AN ABSTRACT OF THE THESIS OF

Mohammad Arif Awan for the degree of Master of Science in Veterinary Science presented on September 5, 1997. Title: Systemic Bacterial Infections in Broiler Chickens.

Abstract approved: Redacted for Privacy

Masakazu Matsumoto

In broiler operations, various health problems develop during the final two weeks of the growing period, resulting in increased mortality and condemnation losses. At this stage, sickly birds were found to be systemically infected by various bacteria regardless of varied clinical signs. The main objective of the present study was to determine the prevalence and nature of systemic bacterial infections in unthrifty commercial broiler chickens and to establish a reproducible infection model in the laboratory.

Thirty-one unthrifty 6-week-old broilers were obtained from three farms, and bacterial isolations were conducted on blood, liver, and hock joint. Bacteria were isolated from 87, 90, and 71% of the blood, liver and hock joint samples, respectively. Mean bacterial counts ($\log_{10}$ CFU/ml or g) of the blood and liver were 2.15 and 2.93, respectively. Among 132 bacterial isolates, major species were; *Staphylococcus* (60%), *Corynebacterium* (18%), *Escherichia coli* (5%), and *Stomatococcus* (4%). Among 79 *Staphylococcus* isolates, 77 were coagulase-negative. Major species of staphylococci

were; *S. lentus* (19%), *S. simulans* (18%), *S. cohnii* (13%), *S. gallinarum* (10%) and *S. captis* (7%). In addition, 6 species of gram-positive and 5 species of gram-negative organisms were isolated. Apparently systemic infections were not caused by predominant pathogenic bacterial species, and adequately described as mixed infections. However, there were some significant relationships between isolated bacterial species and sampling sites, suggesting that certain organisms were abundant in the environment of a particular poultry house. These results indicate that systemic infections in market age broilers are caused by mixed bacterial species and suggest that they are caused by suppressed host antibacterial systems rather than pathogenic factors of microorganisms. Antibiotic susceptibility results showed 100% susceptibility of staphylococcal isolates (n= 69) against vancomycin and enrofloxacin. Of these coagulase negative staphylococci showed 19% and 73% resistance against methicillin and penicillin G, respectively. There was also heterogeneity in antibiogram profiles within species of coagulase-negative staphylococci.

Pathogenicity of representative field isolates from the above described study was tested in 5-day-old embryonated eggs and in 3- week-old broiler chicks. Consistent lethality was demonstrated with *S. aureus* in embryos. *Staphylococcus intermedius* or *S. lentus* demonstrated some pathogenicity, while *S. gallinarum* or *Corynebacterium* were non-pathogenic in embryos. In 3-week-old broilers, however, only *S. aureus* caused septicemia and death; other bacterial species mentioned above caused neither clinical signs of acute or chronic staphylococcosis nor mortality.

The effect of stressed conditions on the induction of spontaneous systemic infections were evaluated in 65 broiler chickens up to 7 week of age. The birds in stressed group were exposed to lowered temperature, poor ventilation, and wet litter. Birds were examined daily for any clinical sign or abnormality until the termination of experiment. Total mortality in the stressed was 4% compared with no mortality in the control group. At necropsy, significant difference ($P<0.05$) was observed only in the occurrence of hypertrophy of the heart between the two groups. There was a significant difference ($P<0.05$) in the frequency of bacterial isolations from the liver, between the two groups. Therefore, although overt signs of systemic bacterial infections were not induced, the significantly higher isolation rates in the liver of the stressed birds suggest that environmental/management factors may be associated with the occurrence of systemic bacterial infections in the market age broilers.

Systemic Bacterial Infections in Broiler Chickens

by

Mohammad Arif Awan

A THESIS

submitted to

Oregon State University

in partial fulfilment of

the requirements for the

degree of

Master of Science

Presented September 5, 1997

Commencement June 1998

Master of Science in Veterinary Science thesis of Mohammad Arif Awan, Student

presented on September 5, 1997

APPROVED:

Redacted for Privacy

Major Professor, representing Veterinary Science

Redacted for Privacy

Dean, College of Veterinary Medicine

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Mohammad Arif Awan Student, Author

ACKNOWLEDGMENTS

The author humbly and sincerely thanks " Allah Almighty" (God) who created the Universe and accorded the mankind with knowledge and wisdom to search for its secrets. He further supplicate peace for the Prophet Muhammad (Peace be upon him) who is an eternal source of guidance and knowledge for humanity as a whole.

The author expresses, deep gratitude and appreciation to his major professor Dr. Masakazu Matsumoto for his keen interest, inspiring guidance, painstaking efforts and consistent encouragement, without which this work would have never been materialized.

Special thanks go to Dr. Mattson, College of Veterinary Medicine and Dr. Tom Savage, Department of Animal Sciences, Oregon State University, for their critical review and valuable suggestions in preparation of this manuscript.

Author cannot forget to express his cordial thanks to the officials of Agriculture Research Project II, and Livestock Department, Government of Baluchistan for granting him fellowship which provide him an impetus for higher studies.

Author is highly obliged to his friends Dr. Shakeel Babar, Maqsood Qureshi, Dost Baluch, and Zafar Malik for their moral support, generous help and cooperation.

Special thanks are extended to my brother in laws, Mohammad Ayyub and Dr. Mubarik Masood for their inspiration, moral support, tremendous cooperation and unfailing help rendered whenever it was needed.

Finally, author wish to express his thanks to his mother, mother in law, aunt, brothers, wife and children for their love and prayers for his success.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

Table Page

Dedication

This thesis is dedicated to my wife, Talat and to my children Ahmed, Laayeba, and Sidrah for their love, patience and understanding.

Systemic Bacterial Infections in Broiler Chickens

Chapter 1

Introduction

The present thesis research has been initiated to investigate a health problem of chickens at a local poultry farm. High mortality was observed in four to six week-old broiler chickens. While some of the affected birds showed arthritis of hock joints or ascites , the majority lacked such distinctive signs/lesions. Microbiological investigations by this laboratory, however, demonstrated that 75% to 100% of the affected birds were systemically infected with various bacteria, most notably by coagulase-negative staphylococci. Investigations on several other broiler farms confirmed the findings, and it appeared that this condition was prevalent in the local area (Awan and Matsumoto, 1997a).

The major objective of the present research was to conduct and document microbiological investigations of the field problem, and to establish a model infection in the laboratory. In Chapter 3, the results of microbiological investigations are presented. Various efforts have been made to develop an infection model in chickens to simulate the field problem. These attempts unfortunately have not resulted in establishing a model, but some useful information have been obtained and presented in Chapter 4.

The literature review (Chapter 2) covers most up-to-date information on staphylococcus infections in man and animals as well as those in poultry. As presented in Chapter 3, the systemic infection in broiler chickens are mostly caused by staphylococci.

Chapter 2

Literature Review

In this chapter; *Staphylococcus*, methicillin-resistant *Staphylococcus aureus,* coagulase-negative staphylococci, and staphylococcal infections in poultry are described under the following subheadings.

2. 1. ***Staphylococcus***

- 2. 1. 1. Classification
- 2. 1. 2. Habitat
- 2. 1. 3. Virulence/ Pathogenicity
- 2. 1. 4. Antibiotic Resistance
- 2. 1. 5. Enterotoxins
- 2. 1. 6. Public Health Significance

2. 2. **Methicillin-Resistant *Staphylococcus aureus***

- 2. 2. 1. Microbiology
- 2. 2. 2. MRSA: Current Research
- 2. 2. 3. MRSA: Control Measures

2. 3. **Coagulase-negative Staphylococci**

2. 4. **Staphylococcal Infections in Poultry**

- 2. 4. 1. Osteomyelitis and Synovitis
- 2. 4. 2. Gangrenous Dermatitis of Chickens
- 2. 4. 3. Bumble Foot.

2. 1. *Staphylococcus*

2. 1. 1. Classification

The genus *Staphylococcus*, is the most important genus in the family *Micrococcaceae*, and is currently composed of thirty two species. The members of this group are gram-positive, spherical in shape, non-motile, non-spore forming with limited capsule formation (Kloos and Bannerman, 1995). These bacteria grow well on most routine laboratory media at 37°C (Jawatz et al., 1984). Most colonies of the staphylococci on solid media are circular, opaque, smooth, raised, with white to pigments of different colors (Joklik et al., 1992). Staphylococci are facultative anaerobes, usually catalase positive and oxidase negative (Kloos and Bannerman, 1995). Coagulase production is the most widely used and generally accepted criterion for the identification of pathogenic staphylococci (Quinn et al., 1994; Skeeles, 1997). Pathogenic staphylococci often hemolyze blood, but the hemolytic pattern depends on both the staphylococcal strain and the source of the blood (Novick, 1990). The biochemical characteristic of different species of staphylococci are well documented (Kloos and Bannerman, 1995; Quinn et al., 1994).

2. 1. 2. Habitat

Staphylococci are ubiquitous in nature. The natural habitat of staphylococci, including coagulase-negative species is the body surfaces of mammals and birds (Anderson, 1986). Sometimes they are also present in blood, upper respiratory tract, genitourinary tract, intestines, and other body organs. Staphylococci are common bacteria

in the environment where poultry are hatched, reared, and processed (Devriese, 1980; Skeeles, 1997). They are also recovered from the skin and nares (Harry, 1967a, b), beak (Witte et al., 1977), and feet (Cooper and Needham, 1976) of apparently healthy chickens. The external tissues of poultry are heavily colonized with non-virulent staphylococci, mostly *S. epidermidis*, and *S. simulans*. The coagulase-negative species are also reported to be recovered in low concentration from the liver and synovial fluid of some healthy birds (Fraser, 1964).

2. 1. 3. Virulence/Pathogenicity

Virulence of *Staphylococcus aureus* can not be explained in terms of a single factor, several factors have similar biologic effects and it is the interaction of the total armory of the organism that makes it virulent (Anderson, 1976; Sherris, 1984). Pathogenic staphylococci have different cell surface structures (Johnsson and Wadstrom, 1993; Scanlan, 1988), as well as producing a variety of exotoxins and extracellular enzymes (Murray et al., 1994; Quinn et al., 1994). *Staphylococcus aureus* and coagulase negative staphylococci interact with various serum and connective tissues proteins such as fibronectin, vitronectin, different types of collagens, and lamins. These interactions may be important in the pathogenesis of wound and foreign body infections (Holderbaun et al., 1987; Wadstrom et al.,1987; Herrmann et al., 1988). It is further reported that almost all *S. aureus* strains recovered from human cases of osteomyelitis and septic arthritis involving bone and joint tissues were found having collagen adhesin (Holderbaun et al., 1987; Switalski et al., 1993).

Staphylococcus aureus and certain coagulase-negative staphylococci are the etiological agents of diverse clinical diseases in human (Joklik et al., 1992; Murray et al., 1994; Boyed, 1995) and in animals (Quinn et al., 1994; Carter and Chengappa, 1991; Johnsson and Wadstrom, 1993).

2. 1. 4. Antibiotic Resistance

Resistance rapidly developed in staphylococci after penicillin was introduced in clinical medicine, and today fewer than 10% of the *S. aureus* strains are susceptible to this antibiotic (Finegold and Baron, 1986; Murray et al., 1994).

Resistance to the penicillins is due to three different mechanisms (Sabath, 1979), such as enzymic destruction due to β-lactamase production (Poston, and Naidoo, 1983), intrinsic resistance typically shown by methicillin-resistant strains of staphylococci (Hartmann and Tomasz, 1984), and tolerance (Sabath, et al., 1977). Genetic studies have established that penicillinase mediated resistance in staphylococci is usually plasmid mediated (Richmond, 1972a). The transmissible plasmids facilitate the rapid dissemination of resistance among staphylococci (Murray et al., 1994).

Penicillin resistance in *Staphylococcus aureus* was followed by resistance to macrolide antibiotics, aminoglycosides, and tetracyclines. During the succeeding years, the frequency of these resistant strains increased rapidly, and in many clinical setting multiple antibiotic resistance is now the rule (Novick, 1990). Multiple-antibiotic resistance is often associated with high levels of penicillinase production (Richmond et al., 1964). Resistance to other antibiotics, such as erythromycin and fusidic acid, may

sometimes also be controlled by genes located on the penicillinase plasmid (Poston and Naidoo, 1983).

Many coagulase-negative staphylococci are also resistant to several antibiotics including the penicillin, gentamicin, erythromycin, and chloramphenicol (Boyed, 1995). Despite the inclination of staphylococci to develop resistance to antibacterials, almost all strains are uniformly susceptible to vancomycin (Hackbarth and Chambers, 1989; Murray et al., 1994; Boyed, 1995).

2. 1. 5. Enterotoxins

Staphylococcal enterotoxins are a series of low molecular weight proteins which are heat-stable in nature. They are classified as enterotoxins because of their emetic reaction when given intragastrically in monkeys. The enterotoxins are the only known biologically active substances produced by the staphylococci whose toxic properties are resistant to proteolytic enzymes (Bergdoll, 1983). Staphylococcal food poisoning, which is an intoxication not an infection (Sherris, 1984) is the most common form of bacterial food poisoning which is due to ingestion of food containing preformed toxin rather than to ingestion of bacteria (Abeyounis, 1982). Animals species except monkeys are reported to be highly resistant against staphylococcal enterotoxins (Jonsson and Wadstrom, 1993). These enterotoxins are mainly produced by 30 to 50% of all *S. aureus* strains whereas *S. epidermidis* was also reported to form these toxins (Murray et al., 1994). It is reported that less than 1 μg enterotoxin may be sufficient to make a sensitive person ill (Bergdol, 1973). The staphylococcal enterotoxins have been serologically classified into seven

groups: A, B, C1, C2, C3, D, and E (Wieneke, 1991). Enterotoxin A is most commonly associated with food poisoning. Enterotoxins C, D and B are associated with contaminated milk products, and staphylococcal pseudomembranous enterocolitis respectively (Murray et al., 1994). The genetic control of staphylococcal enterotoxins has not been clearly defined. However the gene for its production may be on the chromosome, but a plasmid may carry a regulatory protein for active toxin production (Joklik et al., 1992)

Outbreaks of food poisoning occur when food is contaminated by a nasopharyngeal carrier or, more often by a person with a staphylococcal lesion of the skin, especially of hands. The contaminated food if kept at inappropriate temperature results in staphylococcal multiplication with a yield of heat-stable enterotoxins. Food poisoning is characterized by vehement vomiting, cramps, diarrhea, and prostration two to eight hours after ingestion. This type of intoxication is rarely fatal and patient fully recovered within 24 to 48 hours (Boyed, 1995).The mechanism of action of staphylococcal enterotoxins has not been well defined. . Besides the stimulation of intestinal peristalsis, the emetic effect of enterotoxin also probably causes stimulation of the central nervous system (vomiting center) after the toxin acts on neural receptors in the gut. Except for the man the only reliable experimental animal for testing enterotoxin activity is the monkey (Murray et al., 1994).

It is reported that 48.5% of *S. aureus* strains isolated from meat products including poultry produced enterotoxin A alone or in combination with other enterotoxins (Payne and Wood, 1974). Enterotoxin C as a cause of staphylococcal food poisoning was

associated with chickens and minced beef in Kenya. The highest isolation rate as well as highest percentage of enterotoxigenic strains of *Staphylococcus aureus* were found in chickens (Ombui et al., 1992). In another situation, strains of *S. aureus* were assayed for the production of enterotoxin in United kingdom. Of the 359 outbreaks and sporadic cases of staphylococcal food poisoning, seventy nine percent of the strains associated with these incidents produced enterotoxin A alone or together with another enterotoxin (Wieneke et al., 1993). More recently in a survey, 26% of the workers in a restaurant were reported as nasal carriers for *S. aureus*. Of these isolates, 86.6% were enterotoxigenic in nature which produced enterotoxins type A (28.5%), type B (28.5%), type C (16.4%), and type D (3.5%) respectively (Bustan et al., 1996).

For the detection of staphylococcal enterotoxins in food samples, enzyme linked immunosorbent assay (Wieneke, 1991; Bennett and McClure, 1994) and latex agglutination have been described (Wieneke and Gilbert, 1987). Recently a reversed-passive-latex-agglutination method for the detection of enterotoxins was reported (Ko and Chang, 1995). By this test the most frequently found staphylococcal enterotoxin was type A, which was detected in 50% (9/18) of the isolates from market food, whereas in 81% (34/42) of the isolates from food poisoning samples. Although test detected enterotoxin as low as 5 ng/g of food samples, but the researchers claimed that recovery would had been better if staphylococcal enterotoxin A was present in foods at a concentration between 10-40 ng/g . More recently a commercially available rapid enzyme-immuno-essay was approved by 13 laboratories for detection of staphylococcal enterotoxins A to

E in foods. The five enterotoxin serotypes were detected at a level of 0.8 ng/g in meat and at different levels in raw milk cheese, mushrooms, and milk (Lapeyre et al., 1996).

Studies on enterotoxins described their function as biologic response modifiers, which affect host immune defense mechanisms. On account of this, enterotoxin are designated as superantigens. They influence T-cell functions by controlling their repertoire, their cytokines production and their modulation of the immune response (Micusan and Thibodeau, 1993).

Recent studies have been focused on several different aspects of these enterotoxins. In one study Niedergang et al., (1995) described, that how the *S. aureus* enterotoxin (superantigen) induced changes in the dynamics of surface T cell receptors. Further, crystal structures of the enterotoxin types A (Sundstrom et al., 1996) and C2 and their interaction with class 11 major histocompatibility complex (MHC) were also described (Papageorgiou et al., 1995). It is reported, that *S. aureus* enterotoxin-B-anti-tumor antibody conjugates represented a potentially powerful approach for better tumor immuno-therapy (Ochi et al., 1993). More recently, Barton et al., (1996) described, the inhibitory action of interlukin-6 and 11 against, superantigen induced T-cell activation. They suggested that further experiments are needed to determine the clinical role of these interlukins. The preventive role of immunoglobulin Y from hen's-egg in the inhibition of *S. aureus* enterotoxin-A and some other bacterial diseases was also reported (Sugita et al., 1996).

2. 1. 6. Public Health Significance

Pathogens often involved in bacterial food poisoning are *S. aureus, Salmonella typhi, Shigella species, Streptococcus pyogenes, Escherichia coli,, Clostridium perfringens, Clostridium botulinum, Vibrio parahemolyticus*, and *Bacillus cereus* (Snyder and Matthews, 1996). Staphylococcal food poisoning is probably the leading cause of food borne illness in the world (Bergdoll, 1983). *Staphylococcus aureus* produces thermostable enterotoxins which cause food poisoning in humans (Murray et al., 1994). Almost 50 percent of the typical and atypical enterotoxigenic strains of *S. aureus* are responsible for human food poisoning (Gibbs et al., 1978; Raska et al., 1981; Harvey et al., 1982; Evans et al., 1983).

In the USA, most cases of *S. aureus* associated food poisoning have occurred due to the consumption of meat, and poultry (Meehan et al., 1992; Wieneke et al., 1993). The other sources of foods such as salad (potato, egg, other), baked foods, dairy (milk, cheese, butter), shellfish, vegetables, fruits, and multiple other sources, in staphylococcal food poisoning have been reported (Halpin and Marth, 1989; Murray et al., 1994). Many reports of food poisoning especially due to the consumption of chicken, minced beef (Ombui et al., 1992), and ham (Richards et al., 1993) from other parts of the world are also documented. Todd (1978) based on data from six countries indicated that up to 22.9% of all outbreaks of food borne disease are associated with poultry. Wieneke (1974) also reported that enterotoxin A was the most common (73%) type in food incriminated for staphylococcal food poisoning outbreaks, which might indicate a rather greater public health hazard from *Staphylococcus aureus* strains of poultry.

Investigations revealed, that in staphylococcal food poisoning, mostly the raw food materials were contaminated by the enterotoxic strains of *S. aureus* either from the abattoir, food processing plants, in whole sale or retail outlets (Meehan et al., 1992; Cowden et al., 1995), or from plant machinery during defeathering and evisceration (Porto and Silva, 1995). It is further concluded that workers in the processing plant are also the main sources for the contamination of dressed poultry (Notermans et al., 1982). In one study *S. aureus* was present in only small numbers (10CFU/ g) on the skin of broiler chickens before processing. Whereas during processing mainly in plucking and evisceration, contamination of carcases with this organism increased to > 10^3 CFU/g of skin (Notermans et al., 1981). The combined use of spray cleaner and spin chiller were found satisfactory in reducing *S. aureus* counts on the contaminated carcases. In another occasion, it was observed by Gibbs, Patterson, and Thompson (1978b) that defeathering machines provided a warm, and moist environment which subsequently favored microbial growth. They suggested that from the hygiene standpoint more attention needs to be given to the design of such machines and to search for more suitable material to replace rubber for the plucking 'fingers' which rapidly become worn and cracked and hence difficult to clean and disinfect properly. Harvey et al., (1982) also indicated that the multiplication of *S. aureus* did not occur on the skin of either commercially processed or hand-plucked hen carcases stored at 10°C, whereas at 15°C it significantly occurred on the processed carcases. Similar results on storage temperature in turkeys have been reported by Yang et al., (1988).

Many food borne staphylococcus associated diseases are caused by food handlers who have staphylococcal lesions of skin, especially of the hands, or are nasopharyngeal carriers (Boyd, 1995; Cowden et al., 1995). In a study, of the 26.6% of *S. aureus* nasal carriage among restaurant workers, 86.6% of the isolates were enterotoxigenic (Bustan et al., 1996). It is also concluded that increased handling of chicken meat and minced beef by the food workers resulted in outbreak of *S. aureus* food poisoning (Ombui et al., 1992). In another situation the same type of food poisoning due to the consumption of contaminated ham was connected to the improper refrigeration, prolonged handling, and inadequate reheating of the food material (Richards et al., 1993). Multiple-organism outbreaks of food poisoning after eating turkey meat was reported by Meehan et al., (1992). In this situation workers who upon investigation were found carriers of *S. aureus* strains did not follow the proper food handling practices. More recently, Cowden et al., (1995) uncovered that of the 458 cases almost 24% were due to *S. aureus* and most commonly cross contamination, improper storage, and inadequate heat treatment of the food stuffs, resulted into outbreaks of food poisoning

Novick (1990), suggested that a food handler who is a carrier of or has an open infection with an enterotoxin-producing strain of *S. aureus*, as such persons may be implicated in outbreaks of food poisoning, they should not be permitted to handle food until active dissemination of the organism has stopped. Boyd, (1995) further concluded that it is impossible to eliminate permanently the nasal carrier. In such situations topical applications of neomycin, bacitracin, and gentamicin ointment have been successful, especially for those who repeatedly experience staphylococcal infections. Sherris, (1984)

on the other hand described the significance of proper refrigeration of food stuffs particularly at homes, restaurants, hospitals and other places, as inappropriate storage may lead to staphylococcal multiplications up to 10^5 or more CFU/ gram. Cowden et al., (1995) further, suggested that cross-contamination of food materials should be avoided in order to avoid food poisoning.

Conventional methods have been in use for the detection of *S. aureus* strains in food samples for the last many years (Niskanen et al., 1991). Recently, Tsen et al., (1994) reported polymerase chain reaction (PCR), in which primers specific for *S. aureus* enterotoxin genes were used for the detection of enterotoxigenic strains of *S. aureus* in foods. More recently reversed-passive-latex-agglutination test was used for the detection of enterotoxigenic strains of *S. aureus* (Ko and Chang, 1995).

Staphylococcal food poisoning is still a common problem throughout the world. Among the main contributing factors for staphylococcal food poisoning are the poultry farm, slaughter house, and kitchen. An access to a rapid, sensitive, and economical pre-slaughter screening test for staphylococcal bacteremia would be helpful in culling the infected birds and subsequently reduce the public health hazards. Poultry carcass examination should be stringent. The plucking and eviscerations units of the processing plant should not be prone to breakage for complete disinfection of the plant. Wearing of aprons, gloves, masks, and caps should be the routine practice. Storage conditions should be optimal. The food handlers should get a wide range of training particularly in food hygiene, and kitchen sanitary measures. Health status of workers should be routinely monitored. Laboratory tests on randomly selected food samples should be performed for

the presence of *S. aureus* and enterotoxins. These measures would help in reducing public health concerns regarding food poisoning implicated by *S. aureus*.

2. 2. Methicillin-Resistant *Staphylococcus aureus*

2. 2. 1. Microbiology

The introduction of penicillinase-resistant penicillin derivatives such as methicillin was considered to solve the problem but methicillin-resistant strains of *S. aureus* appeared within few years (Barber, 1961), and are designated as methicillin-resistant *S. aureus* (MRSA). No antibiotic resistance marker has distinguished a species more than methicillin resistance has for *S. aureus*.

Methicillin-resistant *S. aureus* strains are important nosocomial pathogens, and outbreaks of MRSA have been reported worldwide (Maple et al., 1989; Wenzel, 1982). The emergence of methicillin resistance and its subsequent spread throughout the world have created therapeutic problems for physicians, management difficulties for nurses, and confusion for infection control practitioners (Mulligan et al., 1993). Today, most authorities consider MRSA a serious threat and it continues to be at the forefront of those organisms that seriously challenge modern technological medicine and surgery (Gould and Chamberlaine, 1995). Methicillin-resistant *S. aureus* has been recognized as a nosocomial pathogen in North America and Europe for three decades (Johnston, 1994). Additionally, a single strain of MRSA, referred as epidemic-methicillin-resistant *S. aureus* (EMRSA), is reported to be the cause of large outbreaks of infection in many hospitals in the USA, and in some other countries. These strains were sometimes also

responsible for severe postoperative sepsis in certain patients (Spicer, 1984; Duckworth et al., 1988). Emergence of methicillin and gentamicin resistant, epidemic strains of *S. aureus*, a cause of an outbreak of hospital infection have been documented (Shanson et al., 1976). More recently, MRSA of enhanced epidemicity, particularly EMRSA-16 is also reported in several hospitals of United Kingdom (Casewell, 1995). The relative virulence of MRSA when compared with methicillin-sensitive *S. aureus* (MSSA) remains to be defined, although there are documented reports that persistence MRSA nasal carriage is more likely to result in serious staphylococcal infection than MSSA carriage (Muder et al., 1991).

Three mechanisms for methicillin resistance have been described. The first mechanism for methicillin resistance is based on the production of unique penicillin-binding-protein 2a (PBP2a). This is the most common mechanism for resistance and is designated as intrinsic resistance (Jorgensen, 1991), which differentiates it from resistance due to β-lactamase production. The production of PBP2a is linked to the presence of a chromosomal gene ending within a region called *mec*, which is regulated by other chromosomal genes including a putative repressor gene called *mecR* (Gustafson et al., 1992; Sakumoto et al., 1996). Further within MRSA there are population of cells which express heterogeneous to homogeneous resistance (Tomasz et al., 1991), and very few cells actually express methicillin resistance (Hackbarth and Chambers, 1989). The second mechanism for methicillin resistance is due to hyper-production of the penicillinase (McDougal and Thornsberry, 1986) and is called borderline-resistant. These strains have lower minimal inhibitory concentrations for β-lactams than do

heteroresistant strains. The third type is termed as methicillin-intermediate resistance in which *S. aureus* instead of producing PBP2a yielded normal penicillin-binding-proteins (PBPs) with reduced affinity for β-lactam antibacterials (Tomasz et al., 1989). These strains are designated as modified *Staphylococcus aureus* (Johnston, 1994).

The principal mode of MRSA transmission within an institution is from patient to patient via the transiently colonized hands of hospital personnel who acquire the organism after direct patient contact or after handling contaminated materials (Peacock et al., 1981). Other routes of transmission such as infected or colonized health care workers who disseminate the organism directly (Ward et al., 1981), from air (Boyce et al., 1983) or, environmental surfaces (Bartzokas et al., 1984) which are usually less important (Mulligan et al., 1993). Recently Jernigan et al., (1995) reported that an increase in the incidence of nosocomial MRSA infection was associated with an increase frequency of transfer of colonized patients from nursing homes and other hospitals. Crowcroft et al., (1996) analyzed an outbreak of MRSA in a general hospital. The risk factors such as length of stay in hospital, pressure sores, physiotherapy, and surgical procedures were associated with a significantly increased risks of acquiring MRSA.

Methicillin-resistant *S. aureus* have been reported to be the cause of different disease conditions specially in certain types of patients. Johnston (1994) delineated MRSA as the cause of pneumonia in long-term care facilities with relatively high mortality of 20% to 84%. Rumbak and Cancio (1995) accounted, MRSA as a cause of ventilator-associated pneumonia in long-term acute care ventilator patients. Kluytmans et al., (1995) reported a case of septicemia due to MRSA. Genetic analysis showed that this

outbreak strain was more virulent and more transmissible than other MRSA strains. Recently Kawahira et al., (1996) described a case of infective endocarditis affecting mitral valve due to MRSA.

For the identification of MRSA, three accepted *in vitro* susceptibility testing methods such as broth-microdilution minimum inhibitory concentration (MIC), Oxacillin agar screen (Thornsberry and McDougal, 1983), and disk-diffusion tests (National Committee for Clinical Laboratory Standards, 1990) are mostly widely used.

Reports of MRSA resistance against other members of the penicillinase-resistant penicillins are emerging. In one study, 11% of MRSA isolates associated with nosocomial infections were also resistant to oxacillin, or nafcillin (Panlilio et al., 1992). There have been reports on MRSA treatment failure due to cephalosporins (Barret et al., 1968; Locksley et al., 1982; Piercy et al., 1989). In one study majority of MRSA strains showed resistant to multiple other classes of antibiotics (Lyon et al., 1987), especially fluoroquinolones such as ciprofloxacin, and norfloxacin (Harnett et al., 1991). Based on these reports it is a general consensus that MRSA should be considered resistant to all classes of β-lactam antibiotics (Chambers, 1991). Erythromycin, sulfas, clindamycin, and tetracyclines have generally shown poor activity against MRSA (Maple et al., 1989; Hackbarth and Chambers, 1989). Of the several antibacterials, generally vancomycin is the antibiotic of choice for the treatment of MRSA infections (Milatovic, 1986; Johnston, 1994; Pulimood et al., 1996). In some instances, where vancomycin is not effective (Johnston, 1994), antibiotic combinations after in-vitro testing were reported to be effective (Hackbarth and Chambers, 1989; Elwell et al., 1986). The use of β-lacatmase

inhibitors such as clavulante, sulbactam, and tazobactam combined with highly active β-lactam-antibiotics were reported to be effective against MRSA encountered in surgical infections (Neu, 1994) however, antibiotic resistance among MRSA is increasing. Vancomycin-resistance from *Enterococcus faecium* was successfully transferred to a laboratory strain of *S. aureus* (Casewell, 1995).

Several antibiotic agents which are effective *in vitro* against *S. aureus* are usually used for the treatment of nasal carriers, but few are clinically effective. Topical treatment with mupirocin ointment is usually effective for nasal carriage in hospital personnel (Reagan et al., 1991) and colonized patients (Rumbak and Cancio, 1995), however, mupirocin resistance in MRSA strains is increasing markedly especially in long-term application cases (Miller et al., 1996).

2. 2. 2. MRSA: Current Research

Early detection of infections due to MRSA has been a main concern. Recently, polymerase-chain-reaction (PCR) method for the diagnosis of MRSA associated bacteremia in postoperative patients has been reported (Kitagawa et al., 1996). This method is based on the detection of *mecA* and toxic shock syndrome toxin-1 genes within 3 to 4 hours after blood collection. It is claimed that this test is superior than the conventional blood culture method which usually needs 48 hours for MRSA identification. Researchers have anticipated that this rapid and sensitive method would be helpful in the prevention of cross infection and for early determination of suitable treatment for MRSA infected patients.

Among the many topical antibiotics, mupirocin has been most widely used in the control of MRSA colonized patients. Netto et al., (1996) and Miller et al., (1996) reported that extensive use of mupirocin as a topical ointment for the control of MRSA colonization resulted in high level of resistance against it. In one study, of the 114 MRSA isolates, 63% showed resistant against mupirocin, whereas in another situation resistance increased from 2.7% to 65% within three years.

The emerging problem with MRSA is the expression of heterogeneous resistance or inconsistent susceptibility against antibiotics. Quantitative antibiogram method was reported by Blanc et al., (1996), which is based on multivariate analysis of inhibition zone diameters of antibiotics in disk-diffusion tests. Researchers claimed that this test is equivalent to ribotyping and suggested its suitability in the surveillance and control of MRSA, particularly for those hospitals which lack molecular typing facilities. Recently, Resende and Figueiredo, (1997) delineated, that besides the heterogeneous resistance within MRSA isolates, agar medium with methicillin 25 mg/L was found suitable for the determination of methicillin resistance in *S. aureus* isolates over agar with less concentration of methicillin or oxacillin.

Reports on the increasing incidence of MRSA in nosocomial infections have been emerging. In a study it is described that these prevalent MRSA were characteristically β-lactamase negative, type-11 coagulase producing, and highly methicillin-resistant in nature. It is considered that all of these factors have contributed in constitutive PBP2 production which resulted in their increased colonizing capabilities and epidemic potential (Yokoyama et al., 1996).

Variety of disinfectants are in use for the last many years to contain the spread of MRSA from inanimate or other sources in various institutions. Recently the efficiency of superoxidized water as a disinfectant was evaluated in a study (Tanaka et al., 1996). It is claimed that superoxidized water which is similar to 80% ethanol but superior to 0.1% chlorhexidine and 0.02% povidone iodine, is a low cost but powerful disinfectant.

2. 2. 3. MRSA: Control Measures

On the basis of evidences from countries where MRSA is not a problem it has been suggested that early detection, effective infection control measures, and rationale antibiotic use may limit the transmission of these organisms; however spread is still increasing in many countries (Ayliffe, 1997). Generally, control measures for MRSA varies from situation to situation. Recent studies on these control measures have focused on rapid methods of detection for MRSA in patients/healthy carriers (Blanc et al., 1996; Kitagawa et al., 1996), extent of infection (Mizushima et al., 1996; Kawahira et al., 1996), selection of antibiotic therapy (Olona et al., 1996; Meier et al., 1996; Evans and Kortas, 1996; Pulimood et al., 1996), exercise of barrier precautions to contain the spread (Back et al., 1996;), problem-oriented education of the personnel involved in health care institutions (Vandenbroucke, 1996), or on understanding of predisposing factors for MRSA infections (Flaherty and Weinstein, 1996).

Education of the hospital individuals is one of the essential elements in a MRSA control program. This should be specifically focussed on the degree of the problem,

modes of spread, methods to prevent person to person transmission, isolation procedures, universal precaution regarding wounds, and cleaning procedures (Mulligan et al., 1993).

Surveillance is required to ascertain the degree and extent of the hospital reservoir specially in endemic or outbreak establishments. The strategies are usually dependent on the magnitude of the noticed problem. For clinical infections, the presence of MRSA is usually determined by routine cultures of infections from hospitalized patients. However these cultures are reported to detect only one third of the patients (Tufnell et al., 1987) . An additional one third of the patients having MRSA in hospital setting can be detected by culturing wounds, tracheostomy sites and sputum from intubated patients (Sanford et al., 1994). The final one third of MRSA infected or colonized patients can be detected through an admission identification list which contains the names of all patients known to be harboring MRSA. In an outbreak situation newly transferred patients from other hospitals or nursing homes with open wounds should be cultured in order to recognize the resistant organism and potential pathogens including MRSA. Medical worker should be cultured only if epidemiologic data implicate them as a likely source of spread of MRSA (Mulligan et al., 1993).

Strategies for the eradication of carrier state depends on different situations . In an out break situation, if only an individual is involved then temporarily removal and treatment of the carrier state is wise. However in most cases several persons are responsible, then cultures of patients for *S. aureus* and onward therapy to attempt elimination of the carrier state is reasonable (Meier et al., 1996). In non-out break situation, MRSA eradication attempts are linked with the emergence of resistance

(Blumberg et al., 1991), side effects of the used agents and extra monitoring cost (Cohen et al., 1991).

Guidelines for the management of patients with infection and colonization due to MRSA is dependent on different factors like different institutes with different prevalence of MRSA, diverse patient populations, and resources. For most institutions, therefore policies aimed at the control of MRSA should have the goals of minimizing intra and inter-institutional transmission, and the prevention of outbreaks (Mulligan et al., 1993). In one study, round re-ordering in hospital wards seemed to help prevent nosocomial infection due to MRSA. In this situation ward rounds for patients who have had gastroenterologic surgery were proceeded from compromised host to stable patients, and then isolated patients (Yoshida et al., 1995).

Precautions for patients infected or colonized with MRSA are very important. Both "contact" isolation and "strict" isolation are usually applied for these patients depending upon the body site involved and the likelihood of nosocomial spread (Cohen et al., 1991). It is reported that besides the small outbreaks of MRSA , strict isolation of all patients in single rooms was found promising in controlling MRSA (Vandenbroucke-Grauls, 1996). However in one study, poor compliance to contact isolation precautions was observed in all hospitals in France (Richet et al., 1996). Cohorting which is the physical separation of patients who are infected or colonized with MRSA, from those who are neither colonized nor infected with the organism is accounted to be critical infection control maneuver in both endemic (Murray et al., 1990) and epidemic (Meier et al., 1996) settings.

Barrier precautions for all patients should be enforced in containing the spread of MRSA from patient to patient via the hands of health care personnel. Precautions should be used for the care of all wound, regardless of known colonization with MRSA or other organisms. The resistant organism is reported to be easily recovered from the hands of health care workers immediately after dressing infected wounds (Thompson, et al., 1982). Hand washing is reported to be more useful than protective clothing (Gould and Chamberlaine, 1995), especially with chlorhexidine (Rumbak and Cancio, 1995). Drainage/secretion precautions for small wounds whereas contact isolation for larger wounds, major burns, and severe dermatitis are found useful (Garner and Simmons, 1983).

Control of MRSA in intensive care units, burns units, outpatient settings, long-term-care facilities, and outbreak situations are of paramount importance. In intensive care units patients present special problems for MRSA control because of increased susceptibility of patients; being cared for by busy physicians, nurses, and technicians; exposure to multiple invasive procedures, and frequent use of broad spectrum antibiotics. In a study encountering the same factors effective control strategy was based on routine unit-based surveillance for antimicrobial resistance, improved compliance with hand washing, and barrier-precautions (Flaherty and Weinstein, 1996). Further, hospital personnel and if possible equipments like blood pressure cuffs and tourniquets should also be specified for such individual patients (Mulligan et al., 1993). In an epidemic of MRSA in neonatal-intensive-care unit, intensive microbiologic surveillance and isolation

of the patients were reported to be effective over the traditional intravenous therapy and mupirocin (Back et al., 1996).

In burns units patients are significantly problematic. Large surface area of burns are good site for the bacterial multiplication and subsequent transmission to other patients via the hands of health worker. Moreover environmental contamination due to the organism is very likely another contributing factor in the spread of the MRSA (Rutala et al., 1983). Barrier precautions should be used for the patients with large burn area. These patients should be kept under strict isolation with gowns, masks, and gloves. Regular changes of intravascular catheters should be on priority. Septic patients should be initially treated with vancomycin until the pathogen is clearly defined (Mulligan et al., 1993).

Sporadic outbreak usually occur in outpatients settings involving patients associated with intravenous drug abuse, long-term hemodialysis, and chronic antibiotic use (Reboli et al., 1990). Careful infection control precautions with the proper disinfection of surgical and medical equipment should be observed in all locations where outpatients are commonly encountered (Mulligan et al., 1993). Visiting nurses and home health care personnel should practice the barrier infection control precautions (Simmons et al., 1990). The prevalence of MRSA in long-term-care facilities are as great as in acute-care hospital settings (Hsu, 1991). Patients should not be excluded from a long-term-care facility just because they are colonized with MRSA. Practices such as hand washing between patients contacts, barrier precautions during wound care should be routinely followed. Unless an outbreak has occurred, the routine use of isolation and cohortation is not encouraged . Moreover surveillance cultures and attempts at

decolonization should not be routinely performed (Mulligan et al., 1993). In an outbreak situation, simple reemphasis on routine infection control measures was found beneficial (Guiguet et al., 1990). On the other hand in most cases an outbreak required extensive modification of local infection control practices (Bitar et al., 1987). However total elimination of MRSA is extremely rare (Goetz et al., 1992; Boyce et al., 1983).

Other general precautionary measures for the control of MRSA are also very important. Careful cleaning of the environment in hospitals with MRSA infections by housekeeping personnel is important (Maki et al., 1990). Equipments and instrument for MRSA patients should not be used for non-MRSA patients unless properly disinfected. Routine cleaning process of the inanimate objects circulated from patients to patients should be inspected periodically (Mulligan et al., 1993). Early discharge of MRSA colonized patients is an effective method of limiting the spread of the organism within the hospital setting (Bell, 1982; Pearman et al., 1985). However, endorsement of the patients and extent of MRSA colonization in hospital record should be made (Platt et al., 1989). From an infection control perspective, the home care setting is ideal for such patients (Mulligan et al., 1993). Although many MRSA colonized and infected patients harbor multiple illnesses and need skilled nursing assistance when discharged from the hospital. Further, the transferring hospital should inform the nursing facility about the patients's MRSA status along with the sites of colonization (Archer and Pennel, 1990).

In summary, MRSA is an accelerating problem in hospitals, and other institutions. The reservoir for MRSA includes recognized and unrecognized colonized or infected patients, as well as previously colonized or infected patients readmitted to the hospital.

Early detection and suitable infection control measures are basic factors to reduce MRSA transmission and to control the hospital reservoir.

2. 3. Coagulase-negative Staphylococci

Besides the other well documented bacteria, coagulase-negative staphylococci are also increasingly important causes of infection particularly in cardiovascular surgery, where they may cause serious postoperative complications. Like *S. aureus*, primarily *S. epidermidis* also produce β-lactamases and can be resistant to all β-lactam antibiotic (Neu, 1994). Coagulase-negative staphylococci are the most frequently isolated organisms in prosthetic-valve endocarditis and inflict 5% of infections involving native valves. Infection is frequently complicated by valvular insufficiency with congestive heart failure, local tissue invasion or synthetic embolization, making surgical intervention necessary in most cases (Whitener et al., 1993). High mortality rate in endocarditis due to *S. lugdunensis* is reported to be increasing. Mitral-valve endocarditis due to this organism in old women (Koh et al., 1996) and another four cases of native-valve endocarditis (Lessing et al., 1996) have been recently reported, all of which were later surgically treated.

The occurrence of wound infections following cardiothoracic surgery has significant implications. In one study overall bacterial infection rate was 11.7%. Of the other bacterial species, 27.4% were identified as coagulase-negative staphylococci. Adverse outcomes were re-explorations and death (4.3%) which were associated with deep chest infections (L' Ecuyer et al., 1996). Coagulase-negative staphylococci are

reported as commonly isolated bacteria from wounds of patients after median sternotomy in open heart procedures (Mossad et al., 1997). Of the 436 sternal wound infections, 100 (23%) were due to the coagulase- negative staphylococci. Ninety-two percent of these isolates were methicillin-resistant. These encountered bacteria inflicted additional cost to the patients due to the further necessary operative procedures and prolonged antibiotic therapy. Insulin-dependent diabetes mellitus was considered, the only risk factor significantly associated with these infections

Among the organisms isolated from bacteremic human-immunodeficiency virus (HIV) infected children, coagulase-negative staphylococci were the most common type of bacteria. These HIV-infected children showed increased risk of bacteremia with an overall mortality of 17% (Nathoo et al., 1996).

Epidemic of postoperative-endoopthalmitis due to bacterial infection have been reported by Arsan et al., (1996). Of the 10 intraocular eye cultures, 3 revealed coagulase-negative staphylococci, whereas 4 were positive for *Pseudomonas aeruginosa*. These complications were considered due to individual risk factors, and delayed subconjunctival antibiotic therapy

Coagulase-negative staphylococci in particular *S. epidrmidis* and *S. haemolyticus* have been reported in patients undergoing chronic ambulatory peritoneal dialysis (Gemmell, 1996). These bacteria survived intracellulary within peritoneal macrophages and exhibited resistant to antibiotic therapy

Late-onset sepsis is an important problem which is a cause of mortality in very low-weight infants. In a surveillance study, of the 6911 infants 1696 (25%) had one or

more episodes of blood culture-proven sepsis. Gram-positive organisms were mainly (73%) involved, of which 55% were due to coagulase negative staphylococci. Forty five percent of deaths in the first weeks were related to the infections (Stoll et al., 1996). It is further reported that neonates are high risk populations in regard to coagulase-negative staphylococci infections because of certain risk factors such as degree of immunosuppression, routine use of central venous catheters, broad spectrum antibiotic therapy (Hubner and Kropec, 1995). As these infants have no marked physiological skin flora they are easily colonized by multiresistant bacteria .

2. 4. Staphylococcal Infections in Poultry

Staphylococcal infections are common in poultry and most of these diseases are caused by *S. aureus*, a recognized pathogen in many animal species (Kibenge et al., 1982). Arthritis and synovitis are the two most early documented conditions (Lucet, 1892; Hole and Purchase, 1931; Jungherr, 1933; Gwatkin, 1940). Osteomyelitis, tendinitis, arthritis, and synovitis are commonly occurring infections (Skeeles, 1997), whereas, vesicular dermatitis (Hoffman, 1979), omphalitis (Williams and Daines, 1942), endocarditis (Bergmann et al., 1980), spondylitis (Carnaghan, 1966), acute fibrinopurulent blepharitis (Cheville et al., 1988), gangrenous dermatitis (Frazier et al., 1963), scabby-hip dermatitis of broilers (Page, 1974), and granulomas in liver and lungs (Arp et al., 1983; Munger and kelly, 1973) are less frequent infections.

2. 4. 1. Osteomyelitis and Synovitis

Of the many staphylococci, *S. aureus* is the predominant cause of osteomyelitis, synovitis and systemic infection, in broiler chickens (Mutalib et al., 1983), and turkeys (Narin, 1973). In broiler and breeder chickens, considerable economic losses are sustained by decreased weight gain, decreased egg production (Sompolinsky et al., 1985), and condemnations related to staphylococcal tenosynovitis (Herenda and Franco, 1996). Several studies on these diseases have been reported in chickens and turkeys. In most of the cases the experimental disease has been reproduced in these species of bird. In acute cases usually the septicemic phase is the rule, otherwise bacteremia followed by osteomyelitis and arthritis involving synovial membranes, articular surfaces of the joints, or tendon sheaths are usually the main consequences.

Arthritic disease was intravenously reproduced in 8 week-old chickens with a dose of 2×10^7 colony-forming-units (CFU) of *S. aureus* that closely resembled the severe form of the naturally occurring disease (Smith, 1954). No disease was reproduced through intraperitoneal or subcutaneous routes of administration or challenge. Death occurred within 2-10 days, after the onset of symptoms. The first sign was lameness due to unilateral or bilateral involvement of the joints mostly hock. The sick birds were reluctant to move, and preferred a recumbent posture. Diarrhea from the onset of the disease was a constant feature. Extreme emaciation in chickens surviving more than 4-5 days after staphylococcal disease was noticed. Lameness disappeared in most cases by 21 days after the onset of disease, but in few cases it was a chronic feature. At necropsy there was no apparent lesions in acutely died birds. Long-lived birds were extremely emaciated

and a few with swollen joints were observed. The articular surfaces in these joints were acutely inflamed with a thin white purulent fluid in the synovial capsule. In chronically lame birds joints, articular surfaces and tendon sheaths exhibited more pronounced lesions. No internal abscesses were found in the birds.

Clinical incidence of *S. aureus* associated osteomyelitis and synovitis in 4-8 week old meat chickens may reach up to 50% with 5% mortality (Narin and Watson, 1972). These conditions in chickens have been reproduced by the parenteral inoculation of *S. aureus*. It is further, considered that through the hematogenous route the organisms reach the cartilaginous growth plate of the long bones and proliferate accordingly. Afterward these bacteria disseminate to the joint and tendon sheath. The broiler with osteomyelitis and synovitis shows a characteristic hopping or a limping gait and were reluctant to move in the advanced stages of the disease.

A study on the experimental osteomyelitis in 6-week-old broiler chickens using different routes was conducted by Mutalib et al., (1983). A single dose of $5x10^6$ CFU of *S. aureus* intravenously, caused mortality in these birds due to acute septicemia. Low doses through the same route, caused osteomyelitis and lameness. A single or repeated doses of $5x10^{11}$ CFU of *S. aureus* intratracheally, inflicted osteomyelitis in small populations of chickens. On the contrary, the aerosol route was found ineffective to cause the disease. It is assumed that under normal conditions lungs are capable to clear a large number of staphylococci, whereas impairment in this function makes the lung, to be route of bacterial entry.

Experiments on the effect of stresses on experimentally induced osteomyelitis in chickens have been conducted (Mutalib et al., 1983). Of these, feed restriction, debeaking, and hormonal injection did not show any effect on the susceptibility of the birds to staphylococci. Conversely, delayed appearance in disease with less severe lesions were observed in stressed birds. Clinically, progressively severe signs were observed in birds with experimental osteomyelitis. By 72 hours (hr) post-inoculation (PI) birds had ruffled feathers, a reluctance to move, and were warm. By 96 hr PI, they were found heads down, eyes closed, sitting on the hock joints and less responsive. Mortality was observed within 3-5 days after the appearance of clinical signs of the disease. A high dose of $2x10^7$ CFU inflicted earlier signs of the disease with early mortality than a low dose ($1\text{-}3x10^5$ CFU) injected birds, which were remained alive at the end of experiment. At necropsy, hock joints were found mostly swollen, and bruised posteriorly with fluid of varying consistency. The livers were small with yellow-white foci in some cases, and no feed in the digestive tract. Acutely died birds with high dose were in good body condition and showed severe lesions in liver and spleen..

Bacteriological studies on arthritis or tenosynovitis in broilers from the field cases of femur head necrosis have been conducted (Griffiths et al., 1984). *Staphylococcus aureus* was recovered from the synovial fluid of the affected joints. Further disease is experimentally reproduced by injecting 10^5 to 10^7 CFU of *S. aureus* in two groups of 35-day-old healthy commercial broilers respectively. Initially, paralysis associated with vertebral osteomyelitis at the thoraco-lumber junction was shown in one bird, 3-days PI.

By 6-days PI, another 5 birds exhibited clinical leg weakness and at necropsy synovitis of hock joint (4 birds), synovitis of stifle joint (2 birds), and mis-shapen femoral heads (3 birds) were noted. *S. aureus* was recovered from heart blood and liver in all the cases.

Staphylococcus aureus associated tenosynovitis in one-day-old male broiler chickens was experimentally produced with a dose of $8.5x10^5$ CFU (Hill et al., 1989). The injected birds were comparatively smaller than the control chickens with a few of them dehydrated and emaciated. They were showing the peculiar signs of the disease 48 hours post-inoculation (PI) with 50 % reluctant to move. High mortality and morbidity within 10 days PI was observed. Low incidence of lameness was the sequelae in birds surviving the acute septicemia. Grossly tendon above the hock was firm and swollen and causing unilateral or bilateral hyperextension of hock joints. Necropsy revealed hepatitis and splenomegaly, with no ascites. Mild swelling and discoloration of the gastrocnemius and digital flexor tendons in 20 % of the *Staphylococcus aureus* injected birds, up to 5 weeks PI were observed. Slightly increased amount of yellow-colored fluid was occasionally noted in the hock joints. *Staphylococcus aureus* from the hock joints of four of 10 injected birds were isolated at 1 and 5 weeks PI

Experiments to observe the resistance in certain leghorn lines of chickens against staphylococcal disease have been reported (Cotter, 1992). Two strains of *S. aureus* (known to be pathogenic in chickens) at a dose of $3x10^7$ (intra-cardiac) and $3x10^8$(intra-venous) CFU in each of 3-day, and 6-weeks-old chickens were injected respectively. Mortality in younger birds was observed after 24 hours post-inoculation which later became maximum within one week. These birds were considered to be died of evident

septicemia as shown by hepatitis and splenomegaly with necrotic foci. Whereas in older birds there was septicemia with few death, but mostly lameness alone or lameness followed by recumbency.

In turkeys, usually the clinical staphylococcosis appears between 9 and 20 weeks of age, whereas predisposition to stress such as other diseases or adverse weather could result in frequent occurrences. Mortality due to synovitis in range turkeys was noted to be 5 to 6 % (Warnick, 1982). A study on the pathogenesis of staphylococcal synovitis in turkeys was reported by Smart et al., (1968). Turkeys of different age groups were injected with *S. aureus* in varying doses. Certain number of turkeys were randomly selected from each group at different intervals post-injection for gross lesions, bacteriological culture, and microscopic examination of different tissues. The severe to moderate clinical signs particular to synovitis, involving the hock joints were observed in the infected birds. The bacterial recovery results showed that bacteremia was prerequisite for the infection, and 48 hours post-inoculation organisms were always detected in most organs, except joints and tendon sheaths in which they usually occurred in chronic phase of the disease.

Narin (1973) reported the results of naturally occurring and experimentally induced osteomyelitis and synovitis of turkeys. Of the 149, five to twenty five week-old field turkeys, 56% were related to osteomyelitis and synovitis which were rated as a single disease entity. There was mortality in the affected turkeys which is suggested due to the bacterial septicemia. The disease conditions were most commonly associated with *S. aureus* followed by *Escherichia coli*. Further experimental disease is reproduced in 8,

11-weeks-old turkeys through intravenous injection of *S. aureus* at a dose of 10-20 million CFU/ml. Within 24 hours of inoculation a number of birds were limping. By 72 hours PI, all of them were reluctant to walk and the hock joints were observed to be swollen. There was no mortality in the experimental birds. Bacteriological results showed *Staphylococcus aureus* from 15 joints with synovitis, 12 bones with osteomyelitis, and all 8 livers.

Studies on the isolation of staphylococci from clinical cases, *in vitro* adherence of staphylococci, *in vivo* colonization of turkeys by aerosol and isolation of staphylococci from naturally colonized market-age turkeys were conducted (Jensen et al., 1987). Clinical cases revealed 59 staphylococcal isolates from turkeys showing signs of staphylococcosis; 18 were coagulase-positive staphylococci (*S. aureus*), whereas 41 were coagulase negative staphylococci. Researchers were unable to reproduced the experimental clinical disease (synovitis) in artificially stressed turkeys, through airborne route. They concluded that initiation of the disease under field conditions might be dependent on certain predisposing factors. It is concluded that staphylococcosis is an opportunist infection in turkeys, which is contrary to the assumptions that staphylococci primarily cause infection in cases of injury or insect bites. Isolation of staphylococci from samples of liver, synovial fluid from hock joints, and tracheas of 40 naturally colonized market-aged turkeys from each of 10 randomly selected flocks was made. Coagulase negative staphylococci were predominantly recovered from the samples. Of these, *S. simulans* and *S. epidermidis* were the main species. No selective distribution of these bacteria either in the tissues or among the farms was noted. Based on these data it is

supposed that in natural conditions, the respiratory tract is the major route for *S. aureus* to cause staphylococcosis in turkeys. Natural infections occur when birds inhale airborne *S. aureus*, which is able to colonize tissues such as lungs, and air sacs.

2. 4. 2. Gangrenous Dermatitis of Chickens

Gangrenous dermatitis is commonly seen in broiler chickens. The wingtips and dorsal pelvic area are the sites most commonly affected (Jordon and Pattison, 1996). Other less frequently affected surfaces are thigh, breast, wattles, and feet (Frazier et al., 1964). Scabby-hip dermatitis of broilers is an important economic problem for the poultry industry. The skin lesions are characterized by dry scabs, resulting in downgrading and trimming of carcases at processing (Page, 1974). In one study, Frazier et al., (1964) reported cases of gangrenous dermatitis in chicken from 20 different flocks. *Staphylococcus aureus* and *Clostridium septicum* were isolated from the lesions. Experimental studies revealed that both organisms were capable of inflicting such lesions. Trauma to the skin could be a predisposing factor for this condition.

A case report on staphylococcal gangrenous dermatitis secondary to infectious bursal disease (IBD) vaccination was presented by Cervantes et al., (1988). Sudden signs of depression, dermatitis, and mortality in 17-day-old broilers in a large scale flock were observed. At necropsy extensive subcutaneous fluid accumulation over the pectoral muscle, with lesions in liver, intestines, and Bursae of Fabricius were noted. Bacteriological examination revealed luxuriant growth of *S. aureus* from the affected tissues and liver. The researchers assumed that staphylococci may have initiated infection

by a noncutaneous route, supported by the compromised immune system of chickens earlier infected with the IBD virus.

Scanlan and Hargis, (1989) also isolated staphylococci from 5 week-old broilers with a 10-60% incidence of scabby-hip lesions. Of the 27 specimens cultured *staphylococcus* species were isolated in pure culture from 10 lesions and *Clostridium perfringens* from 4 lesions. Thirteen scabby-hip lesions were culture-negative. Heterogenous species of staphylococci were isolated from these specimens. Like lesions samples, cutaneous specimens were also positive for a variety of coagulase-negative staphylococci. Interestingly only a few isolates were coagulase positive other than S. aureus, from these sources.

2. 4. 3. Bumble Foot

Subdermal staphylococcal, planter abscesses (bumble foot) may develop when phalangeal joints are affected (Jordan and Pattison, 1996). It is a common infection in mature chickens which leads to massive swelling of the foot and subsequent lameness (Skeeles, 1997). *Staphylococcus aureus* have been isolated from 26 of 30 one-year-old chickens with bumble foot infection (Sarmah, 1995). Antibiogram results showed their resistance against penicillin, ampicillin and metronidazole.

Chapter 3

Heterogeneity of Staphylococci and other Bacteria Isolated from 6-week-old broiler Chickens

3. 1. Introduction

Staphylococcus species are normal inhabitants of the skin and mucous membrane of animals. In poultry, staphylococci including *S. aureus* is known to cause various diseases from acute septicemia to chronic osteomyelitis (Skeeles, 1997). Although staphylococci possess a variety of pathogenic and/or virulence factors such as capsule, protein A, coagulase, exotoxins, etc., they are generally considered as an opportunistic or secondary pathogen since some predisposing conditions are required to allow them to enter into and multiply within a host (Jonsson and Wadstrom, 1993).

Osteomyelitis and synovitis in young adult turkeys were found to be most commonly associated with *S. aureus* followed by *Escherichia coli* infection and both organisms were isolated at high frequencies from the bones and livers as well as from joint fluids of lame turkeys (Nairin, 1973). The intravenous inoculation of *S. aureus* into turkeys resulted in the immediate localization of the organism in the liver and spleen, in other parenchymal organs at 48 h, and in the synovial sacs 72 h after the inoculation (Smart *et al.*, 1968). An aerosol exposure of 2 week-old turkey poults to *S. aureus* or coagulase-negative *Staphylococcus* for 2 h resulted in the localization of the organisms in the lungs and in the liver (Jensen *et al.*, 1987). These authors hypothesized that staphylococci colonize and invade the respiratory tract tissue to cause hematogenic or lymphatic spread to parenchymal organs and leg joints. Subsequently

they reported competitive inhibition of *S. aureus* colonization in the turkey respiratory tract by coagulase-negative *S. epidermidis* (Meyers and Jensen, 1987).

Staphylococcal osteomyelitis has been recognized as one of the major problems in broiler chickens (Nairn and Watson, 1972; Skeeles, 1997). Osteomyelitis accompanying synovitis was consistently reproduced in 6-week-old broiler chickens with the intravenous inoculation of *S. aureus* (Mutalib *et al.*, 1983). Signs of a systemic infection preceded the development of joint lesions. The disease, however, was self-limiting as it did not spread to control birds housed together. Evidence is lacking on the pathogenic roles of other bacteria in leg abnormalities of broiler chickens.

Lately, one of the major concerns of commercial broiler growers has been an increased mortality during the final two weeks of the growing period (Awan and Matsumoto, 1997). When sick birds between 6 and 7 weeks of age were examined from problem flocks, ascites and hock joint infections were commonly found. When the blood of these birds was aseptically collected and examined bacteriologically, *Staphylococcus* sp. were isolated at high frequencies. The onset of the systemic infection appeared to be between three and four weeks of age. The purpose of the present investigation was to confirm these observations and to find out whether a particular strain of *Staphylococcus* is associated with the bacteremia occurring in broilers of 6 weeks of age.

3. 2. Materials and Methods

3. 2. 1. Chickens

Ten or eleven unthrifty chickens were obtained from each of three locations, Farm A, B or C. Ten forty-two days-old chickens were taken from a flock of approximately 30,000 at commercial farm A, and eleven from another flock of approximately 20,000 commercial broilers at farm C. Farm B was an experimental poultry farm belonging to the Department of Animal Sciences, Oregon State University. Ten sickly broiler chickens at the age of 44 days were removed from a flock of 2,900. All the chicks were originated from a single source of hatching eggs. Commercial broilers at farm A and C were hatched at a commercial hatchery and injected subcutaneously with Marek's disease vaccines (HVT and SB-1 strain[1]) and 0.1 mg/chick of sarafloxacin hydrochloride[2] at day-old. In addition, at Farm C, they were vaccinated via drinking water with an infectious bursal disease vaccine[3] at the ages of 8 and 14 days. Broiler chickens at farm B were hatched at a university experimental hatchery and raised without any medications or vaccinations. At all three farms, the cumulative mortality at 7 weeks of age was 6% or higher, and the major loss occurred between 5 and 7 weeks of age.

3. 2. 2. Necropsy and Bacterial Isolation

Birds were briefly stunned by electricity at 120 V, the ribs were cut, the sternum was reflected to expose the thoracic and abdominal cavities, and 10 ml blood was collected from the heart with sterile syringes and 21 ga. needles. A portion of

blood, 0.5 ml, was spread each onto sheep blood and MacConkey agar plates[4] , and incubated at 37 C. The remaining blood was allowed to clot for serum samples. A piece of the liver (approximately 4 g) from each bird was cut, placed separately into sterile plastic bags, which were kept on ice. The net weight of cut pieces of the liver was determined, and PBS (0.05 M phosphate, 0.15 M NaCl) was added in 10 ml into each bag followed by homogenization[5]. The liver homogenate in 0.5 ml was plated out onto sheep blood and MacConkey agar plates. The plates were examined at 24 and 48 h after incubation at 37 C to enumerate colonies. The outer surface of hock joints were seared with a soldering iron, cut with a pair of sterile scissors and a swab[6] was inserted into the articular space. Swabs were subsequently streaked onto blood and MacConkey agar plates. After 48 h of incubation at 37 C, representative colonies were subcultured and identified.

3. 2. 3. Bacterial Identification

Representative colonies were gram-stained and identified based on their biochemical characteristics (Carter and Cole, 1990). *Staphylococcus* isolates were speciated based on further biochemical reactions (Kloos and Bannerman, 1995; Schleifer, 1986). Those isolates which did not show typical patterns of reactions were additionally tested with a commercial differentiation kit.[7]

3. 2. 4. Antibiotic Susceptibility Testing

The majority of the staphylococcal isolates were tested for their susceptibilities to the antibiotics procured from Difco and BBL, following the standard disk-diffusion

method (Woods and Washington, 1995). Briefly, four to five well isolated colonies each of reactivated isolate on Brain heart infusion agar was used for the inoculum preparation in Brain heart infusion broth. Turbidity of the inoculum was standardized in comparison to half the density of McFarland no. 1 standard (optical density of 0.08 to 0.10 at 625 nm). Further Muller Hinton agar plates were cultured with each of a inoculum using sterile cotton swabs prior to antibiotic disks. Inhibition zones on the plates were then measured with the help of calibrated view enlarger after overnight incubation at 35°C.

3. 2. 5. Serology

Serum samples obtained at necropsy were assayed for antibodies against infectious bursal disease virus (IBDV; D-78 strain), infectious bronchitis virus (IB; Mass 41 strain), and Newcastle disease virus (NDV) by an enzyme-linked immunoassay.[8]

3. 2. 6. Statistics

The Chi-square test (at 5% level of significance) was used to test differences between isolation frequencies and farm locations or origins of tissues. Student's *t*-test was used to compare mean bacterial counts from blood ($\log_{10}$ CFU/ml) and liver ($\log_{10}$ CFU/g) samples of birds in farms A, B, and C.

3. 3. Results

3. 3. 1. Clinical Signs and Gross Lesions

Affected birds appeared depressed and were reluctant to walk, but were generally alert. At necropsy, feed materials were absent in the crop and proventriculus and little was found in the gizzard in most of the birds. Ascites lesions of various degrees including right ventricular hypertrophy (Becker *et al.*, 1995) were found in 0, 50, and 64% of the birds taken from Farm A, B, and C, respectively. Unilateral swollen hock joints were found in 50, 30, and 9% of the birds taken from Farm A, B, and C, respectively.

3. 3.2. Viable Bacteria in the Blood or Liver

Viable bacteria were detected in 73% to 100% of the blood and/or liver samples (Table 1). The mean counts were 2.15 $\pm$ 0.76 (m $\pm$ s. d., $\log_{10}$ CFU) /ml in the blood and 2.93 $\pm$ 0.57 (m $\pm$ s. d., $\log_{10}$ CFU) /g in the liver. There was no significant difference ($P > 0.05$) in the mean bacterial counts of blood ($\log_{10}$ CFU/ml) and liver ($\log_{10}$ CFU/g) samples among farms A, B, and C, except; significantly ($P < 0.01$) higher mean bacterial count was observed in blood of birds in farm A as compared to birds in farm C. Seventy-four per cent had bacteria both in the liver and blood, and 100% of the birds had bacteria in one of the two organs. Seventy-one per cent of the birds yielded bacteria from the hock joint.

3. 3. 3. Identification of Bacterial Isolates

A total 79 staphylococcus isolates represented 60% of all bacterial isolates (Table 2). Only one each of coagulase-positive *S. aureus* and *S. intermedius* was isolated; both from the hock joint. Of the total 77 coagulase-negative isolates, no predominance of a particular species was noted; *S. lentus* was most frequently isolated at 18% (of the total staphylococcus isolates) followed by 13 other species. No affinity of certain species to one of the three tissue/organ sites was apparent. Some species, however, were isolated at higher frequencies in a particular farm than others, although there was no significant difference ($P > 0.05$) in all bacterial isolation numbers among the three farms. For example, *S. simulans* was isolated predominantly from Farm A ($P < 0.05$). *Staphylococcus cohnii* subsp. *urealyticus* was isolated only from Farm C. Significantly lower numbers ($P < 0.01$) of coagulase-negative staphylococci were isolated in birds from Farm B than Farm A or C, but only from the blood and liver.

Totally 53 organisms other than staphylococci were also isolated (Table 3). Of these isolates, 24 were *Corynebacterium* sp.; a single, predominant species only next to staphylococci. However, 63% of this species was isolated from Farm A ($P < 0.01$). Fifteen gram-negative bacteria were isolated. Significantly more gram-negative organisms were isolated from Farm B than A or C ($P < .01$). *Escherichia coli*, *Moraxiella* sp. and *Proteus* sp. were most frequently isolated gram-negative bacteria.

Table 1. The results of viable bacterial isolations/ counts from the blood, liver, or hock joint of 6-week-old broiler chickens

	Blood		Liver		Hock joint
Farm	Pos./total	Log_{10} (CFU/ml)	Pos./total	Log_{10} (CFU/g)	Pos./total
A	9/9	$2.60^{a} \pm 0.68$[1]	8/10	$2.80^{a} \pm 0.62$	8/10
B	9/10	$2.11^{ab} \pm 0.86$	9/10	$3.50^{a} \pm 0.50$	9/10
C	8/11	$1.75^{bc} \pm 0.73$	11/11	$2.50^{a} \pm 0.60$	5/11
Total	26/30	2.15 ± 0.76	28/31	2.93 ± 0.57	22/31

[1]m±s. d.
significantly ($P < 0.01$) higher mean blood bacterial count in birds of farm A than farm C.

Table 2. *Staphylococcus* **sp. isolated from the blood (B), liver (L), and hock joint (J) of broiler chickens taken from Farm A, B, or C**

Bacteria	Farm A				Farm B				Farm C				Total
	B	L	J	T[1]	B	L	J	T	B	L	J	T	
Coagulase-pos.													
S. aureus	0	0	0	0	0	0	1	1	0	0	0	0	1
S. intermedius	0	0	1	1	0	0	0	0	0	0	0	0	1
Coagulase-neg.													
S. lentus	1	2	4	7	0	0	0	0[*2]	3	1	4	8	15
S. simulans	4	3	3	10[*]	0	0	2	2	1	1	0	2	14
S. cohnii[3]	0	0	0	0	0	0	0	0	2	7	1	10[**]	10
S. gallinarum	2	3	1	6[*]	1	0	0	1	1	0	0	1	8
S. capitis[3]	1	1	0	2	1	0	1	2	1	1	1	3	7
S. xylosus	0	1	0	1	0	1	0	1	1	2	1	4	6
S. hominis	0	0	0	0	1	1	1	3	1	1	0	2	5
S. auricularis	1	2	0	3	0	1	0	1	0	0	0	0	4
S. carnosus	2	0	0	2	0	0	0	0	0	0	0	0	2
S. caseolyticus	0	0	2	2	0	0	0	0	0	0	0	0	2
S. kloosi	1	0	0	1	0	0	0	0	0	0	0	0	1
S. epidermidis	1	0	0	1	0	0	0	0	0	0	0	0	1
S. arlettae	0	0	0	0	0	0	0	0	0	1	0	1	1
S. pisifermentans	0	0	0	0	0	0	0	0	0	1	0	1	1
Total	13	12	10	36	3	3	4	11[**]	10	15	7	32	79

[1]subtotal

[2]Chi-square-tests were performed to test differences of subtotals among farm origins. *; $P<0.05$; **; $P<0.01$

[3]Subspecies *urealyticus*

Table 3. Non-staphylococcal bacteria isolated from the blood (B), liver (L), or hock joint (J) of broiler chickens originated from Farm A, B, or C

Bacteria	Farm A				Farm B				Farm C				Total
	B	L	J	T[1]	B	L	J	T	B	L	J	T	
Gram-positive													
Corynebacterium sp.	6	4	5	15**	3	1	2	6	0	1	2	3	24
Stomatococcus sp.	0	1	0	1	0	1	0	1	1	1	1	3	5
Micrococcus sedentarius	0	0	0	0	1	1	1	3*	0	0	0	0	3
Micrococcus varians	0	0	0	0	0	1	1	2	0	0	0	0	2
Micrococcus luteus	0	0	0	0	0	0	1	1	0	0	1	1	2
Streptococcus sp.	0	0	1	1	0	0	0	0	0	0	0	0	1
Lactobacillus sp.	0	0	1	1	0	0	0	0	0	0	0	0	1
Total	6	5	7	18	4	4	5	13	1	2	4	7	38
Gram-negative													
Escherichia coli	0	0	0	0	2	2	1	5*	0	1	0	1	6
Moraxiella sp.	0	0	0	0	0	2	0	2	0	1	0	1	3
Proteus mirabilis	0	0	0	0	1	1	1	3*	0	0	0	0	3
Acinetobacter sp.	0	0	0	0	0	1	0	1	0	0	0	0	1
Pseudomonas sp.	0	0	0	0	0	1	0	1	0	0	0	0	1
Yersinia sp.	0	0	0	0	0	0	0	0	0	1	0	1	1
Total	0	0	0	0	3	7	2	12**	0	3	0	3	15

[1]Subtotal

[2]Chi-square-tests were performed to test differences of isolation subtotals among farm origins. *; $P<0.05$, **; $P<0.01$

3. 3. 4. Antibiotic Susceptibility

Susceptibility tests of the staphylococci showed that all the staphylococcal isolates (n= 69) were susceptible to vancomycin and enrofloxacin . An isolate of *S. aureus* was susceptible to all antibiotics except lincomycin and polymyxin B. Whereas *S. intermedius* was susceptible to all antibacterials except novobiocin, penicillin G, chlortetracycline and oxytetracycline. For the coagulase negative staphylococcal species 97% were susceptible to polymyxin B, 94% to gentamicin, and 87% to neomycin. This group of bacteria showed 73% and 19 % resistance against penicillin G and methicillin respectively. The results for the other antibacterial drugs tested are listed in Table 4. There was also heterogeneity in antibiogram profiles within species of coagulase- negative staphylococci.

Table 4. Antimicrobial susceptibility test results of staphylococci isolated from 6-7 week-old broiler chickens

Drug	Number of susceptible isolates		
	S. aureus (n= 1)	*S. intermedius* (n=1)	Coagulase (-) *Staphylococcus* species (n= 67)
Novobiocin	1	0	8
Methicillin	1	1	54
Lincomycin	0	1	9
Triple sulfa.	1	1	40
Penicillin G	1	0	18
Vancomycin	1	1	67
Enrofloxacin	1	1	67
Gentamicin	1	1	63
Chlortetracycline	1	0	19
Neomycin	1	1	58
Erythromycin	1	1	25
Oxytetracycline	1	0	20
Polymyxin B	0	1	65

3. 3. 5. Serology

Samples from Farm A (N=9) were negative for antibodies against NDV, one positive for IBV and two positive for IBDV. Farm B samples (N=10) were uniformly negative for the three antigens tested. Samples from Farm C (N=11) were all positive for antibodies against IBDV with $\log_{10}$ titers of 3.36±0.36 (m±s. d.), while negative for antibodies against IBV and NDV.

3. 4. Discussion

Our previous investigation suggested that the systemic infection by *Staphylococcus* is common among sickly broilers of market age (Awan and Matsumoto, 1997). The present study confirmed the preliminary findings as to the high rate of systemic bacterial infections in 6-week-old unthrifty birds. As expected, coagulase-negative staphylococci were the major organisms isolated, but others such as gram-positive *Corynebacterium* or some gram-negative organisms were also found. As the results of species identification indicate, staphylococci and other bacteria present in tissues were highly heterogeneous, and there was no apparent association between the presence of some species or groups of species at tissue sites, or between the latter and ascites or arthritic conditions. Therefore, these systemic infections are best described as mixed bacterial infections.

The analysis of bacterial isolations (Table 2 and 3) shows some significant relationships between bacterial species and the isolation site (premises). Thus, *S. simulans* and *Corynebacterium* are isolated at significantly higher rates at Farm A, *S.*

cohnii subsp. *urealyticus* from Farm C, and gram-negative bacteria from Farm B. Probably, these organisms were abundant in the environment of each poultry house. These results are consistent with the hypothesis that the systemic bacterial infection is caused as the result of suppressed host antibacterial systems rather than by primary pathogenic/virulence factors of bacteria. Bacteria in the environment constantly colonize the respiratory tract of poultry and invade the bloodstream. The exact portal of entry has not been elucidated, but suggested as the lower respiratory tract (airsacs and lungs) (Jensen *et al.*, 1987). If any of the chemotactic, phagocytic or bactericidal mechanism in the blood or reticuloendothelial organs (liver, spleen, bone marrow, etc.) is disturbed, multiplying bacteria would be disseminated systemically.

Susceptibilities of 69 staphylococcal isolates to 13 antimicrobial agents by disk diffusion method showed that all staphylococci were sensitive to vancomycin and enrofloxacin. Except enrofloxacin this is in agreement with the findings of Scanlan and Hargis, (1989), who described complete susceptibility of staphylococcal isolates to vancomycin. Among coagulase positive, *S. aureus* isolate was sensitive to almost all common antibiotics, especially penicillin and methicillin. This finding is also similar with the findings of Narin and Watson (1973) who reported varying degree of susceptibilities among *S. aureus* isolates of poultry origin to penicillin, erythromycin, tetracycline, and novobiocin. Keeping in view the current resistance patterns of *S. aureus*, however this finding could be inconsistent, if large number of *S. aureus* isolates would have been tested. Whereas *S. intermedius* which is not a primary

poultry pathogen showed resistance against penicillin. Cox et al., (1984) reported that 59 percent of *S. intermedius* from different animal species were positive for β-lactamase production. Coagulase negative staphylococci in this study showed varying degree of resistances to different classes of antibacterials. However fairly high resistance against penicillin and increasing resistance to methicillin by these coagulase negative staphylococci is observed. This is in agreement with the findings of Kawano et al., (1996) who reported 25.7% methicillin resistance among coagulase negative staphylococci isolated from chickens. Further, these isolates also had shown fairly high resistance to β- lactams, and few against macrolide as well as aminoglycosides antibiotics. This methicillin resistance among coagulase negative staphylococci is alarming, both in terms of poultry hygiene as well as of public health significance.

Systemic bacterial infections in poultry are caused by such acute pathogenic bacteria as *Pasteurella multocida* (Rimler and Glisson, 1997), some species of *Salmonella* (Gast, 1997), etc., or as secondary infections such as *Escherichia coli* infection. According to Barnes and Gross (1997), the most frequent causes of *E. coli* infections are infectious bronchitis infection in chickens, hemorrhagic enteritis infection in turkeys, and exposure to excess ammonia resulting from poor ventilation and/or overcrowding. These primary factors disrupt mucosal barriers, impair antibacterial defense systems, and/or interfere normal immune responses to cause secondary *E. coli* infections. Under these conditions, if a strain of *E. coli* has some virulence/pathogenic factors, it spreads preferentially over other organisms, but altered host susceptibility factors are underlying causes for septicemia and other colibacillosis lesions. In the

present study, however, no apparent viral infections or stress factors were identified. There was no apparent associations between the occurrence of the systemic bacterial infection and evidences of three major viral infections. Antibody responses against IBV and NDV were negative in birds from all the three farms (except two positive samples against IB at Farm A) as well as no antibody responses against IBDV in Farm A and B; in Farm C, despite repeated vaccinations, mortality loss was no lower than at Farm A or B. No other stress factors stemming from the environment or management, which were present at all the three locations, were identified. It is noteworthy that both ascites and the systemic bacterial infection occur during the last two to three weeks of the growing period when birds were fed with a high calory diet under 24 hour lighting. As noted in the 'result' section, however, there is no direct correlation between these two clinical events. It is possible to speculate that a genetic factor in combination with a nutritional stress may be a underlying cause of both events. This possibility should be investigated in the future studies.

It is unknown how frequently the systemic bacterial infection occurs in market ages of normal broilers under the current management practice. A preliminary experiment in this laboratory showed that around 5% of "normal" broilers are bacteremic at 6 weeks of age (unpublished data). However, it is unknown whether bacteremia represents transient or perpetual type of infections. Before considering these elements of infection, one must carefully define 'systemic infection' based on established mechanisms of infection. For example, in *Pasteurella multocida* infection (fowl cholera) in turkeys, the organism invades the respiratory tissue to enter the

bloodstream (Tsuji and Matsumoto, 1989; Matsumoto *et al.*, 1991). Within a short period of time, however, >99% of the organism will localizes in the liver and spleen. The organism then either multiplies or being killed in these organs, and, if it propagates, it will reappear in the blood in 3 to 4 h, resulting in a systemic infection. This mechanism may not apply to other bacterial infections since *P. multocida* possesses highly pathogenic factors such as toxic LPS, thick hyaluronic acid capsule, etc. (Rimler and Glisson, 1997), which may play a role in evading such defense mechanisms as complement-mediated bacteriolysis or opsonophagocytic activity of blood heterophils. Hence, further investigations are needed to elucidate host-parasite interactions in each bacterial infection in the laboratory as well as more investigations to find causative agents/mechanisms of systemic bacterial infections in market age broilers.

3.5. End Notes

[1]Select Laboratories, Gainesville, GA 30501

[2]Saraflox®, Abbott Lab., North Chicago, IL 60064

[3]Bursine 2®, Solvay Laboratories, Mendota Heights, MN 55120

[4]Difco Laboratories, Detroit, MI 48232

[5]Stomacher™ 80, Tekmar, Cincinnati, OH 45222

[6]Calcium alginate urethral swab, Fisher Scientific, Houston, TX 77251

[7]API® Staph, Biomérieux Vitek, Hazelwood, MO 63042

[8]Proflock®, Kirkegaard & Perry Lab., Gaithersburg, MD 20879

[9]BBL

3.6. Acknowledgments

This paper is a part of the senior author's M. S. thesis. The project was partly supported by the Oregon Agricultural Experiment Station, OAES 00168, and published as a Technical Paper No. . The authors wish to recognize the assistance of Peterson's Fryer Farms, Tacoma, WA and Fircrest Farms, Creswell, OR.

Chapter 4

In Vivo Pathogenicity of Staphylococci and Other Bacteria in Chick Embryos and in Broilers

4. 1. Introduction

In the previous investigations presented in the chapter 3, various bacteria were isolated from broilers in the field. In experiments described in this chapter, pathogenicity of representative isolates, majority of which were staphylococci, was tested in embryos and in broiler chicks.

Three experiments were conducted. Experiment 1 and 2 were conducted to determine *in vivo* pathogenicity of the field isolates of the first study in 5 day-old embryonated eggs, and 3-week-old broiler chickens, respectively. Experiment 3 was conducted to determine the role of stressed and non-stressed environments in acquiring natural staphylococcal infections in broiler chickens.

4. 2. Materials and Methods

4. 2. 1. Experiment 1

Fertile eggs were purchased from a local hatchery. These were disinfected with quaternary ammonium compounds (0.125% Roccal-D Plus™; Up john) and incubated at 99.5°F for 5 days prior to inoculation. Eggs were checked for viability of embryos and then only viable embryos were used in each inoculation. Ten colonies each of *S. aureus,*

S. intermedius, S. lentus, S. gallinarum or *Corynebacterium sp.* were picked from overnight grown culture on blood agar plates and transferred into 100 ml of brain heart infusion (BHI) broth, followed by 10 fold dilutions of each suspension in BHI broth. Each diluted culture was inoculated into 4 or 5 embryos/dilution. Prior to inoculation, the top areas of the egg shell were disinfected with 70% ethanol, and a small hole into egg membrane was made with the help of disinfected egg driller. Each culture suspension in 0.1 ml was inoculated into the yolk-sac with a 21G needle and a 1 ml disposable syringe. The outer pierced area of each egg was then sealed with sealant, and eggs were kept in an egg incubator at 99.5°C. Eggs were candled daily. Yolk was harvested from dead or live embryos at 24 hrs. at the 1st through 5th and at 36 hrs. at the 6th passage; the top of egg shell was disinfected and egg shell area over air sac was cut and the shell removed in a circular fashion with a pair of sterile scissors. Exposed egg membrane was then flipped off to one side with sterile forceps. Approximately 2 to 5 ml pooled egg yolk sample from each group of selected eggs was aspirated separately into sterile test tubes with 18G needles and 1 ml disposable syringes. The harvested yolk was inoculated into embryos for further passages, or lyophilized for storage.

4. 2. 2. Experiment 2

Sixty-four one-day-old, unvaccinated, unmedicated broiler chicks were donated by the courtesy of Fircrest Farms, Oregon and raised under the normal conditions of feeding, temperature, and ventilation in battery cages to the 21 days of age. The birds were then moved into an isolation unit and immediately used for the experiment. For bacterial inocula, freshly grown colonies on blood agar plates were suspended in BHI

broth, and the concentration was adjusted in such that OD_{625} of a suspension culture was 0.5. The viable count for each inoculum was made by the dilution and plate-out method. Eleven birds in each group were intravenously inoculated with 1 ml of inoculum. Nine birds without any inoculation were kept as a control. All the birds were observed for 7 days for any untoward signs, and any dead chick found was examined at necropsy, whereas those alive in the end of experiment were also examined at necropsy. Viable bacterial counts for the blood (3 and 7 days), and liver (7-days post-inoculation), or bacterial isolation and identification were performed as described in Chapter 3.

4. 2. 3. Experiment 3

Sixty-five chicks of one day of age were obtained. They were embryo-vaccinated at 18 days of incubation with HVT and SB-a Marek's disease vaccine with 2 mg/chick of gentamicin sulfate. Fifteen birds were raised in brooder battery cages under the normal conditions (control group). The room was maintained between 70 to 80 F and ventilated with electric fans. At 25 days of age, they were moved to a clean compartment (2.7 square feet/ bird) with wood shavings as litter. Fifty chicks were raised on the floor (0.8 square feet) with chopped fescue grass straw as litter. Temperature of the compartment was kept approximately 10 F lower than temperature required for their optimal growth. Starting at 17 days of age, cold water in 2 to 4 litters was sprayed daily on the litter in stressed group birds. No forced ventilation was used (stressed group). At the age of 28, 35 and 42 days, all the birds were bled. The skin surface was scrubbed with soap water followed by disinfection with 70% ethanol. Approximately 1 ml of blood was collected from the brachial vein into a syringe containing 10 units of sodium heparin. Syringes

containing blood was immediately placed on ice. Viable counting and bacterial isolation was done as described in Chapter 3. At 49 days of age, all the birds were bled, sacrificed, and tissue samples were taken from the liver and hock joints. These samples were processed for viable counting and bacterial isolations as described in Chapter 3.

4. 3. Results

4. 3. 1. Experiment 1

Highest mortalities of 80% and 100% were shown by *S. aureus* in first, and second egg passages (Table 5). *Staphylococcus intermedius* and *S. lentus* showed varying degrees of mortalities up to the 6th passage. The other two isolates did not cause consistent mortality in the embryos.

4. 3. 2. Experiment 2

The birds inoculated with *S. aureus* showed progressing signs of septicemia. By 24 hr PI, most of the birds in this group were depressed. Cumulative mortality was 55, 73, 82, and 91% at 2, 3, 5, and 7 days post-inoculation, respectively. At necropsy (1 to 7 days PI) birds in *S. aureus* group showed enlarged heart (55%) with yellow colored fluid in the pericardial sac, enlarged liver (27%) with necrotic foci , and swollen hock joint (18%). In contrast, there was no mortality and no gross lesion in internal organs of birds in control and other groups. All the birds in all groups were negative for ascites (Table 6).

Overall. *S. aureus* was detected in 91% in each of the heart, liver and hock joint samples of birds died in *S. aureus* injected group of birds. Further percentages for concurrent bacterial isolation and gross lesions in the dead birds were similar as described

earlier for the lesions alone. The mean bacterial count of the liver in this group of birds was 5.19 ± 0.62 (m ± s.d., $\log_{10}$ CFU) per gram of tissue. One bird necropsied at 7 days PI in the same group, was bacteriologically negative in blood, liver, and hock joint cultures. Conversely, by 7 days PI, all the samples (blood, liver, and hock joint) from birds in the remaining groups were bacteriologically negative.

Table 5. *In vivo* pathogenicity of field isolates in 5-day-old embryonated eggs (Experiment 1)

	Egg passage number					
Inoculum	1	2	3	4	5	6
S. aureus	4/5[1]	4/4	-	-	-	-
S. intermedius	0/5	3/5	3/5	1/8	3/8	4/7
S. lentus	1/4	0/5	3/5	5/9	3/8	7/7
S. gallinarum	1/5	0/5	1/5	0/8	0/7	0/6
Corynebacterium sp.	0/5	0/5	1/5	1/8	0/7	0/6

[1]Dead/total inoculated

Table 6. *In vivo* pathogenicity of bacterial isolates in 3-week old broiler chickens (Experiment 2)

			Gross lesions and bacterial isolations		
Inoculum	Dose (CFU/ml)	Dead/ total	Enlarged Heart	Enlarged Liver	Swollen Hock Joint
S. aureus	6.4 x10^6	10/11	6	3	2
S. gallinarum	2.5 x10^6	0/11	0	0	0
S. intermedius	3.5 x10^6	0/11	0	0	0
S. lentus	7.5 x10^6	0/11	0	0	0
Corynebacterium sp.	4.0 x10^6	0/9	0	0	0
Control	0	0	0	0	0

4. 3. 3. Experiment 3

Birds in the stressed group were inclined to sit down and reluctant to move, but, otherwise they were alert. Cumulative mortality in the stressed group was 4% compared with no mortality in the control group. At necropsy, some gross lesions were observed, but a significant difference ($P<0.05$) was observed only in the occurrence of hypertrophy of heart between the two groups (Table 7). There was no significant difference in frequencies of bacterial isolations ($P>0.05$) from or in the viable counts in the blood between the two groups when they were examined at 4, 5, 6 or 7 weeks of age (Table 8). . In the frequency of bacterial isolations from the liver, there was a significant difference ($P<0.05$) between the two groups although no significant difference was noted in their viable counts (Table 9).

Table 7. Comparison of different lesions in between stressed and control broiler chickens (Experiment 3)

	Lesions[a]					
Treatment	Hypertrophy[b]	Enlarged	Joint	Breast	Air	Ascites
Stressed (n= 48)	2	1	11	2	4	0
Control (n=15)	7[c*]	0	6	0	0	0

[a] no. of positive birds
[b] with the accumulation of watery fluid
[c]Chi-square test applied to test differences in the incidence of various lesions, *$P<0.05$

Table 8. Bacterial isolation results from the blood of birds in the stressed or control group at 4, 5, 6 or 7 weeks of age (Experiment 3)

	Age in days							
	28		35		42		49	
Treatment	Pos[a]	Log_{10} CFU/ml	Pos.	Log_{10} CFU/ml	Pos.	Log_{10} CFU/ml	Pos.	Log_{10} CFU/ml
Stressed[c] (n=48)	0	0	4	3.8±0.5[b]	2	2.7±1.2	2	2.5±0.35
Control[c]	1	4.3	1	4.3	1	1.95	1	2.5

[a]Birds positive for bacteremia.
[B] m± s.d.
[C] birds

Table 9. Comparison of bacterial isolation from different samples from two groups of 7-week-old broiler chickens (Experiment 3)

	Samples				
	Blood		Liver		Hock joint
Treatment	Pos.	Log_{10} (CFU/ml)	Po	Log_{10}(CFU/gm)	
Stressed birds	2	2.53±0.35[a]	16	2.87±0.69	2
Control birds	1	2.54	1	2.02	0

[a] m± s.d.
[b] Chi-square test applied to test differences in the incidence of bacterial isolations from different samples $^{*}P < 0.05$

4. 4. Discussion

In our previous study, different species of bacteria particularly coagulase-negative staphylococci were isolated from unthrifty commercial broiler chickens (Awan and Matsumoto, 1997). In the present study attempts were made to determine pathogenicity of some of those field isolates in embryonated eggs and broiler chickens. Further effect of stressed and non stressed environments in acquiring natural bacterial infection were also undertaken.

In vivo study in embryonated eggs showed that field isolates had none to varying degrees of pathogenic potentials. Especially *S. aureus* showed highest level of mortality in egg embryos, whereas *S. intermedius*, *S. lentus*, *S. gallinarum*, and *Corynebacterium sp.* did not demonstrate a uniform pattern of mortality in eggs. Among these, however, *S. intermedius* and *S. lentus* showed increasing levels of lethality. The pathogenicity of *S. aureus* in laboratory animals and susceptible field hosts is well documented (Mutalib et al., 1983; Hill et al., 1989; Jonsson and Wadstrom, 1994), but similar studies in chicken embryos are lacking. *Staphylococcus intermedius* is a pathogen in dogs, but its pathogenicity in poultry has not been established. Similarly, no reports regarding disease causing capability of *S. lentus* in poultry have been recorded, except their isolation from broiler chickens as presented in the previous chapter.

In vivo experiments in 3-week-old broiler chickens in order to determine the pathogenicity of field bacterial isolates are conclusive. Only *S. aureus* was found highly pathogenic in injected birds. Most of the birds died within 72 hrs PI, were found without signs of staphylococcal disease. Chronic complications involving hock joints (arthritis

and lameness) were seen only in a few birds which survived for 4 to 5 days after the inoculation. Only, gross lesions in liver and particularly in heart are suggestive for acute, septicemic disease pattern. *Staphylococcus aureus* was recovered as a pure culture from different tissues including particularly pericardial fluid. These findings are in agreement with the findings of Mutalib et al., (1983) who described that high dose ($2x10^7$ CFU) of *S. aureus* inflicted earlier mortality within 2 to 4 days PI with less severe signs due to acute septicemic disease. Smith et al., (1954) and Cotter (1992) reported same pattern of septicemic staphylococcal disease in experimental chickens. Conversely, up to 7 days PI, the coagulase-negative staphylococci and *Corynebacterium* isolates were found non-pathogenic in experimental birds. Bacterial isolations from different tissue samples were uniformly negative. There is no report in the literature on the pathogenicity of *S. intermedius*, *S. lentus*, *S. gallinarum* and *Corynebacterium* sp. in poultry including broiler chickens. Based on these findings it may be said that micro-organism such as *S. aureus* is capable of causing septicemic chronic disease even in the healthy birds, whereas coagulase-negative staphylococci do not cause systemic disease unless general host defense system or local skin or mucosal barriers are impaired. The *in vivo* study, in which the effect of stress condition was tested, provided some useful information. Bacteremia in stressed birds was observed almost one week later than in the control birds, although there was no significant difference. It may be due to increased numbers of heterophils particularly in stressed birds, which contained bacteria to a certain limit. In one study Mutalib et al., (1983) also reported that stressed broiler chickens of 6 week age were comparatively more resistant to *S. aureus* than the control birds.

The rate of bacterial recovery in stressed birds was highest at 5 weeks of age than at 6 or 7 weeks of age. This may suggest that after 5 week birds were immunologically more capable to clear the organisms than 5 week of age or younger. Data also indicated that bacteremic phase is transient and usually clears within one week. All of these findings are consistent with the hypothesis that due to some reasons the host immune system becomes impaired, which resulted into transient bacteremia by these environmental non-virulent microorganisms via certain portal of entry. Among the results of various cultured tissues, significantly ($P < 0.05$) high isolation rates only from liver of the stressed birds was observed. This finding is some how in agreement with the hypothesis (Mutalib et al., 1983) that septicemia followed by death or localization of *S. aureus* in different tissues was the outcome of interplay in between ratio of the number of inoculated organisms and the speed with which new phagocytic cells mobilized or number of already present phagocytic cells.

Necropsy findings of different internal organs, tissues, and hock joints have shown significantly ($P < 0.05$) high incidence in hypertrophy of heart only in the control birds, although swab culture results from these samples were bacteriologically negative. On the contrary low heart associated lesions were seen in stressed birds. The reason for this increased incidence of heart lesions could be multifactorial. The accumulation of extra pericardial fluid may be an early sign before the actual development of ascites lesions. Reports documented on ascites suggested, that atmospheric hypoxia, rapid growth rate, high-energy rations, excess sodium in the diet, mycotoxins, and poor housing ventilations are the main contributing factors (Anderson et al., 1986; Julian et al.,

1987; Wideman, 1988). The role of some viral, or some unknown etiological factors may be highly unlikely in this study because stressed birds have shown low incidence of heart lesions.

In summary *S. aureus* was found pathogenic both in 5-days embryonated eggs and 3 week old broiler chickens and inflicted high levels of mortality. Conversely, coagulase negative staphylococci and corynebacterium sp., though exhibiting doubtful pathogenicity in egg embryos, were found unable to cause systemic disease in broiler chickens. Lastly birds raised under stressed and normal conditions were found almost equal in getting staphylococcal or other bacterial infections with the exceptions of high incidences of bacterial isolation from the liver and hypertrophy of heart in the former and later groups respectively. However in field conditions some other predisposing conditions /factors other than of our study would be responsible for consistent staphylococcal bacteremia and various tissues localizations (Awan and Matsumoto, 1997).

Chapter 5

Conclusions

1. There was an incidence of high rate of systemic bacterial infections in unthrifty commercial broiler chickens in the Northwest.
2. These infections can be rated as mixed bacterial infections; mostly due to coagulase-negative staphylococci, few coagulase positive staphylococci, and some other bacterial species.
3. Statistically there was no association between the prevalence of bacterial species and their localization in different tissues.
4. Statistically there was significant ($P < 0.05$) relationship between occurrence of bacterial species and premises (farms).
5. Heterogenous profiles of antibacterial resistance were observed among coagulase-negative staphylococci; the presence of methicillin-resistant staphylococci is alarming.
6. There was no apparent association between the above-noted bacterial infections and tested viral (IBDV, NDV, IBV) etiologies.
7. *In vivo* experiment in embryonated eggs showed high pathogenicity of *S. aureus*, while few coagulase-negative staphylococci (*S. intermedius*, and *S. lentus*) showed variable pathogenic potentials and others (*S. gallinarum*, and *Corynebacterium sp.*) were found non-pathogenic.

8. All experiments on *in vivo* pathogenicity in 3-weeks old broiler chickens were only promising for *S. aureus*, whereas rest of all the tested isolates were failed to inflict systemic or local infections.

9. The experiment on the effect of stressed and controlenvironmentss apparently showed equal susceptibility in the initiation of spontaneous bacterial infections particularly staphylococcosis. However, a significantly ($P<0.05$) high frequency of bacterial isolations from liver of birds in the stressed group, and more heart lesions in the control birds could be an early stages of disease conditions probably associated with the environmental/ managemental factors.

Extensive studies are needed to further understand the cause of avian systemic bacterial infections and pathogenicity of staphylococci particularly coagulase-negative.

Bibliography

Abeyounis, C. J. 1982. *Staphylococcus*. In Milgrom, F. and T. D. Flanagan (eds), *Medical Microbiology*, pp.251-261. Churchil Livingstone, New York.

Anderson, J. C. 1986. Staphylococcus. *In* Gyles, C. L., and C. O. Thoen (eds), *Pathogenesis of Bacterial Infections in Animals*, pp. 14-20. Iowa State University Press, Ames, Iowa.

Anderson, J. C. 1976. Mechanisms of staphylococcal virulence in relation to bovine mastitis. *Brit. Vet. J.* 132:229-245.

Anderson, L. S., M. Gleeson, A. L. Haigh, and V. Molong. 1986. Respiratory responses of the domestic fowl to low level carbon dioxide exposure. *Res. Vet. Sci.* 40:99-104

Archer, G. L., and E. Pennel. 1990. Detection of methicillin-resistance in staphylococci using a DNA probe. *Antimicrob. Agents chemother.* 34:1720-1724.

Arp, L. H., I. M. Robinson, and A. E. Jensen. 1983. Pathology of liver granulomas in turkeys. *Vet. Pathol.* 20:80-89.

Arsan, A. K., A. Adisen, S. Duman, B. Aslan, and I. Kocak. 1996. Acute endoopthalmitis outbreak after cataract surgery. *J. Cataract. Refract. Surg.* 22:1116-1120.

Awan, M. A. and M. Matsumoto, 1997. The systemic infection with staphylococcal organisms in broiler chickens. Pages 61-62 In: *Proceedings of the 46th Western Poultry Disease Conference Sacramento, California U. S. A.*

Ayliffe, G. A. 1997. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. *Clin. Infect. Dis.* 24: Suppl. 1. S74-79.

Back, N. A., C. C. Jr. Linnemann, J. L. Staneck, U. R. Kotagal. 1996. Control of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit: use of intensive microbiologic surveillance and mupirocin. *Infect. Control Hosp. Epidemiol.* 17:227-231.

Barber, M. 1961. Methicillin-resistant staphylococci. *J. Clin. Pathol.* 14:385-393.

Barnes, H. J. and W. B. Gross, 1997. Colibacillosis. Pages 131-141 In: *Diseases of Poultry*, 10th ed. B. W. Calnek, ed., Iowa State Univ. Press, Ames.

Barret, F. F., R. F. McGehee, M. Finland. 1968. Methicillin-resistant *Staphylococcus aureus* at Boston city hospital: bacteriologic and epidemiologic observations. *N. Engl. J. Med.* 279:441-448.

Barton, B. E., J. Shortall, and J. V. Jackson. 1996. Interlukins 6 and 11 protect mice from mortality in a staphylococcal enterotoxin-induced toxic shock model. *Infect. Immun.* 64:714-718.

Bartzokas, C. A., J. H. Paton, M. R. Gibson, et al. 1984. Control and eradication of methicillin resistant *Staphylococcus aureus* on a surgical unit. *N. Eng. J. Med.* 311:1422-1425.

Becker, A., S. L. Vanhooser, and R. G. Teeter. 1995. Effect of oxygen level on ascites incidence and performance in broiler chicks. *Avian Dis.* 39:285-291.

Bell, S. M. 1982. Recommendations for control of the spread of methicillin-resistant *Staphylococcus aureus* infection. Based on 18 years' experience in a group of teaching hospitals. *Med. J. Aust.* 1:472-474.

Bennett, R. W., and F. McClure. 1994. Visual screening with enzyme immunoassay for staphylococcal enterotoxins in foods: Collaborative study. *J. AOAC. Int.* 77:357-364.

Bergdol, M. S. 1973. Enterotoxin detection. In Hobbs, B. C. and J. H. P. Christian (eds.), *The Microbiological safety of food.* pp. 287-292. New York and London. Academic Press.

Bergdoll, 1983. Enterotoxins. In Easmon C.S. F and C. Adlam (eds.), *Staphylococci and Staphylococcal infection.* Vol. 2. pp. 559-598. Academic Press. London.

Bergmann, V., B. Kohler, and K. Vogel. 1980. *Staphylococcus aureus* infections of fowls on industrialized poultry units. 1. Types of infection. *Arch. Exp. Vet* 34:891-903.

Bitar, C. M., C. G. Mayhall, V. A. Lamb, T. J. Bradshaw, A. C. Spadora, and H. P. Dalton. 1987. Outbreak due to methicillin-resistant *Staphylococcus aureus*: epidemiology and eradication of the resistant strain from the hospital. *Infect. Control.* 8:15-23.

Blanc, D. S., C. Petignat, P. Moreillon, A. Wenger, J. Bille, and P. Francioli.1996. Quantitative antibiogram as a typing method for the prospective epidemiological surveillance and control of MRSA: comparison with molecular typing. *Infect. Control Hosp. Epidemiol.* 17:654-659.

Blumberg, H. M., D. Rimland, D. J. Carroll, P. Terry, I. K. and Wachsmote. 1991. Rapid development of ciprofloxacin resistance in methicillin susceptible and resistant *Staphylococcus aureus*. *J. Clin. Dis*. 163:1278-1285.

Boyce, J. M., R. L. White, W. A. Causey, W. R. Lockwood. 1983. Burn units as a source of methicillin-resistant *Staphylococcus aureus*. *J. Am. Med. Assoc*. 249:2803-2807.

Boyed, R. F. 1995. The Gram-Positive Cocci. In Boyed, R. F. *Basic Medical Microbiology*, 5th Ed, pp.246-262. Little, Brown and company, Inc. Boston.

Bustan, Al. M. A., E. E. Udo, and T. D. Chugh. 1996. Nasal carriage of enterotoxin-producing *Staphylococcus aureus* among restaurants workers in Kuwait city. *Epidemiol. Infect*. 116:319-322.

Carnaghan, R. B. A. 1966. Spinal cord compression in fowls due to spondylitis caused by *Staphylococcus pyogenes*. *J. Comp. Pathol*. 76:9-14.

Carter, G. R. and J. R. Cole, 1990. In. *Diagnostic Procedures in Veterinary Bacteriology and Mycology* , 5th ed. Academic Press, New York, N. Y.

Carter, G. R., and M. M. Chengappa (eds.) 1991. Staphylococci. In *Essentials of Veterinary Bacteriology and Mycology*, 4th Ed, pp.115-119. Lea and Febiger, Malvern, Pennsylvania.

Casewell, M. W. 1995. New threats to the control of methicillin-resistant *Staphylococcus aureus*. *J. Hosp. Infect*. 30: suppl. 465-471.

Cervantes, H. M., L. L. Munger, D. H. Ley, and M. D. Ficken. 1988. *Staphylococcus*-induced gangrenous dermatitis in broilers. *Avian Dis*. 32:140-142.

Chambers, H. F. 1991. Treatment of infection and colonization caused by methicillin-resistant *Staphylococcus aureus*. *Infect. Control. Hosp. Epidemiol*. 12:29-35.

Cheville, N. F., J. Tappe, M. Akermann, and A. Jensen. 1988. Acute fibrinopurulent blepharitis and conjunctivitis associated with *Staphylococcus hyicus, Escherichia coli*, and *Streptococcus sp*. In chickens and turkeys. *Vet. Pathol*. 25:369-375.

Cohen, S. H., M. M. Morita, M. Bradford. 1991. A seven-year experience with methicillin-resistant *Staphylococcus aureus*. *Am. J. Med. Suppl*. 3B: 233S-237S.

Cooper, J. E., and J. R. Needham. 1976. An investigation into the prevalence of *Staphylococcus aureus* on avian feet. *Vet. Rec.* 98:172-174.

Cotter, P. F. 1992. Differential resistance to *Staphylococcus aureus* challenge in major histocompatibility (B) complex congenic lines. *Poult. Sci.* 71:1873-1878.

Cowden, J. M., P. G. Wall, G. Adak, H. Evans, S. Le-Baigue, and D. Ross. 1995. Outbreaks of food borne infectious intestinal disease in England and Wales: 1992 and 1993. *Commun. Dis. Rep. C. D. R. Rev.* 5:R109-117.

Cox, H. U., S. S. Newman, A. F. Roy, and J. D. Hoskins. 1984. Species of *Staphylococcus* isolated from animal infections. *Cornell. Vet.* 74:124-135.

Crowcroft, N., H. Maguire, M. Fleming, J. Peacock, and J. Thomas.1996. Methicillin resistant *Staphylococcus aureus* : investigation of a hospital outbreak using a case control study. *J. Hosp. Infect.* 34:301-309.

Devriese, L. A. 1980. Pathogenic staphylococci in poultry. *World's Poult. Sci. J.* 36:227-236.

Duckworth, G. J., J. L. E. Lothian, and J. D. Williams. 1988. *J. Hosp. Infect.* 11:1.

ElwellL. P., H. R. Wilson, V. B. Knick, et al., 1986. In vitro and in vivo efficacy of the combination trimethoprim-sulfamethoxazole against clinical isolates of methicillin-resistant *Staphylococcus aureus* . *Antimicrob. Agents. Chemother.* 29:1092-1094.

Evans, J, B., G. A. Ananaba, C. A. Pate, and M. S. Bergdol. 1983. Enterotoxin production by atypical *Staphylococcus aureus* from poultry. *J. Appl. Bacteriol.* 54:257-261.

Evans, M. E. and K. J. Kortas. 1996. Vancomycin use in a university medical center: comparison with hospital infection control practices advisory committee guidelines. *Infect. Control. Hosp. Epidemiol.* 17:356-359.

Finegold, S. M., and E. J. Baron.1986. Micrococcaceae: Staphylococci and Micrococci. In Carson, D. C.(ed.), *Baily and Scott's, Diagnostic Microbiology*, 7th Ed., 355- 365. The C. V. Mosby Company, St. Louis, Missouri.

Flaherty, J. P., and R. A. Weinstein. 1996. Nosocomial infection caused by antibiotic-resistant organism in the intensive-care unit. *Infect. Control. Hosp. Epidemiol.* 17:236-248.

Fraser, C. M., 1991. Staphylococcosis. In Fraser, C. M. (ed.), *The Merk Veterinary Manual*, 7th Ed, pp.1599-1600. Merck and CO., Inc. Rahway, N. J.

Frazier, M. N., W. J. Parizek, and E. Garner. 1964. Gangrenous dermatitis of chickens. *Avian Dis.* 8:269-273.

Garner, J. S., and N. P. Simmons. 1983. Guideline for isolation precautions in hospitals. *Infect. Control.* 4:245-248.

Gast, R. K., 1997. Paratyphoid infections. Pages 97-121 In: *Diseases of Poultry*, 10th ed. B. W. Calnek, ed. Iowa State Univ. Press, Ames, IA.

Gwatkin, R. 1940. An out break of staphylococcal infection in Barred plymouth Rock males. *Can. J. Comp. Med.* 4:294-296.

Gemmell, C. G. 1996. Does the appearance of drug resistance during therapy alter bacterial susceptibility to opsonophagocytosis? *Drugs. Exp. Clin. Res.* 22:51-55.

Gibbs, P. A.,J. T. Patterson, and J. K. Thompson. 1978. Characterization of poultry isolates of *Staphylococcus aureus* by a new set of poultry phages. *J. Appl. Bacteriol.* 44:387-400.

Gibbs, P. A., J. T. Patterson, and J. K. Thompson. 1978b. The distribution of *Staphylococcus aureus* in a poultry processing plant. *J. Appl. Bacteriol.* 44:401-410.

Goetz, M. B., m. E. Mulligan, R. Kowok, H. O. Brien, C. Caballes, J. P. Garcia. 1992. Management and epidemiological analysis of an outbreak due to methicillin-resistant *Staphylococcus aureus*. *Am . J. Med.* 92:607-614.

Gould, D., and A. Chamberlaine, 1995. *Staphylococcus aureus*: a review of the literature. *J. Clin. Nurs.* 4:5-12.

Griffiths, G. L., W. I. Hopkinson, and J. Lloyd. 1984. Staphylococcal necrosis of the head of the femur in broiler chickens. *Aust. Vet. J.* 61:293.

Guiguet, M., C. Rekacewicz, B. Laclercq, Y. Brun, B. Escudier, and A. Andermont. 1990. Effectiveness of simple measures to control an outbreak of nosocomial methicillin-resistant *Staphylococcus aureus* infections in an intensive care unit. *Infect. Control. Hosp. Epidemiol.* 11:23-26.

Hackbarth, C. J., and H. F. Chambers. 1989. Methicillin- resistant Staphylococci: Genetics and mechanisms of resistance. *Antimicrob. Agents. Chemother.* 33:991-994.

Hackbarth, C. J., and H. F. Chambers. 1989. Methicillin-resistant staphylococci: detection methods and treatment of infections. *Antimicrob. Agents Chemother.* 33:995-999.

Halpin- Dohnalek, M. I., and E. H. Marth. 1989. *Staphylococcus aureus*: production of extracellular compounds and behavior in foods-a review. *J. Food. Protect.* 52:267-282.

Harnett, N., S. Brown, and C. Krishnan. 1991. Emergence of quinolone resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in Ontario, Canada. *Antimicrobial Agents Chemother.* 35:1911-1913.

Harry, E. G. 1967a. The characteristics of *Staphylococcus aureus* isolated from cases of staphylococosis in poultry. *Res. Vet. Sci.* 8:479-489.

Harry, E. G. 1967b. Some characteristics of *Staphylococcus auerus* isolated from the skin and upper respiratory tract of domesticated and wild (feral) birds. *Res.Vet. Sci.* 8:490-499.

Hartmann, B. J., and A. Tomasz. 1984. *J. Bacteriol.* 158:513.

Hartmann, b. J., and A. Tomasz, 1986. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus. Antimicrob. Agents Chemother.* 29:85-92.

Harvey, J., J. T. Patterson, and P. A. Gibbs. 1982. Enterotoxigenicity of *Staphylococcus aureus* strains isolated from poultry: raw poultry carcases as a potential food-poisoning hazard. *J. Appl. Bacteriol.* 52:251-258.

Herenda, D. C., and D. A. Franco (eds.). 1996. Musculoskeletal system. In *Poultry diseases and meat hygiene.* pp. 115-149. Iowa State University Press, Ames Iowa.

Herrmann, M., P. E. Vaudeaux, D. Pittet, R. Auckenthaler, P. D. Leew, F. Schumaker-Perdreau, G. Peters, and F. A. Waldvogel. 1988. Fibronectin, Fibrinogen, and lamin acts as mediators for adherence of clinical staphylococcal isolates of foreign material. *J. Infect. Dis.* 158:693-701.

Hill, J. E., G. N. Rowland, j. R. Glisson, and P. Villegas. 1989. Comparative microscopic lesions in reoviral and Staphylococcal tenosynovitis. *Avian Dis.* 33:401-410.

Hoffman, H. A. 1979. Vesicular dermatitis in chickens. *J. Am. Vet. Med. Assoc.* 48:329-332.

Hole, N., and H. S. Purchase. 1931. Arthritis and periostitis in pheasants caused by *Staphylococcus pyogenes aureus*. *J. Comp. Pathol. Ther.* 44:252-257.

Holderbaun, D., T. Spech, A. Ehrhart, T. Keys, and G. S. Hall. 1987. Collagen binding to clinical isolates of *Staphylococcus aureus*. *J. Clin. Microbiol.* 25:2258-2261.

Hsu, C. C. S. 1991. Serial survey of methicillin-resistant *Staphylococcus aureus* nasal carriage among residents in a nursing home. *Infect. Control. Hosp. Epidemiol.* 12:416-421.

Hubner, J. and A. Kropec. 1995. Cross infections due to coagulase negative staphylococci in high-risk patients. *Zentralbl. Bakteriol.* 283:169-174.

Jawetz, E., J. L. Melnick. and E. A. Adelberg. 1984. Pyogenic Cocci. In *Review of Medical Microbiology*, 16th Ed., pp. 197-201. Lange Medical Publications, Los Altos, California.

Jensen, M. M., W. C. Downs, J. D. Morrey, T. R. Nicoll, S. D. Lefevre, and C. M. Meyers.1987. Staphylococcosis of turkeys. 1. Portal of entry and tissue colonization. *Avian Dis.* 31:64-69.

Jernigan, J. A., M. A. Clemence, G. A. Stott, M. G. Titus, C. H. Alexander, C. M. Palumbo, and B. M. Farr. 1995. Control of methicillin-resistant *Staphylococcus aureus* at a university hospital: one decade later. *Infect. Control. Hosp. Epidemiol.* 16:686-696.

Johnston, B. L. 1994. Methicilin-resistant *Staphylococcus aureus* as a cause of community-acquired pneumonia- a critical review. *Semins. Resp. Infect.* 9:199-206.

Joklik, W. K., H. P. Willett. D. B. Amos. and C. M. Wilfert. 1992. Staphylococcus. In *Zinsser Microbiology*, 20th Ed., pp.401-415. Appleton and Lange, Norwalk, Connecticut / San Mateo, California.

Jonsson, P., and T. Wadstrom. 1993. *Staphylococcus*. In Gyles, C. L., and C. O. Thoen (eds.), *Pathogenesis of Bacterial Infections in Animals*, 2nd Ed., pp. 21-35. Iowa State University Press, Ames, Iowa.

Jorden, F. T. W, and M. Pattison (eds). 1996. Staphylococci. In *Poultry diseases*, fourth Ed. pp. 66-69. W. B. Saunders Company Ltd. London. Philadelphia.

Jorgensen, J. H. 1991. Mechanisms of methicillin resistance in *Staphylococcus aureus* and methods for laboratory detection. *Infect. Control. Hosp. Epidemiol.* 12:14-19.

Julian, R. J., G. W. Friars, H. French, and M. Quinton. 1987. The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. *Avian Dis.* 31:130-135.

Jungherr, E. 1933. Staphylococcal arthritis in turkeys. *J. Am. Vet. Med. Assoc.* 35:243-249.

Kawahira, T., N. Wakita, H. Nohara, and T. Shida. 1996. A case report of an infective endocarditis caused by methicillin-resistant *Staphylococcus aureus* with successful mitral valve replacement. *Kyobu. Geka.* 49 (8 Suppl): 680-683.

Kawano. J., A. Shimizu, Y. Saitoh, M. Yagi, T. Saito, and R. Okamoto. 1996. Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. *J. Clin. Microbiol.* 34: 2072-2077

Kibenge, F. S. B., G. E. Wilcox, and D. Perret. 1982. *Staphylococcus aureus* isolated from poultry in Australia 1. Phage typing and cultural characteristics. *Vet. Microbiol.* 7:471-483.

Kitagawa, Y., M. Ueda, N. Ando, M. Endo, K. Ishibiki, Y. Kobayashi, T. Arai, and M. Kitajima. 1996. Rapid diagnosis of methicillin-resistant *Staphylococcus aureus* bacteremia by nested polymerase chain reaction. *Ann. Surg.* 224:665-671.

Kloos, W. E., and T.L. Bannerman. 1995. *Staphylococcus* and *Micrococcus*. In Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R.H. Yolken (eds.), *Manual of Clinical Microbiology*, 6th Ed., pp. 282-298. Am Soc Microbiol, Washington, DC.

Kluytmans, J., W. Van-Leeuwen, W. Goessens, R. Hollis, and S. Messer. 1995. Food- initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno-and genotyping. *J. Clin. Microbiol.* 33:1121-1128.

Ko, H. C. and T. Y. Chang. 1995. Using reversed passive latex agglutination method to detect enterotoxigenic Staphylococcus aureus and enterotoxin in foods. *J. Food. Drug. Analy.* 3:57-63.

Koh, T. W., S. J. Brecker, and C. A. Layton. 1996. Successful treatment of *Staphylococcus lugdunensis* endocarditis complicated by multiple emboli: a case report and review of the literature. *Int. J. Cardiol.* 55:193-197.

Lapeyre, C., M. N. De-Solan, and X. Drouet.1996. Immunoenzymatic detection of staphylococcal enterotoxins: international interlaboratory study. *J. A. O. A. C. Int.* 79:1095-1101.

Lessing, M. P., D. W. Crok, I. C. Bowler, and B. Gribbin. 1996. Native-valve endocarditis caused by *Staphylococcus lugdunensis*. *Q. J. M.* 855:8.

Locksley, R. M., M. L. Cohen, and T. C. Quinn, et al., 1982. Multiple antibiotic-resistant *Staphylococcus aureus*: introduction, transmission, and evolution of nosocomial infection. *Ann. Intern. Med.* 97:317-324.

Lucet, A. 1892. De I' osteo-arthrite aigue infectieuse des jeunes oies. *Ann. Inst. Pasteur* (Paris) 6:841-850.

Lyon, B. R., J. L. Iuorio, J. W. May, and . A. Skurray. 1987. Molecular epidemiology of multiresistant *Staphylococcus aureus* in Australian hospitals. *J. Med. Microbiol.* 17:79-89.

L' Ecuyer, P. B., D. Murphy, J. R. Little, V. J. Fraser. 1996. The epidemiology of chest and leg wound infections following cardiothoracic surgery. *Clin. Infect. Dis.* 22:424-429.

Maki, D. G., R. D. McCormick, M. A. Zilz, S. M. Stolz, and C. J. Alvarado. 1990. A MRSA outbreak in an SICU during universal precaution: new epidemiology for nosocomial MRSA downside for universal precautions (abstract 473). *30th Interscience Conference on Antimicrob. Agents and Chemother. Atlanta.*

Maple, P. A. C., J. M. T. Hamilton-Miller, W. Brumfitt.1989. World wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. *Lancet* i:537-540.

Matsumoto, M., J. G. Strain and H. N. Engel. 1991. The fate of *Pasteurella multocida* after intratracheal inoculation into turkeys. *Poult. Sci.* 70:2259-2266.

McDougal, L. K. And C. Thornsberry. 1986. The role of B-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *J. Clin. Microbiol.* 23:832-839.

Meehan, P. J., T. Atkeson, D. E. Kepner, and M. Melton. 1992. A food borne outbreak of gastroenteritis involving two different pathogens. *Am. J. Epidemiol.* 136:611-616.

Meier, P. A., C. D. Carter, S. E. Wallace, R. J. Hollis, M. A. Pfaller, and L.A. Herwaldt. 1996. A prolonged outbreak of methicillin-resistant *Staphylococcus aureus* in the burn unit of a tertiary medical center. *Infect. Control Hosp. Epidemiol.* 17:798-802.

Meyers, C. M., and M. M. Jensen. 1987. Staphylococosis of turkeys. 3. Bacterial interference as a possible means of control. *Avian Dis.* 31:74-79.

Micusan, V. V., and J. Thibodeau. 1993. Superantigens of microbial origin. *Semin. Immunol.* 5:3-11.

Milatovic, D. 1986. Vancomycin for treatment of infections with methicillin-resistant *Staphylo coccus aureus* : are there alternatives ? *Eur. J. Clin. Microbiol.* 5:689- 692.

Miller, M. A., A. Dascal, J. Portnoy, and J. Mendelson. 1996. Development of mupirocin resistance among methicillin resistant *Staphylococcus aureus* after widespread use of nasal mupirocin ointment. *Infect. Control Hosp. Epidemiol.* 17:811-813.

Mizushima, Y., H. Li, I. Yoshida, K. Sassa, T. Hamazaki, and M. Kobayshi. 1996. Nosocomial septicemias due to methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in university hospital over a 12-year period--the same intractable infections. *Infect.* 24:238-241.

Morita, M. M. 1993. Methicillin-resistant *Staphylococcus aureus*. Past, present, and future. *Nurs. Clin. North. Am.* 28:625-637.

Mossad, S. B., J. M. Serkey, D. L. Longworth, D. M. Cosgrove, and S. M. Gordon. 1997. Coagulase-negative staphylococcal sternal wound infections after open heart operations. *Ann. Thorac. Surg.* 63:395-401.

Muder, R. R., C. Brennen, M. Wagener, et al. 1991. Methicillin-resistant staphylococcal colonization and identification in long-term care facility. *Ann. Intern. Med.* 114:107-112.

Mulligan, M. E., K. A. Murray-leisure, B. S. Ribner, H. C. Standiford, J. A. Korvick, C. A. Kauffman, and V. Yu. 1993. Methicillin-resistant *Staphylococcus aureus*: A consensus review of the microbiology, pathogenesis and epidemiology with implications for prevention and management. *Am. J. Med.* 94:313-328.

Munger, L. L., and B. L. Kelly. 1973. Staphylococcal granulomas in a leghorn hen. *Avian Dis* 17:858-860.

Murray, P. R., G.S. Kobayashi. M. A. Pfaller and K. S. Rosenthal. 1994. *Staphylococcus*. In. Farrel, R (ed.), *Medical Microbiology*, 2nd Ed., pp.166-179. Mosby-Year Book, Inc. St. Louis, Missouri.

Murray, L. A., S. Geib, D. Gracely, et al., 1990. Control of epidemic methicillin-resistant *Staphylococcus aureus* . *Infect. Control. Hosp. Epidemiol.* 11:343-350.

Mutalib, A., C. Riddel, and A. D. Osborne. 1983. Studies on the pathogenesis of Staphylococcal osteomyelitis in chickens. 11. Role of the respiratory tract as a route of infection. *Avian Dis.* 27:157-160.

Mutalib, A., C. Riddel, and a. D. Osborne. 1983. Studies on the pathogenesis of Staphylococcal osteomyelitis in chickens. 1. Effect of stress on experimentally induced osteomyelitis. *Avian Dis.* 27:141-156.

Nairin, M. E., 1973. Bacterial osteomyelitis and synovitis of the turkey. *Avian Dis.* 17:504-517.

Nairin, M. E. and A. R. A. Watson, 1972. Leg weakness of poultry - A clinical and pathological characterization. *Aust. Vet. J.* 48:645-656.

Nathoo, K. J., S. Chigonde, M. Nhembe, M. H. Ali, and p. R. Mason. 1996. Community-acquired bacteremia in human immunodeficiency virus-infected children in Harare. *Pediatr. Infect. Dis. J.* 15:1092-1097.

Netto Dos Santos., K. R., L. De Souza Fonseca, and P. P. Gontijo Filho.1996. Emergence of high level mupirocin resistance in methicillin-resistant *Staphylococcus aureus* isolated from Brazilian university hospital. *Infect. Control. Hosp. Epidemiol.*17:813-816.

Neu, H. C. 1994. Emerging trends in antimicrobial resistance in surgical infections. A review. *Eur. J. Surg. Suppl.* 573:7-18.

Nicoll, T. R., and M. M. Jensen. Preliminary studies on bacterial interference of staphylococcosis of chickens. *Avian Dis.* 31:140-144.

Niedergang, F., A. Hemar, C. R. A. Hewitt. M. J. Owen, A. D. Varsat, and A Alcover. 1995. The *Staphylococcus aureus* enterotoxin B superantigen induces specific T cell receptor down-regulation by increasing its internalization. *J. Biologic. Chemist.* 270:12839-12845.

Niskanen, A., H. Korkeala, M. Manninen, M. Vuento, and P. Kuusela. 1991. Evaluation of three slide agglutination tests for rapid identification of *Staphylococcus aureus*. *Acta. Vet. Scand.* 32:543-549.

Notermans, S., J. Dufrenne, and W. J. Van Leeuwen, 1982. Contamination of broiler chickens by *Staphylococcus aureus* during processing: incidence and origin. *J. Appl. Bacteriol.* 52:275-280.

Notermans, S., E. H. W. Van Erne, H. J. Beckers, and J. Oosterom. 1981. The assessment of the bacteriological conditions of fresh poultry in shops and market places. *Fleischwirtschafi.* 61:101-104.

Novick, R. P. 1990. Staphylococci. In Davis, B. D. et al., (eds), *Microbiology*, 4th Ed., pp. 539-550. J. B. Lippincott Company, Philadelphia.

Ochi, A., K. Migita, J. Xu, and K. Siminovitch. 1993. In vivo tumor immunotherapy by bacterial superantigen. *J. Immunol.* 151:3180-3186.

Ogston, A. 1880. Uber Abscesse. *Arch. Klin. Chir.* 25:588-600.

Olona, C. B., N.T. Falguera, L. R. Garceran, O. D. V. O. Ortiz, T. C. Verdu and L. F. Garcia 1996. Methicillin-resistant *Staphylococcus aureus*: a four-year experience in a spinal cord injury unit in Spain. *Spinal Cord.* 34:315-319.

Ombui, J. N., S. M. Arimi, and M. Kayihura. 1992. Beef and dressed chickens as sources of enterotoxigenic *Staphylococcus aureus* in Nairobi. *East. Afr. Med. J.* 69:606-608.

Page, R. K. 1974. Scabby-hip disease in broilers. *Poult. Dig.* 33:431-432.

Panlilio, A. L, D. H. Culver, R. P. Gaynes, S. Banerjee, T. S. Henderson, J. S. Tolson, and W. J. Martone. 1992. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975-1991. *Infect. Control. Hosp. Epidemiol.* 13:582-586.

Papageorgiou, A. C., K. R. Acharya, R. Shapiro, E. F. Passalacqua, R. D. Brehm, and H. S. Tranter. 1995. Crystal structure of the superantigen enterotoxin C2 from *Staphylococcus aureus* reveals a zinc-binding site. *Struct.* 3:769-779.

Payne, D. N., and J. M. Wood. 1974. The incidence of enterotoxin producing strains of *Staphylococcus aureus* isolated from foods. *J. Appl. Bacteriol.* 37:319-325.

Peacock, J. E., D. Moorman, R. P. Wenzel, and G. L. Mandell. 1981. Methicillin-resistant *Staphylococcus aureus:* microbiological characteristics, antimicrobial susceptibility, and assessment of virulence of an epidemiologic strain. *J. Infect. Dis.* 144:575-582.

Pearman, J. W., J. Christensen, D. I. Annear, et al., 1985. Control of methicillin-resistant *Staphylococcus aureus* (MRSA) in an Australian metropolitan teaching hospital complex. *Med. J. Aust.* 142:103-108.

Performance standards for antimicrobial disk susceptibility tests. 1990. (ed. 4), In *National Committee for Clinical Laboratory Standards*. pp. M2-A4. Villanova, PA.

Piercy, E. A., D. Barbaro, J. P. Luby, et al., 1989. Ciprofloxacin for methicillin-resistant *Staphylococcus aureus* infection. *Antimicrob. Agents Chemother.* 33:128-130.

Platt, C. G., J. F. John, R. A. Boude, T. A. Cappello, 1989. *Veterans Administration Medical Center, Charleston, South Carolina, Infection Control Guidelins.* Methicillin-resistant *Staphylococcus aureus* infections.

Porto, E., and E. N. Silva. 1995. *Staphylococcus aureus* in a broiler processing plant: origin, dissemination and thermal resistance of strains isolated from carcases. *Arquivo. Brasileiro. De. Medicina. Veterinaria. E. Zootecnia.* 47:417-433.

Poston, S. M., and J. L. Naidoo. 1983. Genetics and antimicrobial drug resistance in the staphylococci. In. Easmon, C. S. F. And C. Adlam(eds.), *Staphylococci and staphylococcal infections*. Vol. 1. pp.63-119.Academic Press. Inc. (London) Ltd.

Pulimood, T. B., M. K. Lalitha, M. V. Jesudason, R. Pandian, J. Selwyn, and T. J. John.1996. The spectrum of antimicrobial resistance among methicillin resistant *Staphylococcus aureus* in a tertiary care center in India. *Indian J. Med. Res.* 103:212-215.

Quinn, P. J., M. E. Carter, B. K. Markey, and G. R. Carter (eds.). 1994. *Staphylococcus species*. In *Clinical Veterinary Microbiology*, pp.118-126.Mosby -Year Book Europe Limited, London.

Raska, K., V. Matejovska, D. Matejovska, M.S. Bergdol, and P. Petrus. 1981. To the origin of contamination of foodstuffs by enterotoxigenic staphylococci. In J. Jeljaszewicz (ed.). *Staphylococci and Staphylococcal infections*. Gustav. Fischer. Verlag, Stuttgart, pp. 381-385.

Reagan, D., B. Doebbeling, M. A. Pfaller, et al., 1991. Elimination of coincident *Staphylococcus aureus* nasal and hand carriage with intranasal application of mupirocin calcium ointment. *Ann. Intern. Med.* 114:101-106.

Reboli, A. C., J. F. John, C. G. Platt, R. Cantey. 1990. Methicillin-resistant *Staphylococcus aureus* outbreak at a veteran'Affairs medical center: importance of carriage of the organism by hospital personnel. *Infect. Control. Hosp. Epidemiol.* 11:291-296.

Resende, C. A., and A. M. Figueiredo.1997. Discrimination of methicillin-resistant *Staphylococcus aureus* from borderline-resistant and susceptible isolates by different methods. *J. Med. Microbiol.* 46:145-149.

Richards, M. S., M. Rittman, T. T. Gilbert, S. M. Opal, B. A. DeBuono, R. J. Neil, and P. Gemski. 1993. Investigation of a staphylococcal food poisoning outbreak in a centralized school lunch program. *Public. Health. Rep.* 108:765-771.

Richet, H., m. Wiesel, F. L. Gallou, B. A. Richet, and E. Espaze. 1996. Methicillin-resistant *Staphylococcus aureus* control in hospitals: the french experience. *Infect. Control Hosp. Epidemiol.* 17:509-511.

Richmond, M. H.,1964. Lancet 1:293.

Richmond, M. H. 1972a. Plasmids and extrachromosomal genetics in *Staphylococcus aureus*. In. Cohen, J. O(ed.), *The staphylococci*. pp.159-186. John Wiley and Sons, Inc. New York.

Rimler, R. B. and J. R. Glisson, 1997. Fowl cholera. Pages 143-159 In: *Diseases of Poultry*. 10th ed. B. W. Calnek, ed. Iowa State Univ. Press, Ames, IA.

Rumbak, M. J., and M. R. Cancio. 1995. Significant reduction in methicillin-resistant *Staphylococcus aureus* ventilator-associated pneumonia associated with the institution of a prevention protocol. *Crit. Care. Med.* 23:1200-1203.

Rutala, W. A., E. B. S. Katz, R. J. Sheretz, and F. A. Sarubbi. 1983. Environmental study of a methicillin-resistant *Staphylococcus aureus* epidemic in a burn unit. *J. Clin. Microbiol.* 18:683-688.

Sabath, L. D. 1979. In. Schlessinger, D.(ed.) *Microbiology*. pp.299-303. American Society of Microbiology.

Sabath, L. D., N. Wheeler, M. Laverdiere, D. Blazevic, and B. J. Wilkinson. 1977. *Lancet*, i:443-447.

Sanford, M. D., A. F. Widmer, M. J. Bale, R. N. Jones, and R. P. Wenzel. 1994. Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*. *Clin. Infect. Dis.* 19:1123-1128.

Sarmah, R. 1995. Bumble foot in poultry: isolation and drug sensitivity studies on bacterial isolate. *Indian Vet. J.* 72:637-638.

Scanlan, C. M. 1988. Genus *Staphylococcus*. In *Introduction to Veterinary Bacteriology*, 1st Ed. pp.72-76. Iowa State University Press, Ames, Iowa.

Scanlan, C. M., and B. M. Hargis. 1989. A bacteriologic study of scabby-hip lesions from broiler chickens in Texas. *J. Vet. Diagn. Invest.* 1:170-173.

Schleifer, K. H., 1986. Gram-positive cocci. Pages 999-1103 In: *Bergey's Manual of Systematic Bacteriology*, vol. 2. Sneath, P. H. A. *et al.* eds., Williams and Wilkins, Baltimore, MD.

Shanson, D. C., J. G. Kensit, and R. Duke. 1976. Outbreak of hospital infection with a strain of *Staphylococcus aureus* resistant to gentamicin and methicillin. *Lancet*:1347-1348.

Sherris, J. C. 1984. Staphylococci. In Sherris, J. C., et al (eds.), *Medical Microbiology* pp.150-161. Elsevier Science Publishing Co., Inc. New York.

Simmons, B., M. Trusler, J. Roccaforte, P. Smith, R. Scott. 1990. Infection control for home health. *Infect. Control. Hosp. Epidemiol.* 11:362-370.

Skeeles, J. K. 1997. Staphylococcosis. In Calnek, B. W., H. J. Barnes, C. W. Beard, L. R. MacDougald, and Y. M. Saif. (eds), *Diseases of Poultry*, pp.247-253. Iowa State University Press, Ames, Iowa.

Smart, R. A., M. L. Miner and R. V. Davis, 1968. Pathogenesis of staphylococcal synovitis in turkeys: Cultural retrieval in experimental infection. *Avian Dis.* 12:37- 46.

Smith, H. W. 1954. Experimental staphylococcal infection in chickens. *J. Pathol. Bacteriol.* 67:81-87.

Snyder, O. P., and M. E. Matthews. 1996. Food safety: review and implications for dietitians and dietetic technicians. *J. Am. Diet. Assoc.* 96:163-168.

Sompolinski, E., Z. Samra, W. W. Karakwa, W. F. Vann, R. Schneerson, and Z. Malik. 1985. Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. *J. Clin. Microbiol.* 22:828-834.

Spicer, W. J. 1984. *J. Hosp. Infect.* 5:suppl.A:45.

Stoll, B. J., T. Gordon, S. B. Korones, S. Shankaran, J. E. Tyson, C. R. Bauer, A. A. Fanaroff, J. Lemons, E. F. Donovan, W. Oh, D. K. Stevenson, R. A. Ehrenranz, L. A. Papile, J. Verter, L. L. Wright. 1996. Late onset sepsis in very low birth weight neonates: a report from the national institute of child health and human development neonatal research network. *J. Pediatr.* 129:63-71.

Sugita, K. Y., K. Shibata, S. S. Yun, Y. K. Hara, K. Yamaguchi, and S. Kumagai. 1996. Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. *Biosci. Biotech. Biochemist.* 60:886-888.

Sundstrom, M., D. Hallen, A. Svensson, E. Schad, M. Dohlsten, L. Abrahmsen. 1996. The co-crystal structure of staphylococcal enterotoxin type A with $Zn2^{+}$ at 2.7 A resolution. Implications for major histocompatibility complex class II binding. *J. Biol. Chem.* 271:32212-32216.

Switalski, L. M., J. M. Patti, W. Butcher, A. G. Gristina, P. Speziale, and M. Hook. 1993. A collagen receptor on *Staphylococcus aureus* strains isolated from patients with septic arthritis mediates adhesion to cartilage. *Mol. Microbiol.* 7:99-107.

Tanaka, H., Y. Hirakata, M. Kaku, R. Yoshida, H. Takemura, R. Mizukane, K. Ishida, K. Tomono, H. Koga, S. Kohno, S.Kamihira. 1996. Antimicrobial activity of superoxidized water. *J. Hosp. Infect.* 34:43-49.

Thompson, R. L., I. Cabezudo, and R. P. Wenzel. 1982. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* . *Ann. Intern. Med.* 97:309-317.

Thornsberry, C., and L. K. McDougal. 1983. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. *J. Clin. Microbiol.* 18:1084-1091.

Todd, E. C. D. 1978. Food borne diseases in six countries- a comparison. *J. Food. Protect.* 41:559-565.

Tomasz, A., H. B. Drugeon, H. M. DeLencastre, et al., 1989. New mechanism for methicillin resistance in *Staphylococcus aureus*. Clinical isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity. *Antimicrob. Agents. Chemother.* 33:1869-1874.

Tomasz et al., S. Nachman, and H. Leaf, 1991. Stable classes of phenotypic expression in methicillin-resistant clinical isolates of staphylococci. *Antimicrob. Agents. Chemother.* 35:124-129.

Tsen, H. Y., T. R. Chen, and G. K. Tu. 1994. Application of polymerase chain reaction (PCR) for the specific detection of enterotoxigenic *Staphylococcus* in various food samples. *J. Food. Drug Analy.* 2:217-224.

Tsuji, M. and M. Matsumoto. 1989. Pathogenesis of fowl cholera: Influence of encapsulation n the fate of Pasteurella multocida after intravenous inoculation into turkeys. *Avian Dis.* 33:238-247.

Tufnell, D. J., R. S. Croton, D. M. Hemingway, et al., 1987. Methicillin-resistant *Staphylococcus aureus*: The role of antisepsis in the control of an outbreak. *J. Hosp. Infect.* 10:255-259.

Vandenbroucke-Grauls, C. M. 1996. Methicillin-resistant *Staphylococcus aureus* control in hospitals: the Dutch experience. *Infect. Control. Hosp. Epidemiol.* 17:512-513.

Wadstrom, T., P. Speziale, F. Rozgoni, A. Ljungh, I. Maxe, and C. Ryden. 1987. Interactions of coagulase-negative staphylococci with fibronectin and collagen as possible first step of tissue colonization in wounds and other tissues trauma. *Zbl. Bakt. Suppl.* 16:83-91.

Ward, T. T., R. E. Winn, A. L. Harstein, and D. L. Sewell. 1981. Observations relating to an inter-hospital outbreak of methicillin resistant *Staphylococcus aureus*: Role of antimicrobial therapy in infection control. *Infect. Control.* 2:453- 459.

Warnick, R. E. 1982. *Research report 87*, Snow Field Station, Utah Agriculture Experiment Station, Logan, Utah.

Wenzel, R. P. 1982. The emergence of methicillin-resistant *Staphylococcus aureus*. *Ann. Intern. Med.* 97:376-378.

Whitener, C., G. M. Caputo, M. R. Weitekamp, and A. W. Karchmer. 1993. Endocarditis due to coagulase-negative staphylococci. Microbiologic, epidemiologic, and clinical considerations. *Infect. Dis. Clin. North. Am.* 7:81-96.

Wideman, R. F., Jr. 1988. Ascites in poultry. *Monsan-to nutrition update* 6 (2):1-7.

Wieneke, A. 1974. Enterotoxin production by strains of *Staphylococcus aureus* isolated from foods and human beings. *J. Hyg. Cambridge*. 73:255-262.

Wieneke, A. A., d. Roberts, and R. J. Gilbert. 1993. Staphylococcal food poisoning in the United Kingdom, 1969-90. *Epidemiol. Infect.* 110:519-531.

Wieneke, A. A. 1991. Comparison of four kits for the detection of staphylococcal enterotoxin in foods from outbreaks of food poisoning. *Int. J. Food Microbiol.* 14:305-312.

Wieneke. A. A., and R. J. Gilbert. 1987. Cimparison of four methods for the detection of staphylococcal enterotoxin in foods from outbreaks of food poisoning. *Inter. J. Food. Microbiol.* 4:135-143.

Williams, R. B. And L. L. Daines. 1942. The relationship of infectious omphalitis of poults and impetigo staphylogenes in man. *J. Am. Vet. Med. Assoc.* 101:26-28.

Witte, W., R. Hummel, W. Meyer, H. Exner, R. Windrak. 1977. Ecology of *staphylococcus aureus*: Characterization of strains from chickens. *Z. Allg. Mikrobiol.* 17:639-646.

Woods, G. L., and J. A. Washington. 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods. In Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R.H. Yolken (eds.), *Manual of Clinical Microbiology*, 6th Ed., pp. 1327-1341. Am. Soc. Microbiol. Washington, DC. F.C.

Wyeth, P. J., 1975. Effect of infectious bursal disease on the response of chickens to S. typhimurium and E. coli infections. *Vet. Rec.* 96:238-243.

Yang, X., R. G. Board, and G. C. Mead.1988. Influence of spoilage flora and temperature on growth of *Staphylococcus aureus* in turkey meat. *J. Food. Protect.* 51:303-309.

Yokoyama, T., J. Honda, T. Kawayama, K. Kajimura, and K. Oizumi. 1996. Increased incidence of beta-lactamase-plasmid negative , high level methicillin-resistant *Staphylococcus aureus* (MRSA). *Kurume. Med. J.* 43:199-206.

Yoshida, J., S. Kuroki, K. Akazawa, K. Chiziiwa, K. Takemori, M. Torisu, and M. Tanaka. 1995. The order of ward rounds influences nosocomial infection. A 2-year study in gastroenterologic surgery patients. *J. Gastroenterol.* 30:718-724.