiansen.** 2004. Comparison of PCR detection of mecA with agar

dilution and E-test for clinical isolates of coagulase negative staphylococci. *Clin. Mirobiol. Infect.* 10: 459-470.

168. **Udo, E. E., M.A. Al-Bustan, L.E. Jacob** and **T.D. Chugh.** 1999. Enterotoxin production by coagulase-negative staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning, *Journal of Medical Microbiology* 48: 819–823.

169. **Ulrich, R. G., S. Sidell,** and **T. J. Taylor.** 1997. Staphylococcal enterotoxin B and related pyogenic toxins. In: *Textbook of Military Medicine. Part I. Warfare, Weaponry and the Casualty.* 3: 621-631.

170. **Valle, J., S. Vadillo, S. Piriz** and **E. Gomez-Lucia.** 1991. Toxic shock syndrome toxin 1 (TSST-1) production by staphylococci isolated from goats and presence of specific antibodies to TSST-1 in serum and milk, *Applied and Environmental Microbiology* 57: 889–891.

171. **Van den Bussche, R.A., J. D. Lyon,** and **G. A. Bohach.** 1993. Molecular evolution of the staphylococcal and streptococcal pyrogenic toxin gene family. *Mol. Phylogenet. Evol.* 2: 281–292.

172. **van Griethuysen, A., I. van Loo, A. van Belkum, C. Vandenbroucke-Grauls, W. Wannet, P. van Keulen ,** and **J. Kluytmans.** 2005. Loss of the mecA gene during storage of methicillin-resistant Staphylococcus aureus strains. *J Clin Microbiol.* 43(3):1361-5.

173. **Varaldo P. E., R. Kilpper-bälz, F. Biavasco, G. Satta** and **K. H. Schleifer.** 1988. *Staphylococcus delphini* sp. nov., a Coagulase-Positive Species Isolated from Dolphins. *Int J Syst Bacteriol.* 38: 436-439.

174. **Vernozy-Rozand, C., C. Mazuy, G. Prevost, C. Lapeyre, M. Bes, Y. Brun** and **J. Fleurette.** 1996. Enterotoxin production by coagulase-negative staphylococci isolated from goats' milk and cheese, *International Journal of Food Microbiology* 30: 271–280.

175. **von Eiff, C., A.W. Friedrich, G. Peters,** and **K. Becker.** 2004. Prevalence of genes encoding for members of the staphylococcal leukotoxin family among clinical isolates of *Staphylococcus aureus, Diagn. Microbiol. Infect. Dis.* 49: 157–162.

176. **von Eiff, C., K. Becker, K. Machka, et al.** 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med.* 344(1):11-6.

177. **Waites, K.B., R. A. Bobo,** and **R. O. Davis, et al.** 1984. Clinically silent polymicrobial amnionitis and intrauterine fetal death associated with a Cu-7 intrauterine contraceptive device. *Am J Obstet Gynecol* 150:998–999.

178. **Weese, J. S., M. Archambault, B. M. Willey, P. Hearn, B. N. Kreiswirth, B. Said-Salim, et al.** 2005. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000–2002. *Emerg Infect Dis* 11:430–5.

179. **Wegener, H. C.** and **E. W. Skov- Jensen.** 1992 Exudative epidermitis. In Straw B.E., D'Allaire S., Mengeling W.L., and Taylor D.J. (eds). Diseases of Swine. 8th Edition. Iowa State University Press, pp 469-474.

180. **Wenzel, R., G. Bearman,** and **M. Edmond.** 2007. Community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA): new issues for infection control. *Int J Antimicro Agt* 30(3): 210-212.

181. **Witte, W.** 1999. Antibiotic resistance in gram-positive bacteria: epidemiological aspects. *J. Antimicrob. Chemother.* 44 (Suppl A): 1-9.

182. **Witte, W., B. Strommenger, C. Stanek,** and **C. Cuny.** 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in Humans and Animals, Central Europe. *Emerg Infect Dis.* 13(2): 255–258.

183. **Wood, C. A., D. L. Sewell,** and **L. J. Strausbaugh.** 1989. Vertebral osteomyelitis and native valve endocarditis caused by *Staphylococcus warneri. Diagn. Microbiol. Infect. Dis.* 12:261.63.

184. **Yarwood J. M., J. K. McCormick, M. L. Paustian, P. M. Orwin, V. Kapur,** and **P.M. Schlievert.** 2002. Characterization and expression analysis of *Staphylococcus aureus* pathogenicity island 3. Implications for the evolution of staphylococcal pathogenicity islands, *J. Biol. Chem.* 277: 13138–13147.

185. **Zecconi A., L. F. Calvinho,** and **K. L. Fox.** 2006. *Stapylococcus aureus* intramammary infections, *IDF Bull.* 408: 42.

186. **Zell, C., M. Resch, R. Rosenstein, T. Albrecht, C. Hertel,** and **F. Götz.** 2008.Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. *Int J Food Microbiol.* 127(3): 246-251.