1988

# Antimicrobial resistance in Staphylococcus aureas : a thesis ...

Marcella Alexandra Morgan
*University of the Pacific*

# ANTIMICROBIAL RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

---

A Thesis

Presented to the Faculty of the Graduate School

University of the Pacific

---

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Marcella Alexandra Morgan

May 1988

**This thesis, written and submitted by**

Marcella Alexandra Morgan

**is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.**

**Department Chairman or Dean:**

Paul A. Richmond

**Thesis Committee:**

Grant Hallas — Chairman

Robert S. Fong

Kishori Chanbal

Alice S. Hunter

**Dated** April 14, 1988

ACKNOWLEDGEMENTS

I am grateful to Dr. Fuad M. Nahhas for giving selflessly of his time, energy, and knowledge throughout my college career. He has supported and encouraged me (even prodded me a little) during the most challenging period of my education, the completion of this thesis. Other members of the faculty and staff have helped in many ways. Dr. Richard Tenaza has given his valuable critisism and his friendship. Dr. Kishori Chaubal has continuously been a joy to work with. Ginger Tibbens has always made it easy to face the day with a cup of hot coffee and a listening ear. I appreciate all who read and commented on the first thesis draft - Dr. Anne Funkhouser, Dr. Paul Richmond, Dr. Steven Anderson, Dr. Alice Hunter, and those who commented anonymously. Thanks to the microbiology department at Dameron Hospital for supplying the clinical specimens used in this project.

I will be thankful always to my mother, Paula. Her constant guidance and support, in every area of my life, have given me the incentive to continue. My grandparents (Nana and Papa) have provided love, inspiration, and generous financial assistance. I appreciate the concern my stepfather, Helmut, has shown, as well as his sense of humor about the whole process. And I thank my sister, Fran, for being the most wonderful, loving sister in the world.

TABLE OF CONTENTS

## ABSTRACT

Susceptibility of 112 strains of Staphylococcus aureus obtained from Dameron Hospital, Stockton, California was tested with 18 antimicrobials. The MIC method was used with the following antimicrobials: tetracycline, oxacillin, penicillin, ampicillin, vancomycin, cefazolin, erythromycin, clindamycin, gentamycin, rifampin, trimethoprim-sulfamethoxazole, chloramphenicol, and cefotaxime. The standard Kirby-Bauer disc diffusion method was used to test neomycin, tobramycin, and amikacin. Methicillin, oxacillin, and nafcillin were tested with a modified Kirby-Bauer method, which included the addition of a 4% salt supplement to the media, incubation at 32C, and readings at both 24 and 48 hours.

Comparing results of this study with those of Hall (1975), suggested that resistance to the following antibiotics has increased: penicillin, ampicillin, erythromycin, neomycin, gentamycin, methicillin, oxacillin, nafcillin, cefazolin, and clindamycin. Resistance to tetracycline has decreased. No resistance to chloramphenicol or vancomycin was encountered in either study.

Of the 112 strains studied, 13.4% were susceptible to all antibiotics tested. Twelve patterns of resistance were identified: 0.9% were resistant to neomycin only, 1.8% to

erythromycin only, 63.9% to both penicillin and ampicillin, and 20.0% were multiply- resistant. Nine patterns of multiple-resistance were found, involving a minimum of three antibiotics and a maximum of nine.

Three MRSA strains were identified from out-patient isolates; no in-patient isolates were methicillin-resistant. The study suggests that MRSA strains are not a problem at Dameron Hospital, but identification of this group would be more accurate if incubation of the MIC panels is maintained for at least 24 hours at ≤35C. It was found that the MIC method of antimicrobial susceptibility testing is more reliable than the Kirby-Bauer disc diffusion method for detection of methicillin-resistance. Problems involved in identification of heteroresistant staphylococci are discussed.

LIST OF TABLES

# I. INTRODUCTION

Staphylococci are Gram positive cocci, 0.5 - 1.5μ in diameter occurring in grape-like clusters or occasionally in tetrads, pairs, and short chains of three to four cells. Staphylococci are currently included with the genera Micrococcus and Planococcus in the family Micrococcaceae. The genus Staphylococcus is presently composed of 23 species, 12 of which occur in humans (Howard and Kloos, 1987). Because Staphylococcus aureus is a pathogen, or potential pathogen, it is recognized as the most important species of the genus. Coagulase production has traditionally been considered a distinctive and unique characteristic of S. aureus. Recent studies (Hajek, 1976; Philips Jr. and Kloos, 1981; Biberstein et al., 1984), however, have shown that the ability to clot plasma (a positive coagulase test) is also characterestic of S. intermedius and S. hyicus, subspecies hyicus. Since S. intermedius and S. hyicus hyicus occur only in nonhuman animals (Kloos and Jorgensen, 1985), it is assumed that any coagulase positive staphylococci isolated from humans is S. aureus. Recent studies (Howard and Kloos, 1987) have found that about 3% of S. aureus are coagulase negative and therefore biochemical characteristics should also be used to identify this species with accuracy.

In the late 1950's and early 1960's S. aureus emerged as a major cause of morbidity and mortality in hospitals. Some

strains of this organism began to show resistance to penicillin due to the production of a ß-lactamase enzyme called penicillinase (Thornsberry, 1984). This enzyme lyses the beta-lactam ring in the "nucleus" of the molecule, inactivating the drug. Resolution of the problem was thought to have been achieved with the introduction of a new group of penicillins known as the penicillinase-resistant penicillins (PRP's). The first, methicillin, was introduced in 1959, followed by nafcillin and oxacillin, shortly after. However, almost concurrently, S. aureus strains resistant to methicillin, nafcillin and oxacillin, were recognized in Britain, throughout Europe, and more recently in the United States (McNeil and Solomon, 1985; Jorgensen and Thornsberry, 1987). These strains came to be referred to as methicillin-resistant or MRSA (methicillin-resistant Staphylococcus aureus). Today it is recognized that resistance to one PRP implies resistance to the other two (Sherris, 1984). The term MRSA for methicillin-resistance is used regardless of whether the susceptibility testing is performed with methicillin, oxacillin or nafcillin. In practice, most clinical laboratories determine methicillin-resistance using oxacillin because of its greater in vitro stability.

MRSA strains were first isolated from patients in large tertiary care hospitals, and later in convalescent homes and rehabilitation facilities (Jorgensen et al., 1984; Thornsberry, 1984). More recently they have appeared in primary care facilities, with estimates of 4.6 - 6.0%

incidence (McGowen, 1988). Many MRSA strains are also resistant to several commonly used antibiotics, besides methicillin (Welch and Southern, 1984; Putland and Guinness, 1985).

The first study of S. aureus from the Stockton community was conducted by Hall (1975). In his survey of 136 strains from patients at Dameron Hospital, Hall investigated the relationship between antibiotic pattern (antibiogram), enzymatic activity, and phage type. To test susceptibility, Hall used the Kirby-Bauer disc diffusion method and 20 antibiotics including methicillin, oxacillin, and nafcillin. One of Hall's most significant findings, relative to the present study, was that all strains were 100% susceptible to the PRP's.

Hall's results, and others obtained prior to 1980, are difficult to evaluate because of the problems involved in testing susceptibility of this organism (Thornsberry, 1984; Robinson et al., 1986). At a meeting of the California Association for Medical Laboratory Technology (April 30, 1986, St. Joseph's Hospital in Stockton, California), Leon Sabath, a visiting lecturer, pointed out that these difficulties reside in the fact that a single MRSA colony may contain both susceptible and resistant subpopulations. Such populations frequently described as "heteroresistant" (Kayser and Muller, 1983; McDougal and Thornsberry, 1984). Sabath indicated that erroneous results are often obtained because test conditions favor the faster growing susceptible

population. He presented a number of observations which can be used to aid in the detection of heteroresistance, including faint growth occurring within the zone of inhibition for any of the PRP's, colonies of the same strain exhibiting varying growth rates, and heterogeneity of resistance within the same strain. In a video entitled "In Vitro Detection of Methicillin Resistant Staphylococcus", prepared by Eli Lilly Co. (Indianapolis, Indiana), Thornsberry of the Centers for Disease Control in Atlanta, Georgia and Jorgensen of the University of Texas Health Science Center, San Antonio, Texas, suggest the use of three additional observations to aid in the detection of MRSA: (1) an intermediate susceptibility result to one or more PRP; (2) concurrent resistance (referred to as multiple resistance) to one or more of several other antibiotics including erythromycin, chloramphenicol, tetracycline, clindamycin and the aminoglycosides; (3) a cephalothin MIC between 1 & 16 mcg/ml.

At the aforementioned meeting of the California Association for Medical Laboratory Technology, it was noted that although all cells of a heteroresistant strain are genotypically resistant to the PRP's, only a minority exhibit phenotypic resistance. Factors promoting expression of phenotypic resistance are: (1) incubation at temperatures lower than 37C (30-35C), (2) incubation for at least 24 hours, but not longer than 48 hours, (3) increasing the content of salt in the medium to between 2-4%, (4)

suspending, rather than growing the inoculum to the standard turbidity of $10^8$ CFU (Colony Forming Units)/ml, and (5) using media incorporating ß-lactam compounds (Jorgensen and Thornsberry, 1987). In Hall's (1975) study, these techniques were not utilized.

The strains used by Hall (1975) were not available for retesting. Whether he would have had any MRSA if he had used the special procedures for detecting these strains cannot be determined. According to Nahhas (personal communication), the extent of resistance of S. aureus strains is not known in the Stockton community because none of the three major hospitals use all the special procedures needed to detect them. The importance of S. aureus as a cause of local infections (skin, eyes, nose, throat, urethra, vagina, and gasterointestinal tract) is well recognized. More serious invasive staphylococcal infections rarely occur in healthy individuals but can occur in individuals whose immune systems are compromised (immune deficiency, leukocyte defects, etc.). Predisposing factors including traumatic wounds, burns, surgical incisions, pacemakers, intravenous catheters, diabetes mellitus, alcoholism, coronary heart disease, and various malignancies, can lead to more serious invasive staphylococcal conditions. With the emergence of methicillin-resistance and implied resistance of the cephalosporin antibiotics (Thornsberry and Jorgensen, 1985), as well as increasing multiple resistance, knowledge of the antibiotic

pattern of local strains of S. aureus becomes important.

It is the purpose of this investigation to:

1. Report on antibiotic susceptibility of S. aureus isolates obtained from Dameron Hospital.
2. Determine if methicillin-resistance exists among these isolates using the recommended procedures.
3. Determine the extent of multiple resistance, i.e. resistance to antimicrobials in addition to the penicillins.
4. Compare results obtained in this study with those of Hall (1975).

# II. MATERIALS AND METHODS

One hundred and twelve strains of Staphylococcus aureus were isolated at the clinical laboratory of Dameron Hospital, Stockton, California, between September 25, 1987 and December 31, 1987. These strains were identified as S. aureus at the hospital microbiology department using Microscan Gram Positive Combo panels or trays (Microscan, Baxter Scientific Products, Sacramento, California).

## Biochemical and Enzymatic Testing

The battery of tests in the panel consists of 27 biochemical and enzymatic tests, 18 of which are used for the identification of staphylococci (Appendix I). The procedures used in these tests are detailed below, and the principles involved are given in Appendix II.

Instructions for inoculation of the tray are as follows: From each primary isolation plate, three to five colonies are selected to inoculate a 5-ml brucella broth tube. The tube is then incubated for four to six hours at 35C which allows the bacteria to grow to the stationary phase of about $10^9$ CFU/ml. One-half ml of the suspension is then pipeted into a tube containing 25 ml sterile distilled water. This 1:50 dilution produces a concentration of approximately $2 \times 10^6$ CFU/ml. The Gram Positive Combo is inoculated by placing a transfer lid of metal prongs into a trough containing the

preparation and situating the lid of prongs over the test panel so that the prongs (each holding 5 mcl of inoculum) dip into the wells containing media and/or antibiotics. The small amount of inoculum contained on each prong is pulled into the media by capillary action. Following inoculation, the tray is incubated for 18-20 hours and read on a scanner (Touchscan) connected to a computer. Positive reactions are recorded by the microbiologists and transferred to the computer memory whose data base identifies the species and translates the information into a biotype or I.D. number (Appendix III).

The panels used in this project were transferred to the microbiology laboratory at the University of the Pacific and reincubated for an additional 24 hours at 32 ± 1C to allow time for slower biochemical reactions to develop. The longer incubation period at a lower temperature was also intended to promote the growth of any existing methicillin-resistant subpopulations. After a total of 42-44 hours, the final results were recorded.

In addition to the tests from the Gram Positive Combo tray, a number of supplemental enzymatic tests were performed at the university, including coagulase, DNAse, and hemolysis. The purpose of these additional tests was to confirm the identity of the isolates and compare results with those of Hall (1975). Each strain was reisolated on mannitol salt agar (Difco - Detroit, Michigan) by dipping a sterile cotton swab into the growth well of the test panel and using it to

apply a drop of bacterial growth to the agar plate. A sterile loop was used to streak the plate after which it was incubated for 24 hours at 37C in an inverted position. Three to five well- isolated 24-hour colonies were selected to prepare a suspension in sterile distilled water with a turbidity (approximately $10^8$ CFU) equal to the 0.5 MacFarland barium sulfate standard purchased from the Remel Co (Lenexa, Kansas). This suspension was used to perform all additional antimicrobial and biochemical testing. Furthermore, a tryptic soy agar slant was inoculated from each suspension and saved as a stock culture. The enzymatic tests are described below.

Coagulase

To test for coagulase production, two drops of the bacterial suspension were transferred to a tube containing 0.5 ml of reconstituted rabbit plasma (Difco). The mixture was incubated for 24 hours at 37C and examined for clot formation, a positive test.

Deoxyribonuclease (DNAse)

This procedure utilizes Difco DNAse test medium containing methyl green. Methyl green combines with DNA in the medium only if the DNA is in its most polymerized state (Smith et al., 1969). If the test organism produces DNAse, the DNA becomes depolymerized, freeing the methyl green. The result

is the formation of a clear zone around the colony.

Hemolysis

Hemolysis was observed on blood agar plates (5% sheep blood in tryptic soy agar). A clear zone around the colonies resulting from complete lysis of the red blood cells indicates a positive result.

Antimicrobial Susceptibility Testing

Most susceptibility testing was accomplished with the Microscan Gram Positive Combo panel. The tray was inoculated at the microbiology lab of Dameron Hospital as described earlier. The test panel, which utilizes the MIC (minimum inhibitory concentration) method of susceptibility, contains 13 antimicrobials in several dilutions (Appendix III). The MIC for a particular antimicrobial is the lowest concentration of that antibimicrobial which inhibits growth.

After the test panels were incubated for 18-20 hours at Dameron Hospital, the results were recorded on the Touchscan computer along with those of the biochemical tests. Identification and biotype generation is done by the computer at this time. Following transport to U.O.P. the trays were reincubated, as indicated earlier, to allow time for the slower growing methicillin-resistant strains to express this characteristic and for slower chemical reactions to develop. The results were interpreted using a chart published by Baxter Scientific Products (Appendix III).

Six antibiotics (Appendix IV - methicillin, oxacillin, nafcillin, neomycin, tobramycin, and amikacin) were tested with the Kirby-Bauer disc diffusion method. The tests were conducted on Difco Mueller-Hinton medium. The medium is standardized so that the only variable in testing is the susceptibility of the test organism to a specific concentration of antibiotic. The agar plates were prepared by rehydrating the media according to the manufacturer's instructions on the bottle and then pouring the molten agar into screw-capped tubes, each holding 20 mls. After sterilizing the media at 121C and 15 p.s.i. for 15 minutes, the liquified agar was aseptically poured into sterile plastic 100 mm petri dishes. The three penicillinase-resistant penicillins (methicillin, oxacillin, and nafcillin) were tested on Mueller-Hinton agar containing 4% sodium chloride which was added to the powdered medium before rehydration. The purpose of using all three PRP's was to determine if susceptibility or resistance is the same for all, under the same standardized conditions. Oxacillin is also on the Gram Positive Combo trays and therefore a comparison between the MIC and disc diffusion methods can be made for that antibiotic.

Each freshly prepared agar plate was streaked with the bacterial suspension using a sterile cotton swab. The plates were evenly streaked in three directions to insure a uniform distribution of bacteria. After allowing the plates to dry for 5 - 15 minutes, paper discs impregnated with antibiotics

were dispensed onto the surface of the plates. Discs containing methicillin, oxacillin, and nafcillin were placed on the agar containing 4% salt. Discs of amikacin, tobramycin, and neomycin were tested on agar without the salt supplement. These last three were tested because Jorgensen and Thornsberry (1987) suggest that results of aminoglycoside susceptibility tests can be useful in determining methicillin-resistance. The plates were inverted and incubated (at 37C for the aminoglycosides and 32C for the PRP's) for 24 hours. Those containing the three PRP's were incubated an additional 24 hours for a total of 48 hours, according to the recommended procedures (Jorgensen and Thornsberry, 1987). The zone of inhibition was then measured in millimeters and interpreted according to Appendix IV.

# III. RESULTS

## Biochemical and Enzymatic Tests

The 24 hour results listed in Table I, with the exception of DNAse, hemolysis, and coagulase were those obtained from the panels that were incubated in the microbiology department at Dameron Hospital. I found that some strains are positive for certain characteristics only after extended incubation. This is particularly true of PGT, urease, raffinose, lactose, and to a lesser extent of arginine, IDX, PYR, arginine, and mannitol.

Table I also shows the results of the three additional tests performed at U.O.P. Hemolysis and coagulase were read at 24 hours, but most DNAse test results were inconclusive at 24 hours and reincubated further, read, and recorded at 48 hours incubation. Ninety-eight percent of the strains were hemolytic, 100% produced DNAse, and 95% were coagulase producers.

## Antimicrobial Susceptibility Tests

The results of these tests were interpreted using the charts in Appendixes III and IV. Those strains showing intermediate results for certain antibiotics were interpreted, and categorized respectively, as either susceptible or resistant depending on how close the results were to each of those categories. This is a common practice in clinical laboratories in order to place an isolate in the

susceptible or resistant category only.

Tables II and IV show raw figures of MIC's of 13 antimicrobials for each in-patient and out-patient isolate derived from computer printouts at the hospital. Tables III and V show the data for the same strains by the Kirby-Bauer disc diffusion method as it relates to six antimicrobials, five of which are not present in the MIC wells of the Gram Positive Combo panel. Table VI is a summary presenting the MIC and disc diffusion results as percent resistance. Table VII shows the various patterns of resistance.

Of the 112 isolates tested (Table VII), 13.4% (8.5% in-patient and 15.4% out-patient) were susceptible to all antimicrobials. There was no resistance (Table VI) by any of the strains to vancomycin (VA), trimethoprin-sulfazoxazole (TXS), chloramphenicol (C), and cefotaxime (Cft). The greatest resistance was to penicillin and ampicillin, each showing 84.8% resistance (89.4% in-patient and 81.5% out-patient). Resistance to erythromycin was seen in 14.3% of the strains (21.3% in-patient and 9.2% out-patient). Three isolates (strains #10284, 11126, and 11299) or 4.6% of out-patient strains were resistant to oxacillin (#11126 showed resistance only after extended incubation), yet no MRSA were found among in-patient isolates. These same isolates were also completely resistant to oxacillin, methicillin, and nafcillin by the Kirby-Bauer disc diffusion method (Table V), although the results here are not so straight forward. Two columns are presented for each of the PRP's because the zone

of inhibition for these antibiotics was read at both 24 and 48 hours. For oxacillin and nafcillin, the additional incubation period resulted in a higher percentage of strains expressing resistance. Only the methicillin results for the disc diffusion method coincide with the oxacillin results for the MIC method (0% resistance in in-patient strains, 4.6% resistant in out-patient strains).

Although Table IV shows MIC's to cefazolin of 8, 1, and 1 respectively for the three MRSA strains 10284, 11126, and 11299, it is standard to report methicillin-resistant strains as resistant to the cephalosporins regardless of in vitro results (NCCLS, M2-A3, 1984). This adjustment was automatically made by the computer and, therefore, was made in compiling Tables VI and VII.

Various patterns of resistance were seen in 86.6% of the isolates (Table VII). Resistance to a single antibiotic was seen in three strains: Two strains (1.8%) were resistant to erythromycin and one strain (0.9%) was resistant to neomycin. Almost 64% were resistant to penicillin and ampicillin only. Multiple resistance is represented by nine patterns. The pattern of multiple resistance encountered most often was found in 7.4% (17.0% in-patient and 1.5% out-patient) of the isolates. This involved resistance to penicillin, ampicillin, and erythromycin.

The three MRSA isolates showed the greatest multiple resistance (penicillin, ampicillin, oxacillin, cefazolin, erythromycin, neomycin, and tobramycin).

# IV. DISCUSSION

My interest in the biochemical profile of S. aureus isolates was only secondary. Since the reading of the tests at the hospital is done after 18-20 hours incubation, I reincubated the panels to determine if any changes occurred. The results suggest that a minimum of 24-hours incubation, and possibly longer, is necessary for PGT, urease, raffinose, lactose, and to a lesser extent arginine, IDX, PYR and mannitol to show a positive reaction. This is important in terms of proper recognition by the data base in the memory of the Touchscan computer. Staphylococcus aureus strains with such negative results are often identified as S. aureus, but also flagged by the computer as "atypical".

The three tests I used to confirm the identity of S aureus were DNAse, hemolysis and coagulase (100%, 98%, and 95% positive, respectively). My results are in complete agreement with Hall (1975) with respect to the DNAse test. The ability to break down DNA has long been considered a characteristic of S. aureus (Barry, 1973).

Eighty-seven percent of Hall's strains tested positive for hemolysis compared with 98% in my study. I cannot explain this discrepancy. Both results are in agreement with the observations of Kloos and Jorgensen (1985) who report that "most" strains of S. aureus are strongly hemolytic.

My results indicate that the use of the coagulase test as the only criterion for identification of S. aureus is

insufficient. In this study, six of 112 strains (5%) were coagulase negative. Kloos and Jorgensen (1987) report 3% of S. aureus strains are coagulase negative. Since Hall (1975) depended entirely on a positive coagulase test to identify S. aureus, it is possible that he excluded a number of strains because of negative results.

The emphasis in this study is on the antibiogram of the isolates. It should be pointed out at this time, that the expression "in-patient isolate" is not necessarily synonymous with "hospital acquired (nosocomial) isolate". These strains may have been acquired during the stay of the patient in the hospital or may have been brought into the hospital with the patient. The records of the in-patients were not availabe to determine how the infection was acquired. In contrast to the above, the terms "out-patient isolate" and "community acquired isolate" are most likely exchangeable. These strains of S. aureus were obtained from either out-patients or emergency room-patients with no history of having an earlier admission to Dameron Hospital. The above clarification is important because of the trend in the literature to compare "hospital acquired" and "community acquired" strains especially as this relates to penicillin resistance.

There appears to have been a general increase in resistance to antimicrobials by S. aureus in the Stockton community since Hall completed his study in 1975. Resistance to the following antimicrobials has increased in the last 12

years: penicillin (79 to 84.8%); ampicillin (79 to 84.8%); erythromycin (3.5 to 14.3%); neomycin (0 to 6.3%); gentamycin (0 to 3.6%); methicillin, oxacillin, nafcillin, cephazolin (0 to 2.7%); and clindamycin (0 to 0.9%). Resistance to both chloramphenicol and vancomycin has remained at 0.0%. The only antimicrobial to which strains of S. aureus have become more susceptible is tetracycline (15 to 3.6%). This observation is in agreement with a trend noted by Hall (1975), that resistance to tetracycline has been decreasing over the years. Statistical significance of changes in resistance to the drugs mentioned are indicated in Table VIII.

Hall (1975) found no significant difference in resistance between in-patient isolates and out-patient isolates. This is also the case in my study, except for erythromycin. Over 21% of in-patient isolates showed resistance to erythromycin but only 9.2% of out-patient isolates expressed resistance. A partial explanation of this difference may be due to the inability to determine if some in-patient strains are "hospital acquired" or "community acquired", as explained earlier. The increase in erythromycin resistance since 1975 (3.5 to 14.3%) is contrary to a trend of decreasing reisistance noted by Hall (1975).

Regarding patterns of resistance, Hall (1975) found no strain resistant to any antimicrobial unless resistant to penicillin and ampicillin. This was not true in my study. Two of the strains I tested were resistant to erythromycin

only, and one strain was resistant to neomycin only. Genes for resistance to these antibiotics (penicillin, ampicillin, erythromycin, and neomycin) may or may not be found on the same plasmid (Tompkins and Falkow, 1986). Therefore, resistance of a strain to various antibiotics without resistance to penicillin and ampicillin may be explained on this basis. Even though resistance to the macrolides (erythromycin, etc.), aminoglycosides (neomycin, tobramycin, gentamycin, amikacin, etc.) and penicillins (excluding the PRP's) may be carried on the same plasmid, mechanisms of resistance to these antimicrobials vary (Tompkins and Falkow, 1986). Resistance to the penicillins and cephalosporins is due to enzymatic hydrolysis of the ß-lactam ring; resistance to the aminoglycosides takes place through interference with transport of the antibiotic into the cell; and resistance to erythromycin is due to modification of 23S RNA (Tompkins and Falkow, 1986).

The percentage of strains resistant to penicillin and ampicillin only, has increased along with increase in overall resistance to antimicrobials. Hall (1975) found 55.1% resistance to penicillin and ampicillin only, compared with my results of 63.9%. Both studies showed 100% cross-resistance between these two drugs. It has long been recognized (Nahhas, personal communication) that resistance to penicillin is always accompanied by resistance to ampicillin.

Multiple resistance occurred in 23.9% of the strains

Hall studied compared to 20.0% of the strains I studied. This does not necessarily mean that multiple resistance in S. aureus strains has decreased in the Stockton community. Streptomycin and/or triple sulfa (SSS) were present in 13.7% of the multiply-resistant strains identified in Hall's study, but were not tested in this study. These drugs are rarely used today. Because of toxicity, those sulfonamides which are used, are administered topically; the exception being short acting sulfonamides in combination with trimethoprim, often utilized in urinary tract infections (Norton, 1986). The sulfa drug tested in the present study was trimethoprim in combination with sulfamethoxazole (TXS). No resistance was found.

The high incidence of multiple resistance is understandable given the variety of mechanisms involved in the transfer of R (resistance) plasmids from one bacterium to another. Tompkin and Falkow (1986) believe that transduction (transmission by a bacteriophage vector) is probably the most common mean of plasmid transmission among staphylococci. Transmission of plasmids may also occur through conjugation. One strand of DNA from the double-stranded plasmid is transferred from the donor to the recipient, followed by synthesis of complementary strands of DNA in each mating partner. Evidence suggests that determinants for methicillin-resistance as well as resistance to other antimicrobials reside in S. epidermidis strains which are part of the normal flora of patients and hospital staff. These determinants

provide a reservoir of resistance genes which can be passed to the more virulent S. aureus through conjugation (Archer (in Neu, 1988)). R plasmids, according to Tompkin and Falkow (1986), can also be obtained by uptake from the surrounding environment, a process known as transformation.

Genes coding for antimicrobial resistance are not always transferred as part of a plasmid. Some plasmodal genes, termed transposition elements or transposons, have the capability to move from one DNA element of a cell to another (from plasmid to plasmid, plasmid to chromosome, or vice versa).

Only three (2.7% total) of the S. aureus isolates studied, were methicillin-resistant. One of these strains was detected only after the additional 24 hour incubation period at 32C. In other words, the standard laboratory procedures used for detecting resistance to antimicrobials at Dameron Hospital were not sufficient to detect methicillin-resistance in the case of strain #11126. The new procedures recommended for detecting MRSA should be used in order to detect all methicillin-resistant strains.

Two of the three MRSA strains (#'s 10284 and 11126) came from patients who were brought to the emergency room of Dameron Hospital about three months apart, but from the same local convalescent home. The third strain (#11299) came from a resident of another convalescent home in Stockton. All three isolates were recovered from decubeti (bed sores) which had become infected at the convalescent homes. They were not

hospital acquired. This is in contrast with national figures reported by J.E. McGowen, Jr. (in Neu, 1988) of Gray Memorial Hospital, Atlanta, Georgia. He states that of all bacterial strains isolated from nosocomial infections in 1984, MRSA accounted for 11.3% of large teaching hospital strains, 6% of nonteaching hospital strains, and 4.6% of small teaching hospital strains. In addition, J.E. McGowen Jr. reports increased methicillin-resistance (28 to 43%) among S. aureus strains isolated from blood cultures at Grady Memorial Hospital between 1984 and 1986.

Trends of increased resistance to methicillin are worldwide. Acar reports that 20-25% of S. aureus strains isolated in French hospitals are methicillin-resistant, and in Italy methicillin-resistance in S. aureus isolates increased from 6% in 1981 to 26% in 1986 as reported by Schito (in Neu, 1988).

All three methicillin (oxacillin)-resistant strains isolated in this study were also resistant to penicillin, ampicillin, cefazolin, erythromycin, neomycin, and tobramycin. The resistance of MRSA to antibiotics in addition to methicillin has been noted by many (Kayser and Santanam, 1975; McNeil and Solomon, 1985; Thornsberry and Jorgensen, 1985) Welch and Southern (1984) report that MRSA almost always shows resistance to the other semisynthetic penicllinase-resistant penicllins as well as penicillin, ampicillin, chloramphenicol, erythromycin, and tobramycin. Although the MRSA strains I studied did not show resistance

to chloramphenicol, they were more multiply-resistant than any of the methicillin-susceptible isolates.

There seems to be a direct correlation between the increase in methicillin-resistance and resistance to other antimicrobials. Dr. Schito (in Neu, 1988) suggests that the significant increase in staphylococcal resistance to practically all antimicrobials (excluding tetracycline) is related to the increasing proportion of methicillin-resistant strains in hospitals. Between 1984 and 1986, resistance to cefoxitin and tobramycin remained essentially unchanged among methicillin-susceptible staphylococci while increasing from 38-53% for cefoxitin and 43-59% for tobramycin among methicillin-resistant strains in Italy. According to R.A. Skurray (in Neu, 1988), MRSA resistance determinants for antimicrobials including methcillin, erythromycin, rifampicin, streptomycin, sulfonamides, tetracycline are chromosomally encoded. Skurry (in Neu, 1988) suggests that some of these determinants were plasmid encoded in strains isolated in Australia prior to 1970, but have subsequently become chromosome encoded through transposition and site-specific-integration.

Kayser and Muller (1983) describe two major mechanisms of staphylococcal resistance to the ß-lactam antibiotics (penicillin, ampicillin, the cephalosporins, and semisynthetic penicillinase-resistant penicillins). These include a plasmid-mediated drug inactivation due to the production of penicillinase, as described earlier, and a

chromosomally-mediated intrinsic resistance. Intrinsically resistant strains have an alternative penicillin binding protein known as either PBP-2 or PBP-2a which shows lesser affinity for ß-lactams (Jorgensen and Thornsberry, 1987). Since the altered PBP has a diminished affinity for all ß-lactam antibiotics, strains with this characteristic should be reported as resistant to all ß-lactams regardless of in vitro results (Jorgensen and Thornsberry, 1987).

Methicillin-resistance due to hyperproduction of penicillinase, has recently been recognized. Overproduction of penicillinase causes moderate hydrolysis of the PRP's, particularly oxacillin. This mechanism of resistance to the PRP's is termed "borderline" or "acquired" resistance and appears to occur only in S. aureus (Jorgensen and Thornsberry, 1987).

Some unusual results for oxacillin and nafcillin by the Kirby-Bauer method are shown in Table VI. For both, the 48 hour results show more resistance than the 24 hour results. Strain #'s 9849 and 11915 from Table III, and 9904 and 12175 from Table V, are examples of this phenomenon. These strains were not resistant by the MIC method. In addition, the 24 hour results for oxacillin and nafcillin indicate a higher percentage of resistance than did the results of the MIC method. Strain #11382 from Table III and #'s 9475 and 12168 from Table V exemplify this. As indicated in the "Results" section, only the methicillin results by the disc diffusion method coincide with the oxacillin results by the Kirby-Bauer

method. Reasons for this are unclear. There still seems to be a great deal of controversy as to which method of susceptibility testing is most reliable. Many scientists are investigating this problem (Thornsberry and McDougal, 1983; Hansen and Freedy, 1984; Jorgensen et al., 1984; Woolfrey et al., 1984; Robinson et al., 1986). Barry and Jones (1987: p.1897) state, "Definition of an optimal, but yet practical method for absolute detection of all staphylococcal resistance to the penicillinase-resistant penicillins seems impossible."

Conflicting results between the two methods compared in this project, may be due to the fact that zone sizes obtained with the Kirby-Bauer method are affected by ß-lactamase production. According to McDougal and Thornsberry (1984), S. aureus strains with the same MIC's, exhibit varying zone sizes depending on whether or not ß-lactamase is produced. Hyperproducers of ß-lactamase often exhibit zones of inhibition at the breakpoint between susceptible and resistant (Campos, 1986), making results difficult to avaluate. An additional complication rests in the possibility that the salt supplement needed to enhance growth of heteroresistant staphylococci, may at the same time promote production and release of ß-lactamase (McDougal and Thornsberry, 1986).

Another factor leading to discrepancies between disc diffusion and MIC results is that the disc potency and zone interpretations set forth by the National Committee for

Clinical Laboratory Standards (NCCLS) do not correlate well with the MIC results obtained by recommended procedures for susceptibility testing (McDougal and Thornsberry, 1984; Coudron et al., 1986). While the NCCLS has approved using the disc potencies and interpretation ranges shown in Appendix IV, McDougal and Thornsberry (1984) recommend 10, 4, and 4 μg with resistance at ≤11, ≤12, and ≤12 mm for methicillin, oxacillin, and nafcillin, respectively. Use of the less potent discs in this study, resulted in smaller zone sizes than would have been obtained by following the procedures recommended by McDougal and Thornsberry (1984).

Results of this study and others indicate that the MIC method of susceptibility testing is more reliable than the Kirby-Bauer method for detecting true heteroresistance in S. aureus. Anyone using the Kirby-Bauer method for detecting methicillin-resistance should limit the incubation period to 24 hours. Mulligan et al. (1987) found that 34% of 139 isolates he tested were resistant at 48 hours with the Kirby-Bauer method, but had MIC's ≤1.0 μg/ml. Coudron et al. (1986) states that the Kirby-Bauer method for detection of MRSA works best if plates are read after 18 hours incubation.

Currently, investigators are placing emphasis on distinguishing between acquired resistance and heteroresistance. It is unclear whether acquired resistance can cause clinical failure of the PRP's and cephaolosporins. Some authors (McDougal and Thornsberry, 1984; Mulligan et al., 1987) state that until investigators know for sure,

serious infections due to MRSA, intrinsic or otherwise, may need to be treated with vancomycin (the drug of choice for treating heteroresistant or intrinsically resistant staphylococci) despite its toxicity and cost.

TABLE I
Biochemical and Enzymatic Activity of
S. aureus Isolates at 24 and 48 Hours

| INCUBATION PERIOD | | | | | | PERCENT POSITIVE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CV | MS | NIT | NOV | P6R | IDX | VP | OPT | PHO | BE | PYR | AR6 | P6T |
| 24 HOURS | 1 | 100 | 96 | 1 | 0 | 95 | 95 | 100 | 99 | 0 | 75 | 91 | 84 |
| 48 HOURS | 1 | 99 | 95 | 1 | 0 | 99 | 96 | 99 | 98 | 0 | 78 | 96 | 92 |
| | | | | | | PERCENT POSITIVE | | | | | | | |
| | | URE | RAF | LAC | TRE | MNS | NaCl | SOR | MAN | DNAse | HEM | COA6 | |
| 24 HOURS | | 67 | 3 | 73 | 99 | 99 | 100 | 1 | 91 | - | 98 | 95 | |
| 48 HOURS | | 88 | 32 | 80 | 98 | 99 | 99 | 1 | 95 | 100 | 98 | 95 | |

TABLE II
MIC's (mcg/ml) of 13 Antimicrobials
(In-Patient Isolates)

| ISOLATE # | TE | OX | AMP | PEN | VA | Cfz | E | Cd | 6M | Rif | TXS | C | Cft |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8804 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8842 | 0.5 | 0.5 | >8 | 8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8859 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8947 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8964 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8986 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8987 | 0.5 | 0.25 | 4 | 8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9110 | 0.5 | 0.25 | 4 | 2 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9235 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9252 | 0.5 | 0.25 | 4 | 4 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9273 | 0.5 | 0.5 | >8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9352 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9474 | 0.5 | 0.25 | 2 | 4 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9600 | 128 | 0.25 | 8 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9757 | 0.5 | 0.25 | 0.25 | 0.25 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9826 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9849 | 0.5 | 0.5 | >8 | >8 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10168 | 0.5 | 0.25 | 8 | >8 | 2 | 1 | 0.5 | 0.25 | >6 | 2 | 2/38 | 8 | 8 |
| 10252 | 0.5 | 0.25 | 4 | 8 | 2 | 1 | 4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10295 | 0.5 | 0.25 | 2 | 4 | 2 | 1 | 0.25 | 0.25 | >6 | >4 | 2/38 | 8 | 8 |
| 10347 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10363 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10399 | 0.5 | 0.25 | 0.25 | 0.12 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10439 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |

TABLE II (cont.)
MIC's (mcg/ml) of 13 Antimicrobials
(In-Patient Isolates)

| ISOLATE # | TE | OX | AMP | PEN | VA | Cfz | E | Cd | GM | Rif | TXS | C | Cft |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10461 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10545 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10748 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10880 | 0.5 | 0.25 | 1 | 0.25 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10941 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11060 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11130 | 0.5 | 0.25 | 2 | 2 | 2 | 1 | 0.5 | 1 | 1 | 2 | 2/38 | 8 | 8 |
| 11142 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11312 | 0.5 | 0.25 | 4 | >8 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11382 | 0.5 | 0.25 | 8 | >8 | 2 | 1 | 4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11383 | 1 | 0.25 | 8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11694 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11728 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 2 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11811 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11878 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11910 | 0.5 | 0.25 | 4 | 2 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11915 | 0.5 | 0.5 | 4 | 8 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11964 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12148 | 8 | 0.25 | 1 | 0.25 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12154 | 0.5 | 0.25 | 8 | 8 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12232 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12241 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| B3459 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| | | | | | | | | | | | | | |

TABLE III
Zones of Inhibition (mm)* to Six Antimicrobials
(In-Patient Isolates)

| ISOLATE # | OX-24 | OX-48 | ME-24 | ME-48 | NA-24 | NA-48 | NEO | TOB | AMIK |
|---|---|---|---|---|---|---|---|---|---|
| 8804 | 14 | 14 | 19 | 19 | 19 | 19 | 23 | 25 | 23 |
| 8842 | 12 | 12 | 15 | 15 | 16 | 16 | 24 | 26 | 23 |
| 8859 | 16 | 16 | 20 | 20 | 17 | 17 | 12 | 24 | 21 |
| 8947 | 14 | 13 | 16 | 15 | 18 | 14 | 24 | 27 | 26 |
| 8964 | 23 | 23 | 23 | 23 | 22 | 22 | 22 | 26 | 25 |
| 8986 | 11 | 10 | 16 | 14 | 17 | 13 | 22 | 25 | 23 |
| 8987 | 19 | 15 | 19 | 15 | 19 | 15 | 21 | 25 | 22 |
| 9110 | 17 | 17 | 19 | 19 | 19 | 19 | 20 | 24 | 23 |
| 9235 | 18 | 18 | 19 | 19 | 18 | 18 | 22 | 24 | 22 |
| 9252 | 20 | 20 | 19 | 19 | 19 | 19 | 21 | 25 | 23 |
| 9273 | 0 | 0 | 14 | 14 | 13 | 13 | 21 | 24 | 23 |
| 9352 | 16 | 16 | 18 | 18 | 17 | 17 | 21 | 24 | 23 |
| 9474 | 19 | 17 | 17 | 17 | 18 | 17 | 21 | 24 | 23 |
| 9600 | 19 | 15 | 17 | 16 | 18 | 16 | 19 | 22 | 20 |
| 9757 | 23 | 23 | 20 | 20 | 20 | 20 | 20 | 23 | 20 |
| 9826 | 23 | 20 | 20 | 18 | 20 | 20 | 20 | 23 | 22 |
| 9849 | 14 | 0 | 14 | 13 | 14 | 10 | 22 | 25 | 24 |
| 10168 | 23 | 23 | 23 | 23 | 23 | 23 | 15 | 12 | 21 |
| 10252 | 22 | 17 | 20 | 18 | 20 | 17 | 21 | 26 | 24 |
| 10295 | 21 | 21 | 22 | 22 | 21 | 21 | 14 | 11 | 19 |
| 10347 | 21 | 15 | 21 | 15 | 21 | 15 | 20 | 24 | 23 |
| 10363 | 20 | 15 | 21 | 17 | 20 | 16 | 21 | 24 | 24 |
| 10399 | 24 | 24 | 23 | 23 | 23 | 23 | 20 | 22 | 22 |
| 10439 | 21 | 19 | 20 | 20 | 20 | 19 | 20 | 23 | 21 |

*Zone Sizes Measured after 24 and 48 Hours Incubation for Methicillin, Oxacillin, and Nafcillin

TABLE III (cont.)
Zones of Inhibition (mm)* to Six Antimicrobials
(In-Patient Isolates)

| ISOLATE # | OX-24 | OX-48 | ME-24 | ME-48 | NA-24 | NA-48 | NEO | TOB | AMIK |
|---|---|---|---|---|---|---|---|---|---|
| 10461 | 20 | 18 | 20 | 16 | 20 | 14 | 19 | 22 | 20 |
| 10545 | 25 | 25 | 22 | 22 | 22 | 22 | 20 | 22 | 20 |
| 10748 | 19 | 19 | 20 | 20 | 16 | 16 | 21 | 23 | 21 |
| 10880 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 23 | 21 |
| 10941 | 24 | 24 | 21 | 21 | 23 | 23 | 21 | 24 | 23 |
| 11060 | 15 | 12 | 14 | 14 | 15 | 14 | 20 | 23 | 22 |
| 11130 | 16 | 14 | 17 | 16 | 18 | 16 | 21 | 24 | 23 |
| 11142 | 17 | 16 | 20 | 17 | 17 | 16 | 20 | 23 | 22 |
| 11312 | 17 | 17 | 19 | 19 | 18 | 18 | 20 | 23 | 21 |
| 11382 | 0 | 0 | 15 | 15 | 14 | 14 | 20 | 24 | 22 |
| 11383 | 17 | 12 | 17 | 14 | 16 | 16 | 21 | 24 | 22 |
| 11694 | 17 | 15 | 19 | 16 | 19 | 16 | 22 | 26 | 15 |
| 11728 | 15 | 13 | 17 | 16 | 18 | 15 | 20 | 23 | 22 |
| 11811 | 23 | 23 | 21 | 21 | 23 | 23 | 23 | 26 | 25 |
| 11878 | 16 | 16 | 18 | 16 | 18 | 16 | 23 | 25 | 24 |
| 11910 | 20 | 16 | 20 | 16 | 20 | 16 | 23 | 25 | 25 |
| 11915 | 14 | 0 | 16 | 0 | 13 | 0 | 21 | 24 | 22 |
| 11964 | 18 | 15 | 19 | 16 | 18 | 15 | 22 | 25 | 23 |
| 12148 | 19 | 14 | 18 | 15 | 18 | 13 | 23 | 25 | 24 |
| 12154 | 12 | 11 | 14 | 13 | 14 | 9 | 25 | 28 | 26 |
| 12232 | 21 | 16 | 19 | 16 | 19 | 15 | 22 | 25 | 24 |
| 12241 | 20 | 15 | 17 | 16 | 17 | 16 | 23 | 25 | 24 |
| B3459 | 25 | 25 | 23 | 23 | 21 | 21 | 20 | 23 | 23 |
| | | | | | | | | | |

*Zone Sizes Measured after 24 and 48 Hours Incubation for Methicillin, Oxacillin, and Nafcillin

TABLE IV
MIC's (mcg/ml) of 13 Antimicrobials
(Out-Patient Isolates)

| ISOLATE # | TE | OX | AMP | PEN | VA | Cfz | E | Cd | GM | Rif | TXS | C | Cft |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8782 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.5 | 1 | 2 | 2/38 | 8 | 8 |
| 8799 | 0.5 | 0.25 | 4 | 8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8898 | 0.5 | 0.25 | 8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8899 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 8920 | 0.5 | 0.25 | 2 | 4 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9033 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9100 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9114 | 0.5 | 0.25 | 8 | 8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9116 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9475 | 0.5 | 0.25 | >8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9487 | 128 | 0.25 | 2 | 4 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9506 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9519 | 0.5 | 0.25 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9538 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9641 | 0.5 | 0.25 | 2 | 2 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9798 | 0.5 | 0.25 | 2 | 4 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9854 | 0.5 | 0.5 | 2 | 2 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9904 | 0.5 | 0.25 | 8 | >8 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9914 | 0.5 | 0.25 | 1 | 0.25 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9925 | 0.5 | 0.25 | 2 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 9987 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | >4 | >4 | 1 | 2 | 2/38 | 8 | 8 |
| 10025 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10097 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10107 | 0.5 | 0.25 | 0.5 | 0.25 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |

TABLE IV (cont.)
MIC's (mcg/ml) of 13 Antimicrobials
(Out-Patient Isolates)

| ISOLATE # | TE | OX | AMP | PEN | VA | Cfz | E | Cd | GM | Rif | TXS | C | Cft |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10112 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10119 | 0.5 | 0.25 | 1 | 2 | 2 | 1 | 0.5 | 0.25 | >6 | 2 | 2/38 | 8 | 8 |
| 10131 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | >4 | 0.5 | 1 | 2 | 2/38 | 8 | 8 |
| 10199 | 0.5 | 0.25 | 0.5 | 0.25 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10205 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 2 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10207 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | >6 | 2 | 2/38 | 8 | 8 |
| 10284 | 0.5 | >8 | 1 | 1 | 2 | 8 | >4 | 0.5 | 1 | 2 | 2/38 | 8 | 8 |
| 10420 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10455 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10477 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10494 | 0.5 | 0.25 | 1 | 0.25 | 2 | 1 | 0.25 | 1 | 1 | 2 | 2/38 | 8 | 8 |
| 10542 | 0.5 | 0.25 | 0.5 | 0.25 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10620 | 0.5 | 0.25 | 0.5 | 0.25 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10638 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10642 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10757 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10784 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10820 | 128 | 0.25 | 4 | 4 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10896 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 10940 | 0.5 | 0.25 | 0.25 | 0.12 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11073 | 0.5 | 0.25 | 0.5 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11122 | 0.5 | 0.25 | 0.5 | 0.25 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11126 | 4 | 0.25, 8 | 4 | 4 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11242 | 0.5 | 0.25 | 2 | 2 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |

TABLE IV (cont.)
MIC's (mcg/ml) of 13 Antimicrobials
(Out-Patient Isolates)

| ISOLATE # | TE | OX | AMP | PEN | VA | Cfz | E | Cd | GM | Rif | TXS | C | Cft |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11299 | 8 | >8 | 4 | 4 | 2 | 1 | >4 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11317 | 0.5 | 0.25 | 1 | 1 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11373 | 128 | 0.25 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11513 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11520 | 0.5 | 0.25 | 0.5 | 0.25 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11527 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11603 | 0.5 | 0.25 | 2 | 4 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11655 | 0.5 | 0.25 | 1 | 0.5 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11696 | 0.5 | 0.25 | 1 | 2 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11760 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11835 | 0.5 | 0.25 | 2 | 2 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 11998 | 8 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12168 | 0.5 | 0.5 | >8 | 8 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12175 | 0.5 | 0.25 | 4 | 2 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12199 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| 12229 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.25 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| B3350 | 0.5 | 0.25 | 0.12 | 0.03 | 2 | 1 | 0.5 | 0.25 | 1 | 2 | 2/38 | 8 | 8 |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |

TABLE V
Zones of Inhibition (mm)* to Six Antimicrobials
(Out-Patient Isolates)

| ISOLATE # | OX-24 | OX-48 | ME-24 | ME-48 | NA-24 | NA-48 | NEO | TOB | AMIK |
|---|---|---|---|---|---|---|---|---|---|
| 8782 | 20 | 20 | 20 | 20 | 21 | 21 | 22 | 24 | 23 |
| 8799 | 17 | 17 | 17 | 17 | 17 | 17 | 25 | 26 | 23 |
| 8898 | 17 | 17 | 20 | 20 | 20 | 20 | 22 | 26 | 24 |
| 8899 | 18 | 18 | 20 | 20 | 21 | 21 | 22 | 27 | 24 |
| 8920 | 19 | 19 | 20 | 20 | 20 | 20 | 23 | 25 | 24 |
| 9033 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 25 | 24 |
| 9100 | 22 | 22 | 20 | 20 | 22 | 22 | 20 | 25 | 23 |
| 9114 | 19 | 16 | 21 | 21 | 19 | 19 | 20 | 25 | 23 |
| 9116 | 14 | 12 | 18 | 17 | 17 | 13 | 20 | 23 | 22 |
| 9475 | 10 | 0 | 17 | 14 | 15 | 10 | 20 | 23 | 20 |
| 9487 | 20 | 17 | 19 | 19 | 19 | 19 | 20 | 22 | 21 |
| 9506 | 23 | 20 | 20 | 20 | 21 | 21 | 21 | 23 | 21 |
| 9519 | 19 | 14 | 19 | 15 | 18 | 14 | 20 | 22 | 20 |
| 9538 | 15 | 12 | 22 | 22 | 23 | 23 | 20 | 22 | 22 |
| 9641 | 18 | 14 | 19 | 19 | 19 | 19 | 20 | 23 | 21 |
| 9798 | 22 | 22 | 22 | 22 | 22 | 22 | 19 | 23 | 21 |
| 9854 | 14 | 0 | 19 | 19 | 17 | 15 | 21 | 24 | 23 |
| 9904 | 12 | 0 | 15 | 13 | 13 | 0 | 18 | 20 | 20 |
| 9914 | 20 | 17 | 20 | 17 | 20 | 17 | 19 | 23 | 20 |
| 9925 | 24 | 19 | 21 | 19 | 23 | 18 | 24 | 28 | 26 |
| 9987 | 19 | 14 | 20 | 14 | 20 | 15 | 21 | 23 | 23 |
| 10025 | 30 | 30 | 27 | 27 | 27 | 27 | 23 | 26 | 25 |
| 10097 | 23 | 23 | 22 | 22 | 21 | 21 | 20 | 23 | 22 |
| 10107 | 31 | 31 | 31 | 31 | 30 | 30 | 25 | 28 | 27 |

*Zone Sizes Measured after 24 and 48 Hours Incubation for Methicillin, Oxacillin, and Nafcillin

TABLE V (cont.)
Zones of Inhibition (mm)* to Six Antimicrobials
(Out-Patient Isolates)

| ISOLATE # | OX-24 | OX-48 | ME-24 | ME-48 | NA-24 | NA-48 | NEO | TOB | AMIK |
|---|---|---|---|---|---|---|---|---|---|
| 10112 | 21 | 19 | 20 | 17 | 20 | 17 | 21 | 25 | 23 |
| 10119 | 21 | 19 | 20 | 20 | 20 | 20 | 15 | 13 | 20 |
| 10131 | 17 | 15 | 20 | 17 | 18 | 16 | 19 | 22 | 20 |
| 10199 | 22 | 22 | 19 | 19 | 20 | 20 | 20 | 23 | 21 |
| 10205 | 26 | 26 | 25 | 25 | 24 | 24 | 14 | 25 | 23 |
| 10207 | 20 | 17 | 21 | 19 | 22 | 17 | 14 | 11 | 19 |
| 10284 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 12 | 21 |
| 10420 | 19 | 16 | 19 | 17 | 19 | 16 | 19 | 23 | 20 |
| 10455 | 19 | 17 | 19 | 19 | 19 | 17 | 18 | 22 | 22 |
| 10477 | 25 | 25 | 24 | 24 | 23 | 23 | 19 | 22 | 21 |
| 10494 | 21 | 21 | 19 | 19 | 20 | 20 | 18 | 22 | 21 |
| 10542 | 21 | 21 | 21 | 21 | 21 | 21 | 23 | 25 | 23 |
| 10620 | 20 | 20 | 19 | 19 | 21 | 21 | 19 | 20 | 22 |
| 10638 | 19 | 14 | 21 | 21 | 21 | 21 | 20 | 24 | 23 |
| 10642 | 23 | 23 | 22 | 22 | 21 | 21 | 20 | 22 | 21 |
| 10757 | 21 | 21 | 20 | 20 | 21 | 21 | 20 | 23 | 22 |
| 10784 | 20 | 20 | 21 | 21 | 20 | 20 | 19 | 23 | 21 |
| 10820 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 23 | 22 |
| 10896 | 22 | 22 | 20 | 20 | 21 | 21 | 21 | 24 | 23 |
| 10940 | 23 | 23 | 21 | 21 | 22 | 22 | 20 | 24 | 23 |
| 11073 | 15 | 14 | 18 | 17 | 16 | 16 | 21 | 25 | 24 |
| 11122 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 25 | 24 |
| 11126 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 12 | 20 |
| 11242 | 20 | 20 | 20 | 20 | 20 | 20 | 21 | 24 | 23 |

*Zone Sizes Measured after 24 and 48 Hours Incubation for Methicillin, Oxacillin, and Nafcillin

TABLE V (cont.)
Zones of Inhibition (mm)* to Six Antimicrobials
(Out-Patient Isolates)

| ISOLATE # | OX-24 | OX-48 | ME-24 | ME-48 | NA-24 | NA-48 | NEO | TOB | AMIK |
|---|---|---|---|---|---|---|---|---|---|
| 11299 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 12 | 21 |
| 11317 | 20 | 20 | 21 | 21 | 21 | 21 | 20 | 23 | 22 |
| 11373 | 23 | 23 | 22 | 22 | 24 | 24 | 23 | 26 | 24 |
| 11513 | 28 | 28 | 26 | 26 | 27 | 27 | 20 | 24 | 23 |
| 11520 | 24 | 24 | 23 | 23 | 24 | 24 | 22 | 25 | 23 |
| 11527 | 25 | 25 | 23 | 23 | 23 | 23 | 20 | 24 | 21 |
| 11603 | 21 | 21 | 20 | 20 | 20 | 20 | 22 | 24 | 23 |
| 11655 | 21 | 21 | 20 | 20 | 20 | 20 | 20 | 24 | 22 |
| 11696 | 23 | 23 | 23 | 23 | 23 | 23 | 22 | 25 | 23 |
| 11760 | 21 | 17 | 21 | 15 | 19 | 18 | 19 | 23 | 20 |
| 11835 | 20 | 16 | 17 | 16 | 19 | 16 | 21 | 25 | 23 |
| 11998 | 25 | 25 | 23 | 23 | 23 | 23 | 22 | 24 | 24 |
| 12168 | 0 | 0 | 11 | 11 | 0 | 0 | 24 | 28 | 26 |
| 12175 | 17 | 14 | 16 | 14 | 17 | 14 | 22 | 19 | 23 |
| 12199 | 24 | 24 | 23 | 23 | 22 | 22 | 25 | 27 | 25 |
| 12229 | 23 | 23 | 21 | 21 | 22 | 22 | 24 | 25 | 24 |
| B3350 | 24 | 24 | 21 | 21 | 21 | 21 | 19 | 22 | 20 |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

*Zone Sizes Measured after 24 and 48 Hours Incubation for Methicillin, Oxacillin, and Nafcillin

## TABLE VI
## Resistance of S. aureus to 18 Antimicrobials

| ISOLATE | | | | | PERCENT RESISTANCE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLASSIFICATIOI | TE | OX | AMP | PEN | VA | Cfz | E | Cd | GM | Rif | TXS | C | Cft |
| In Patient (n 47) | 2.1 | 0.0 | 89.4 | 89.4 | 0.0 | 0.0 | 21.3 | 0.0 | 4.3 | 2.1 | 0.0 | 0.0 | 0.0 |
| Out Patient (n=65) | 4.6 | 4.6 | 81.5 | 81.5 | 0.0 | 4.6 | 9.2 | 1.5 | 3.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total (n=112) | 3.6 | 2.7 | 84.8 | 84.8 | 0.0 | 2.7 | 14.3 | 0.9 | 3.6 | 0.9 | 0.0 | 0.0 | 0.0 |

| ISOLATE | | | PERCENT RESISTANCE | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLASSIFICATION | | | OX-24 | OX-48 | ME-24 | ME-48 | NA-24 | NA-48 | NEO | TOB | AMIK | | |
| In Patient (n=47) | | | 4.3 | 10.6 | 0.0 | 0.0 | 0.0 | 6.4 | 4.3 | 4.3 | 2.1 | | |
| Out Patient (n=65) | | | 6.2 | 10.8 | 4.6 | 4.6 | 6.2 | 9.2 | 7.7 | 7.7 | 0.0 | | |
| Total (n=112) | | | 5.4 | 10.7 | 2.7 | 2.7 | 3.6 | 8.0 | 6.3 | 6.3 | 0.9 | | |

% Resistance to Oxacillin, Methicillin, and Nafcillin Shown at 24 and 48 Hours

# Table V II
## Antimicrobial Resistance Patterns Among In-Patient and Out-Patient Isolates (Staphylococcus aureus)

| | % Susceptible or Resistant to Various Antimicrobial Groups | | |
|---|---|---|---|
| | **Total (n=112)** | **IP (n=47)** | **OP (n=65)** |
| **RESISTANT TO:** | | | |
| **None of the Tested Antibiotics** | 13.4 | 8.5 | 15.4 |
| | | | |
| **Single Antibiotic Only:** | | | |
| E Only | 1.8 | 2.1 | 1.7 |
| NEO Only | 0.9 | 0.0 | 1.7 |
| | | | |
| **PEN & AMP Only** | 63.9 | 61.7 | 66.0 |
| | | | |
| **PEN, AMP, & Others:** | | | |
| PEN, AMP, TE | 3.6 | 2.1 | 4.6 |
| PEN, AMP, E | 7.4 | 17.0 | 1.5 |
| PEN, AMP, E, Cd | 0.9 | 0.0 | 1.7 |
| PEN, AMP, E, NEO | 0.9 | 2.1 | 0.0 |
| PEN, AMP, AMIK | 0.9 | 2.1 | 0.0 |
| PEN, AMP, GM, TOB | 1.8 | 2.1 | 1.7 |
| PEN, AMP, GM, NEO, TOB | 0.9 | 0.0 | 1.7 |
| PEN, AMP, GM, NEO, TOB, Rif | 0.9 | 2.1 | 0.0 |
| PEN, AMP, OX, ME, NA, CEF, E, NEO,TOB | 2.7 | 0.0 | 4.7 |

IP = In Patient, OP = Out Patient

# Changes in Antimicrobial Resistance of S. aureus Strains from Dameron Hospital between 1973-74 and 1987

| ANTIMICROBIALS | PERCENT RESISTANCE | | CHANGE IN | TWO-TAILED |
|---|---|---|---|---|
| | 1973-74 (a) | 1975 (b) | RESISTANCE | PROBABILITY (c) |
| PEN | 79 | 84.8 | + 5.8 | < 0.3 |
| AMP | 79 | 84.8 | + 5.8 | < 0.3 |
| E | 3.5 | 14.3 | + 9.0 | < 0.01 |
| NEO | 0 | 6.3 | + 6.3 | < 0.02 |
| GM | 0 | 3.6 | + 3.6 | < 0.10 |
| ME, OX, NAF, CEF | 0 | 2.7 | + 2.7 | < 0.20 |
| Cd | 0 | 0.9 | + 0.9 | < 0.95 |
| C | 0 | 0 | 0 | - |
| Va | 0 | 0 | 0 | - |
| TE | 15 | 3.6 | - 11.4 | < 0.01 |

a. Data from Hall (1975)
b. Data from present study
c. Probabilities were obtained with the Chi-square 2x2 contingency table method.

# LITERATURE CITED

Acar, F.A. 1988. In Neu, H.C. (ed.), Special issue: highlights of an international symposium, "Issues in Gram-positive infection", Gerenzano, Italy. Challenges in Gram-Positive Infection: a Global Perspective. Healthmark, New York.

Archer, G.L. 1988. In Neu, H.C. (ed.), Special issue: highlights of an international symposium, "Issues in Gram-positive infection", Gerenzano, Italy. Challenges in Gram-Positive Infection: a Global Perspective. Healthmark, New York.

Barry, A.L. and R.N. Jones. 1987. Reliability of high-content discs and modified broth dilution tests for detecting staphylococcal resistance to the penicillinase-resistant penicillins. Jour. Clin. Micro. 25(10):1897-1901.

_____R.V.F. Lachica, and F.W. Atchison. 1973. Identification of Staphylococcus aureus by simultaneous use of tube coagulase and thermonuclease tests. Appl. Micro. 25(3):496-497.

Biberstein, E.L., S.S. Jang, and D.C. Hirsh. 1984. Species distribution of coagulase-positive staphylococci in animals. Jour. Clin. Micro. 19(5):610-615.

Campos, J.M. 1986. Beta-lactamase mediated resistance to penicillinase-resistant penicillins in Staphylococcus aureus. Diagnostic Testing Allert (Atlanta, Georgia). 2(5):17-20.

Coudron, P.E., D.E. Jones, H.P. Dalton, G.L. Archer. 1986. Evaluation of laboratory tests for detection of methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis. Jour. Clin. Micro. 24(5):764-769.

Hajek, V. 1976. Staphylococcus intermedius, a new species isolated from animals. Int. Jour. Syst. Bacteriol. 26:401-408.

Hall, J. 1975. Antibiogram patterns of primary isolates of Staphylococcus aureus in relation to enzymatic activity and phage type. Thesis (M.S.), University of the Pacific, Stockton, California.

Hansen, S.L. and P.K. Freedy. 1984. Variation in the abilities of automated, commercial, and reference methods to detect methicillin-resistant

(heteroresistant) Staphylococcus aureus. Jour. Clin. Micro. 20:494-499.

Howard, B.J. and W.E. Kloos. 1987. Staphylococci. In Howard, B.J. et al. (eds.), Clinical and Pathogenic Microbiology. C.V. Mosby Co., St. Louis.

Jorgensen, J.H., J. Redding, J.E. Johnson, V. Holloway, and R.J. Almeida. 1984. Rapid recognition of methicillin-resistant Staphylococcus aureus by use of automated test systems. Jour. Clin. Micro. 20(3):430-433.

_____and C. Thornsberry. 1987. Progress and pitfalls in Staphylococcus susceptibility testing. Eli Lilly and Co., Indianapolis.

Kayser, F.H. and F. Muller. 1983. Resistance and tolerance of the Staphylococcus to ß-lactam antibiotics. Procedures of the 13th International Congress of Chemotherapy. Symposium Staphylococcal Infections, Part 10, pages 8-14.

_____J. Wust, and P. Santanam. 1975. Genetic and molecular characterisation of resistance determinants in methicillin-resistant Staphylococcus aureus. Jour. Med. Micro. 9:1976.

Kloos, W.E. and J.H. Jorgensen. 1985. Staphylococci. In Lennette, E.H. et al. (eds.), Manual of Clinical Microbiology, Fourth ed. American Society for Microbiology, Washington DC.

McDougal, L.K. and C. Thornsberry. 1984. New recommendations for disc diffusion antimicrobial susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. Jour. Clin. Micro. 19(4):482-488.

_____and C. Thornsberry. 1986. The role of ß-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. Jour. Clin. Micro. 23(5):832-839.

McGowen, J.E. Jr. 1988. In Neu, H.C. (ed.), Special issue: highlights of an international symposium, "Issues in Gram-positive infection", Gerenzano, Italy. Challenges in Gram-Positive Infection: a Global Perspective. Healthmark, New York.

McNeil, M.M. and S.L. Solomon. 1985. The epidemiology of methicillin-resistant Staphylococcus aureus. The Antimicrobic Newsletter. 2(7):49-56.

Mulligan, M.A., D.A. Citron, R.Y.Y. Kwok, J.P. Wheelock, F.K.

Farrohi, J.A. Hindler, and L. Johnston. 1987. Impact of prolonged incubation on disc diffusion susceptibility test results for Staphylococcus aureus. Jour. Clin. Micro. 25(5):840-844.

National Committe for Clinical Laboratory Standards. 1984. Performance standards for Antimicrobial Disc Susceptibility Tests. Approved standard: M2-A3 with Supplements. National Committe for Clinical Laboratory Standards, Villanova, PA.

Norton, C.F. 1986. Microbiology. Second ed. Addison-Wesley Publishing Company, Reading, Massachusetts.

Philips W.E.,Jr. and W.E. Kloos. 1981. Identification of coagulase-positive Staphylococcus intermedius and Staphylococcus hyicus subsp. hyicus isolates from veterinary clinical specimens. Jour. Clin. Micro. 14(6):671-673.

Putland, R.A. and M.D. Guinness. 1985. Autobac susceptibility testing of methicillin-resistant Staphylococcus aureus isolated in an Australian hospital. Jour. Clin. Micro. 22(5):822-827.

Robinson, A., J.E. Mortensen, and S.B. Griffith. 1986. Susceptibility testing of methicillin-resistant Staphylococcus aureus with three commercial microdilution systems. Diag. Microbiol. Infect. Dis. 5:245-253.

Schito, G.C. 1988. In Neu, H.C. (ed.), Special issue: highlights of an international symposium, "Issues in Gram-positive infection", Gerenzano, Italy. Challenges in Gram-Positive Infection: a Global Perspective. Healthmark, New York.

Sherris, J.C. 1984. Staphylococci. In Sherris, J.C. et al. (eds.), Medical Microbiology, an Introduction to Infectious Disease. Elsevier, N.Y.

Skurry, R.A. 1988. In Neu, H.C. (ed.), Special issue: highlights of an international symposium, "Issues in Gram-positive infection", Gerenzano, Italy. Challenges in Gram-Positive Infection: a Global Perspective. Healthmark, New York.

Smith, P.B., G.A. Hancock and D.L. Rhoden. 1969. Improved medium for detecting deoxyribonuclease-producing bacteria. Appl. Micro. 18:991-993.

Thornsberry, C. 1984. Methicillin-resistant (heteroresistant) staphylococci. The Antimicrobic Newsletter. 1(6):43-47.

_____and Jorgensen, J.H. 1985. In Vitro Detection of Methicillin-Resistant Staphylococcus (Video). Eli Lilly and Co., Indianapolis.

_____and L.K. McDougal. 1983. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. Jour. Clin. Micro. 18(5):1084-1091.

Tompkins, L.S. and S. Falkow. 1986. Properties, problems and therapeutic progress: bacterial plasmids. Roche Seminars on Bacteria: Number 3 in a Series. Hoffman-La Roche Inc., Nutley, New Jersey.

Welch, W.D. and P.M. Southern. 1984. Unusual susceptibility of methicillin-resistant Staphylococcus aureus to erythromycin, clindamycin, gentamycin, and tetracycline at 30C but not at 35C. Jour. Clinl. Micro. 22(5):822-827.

Woolfrey, B.F., R.T. Lally, and M.N. Ederer. 1984. Evaluation of the AutoMicrobic system for detection of resistance of Staphylococcus aureus to methicillin. Jour. Clin. Micro. 19(4):464-467.

# APPENDIX I: PRINCIPLES OF BIOCHEMICAL AND ENZYMATIC REACTIONS (GRAM POSITIVE COMBO)

## Crystal Violet (CV)

Growth in the presence of low concentrations of crystal violet is used to distinguish streptococci (positive) from from staphylococci (mostly negative).

## Micrococcus Screen (MS)

Growth in the presence of 0.5 mcg/ml of bacitracin is used to distinguish staphylococci (positive) from Micrococci (negative).

## Nitrate (NIT)

Reduction of nitrate to nitrite is detected by formation of a red color within seconds following addition of one drop of 0.8% sulfonic acid and one drop of 0.5% N,N- dimethyl alpha-naphthylamine. Streptococci are nitrate-negative and most staphylococci are nitrate-positive.

## Novobiocin (NOV)

Resistance to low concentrations (5 mcg) of novobiocin (evidenced by growth) is characteristic of *Staphylococcus saprophyticus*, *S. xylosus*, *S. cohni*, and *S. sciuri*. Other staphylococci are susceptible to

novobiocin.

PNP-B-D-Glucuronide (PGR)

The ability of an organism to produce a specific glycosidase enzyme is detected by the splitting of a beta-napthylamide-carbohydrate complex releasing p-nitrophenol. A positive test is indicated by a yellow color in the well.

Indoxyl Phosphatase (IDX)

Hydrolysis of indoxyl phosphate by the enzyme indoxyl phosphatase results in an insoluble blue compound. Most coagulase and DNase-positive staphylococci are IDX-positive, although the reaction may take more than 18-20 hours to develop.

Vogues-Proskauer (VP)

Actylmethylcarbinol, produced from glucose, reacts with 5% alpha-naphthol in an alkaline environment to form a red complex. One drop of 40% KOH is added to the well to alkalinize the contents, followed by one drop of 0.5% alph-naphthol. A positive reaction appears in 2-15 minutes.

Optochin (OPT)

Susceptibility to optochin is a characteristic of Streptococcus pneumoniae. Other streptococci and

staphylococci are not inhibited by optochin. This test is not essential for the identification of S. aureus or other staphylococci but is required for computer identification in generating a biotype number (This process is explained in the next section.). A positive reaction is indicated by growth.

Phosphatase (PHO)

Alkaline phosphatase splits p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. A positive test is indicated by a yellow color.

Bile Esculin (BE)

Organisms capable of growing in 40% bile and hydrolyzing esculin are detected by the production of a black precipitate resulting from the reaction of the hydrolytic product esculetin with ferric citrate. Group D streptococci, some viridans streptococci, and some Staphylocci are BE-positive.

Pyrroldonyl-Beta-naphthylamide (PYR)

Organisms which produce pyrrolidonase split L-pyrrolidonyl-b-naphthylamide into L-pyrrolidonyl and b naphthylamide. After the addition of one drop of peptidase reagent, a red color develops within two minutes from the combination of b-naphthylamide with peptidase.

Arginine (ARG)

Dehydrolization of arginine results in alkalinization of the medium. A positive test is indicated by a yellow to red color change of the phenol red indicator.

PNP-Beta-D-Galactopyranoside

The splitting of a b-napthylamide-carbohydrate complex denotes the ability of the organism to produce a specific glycosidase enzyme. This is indicated by the formation of p-nitro-phenol which is a yellow color.

Urea (URE)

The enzyme urease splits urea forming ammonia and carbon dioxide. The resulting increase in pH is detected by the phenol red indicator changing from yellow to red.

Carbohydrate Fermentation

Nine sugars and sugar alcohols including raffinose (RAF), lactose (LAC), trehalose (TRE), mannose (MNS), sorbitol (SOR), arabinose (ARA), ribose (RBS), inulin (INU), and mannitol (MAN) are used in fermentation studies. The fermentation of a specific carbohydrate results in acid formation. The consequent drop in pH is detected by the phenol red indicator turning

yellow.

6.5% NaCl (NACL)

Tolerance to 6.5% sodium chloride is demonstrated by growth. Salt tolerance is a characteristic of all staphylococci and the enterococcus group of streptococci.

Bacitracin (BAC)

Susceptibility to low concentrations (0.2mcg?) of bacitracin is indicated by a lack of growth and is characteristic of Streptococcus pyogenes. This test is not essential for identification of staphylococci but is required for computer identification in generating a biotype.

Pyruvate (PRV)

Utilization of pyruvate results in acid formation. The resultant drop in pH is detected by the phenol red indicator turning yellow.

Beta-Lactamase (BL)

Following incubation of the trays for 18-20 hours, a drop of 400,000 units of a penicillin G suspension is added to the BL well and trays reincubated for 30 minutes. A drop of iodine is then added. The disappearance of the blue-black color within 10

seconds indicates ß-lactamase production and therefore a positive reaction. The principle of the test is based on the splitting of the ß-lactam ring forming binding sites which are more competitive for iodine than starch molecules are. If ß-lactamase is present, the iodine binds with the ß-lactam ring to form a colorless reaction. If ß-lactamase is not present, the iodine will combine with the starch molecules and form a blue-black color.

# APPENDIX II: GENERATION OF A BIOTYPE NUMBER

A six digit biotype number is derived from the results obtained for each strain. The 18 biochemical tests are divided into six groups of three. Negative reactions receive a value of zero. Positive results are given a value of one, two, or four depending on their vertical location within each group. A value of four is assigned to those tests in the top horizontal row (CV, NOV, VP, BE, PGT, and LAC) while tests in the middle row receive a value of two (MS, PGR, OPT, PYR, URE, and TRE) and results in the bottom row, a value of one (NIT, IDX, PHO, ARG, RAF, and MNS). The six digit biotype number is obtained by adding the values for the positive tests in each vertical group. Biotype numbers are automatically generated by the computer as an isolate is identified to species.

# APPENDIX III
## MIC Interpretive Chart for 13 Antimicrobials in Several Dilutions*

| | | MICROGRAMS / MILLILITER | | |
|---|---|---|---|---|
| **Antimicrobial or Chemotherapeutic Agent** | **Dilutions** | **Sensitive** | **Intermediate** | **Resistant** |
| Tetracycline (TE) | 0.5 - 8, 128 | ≤0.5 - 4 | 8 | 128 - >128 |
| Oxacillin (OX) | 0.25 - 8 | ≤0.25 - 2 | - | 4 - >8 |
| Ampicillin (AMP) | 0.12 - 8 | ≤0.03 - 0.12 | - | 0.25 - >8 |
| Penicillin (PEN) | 0.03 - 8 | ≤0.03 - 0.12 | - | 0.25 - >8 |
| Vancomycin (VA) | 2 - 16 | ≤2 - 4 | 8 - 16 | >16 |
| Cephazolin (Cfz) | 1 - 16 | ≤1 - 8 | 16 | >16 |
| Erythromycin (E) | 0.25 - 4 | ≤0.25 - 0.5 | 1 - 4 | >4 |
| Clindamycin (Cd) | 0.25 - 4 | <0.25 - 0.5 | 1 - 4 | >4 |
| Gentamycin (GM) | 1 - 4, 6 | ≤1 - 4 | 6 | >6 |
| Rifampin (Rif) | 2, 4 | ≤2 | 4 | >4 |
| Trimethoprim-sulfamethoxazole (TXS) | 8/152, 2/38 | ≤2/38 | 8/152 | >8/152 |
| Chloramphenicol (C) | 8, 16 | ≤8 | 16 | >16 |
| Cefotaxime (Cft) | 8, 32 | ≤8 | 32 | >32 |

*Dilutions represented by a range are actually a series of twofold dilutions. Reproduced from Microscan Gram Positive Combo Interpretive Chart, Baxter Scientific Products, Sacramento, California.

# APPENDIX IV
## Zone Size Interpretive Chart for Six Antimicrobials, Kirby-Bauer Method*

| Antimicrobial or Chemotherapeutic Agent | Disc Potency (mcg) | Zone of Inhibition in Millimeters | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| Methicillin (ME) | 5 | ≥14 | 10-13 | ≤9 |
| Oxacillin (OX) | 1 | ≥13 | 11-12 | ≤10 |
| Nafcillin (NA) | 1 | ≥13 | 11-12 | ≤10 |
| Neomycin (NEO) | 30 | ≥17 | 13-16 | ≤12 |
| Tobramycin (TOB) | 10 | ≥15 | 13-14 | ≤12 |
| Amikacin (AMIK) | 30 | ≥17 | 15-16 | ≤14 |

*Reproduced from Bacto-Susceptibility Discs, Difco Laboratories, Detroit, Michigan.