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# Characterization of gram-positive bacterial isolates from burn victims

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AN ABSTRACT OF THE THESIS of Trisha A. Hannan for the Master of Science in Biology presented July 14, 1981.

Title: Characterization of Gram-Positive Bacterial Isolates from Burn Victims

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*Streptococcus* and *Staphylococcus* were collected from patients hospitalized at the Oregon Burn Center, Emanuel Hospital, Portland, Oregon, between October 27, 1980 and January 1, 1981. Forty-five isolates of *Staphylococcus* and twenty-one isolates of *Streptococcus*, Lancefield Group D, were included in the investigation.

The objectives of the investigation were: (i) to identify to the species level all isolates, (ii) to determine their susceptibility

to antimicrobial agents, (iii) to test the organisms over time for increasing resistance to silver, (iv) to determine, if present, a bacterium harbored within the burn unit (nosocomial bacterium), and (v) to determine any recognizable pattern of successive bacterial colonization which occurred in hospitalized patients.

Biochemical tests were conducted on each isolate to determine species differentiation. Antibiotic susceptibility tests were performed on all isolates. Susceptibility tests to the inorganic salts of arsenate, arsenite and mercury were performed on all species of *Staphylococcus*. The minimal inhibitory concentration (MIC) of silver nitrate was determined for all isolates.

Species of group D streptococci identified were: *Streptococcus faecalis*, *Streptococcus faecium* and *Streptococcus faecium* var. *casseliflavus*. Species of *Staphylococcus* identified were: *Staphylococcus aureus*, *Staphylococcus cohnii*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus simulans* and *Staphylococcus warneri*.

Antibiotic susceptibility tests yielded typically species-specific results. The MICs of silver nitrate ranged from 1.25  $\mu\text{g/ml}$  to 5.0  $\mu\text{g/ml}$ . The MIC data showed a species-specific susceptibility pattern rather than one of increasing resistance. Analysis of all isolates yielded no common, persistent organism within the burn unit. Although *S. aureus* isolates were the most tenacious organisms, no pattern of successive bacterial colonization was indicated by the data collected.

CHARACTERIZATION OF GRAM-POSITIVE BACTERIAL  
ISOLATES FROM BURN VICTIMS

by

TRISHA A. HANNAN

A thesis submitted in partial fulfillment of  
the requirements for the degree of

MASTER OF SCIENCE  
in  
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Portland State University

1981

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## CHAPTER I

### INTRODUCTION

#### Burn Trauma

Thermal burns are among the most common, most extensive and most lethal forms of trauma (1). Persons sustaining severe burns manifest numerous physiological and biochemical alterations.

The vascular response to burn injury is marked. Increased vascular permeability, as a result of the release of vasoactive substances, leads to edema and the formation of blisters (2, 3). Substantial losses of fluid and electrolytes through the burn wound may result in metabolic acidosis (4, 5). Thromboplastins released from damaged tissues cause aggregations of erythrocytes (6) and thrombocytes (5).

Direct upper respiratory tract damage from inhalation of superheated air and smoke leads to head and neck edema. Days later, decreased diffusion of oxygen across the alveolar membrane may occur, and death may result.

Evaporative water loss through eschar (inert and denatured biological matter) results in increased caloric expenditure to maintain thermal equilibrium (7). Excessive protein catabolism as a result of the subsequent energy demand may lead to renal hypertrophy, hyperplasia and semi-permanent enlargement of the kidneys (8).

Thermal injury also stimulates the sympatho-adrenal medullary

system, resulting in an increase in blood levels and urinary output of adrenalin and noradrenalin (9).

The intact epidermis constitutes an effective barrier to the ingress of bacteria from the environment. Burning the skin results in a distinct impairment of this role (1). The impaired condition persists until the eschar is totally separated and granulation tissue formation complete (10). A compromised immune system due to local destruction of phagocytic cells in the burn wound (11, 12) also renders the burn victim susceptible to bacterial invasion and colonization. Thus, infection is a major hazard to life after burn trauma, in which the burn wound plays a primary role (11, 13, 14).

Bacterial colonization of the burn wound is inescapable and as bacteria begin invading the viable, unburned, tissue surrounding the wound, septicemia may result. The indigenous flora of the skin are initially recovered (i.e., *Staphylococcus epidermidis* and other coagulase-negative species of *Staphylococcus*); however, colonization of the wound by pathogenic species (e.g., *Staphylococcus aureus* from nasal passages) will occur within as little as sixteen hours unless prompt burn wound bacteriostasis is begun and maintained (1). Bacteria can reach concentrations of  $10^6$  -  $10^7$  organisms per gram of burn tissue before overt signs and symptoms of systemic sepsis are evident (15).

Thus in addition to early life support care, medical therapy includes the topical application of an antimicrobial substance aimed at preventing burn wound sepsis (16). Table I lists and describes the topical agents in use at the Oregon Burn Center (OBC). The

TABLE I

## TOPICAL AGENTS

AGENT	ANTIMICROBIAL SPECTRUM	ADVANTAGE (S)	DISADVANTAGES
Silver Sulfadiazine (Silvadene®)	Gram-positive, gram-negative bacteria and yeasts	<ol style="list-style-type: none"> <li>1. Painless on application</li> <li>2. No dressings required.</li> </ol>	<ol style="list-style-type: none"> <li>1. Skin allergy</li> <li>2. Poor control of <i>S. aureus</i></li> </ol>
Mafenide Acetate (Sulfamylon®)	Most gram-positive and gram-negative bacteria (aerobes)	<ol style="list-style-type: none"> <li>1. Rapid penetration to sub-eschar tissue</li> </ol>	<ol style="list-style-type: none"> <li>1. Painful on application</li> <li>2. Skin allergy</li> </ol>
Silver Nitrate	Most gram-positive bacteria and most strains of <i>Pseudomonas</i>	<ol style="list-style-type: none"> <li>1. Broad spectrum protection due to non-discriminating action of free silver ion</li> </ol>	<ol style="list-style-type: none"> <li>1. Hypochloremia</li> <li>2. Methemoglobinemia</li> </ol>
Providine-Iodine (Betadine®)	Gram-positive, gram-negative bacteria and fungi	<ol style="list-style-type: none"> <li>1. No dressings required</li> <li>2. Effective against fungi</li> </ol>	<ol style="list-style-type: none"> <li>1. Pain on application</li> <li>2. Hyperiodonemia</li> </ol>

broad-spectrum activity of topical therapy is necessary as gram-positive and gram-negative species of bacteria (both aerobic and anaerobic), and fungi may flourish on, in and below the eschar.

Isolation and identification of the organisms present in the burn victim is of utmost importance. Bacteriologic monitoring of the burn wound takes into account several parameters: type, number, location and antibiotic sensitivity of the bacteria (17).

The following investigation was performed to elucidate the characteristics of the species of *Staphylococcus* and Group D *Streptococcus* isolated from patients hospitalized in the OBC.

#### Genus *Streptococcus*

The streptococci are a heterogeneous group of organisms in the family *Streptococcaceae*. The organisms are gram-positive, catalase-negative, non-spore forming cocci. They possess a fermentative type of metabolism and are classified as facultative anaerobes (18, 19, 20, 21).

The streptococci can be grouped serologically, commonly referred to as the Lancefield groups, designated A - V. The organisms of Lancefield Group D contain a soluble polysaccharide (a teichoic acid) present between the cell wall and the cell membrane (18, 19, 20, 22).

Members of the group D streptococci are differentiated by characteristic biochemical patterns which are used for speciation.

The group D streptococci are normal fecal flora, but can frequently colonize extra-intestinal surfaces (22). Although isolation of group D streptococci from sites other than feces does not in

itself indicate a current infection, organisms found in a burn wound, or in the blood stream, increase the chance for development of septicemia, endocarditis and urinary tract infections.

*Streptococcus faecalis* (and varieties) and *Streptococcus faecium* (and varieties) are the strains most often associated with streptococcal infection (22, 23, 24). These species also are usually quite resistant to antimicrobial agents (25, 26), so eradication of these organisms can be difficult.

#### Genus *Staphylococcus*

The staphylococci are a heterogeneous group of organisms in the family *Micrococcaceae*. The organisms are gram-positive, catalase-positive, non-spore forming cocci. They are aerobic organisms and have both respiratory and fermentative capabilities. Human species of *Staphylococcus* include *Staphylococcus aureus*, *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. simulans*, *S. warneri* and *S. xylosus* (21a, 27, 28, 29).

Historically, the coagulase-positive species of *S. aureus* has been regarded as an opportunistic pathogen, whereas the coagulase-negative species have been generally regarded as non-pathogens (28). However, [*Staphylococcus epidermidis* can produce a number of virulence factors such as deoxyribonuclease and hemolysins which aid in pathogenicity (33)] today it is well documented that coagulase-negative species of *Staphylococci* can be associated with disease (30, 31, 32), and their resistance to many antibiotics makes it important that the isolates be accurately identified (33).

Identification of the species of *Staphylococci* can be accomplished by the results of a series of physiological and biochemical tests.

#### Description of the Investigation

The identification of the species of both the group D streptococci and staphylococci is important with respect to hospital epidemiology. Biochemical parameters can be used to biotype (fingerprint) each bacterial strain isolated. These biotypes then can be used for determination of the presence of a nosocomial (resident) bacterium that infects, or colonizes, each patient in the unit.

Species of both *Staphylococcus* and *Streptococcus* can persist on inanimate objects outside the body, such as dust or surfaces in rooms. These environmental reservoirs (23) are one possible source of nosocomial bacteria, as is cross-contamination of patients via personnel working within the burn unit.

The objectives of the investigation were to: (i) identify all gram-positive bacterial isolates, (ii) determine their susceptibility to antibiotics and antimicrobial agents, (iii) test the organisms, isolated over time, for increasing resistance to silver ion as a result of selective pressure as a result of the topical use of Silvadene® (silver sulfadiazine), (iv) determine if a nosocomial bacterium was being transmitted from patient to patient, and (v) determine if a pattern of successive bacterial colonization occurred in each patient.

## CHAPTER II

### METHODS AND MATERIALS

The bacterial strains used in this investigation were isolates from eighteen patients hospitalized in the OBC, Emanuel Hospital, Portland, Oregon. Cultures were received from the Emanuel Hospital Microbiology Laboratory on agar slants. Stock cultures of all organisms were maintained in a sterile 50 percent glycerol - 50 percent 0.01 M phosphate buffer (pH 7.0 - 7.2) suspension at -20° Centigrade. Control organisms included *S. aureus* (ATCC 29213, ATCC 25923), *S. epidermidis* (ATCC 14990) and *S. faecalis* (ATCC 29212).

All organisms were characterized by routine biochemical procedures and antimicrobial agent susceptibility tests as described below.

#### BIOCHEMICAL TESTS FOR DIFFERENTIATION OF ISOLATES OF GROUP D *STREPTOCOCCUS*

##### Catalase

Isolates were tested for the ability to produce catalase. An individual colony from an overnight plate culture was placed on a clean microscope slide, one drop of 3 percent hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) added, and observed for evolution of bubbles. The test was considered negative if no immediate bubbling occurred (21b).



### Tetrazolium Reduction

The ability of each isolate to reduce 2,3,5-Triphenyltetrazolium chloride (Sigma) to the insoluble compound formazan was assayed by using a modification of the method of Barnes (34).

### Potassium Tellurite

The ability of each isolate to initiate growth in the presence of 0.04 percent potassium tellurite was determined by adding Chapman Tellurite Solution (Difco) to 5 milliliters Brain Heart Infusion broth (BHI, BBL) to a final concentration of 0.04 percent. Tubes were incubated at 37° Centigrade. Tests were considered negative if no black precipitate was evident within seventy-two hours.

### Temperature Tolerance

The ability of each isolate to initiate growth at 10° Centigrade and 45° Centigrade was tested in tubes containing 3 milliliters BHI. Tests were considered negative if no turbidity was visible within seventy-two hours at the stated temperature.

### Growth in 6.5 Percent Sodium Chloride

The ability of each isolate to initiate growth in 6.5 percent sodium chloride (NaCl) was performed in tubes containing 3 milliliters BHI with the appropriate concentration of NaCl added. Tubes were incubated at 37° Centigrade and tests were considered negative if turbidity was not evident within forty-eight hours.

### Other Tests

The ability of each isolate to produce acid in litmus milk

(Difco), ability to hydrolyze gelatin (Difco) and starch (Mallinkrodt) and carbohydrate fermentation tests were conducted using the methods of Facklam (18). Carbohydrates tested were lactose, sorbitol, sucrose, mannitol and arabinose.

#### BIOCHEMICAL TESTS FOR DIFFERENTIATION OF ISOLATES OF *STAPHYLOCOCCUS*

##### Catalase

Isolates were tested for the ability to produce catalase. An individual colony from an overnight plate culture was placed on a clean microscope slide, one drop of 3 percent H<sub>2</sub>O<sub>2</sub> added, and observed for evolution of bubbles. The test was considered positive if immediate bubbling occurred (21b).

##### Glycerol Fermentation

Ability to ferment glycerol in the presence of 0.01 percent erythromycin (Sigma) was tested to eliminate any species of *Micrococcus* which may have been present as a contaminant (35).

##### Lysostaphin

Isolates of *Staphylococcus* were screened for susceptibility or resistance to lysostaphin at a concentration of 50 µg/ml in P Agar (28, 36). (P Agar: Peptone, 10 grams; Yeast Extract, 5 grams; NaCl, 5 grams; Glucose, 1 gram; Agar, 15 grams; and water, 1 litre.)

##### Phosphatase Production

All isolates of *Staphylococcus* were tested for the ability to produce phosphatase enzyme, as described by MacFaddin (21c). The

indicator used was 0.05 percent phenolphthalein diphosphate (Mallinkrodt).

### Nitrate Reduction

The ability of each coagulase-negative isolate to reduce potassium nitrate to nitrite or nitrogen gas was determined in Nitrate Broth (Difco) (21d).

### Vogues-Proskauer Test

Coagulase-negative strains of *Staphylococcus* were tested for the ability to produce a neutral end product, acetoin, from glucose fermentation (21e).

### Carbohydrate Fermentation

The ability of each isolate to utilize and ferment various carbohydrates was tested (28). Coagulase-positive strains were tested for fermentation of mannitol, galactose, trehalose, ribose and xylose. In addition to the aforementioned carbohydrates, coagulase-negative strains were tested for utilization of sucrose, lactose, maltose and mannose.

## ANTIBIOTIC SUSCEPTIBILITY TESTS

### Disc Diffusion (Agar)

Inocula for the antibiotic susceptibility tests were derived from the stock glycerol-phosphate suspensions and grown overnight in 5 milliliters BHI at 37° Centigrade (37). Methods employed were those recommended by the National Committee for Clinical Laboratory Standards (38). Inoculated plates with antibiotic discs (BBL) were

incubated at 37° Centigrade and zone sizes of inhibition measured after twenty-four hours. Species of *Staphylococcus* were tested for susceptibility to cefamandole, penicillin, cephalothin, tetracycline, methicillin, cefoxitin, novobiocin, erythromycin and clindamycin. Species of *Streptococcus* were tested for susceptibility to the above mentioned antibiotics as well as tobramycin, carbenicillin, nitrofurantoin, gentamicin and amikacin.

#### ANTIMICROBIAL AGENT SUSCEPTIBILITY

##### Arsenate, Arsenite and Mercury

Species of *Staphylococcus* were tested for susceptibility to disodium arsenate (0.1 M), sodium arsenite (1.0 M) and mercuric chloride ( $10^{-3}$  M). The tests were performed according to the methods of Novick and Roth (39), utilizing Mueller Hinton Agar (MHA, Difco). Zone sizes of inhibition were measured after twenty-four hours incubation at 37° Centigrade and interpreted as follows: arsenate susceptibility, 11 millimeters and greater; arsenite susceptibility, 10 millimeters and greater; and mercury susceptibility, 9 millimeters and greater.

##### Silver Susceptibility, Agar Dilution

Using sterile technique, silver nitrate ( $\text{AgNO}_3$ ) in two-fold dilutions (concentrations ranged from 156  $\mu\text{g/ml}$  to 0.01  $\mu\text{g/ml}$ ) was pipetted into a nutrient agar [1 percent Tryptone (Difco), 0.5 percent Glucose, 2 percent Agar]. Medium containing each concentration of  $\text{AgNO}_3$  was dispensed into 15 x 100 millimeter plastic petri dishes (40). Cultures were inoculated onto the agar using a replicator apparatus similar to the one described by Steers, et al. (41, 42). Plates were

incubated at 37° Centigrade for twenty-four hours and observed for visible growth.

#### Silver Susceptibility (MIC), Broth-Dilution

Using sterile technique, silver nitrate, in two-fold dilutions, was pipetted into Mueller Hinton Broth (MHB, Difco). Final concentrations of AgNO<sub>3</sub> ranged from 10 µg/ml to 1.25 µg/ml. Two milliliters of the AgNO<sub>3</sub>-MHB were pipetted into sterile tubes, and inoculated with 5 x 10<sup>5</sup> bacteria/ml. Turbidity was checked after twenty-four hours incubation at 37° Centigrade (43). The lowest concentration in each series showing no visible turbidity was reported as the minimum inhibitory concentration (MIC) (44).

#### ANALYSIS OF THE DATA OF SPECIES DIFFERENTIATION

Individual combinations of each isolates' response to all agents tested (biochemical and antimicrobial) were tabulated and assigned a "type number", similar to the methods described by Lapage and Willcox (45). By comparison of "type numbers" within the species, patient-by-patient analysis was also accomplished. The criteria used in defining a nosocomial (resident) bacterium were: exact biochemical phenotypes and exact antimicrobial agent susceptibility patterns. These "exact" bacteria must also have been recovered from at least six patients (33 percent of the sample).

#### CHEMICALS

All carbohydrates were of reagent grade. Mannose, trehalose and ribose were obtained from Sigma. All others were obtained from

Mallinkrodt, as was the NaCl. Lysostaphin (Lot No. 40F-0532) was obtained from Sigma. Silver nitrate, from Mallinkrodt, was of analytical reagent quality.

## CHAPTER III

### RESULTS AND DISCUSSION

#### Species of *Streptococcus*

Using the criteria shown in Table II, isolates of group D streptococci were differentiated into three biochemically distinct species, namely: *S. faecalis*, *S. faecium* and *S. faecium* var. *casseliflavus*. Table III shows the reactions of the aforementioned species on a cumulative percentage basis. In the OBC these isolates were found in the following percentage distribution: *S. faecalis*, 38 percent; *S. faecium*, 38 percent; and *S. faecium* var. *casseliflavus*, 24 percent. These figures are of interest by virtue of the fact that of the group D streptococci, *S. faecalis* is the species most often isolated in infection (18, 22, 46). As seen above, *S. faecium* and *S. faecium* var. *casseliflavus* comprised 62 percent of the isolates of group D streptococci. All twenty-one strains of group D streptococci were isolated from six patients (33 percent of the sample) and were recovered only between the period from November 13, 1980 to December 12, 1980.

*Streptococcus faecalis* was easily differentiated from *S. faecium* by the reactions in tetrazolium, tellurite, sorbitol and arabinose. *Streptococcus faecium* was differentiated from *S. faecium* var. *casseliflavus* by the reactions in tellurite and tetrazolium. The fourteen tests employed proved satisfactory for determining the species of the

TABLE II

REACTIONS USEFUL IN CHARACTERIZATION OF THE SPECIES OF *STREPTOCOCCUS*, LANCEFIELD GROUP D<sup>a</sup>

SPECIES	TEST													
	Catalase	Tetrazolium	Tellurite	10° C (growth)	45° C (growth)	6.5% NaCl	Litmus Milk	Gelatin	Starch	Lactose	Sorbitol	Mannitol	Arabinose	Sucrose
<i>S. faecalis</i>	-	+	+	+	+	+	+	-	-	+	+	+	-	+
<i>S. faecalis</i> var. <i>liquefaciens</i>	-	+	+	+	+	+	+	+	-	+	+	+	-	+
<i>S. faecium</i>	-	-	-	+	+	+	+	-	-	+	-	+	+	+
<i>S. faecium</i> var. <i>casseliflavus</i>	-	v	v	+	+	+	+	-	-	+	-	+	+	+
<i>S. bovis</i>	-	v	-	-	+	-	+	-	+	+	-	v	v	+
<i>S. equinus</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	+

<sup>a</sup>Species not shown: *S. faecalis* var. *zymogenes*, *S. durans*, *S. avium*  
 Symbols: +, positive reaction; -, negative reaction; v, variable reaction



TABLE III

REACTIONS OF IDENTIFIED SPECIES OF *STREPTOCOCCUS*,  
LANCEFELD GROUP D<sup>a</sup>

TEST	SPECIES		
	<i>S. faecalis</i>	<i>S. faecium</i>	<i>S. faecium</i> var. <i>casseliflavus</i>
	NUMBER OF ISOLATES		
	8	8	5
Catalase	100 ( - )	100 ( - )	100 ( - )
Tetrazolium	100 ( + )	100 ( - )	80 ( + )
Tellurite	100 ( + )	100 ( - )	80 ( + )
10° C (growth)	100 ( + )	100 ( + )	100 ( + )
45° C (growth)	100 ( + )	100 ( + )	100 ( + )
6.5% NaCl	100 ( + )	100 ( + )	100 ( + )
Litmus Milk	100 ( + )	100 ( + )	100 ( + )
Gelatin	100 ( - )	100 ( - )	100 ( - )
Starch	100 ( - )	100 ( - )	100 ( - )
Lactose	100 ( + )	100 ( + )	100 ( + )
Sorbitol	100 ( + )	100 ( - )	100 ( - )
Mannitol	100 ( + )	100 ( + )	100 ( + )
Arabinose	100 ( - )	100 ( + )	100 ( + )
Sucrose	88 ( + )	88 ( + )	100 ( + )

<sup>a</sup>Numbers indicate percentage of species exhibiting the reaction shown in parenthesis.

isolates of group D streptococci found in the burn unit.

Table IV shows the antibiotic susceptibility tests results of the three species of *Streptococcus*, Lancefield Group D. As a whole, all of the strains of group D streptococci were resistant to most of the antibiotics tested. The strains of *S. faecalis* were uniformly more resistant to the antibiotics tested than were the other two species of *Streptococcus*. The extreme resistance to antibiotics of *S. faecalis* is well documented (22, 23, 25). The strains of *S. faecium* were more resistant, as a group, than were the strains of *S. faecium* var. *casseliflavus*. The strains of *S. faecium* were 100 percent resistant to ten antibiotics, whereas the strains of *S. faecium* var. *casseliflavus* were 100 percent resistant to six antibiotics.

An attempt was made to correlate a particular antibiotic susceptibility pattern to each of the three species of *Streptococcus*. No definite patterns were found that were broad-based enough to use as a species differentiation criteria. The only antibiotic tested that yielded a clearly species-specific difference was novobiocin. The strains of *S. faecalis* were 100 percent resistant to novobiocin, whereas the strains of both *S. faecium* and *S. faecium* var. *casseliflavus* were 100 percent sensitive. The only strains showing sensitivity to cefamandole, penicillin and cephalothin were the five isolates of *S. faecium* var. *casseliflavus*. All eight isolates of *S. faecium* were resistant to nitrofurantoin, in contrast to the high sensitivity shown by the isolates of *S. faecalis* (88 percent susceptible) and the isolates of *S. faecium* var. *casseliflavus* (100

TABLE IV

ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES OF GROUP D *STREPTOCOCCUS*<sup>a</sup>

ANTIBIOTIC	SPECIES		
	<i>S. Faecalis</i>	<i>S. faecium</i>	<i>S. faecium</i> var. <i>casseliflavus</i>
	NUMBER OF ISOLATES		
	8	8	5
Cefmandole	0	0	60
Tobramycin	0	0	0
Garbenicillin	12	0	80
Penicillin	0	0	80
Cephalothin	0	0	80
Tetracycline	12	75	80
Methicillin	0	0	0
Chloramphenicol	25	100	60
Cefoxitin	0	12	0
Novobiocin	0	100	100
Nitrofurantoin	88	0	100
Gentamicin	0	0	0
Erythromycin	50	0	60
Amikacin	0	0	0
Clindamycin	0	12	0

<sup>a</sup>Numbers represent percentage of each species susceptible to the listed antibiotics.

percent susceptible). Taken as an entire group, tetracycline resistance was found in 48 percent of the twenty-one group D streptococci, erythromycin resistance was found in 66 percent and chloramphenicol resistance noted in 38 percent of the total isolates. In contrast, a 1977 study of clinical isolates of group D streptococci found that 58 percent were resistant to tetracycline, 12 percent were resistant to erythromycin and 14 percent were resistant to chloramphenicol (26). Resistance to these three antibiotics is known to be plasmid-mediated (25, 26), as is resistance to the aminoglycoside antibiotics such as tobramycin and gentamicin (47, 48). All isolates of the group D streptococci found in this investigation were 100 percent resistant to both tobramycin and gentamicin.

The MIC of silver nitrate for the three identified species of *Streptococcus* are shown in Table V. Nothing remarkable was found in the analysis of this data with respect to increasing resistance to silver from the selective pressure of Silvadene®. The effectiveness of silver as an antimicrobial agent is well known (44). It was assumed that as therapy with Silvadene® continued, a pattern of increasing resistance to silver would emerge. The data, however, did not support this assumption. The single strain of *S. faecalis* sensitive to a concentration of silver ion between 0.79 µg/ml and 1.58 µg/ml was eradicated early, and was not followed by any other isolate of the *Streptococcus*. The two sensitive *S. faecium* var. *casseliflavus* were also eradicated early in the course of therapy, and were not succeeded by any other *S. faecium* var. *casseliflavus* that were more resistant. As a whole, 86 percent of the isolates

TABLE V

MINIMUM INHIBITORY CONCENTRATIONS OF SILVER NITRATE (SPECIES  
OF GROUP D *STREPTOCOCCUS*)<sup>a</sup>

SPECIES	CONCENTRATION AgNO <sub>3</sub> (µg/ml)			
	10 <sup>b</sup>	5 <sup>c</sup>	2.5 <sup>d</sup>	1.25 <sup>e</sup>
<i>S. faecalis</i>	0	88	12	0
<i>S. faecium</i>	0	100	0	0
<i>S. faecium</i> var. <i>casseliflavus</i>	0	60	40	0

<sup>a</sup>Numbers represent percentage of each species exhibiting MIC at the listed concentrations.

<sup>b</sup>Corresponds to 6.35 µg/ml silver ion.

<sup>c</sup>Corresponds to 3.17 µg/ml silver ion.

<sup>d</sup>Corresponds to 1.58 µg/ml silver ion.

<sup>e</sup>Corresponds to 0.79 µg/ml silver ion.

of group D streptococci were inhibited by concentrations of silver ion between 1.58 µg/ml and 3.17 µg/ml. No strains of *S. faecium* were inhibited by concentrations of silver ion less than 1.58 µg/ml. The relatively high resistance to silver ion is of little consequence, in that Silvadene® ointment releases much higher quantities of silver ion than the bacteria are resistant to, e.g., up to  $4.31 \times 10^4$  µg/ml ointment.

#### INDIVIDUAL ANALYSIS OF THE ISOLATES OF GROUP D *STREPTOCOCCUS*

##### *Streptococcus faecalis*

The eight strains of *S. faecalis* yielded five distinct biochemical phenotypes and seven different antimicrobial susceptibility patterns. There were two strains exactly alike. Both were isolated from a single patient and were only present for a two week period.

##### *Streptococcus faecium*

The eight strains of *S. faecium* yielded two distinct biochemical phenotypes and six different antimicrobial susceptibility patterns. There were three strains exactly alike. All three "like" strains were isolated from a single patient and were only present for a one week period.

##### *Streptococcus faecium* var. *casseliflavus*

The five strains of *S. faecium* var. *casseliflavus* yielded four distinct biochemical phenotypes and five different antimicrobial susceptibility patterns. There were no isolates exactly alike in this group.

From the above data it does not appear that a particular streptococcal bacterium can be implicated as a resident in the OBC. Although the species of *Streptococcus* were resistant to a wide variety of antibiotics, as well as moderate concentrations of silver, the isolates of the group D streptococci were easily eliminated from each and every patient who became colonized with them, and within two weeks of their detection.

#### SPECIES OF *STAPHYLOCOCCUS*

Using the criteria shown in Table VI, isolates of *Staphylococcus* were differentiated into seven biochemically distinct species, namely: *S. aureus*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. simulans* and *S. warneri*. Table VII shows the reactions of the forty-five species of *Staphylococci* on a cumulative percentage basis. In the OBC these isolates were found in the following percentage distribution: *S. aureus*, 29 percent (twenty-two strains) and *S. epidermidis*, 29 percent (thirteen isolates). The remaining 22 percent (ten isolates) were coagulase-negative strains of *Staphylococcus* other than *S. epidermidis*, and were isolated from six patients only (33 percent of the sample).

The species of *S. aureus* were easily differentiated from other species of *Staphylococcus* by their ability to produce coagulase, their sensitivity to lysostaphin and phosphatase production. Species that satisfied the above criteria were further subjected to a limited number of confirmatory biochemical tests. Species differentiation among the coagulase-negative strains of *Staphylococcus* was much more

TABLE VI

REACTIONS USEFUL IN CHARACTERIZATION OF SPECIES OF *STAPHYLOCOCCUS*

SPECIES	TEST																
	Catalase	Glycerol	Coagulase	Lysostaphin	Phosphatase	Nitrate Reduction	Mannitol	Trehalose	Sucrose	Lactose	Ribose	Mannose	Galactose	Fructose	Voges-Proskauer	Xylose	Maltose
<i>S. aureus</i>	+	+	+	S	+	+	+	+	+	+	+	+	+	+	+	-	+
<i>S. capitis</i>	+	+	-	R	-	+	+	+	-	-	-	+	-	+	+	-	-
<i>S. cohnii</i>	+	+	-	S	-	-	V	+	-	-	-	V	-	+	+	-	V
<i>S. epidermidis</i>	+	+	-	R	+	+	-	+	+	+	-	+	+	+	+	-	+
<i>S. haemolyticus</i>	+	+	-	R	-	+	V	+	V	V	-	V	V	V	+	-	+
<i>S. hominis</i>	+	+	-	R	-	+	-	+	V	-	-	-	V	+	+	-	+
<i>S. saprophyticus</i>	+	+	-	R	-	-	+	+	+	+	-	-	-	+	+	-	+
<i>S. simulans</i>	+	+	-	S	V	+	+	+	+	+	V	+	-	+	+	-	V
<i>S. warneri</i>	+	+	-	R	-	-	V	+	+	-	V	-	-	+	+	-	V
<i>S. xylosus</i>	+	+	-	S	+	+	+	+	+	V	V	+	V	+	+	+	+

Symbols: +, positive; -, negative; v, variable; S, sensitive; R, resistant.



TABLE VII

REACTIONS OF IDENTIFIED SPECIES OF *STAPHYLOCOCCUS*<sup>a</sup>

TEST	SPECIES						
	<i>S. aureus</i>	<i>S. cohnii</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. simulans</i>	<i>S. warneri</i>
Catalase	100	100	100	100	100	100	100
Glycerol	100	100	100	100	100	100	100
Coagulase	100	100	100	100	100	100	100
Lysostaphin	82	100	100	100	100	100	100
Phosphatase	100	100	93	100	100	100	100
Nitrate Reduction	nt <sup>b</sup>	100	92	100	100	100	50
Mannitol	86	100	80	0	100	0	0
Trehalose	95	100	100	100	100	100	100
Sucrose	nt	100	93	100	100	100	100
Lactose	nt	100	100	0	100	100	100
Ribose	100	100	93	0	100	0	100
Mannose	nt	100	60	100	100	100	100
Galactose	91	50	100	100	100	0	100
Fructose	nt	100	100	0	100	100	100
Voges-Proskauer	nt	0	93	0	67	0	50
Xylose	100	100	100	100	100	100	100
Maltose	nt	100	100	100	100	100	100

<sup>a</sup>Numbers represent percentage of each species exhibiting reaction shown in Table VI; where reaction is variable, numbers represent percentage of species exhibiting a positive reaction.

<sup>b</sup>nt: Not Tested.

extensive, as some species are differentiated only among a limited number of carbohydrate fermentation abilities. No strains of *S. capitis*, *S. saprophyticus* or *S. xylosum* were identified. The series of biochemical tests employed to differentiate the coagulase-negative species of *Staphylococcus* was adequate.

Table VIII shows the antibiotic susceptibility test results of the seven identified species of *Staphylococcus*. The strains of *S. aureus*, as a whole, were relatively susceptible to the ten antibiotics tested, although they were 100 percent resistant to both cefamandole and penicillin. Of the coagulase-negative species of *Staphylococcus*, *S. cohnii* was the most sensitive strain isolated, being resistant to only methicillin. The strains of *S. hominis* exhibited the greatest resistance to the antibiotics tested, being 100 percent susceptible to only novobiocin and clindamycin. The *S. epidermidis* were the strains more uniformly resistant to all antibiotics tested than were the other coagulase-negative species, except *S. hominis* as noted above. The relative resistance of the coagulase-negative *Staphylococcus* strains to antibiotics has been previously noted (33, 37).

Methicillin-resistant strains of *Staphylococcus* are considered of importance in the hospital setting and methicillin-resistant *S. epidermidis* are becoming common (32, 33). Methicillin resistance was observed in only one of the twenty-two strains of *S. aureus*, whereas resistance was observed in eight of the thirteen strains of *S. epidermidis*. The *S. cohnii*, *S. hominis* and *S. simulans* strains were 100 percent resistant to methicillin.

TABLE VIII

ANTIBIOTIC SUSCEPTIBILITY OF SPECIES OF *STAPHYLOCOCCUS*<sup>a</sup>

ANTIBIOTIC	SPECIES						
	<i>S. aureus</i>	<i>S. cohnii</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. simulans</i>	<i>S. warneri</i>
Cefmandole	0	100	23	0	0	0	50
Penicillin	0	100	15	0	0	0	50
Cephalothin	100	100	100	100	0	100	100
Tetracycline	86	100	85	0	33	100	100
Methicillin	91	0	38	100	0	0	100
Chloramphenicol	95	100	100	100	0	100	100
Cefoxitin	95	100	54	100	0	100	100
Novobiocin	95	100	100	100	100	100	100
Erythromycin	100	100	77	100	0	100	100
Clindamycin	95	100	85	100	100	100	100

<sup>a</sup> Numbers represent percentage of each species susceptible to the listed antibiotic.

Susceptibility by species to the inorganic salts of arsenate ( $\text{Na}_2\text{AsO}_4$ ), arsenite ( $\text{NaAsO}_2$ ) and mercury ( $\text{HgCl}_2$ ) are shown in Table IX. The discs used in the test were impregnated with the following concentrations of ion: arsenate,  $1.49 \times 10^2 \mu\text{g}$ ; arsenite,  $1.48 \times 10^3 \mu\text{g}$ ; and mercury,  $4.00 \mu\text{g}$ . Only 7 percent of the total strains of *Staphylococci* were resistant to arsenate while 64 percent of the total strains of *Staphylococci* were resistant to arsenite. Resistance to mercury was observed in 38 percent of all strains of *Staphylococci*. The strains of *S. aureus* were much more susceptible to arsenite (55 percent) than were the coagulase-negative species (17 percent). Resistance to these inorganic antimicrobial agents has been plasmid-linked, at least in *S. aureus* (39). Resistance to mercury may be due to the action of a mercuric reductase which volatilizes mercury (49, 50).

Table X details the MIC data of silver for the species *Staphylococcus*. Ninety-five percent of the *S. aureus* isolates were inhibited by a silver ion concentration between  $1.58 \mu\text{g/ml}$  and  $3.17 \mu\text{g/ml}$ , as were the two strains of *S. cohnii*. The strains of *S. epidermidis* showed the greatest variability in their sensitivity to silver ion. The majority of the strains (62 percent) exhibited sensitivities between  $0.79 \mu\text{g/ml}$  and  $1.58 \mu\text{g/ml}$  silver ion. The isolates of *S. epidermidis* were the only strains of *Staphylococcus* (or *Streptococcus*) that exhibited susceptibility to silver ion at a concentration between  $0.4 \mu\text{g/ml}$  and  $0.79 \mu\text{g/ml}$ . As would be expected, these sensitive strains were quickly eliminated from each of the two patients harboring them.

TABLE IX

SUSCEPTIBILITY OF SPECIES OF *STAPHYLOCOCCUS* TO INORGANIC  
SALTS OF ARSENATE, ARSENITE AND MERCURY<sup>a</sup>

SPECIES	INORGANIC SALT		
	Arsenate <sup>b</sup> (Na <sub>2</sub> AsO <sub>4</sub> )	Arsenite <sup>c</sup> (NaAsO <sub>2</sub> )	Mercury <sup>d</sup> (HgCl <sub>2</sub> )
<i>S. aureus</i>	21/22	12/22	17/22
<i>S. cohnii</i>	2/2	0/2	1/2
<i>S. epidermidis</i>	13/13	0/13	4/13
<i>S. haemolyticus</i>	1/1	1/1	1/1
<i>S. hominis</i>	1/3	0/3	1/3
<i>S. simulans</i>	2/2	1/2	2/2
<i>S. warneri</i>	2/2	2/2	2/2

<sup>a</sup>Numbers indicate: susceptible species/number species tested.

<sup>b</sup>Disc impregnated with 0.02 ml of a 0.1 M solution.

<sup>c</sup>Disc impregnated with 0.02 ml of a 1.0 M solution.

<sup>d</sup>Disc impregnated with 0.02 ml of a 10<sup>-3</sup> M solution.

TABLE X

MINIMUM INHIBITORY CONCENTRATIONS OF SILVER NITRATE  
(SPECIES OF *STAPHYLOCOCCUS*)<sup>a</sup>

SPECIES	CONCENTRATION AgNO <sub>3</sub> (µg/ml)			
	10 <sup>b</sup>	5 <sup>c</sup>	2.5 <sup>d</sup>	1.25 <sup>e</sup>
<i>S. aureus</i>	0	95	5	0
<i>S. cohnii</i>	0	100	0	0
<i>S. epidermidis</i>	0	23	62	15
<i>S. haemolyticus</i>	0	100	0	0
<i>S. hominis</i>	0	33	67	0
<i>S. simulans</i>	0	0	100	0
<i>S. warneri</i>	0	50	50	0

<sup>a</sup> Numbers represent percentage of each species exhibiting MIC at the listed concentrations.

<sup>b</sup> Corresponds to 6.35 µg/ml silver ion.

<sup>c</sup> Corresponds to 3.17 µg/ml silver ion.

<sup>d</sup> Corresponds to 1.58 µg/ml silver ion.

<sup>e</sup> Corresponds to 0.79 µg/ml silver ion.

INDIVIDUAL ANALYSIS OF THE ISOLATES OF *STAPHYLOCOCCUS**Staphylococcus aureus*

The twenty-two strains of *S. aureus* yielded seven distinct biochemical phenotypes and fourteen different antibiotic and antimicrobial agent susceptibility patterns. Three of the strains from a single patient were exactly alike, and presumed to be clones. The two strains of *S. aureus* from two different patients were exactly alike, but were eliminated from both patients within one week of their detection. The strains of *S. aureus* were the most homogeneous group in the investigation with respect to biochemical phenotypes.

*Staphylococcus cohnii*

The two strains of *S. cohnii* were different in both biochemical phenotype and antibiotic and antimicrobial agent susceptibility patterns. They were isolated from a single patient, and were presumed to be indigenous skin flora.

*Staphylococcus epidermidis*

The thirteen strains of *S. epidermidis* yielded eight distinct biochemical phenotypes and ten different antibiotic and antimicrobial agent susceptibility patterns. There were no two isolates exactly alike in this group of *Staphylococci*.

*Staphylococcus haemolyticus*

The single strain of *S. haemolyticus* was isolated from one patient and was present in the first culture only. It was presumed to be indigenous skin flora.

*Staphylococcus hominis*

The three strains of *S. hominis* yielded three distinct biochemical phenotypes and two different antibiotic and antimicrobial agent susceptibility patterns. There were no isolates exactly alike in this group.

*Staphylococcus simulans*

The two strains of *S. simulans* found were biochemically alike and had the same antibiotic and antimicrobial agent susceptibility patterns. They were isolated from a single patient, assumed to be clones, and presumed to be indigenous skin flora.

*Staphylococcus warneri*

The two strains of *S. warneri* yielded two distinct biochemical phenotypes and two different antibiotic and antimicrobial agent susceptibility patterns. Thus, these two strains were not alike.

From the above data, the strains of *S. aureus* were the isolates which were most similar in phenotype. The *S. aureus*, however, are also the species of *Staphylococcus* most easily identified by the least number of biochemical tests, so possible permutations of phenotypes were limited. Among the isolates of *Staphylococcus*, no apparent resident bacterium could be identified. The variety of *Staphylococcus* spp. and the range of biochemical phenotypes precluded finding enough "like" isolates to qualify as a nosocomial bacterium.

## SILVER SUSCEPTIBILITY, AGAR-DILUTION

All previously stated results for silver susceptibility (MICs)



were determined in  $\text{AgNO}_3$ -MHB. Susceptibility to silver was also performed in tryptone-glucose agar. This media was initially chosen because of its relative lack of chloride, which combines with silver ion to form the insoluble silver chloride. This series of tests yielded no results. All strains of *Staphylococcus* and *Streptococcus* grew on all plates, including the plate containing the highest concentration of silver ion (9.56  $\mu\text{g/ml}$ ). In view of the data collected previously for the tube dilution method, the results were not acceptable. For those strains inhibited by 3.17  $\mu\text{g/ml}$  silver ion, the agar contained more than three times the inhibitory concentration of silver ion than in the  $\text{AgNO}_3$ -MHB. For the isolates inhibited by 1.58  $\mu\text{g/ml}$  silver ion in the tube-dilution test, the agar contained more than six times the inhibitory concentration of silver ion. A possible explanation for this discrepancy may be that the silver ion combined with the free sulfate groups in the agar, leading to the formation of an insoluble compound, silver sulfate. In this bound form the silver would have been unavailable to interact with, and thus inhibit, the growth of the bacterial species.

#### BACTERIAL SUCCESSION

The change (succession) in burn flora over time was analyzed. Table XI shows the variety of organisms isolated in the first positive bacterial culture of each patient. The fact that several patients exhibited mixed cultures initially made succession study difficult. Species of *Staphylococcus* were isolated first from 89 percent of the burn victims entering the OBC.

TABLE XI

## BACTERIA ISOLATED FROM PATIENTS AS A FIRST CULTURE

ORGANISM(S)	NUMBER <sup>a</sup>
<i>S. aureus</i> only	6
<i>S. epidermidis</i> only	4
Other species of <i>Staphylococcus</i> :	
<i>S. warneri</i> only	1
<i>S. haemolyticus</i> only	1
<i>S. hominis</i> only	1
Group D <i>Streptococcus</i> :	
<i>S. faecalis</i> only	1
<i>S. faecalis</i> + <i>S. faecium</i> var. <i>casseliflavus</i>	1
<i>S. epidermidis</i> + <i>S. simulans</i>	1
<i>S. aureus</i> + <i>S. epidermidis</i> + <i>S. warneri</i>	1
<i>S. epidermidis</i> + Group D <i>S. faecium</i> var. <i>casseliflavus</i>	1

<sup>a</sup>Indicates number of patients whose first culture after entering the burn unit yielded the stated organism(s).

In the average length of stay (nineteen days), eight patients harbored a series of different species of bacteria, i.e., they were colonized at some time by any combination of species of *Streptococcus*, a strain of *S. aureus* and/or a *S. epidermidis* or other coagulase-negative species of *Staphylococcus*. (See Table XII for an example of a typical patient's diverse bacterial flora through time.) Of these eight patients, two were not ever colonized with any of the species of *Streptococcus*, one patient had only different coagulase-negative species of *Staphylococcus*, and one patient had only different strains of *S. aureus*. The four patients with notable series of bacterial succession had a *S. aureus* as the final organism isolated before leaving the burn unit. Of these patients, one began with a strain of *S. aureus*, one began with a mixed culture of both a *S. epidermidis* and *S. faecium* var. *casseliflavus*, one patient began with *S. haemolyticus*, and one began with two different strains of *S. hominis*. All four of these patients had positive cultures of *Streptococcus* during their course of stay at the OBC, but these species were always eliminated before the final colonization with *S. aureus*. The limited number of patients showing successive colonization facilitated analysis of the data. No pattern of colonization was evident which was totally consistent between all four patients, other than the fact that *S. aureus* was the most persistent organism of all the species. Little is known about burn wound ecology which would aid in determining whether the factors involved that favor *S. aureus* colonization in late healing are intrinsic to the wound, or are favored by other, extrinsic factors.

TABLE XII  
 SEQUENTIAL ISOLATION OF BACTERIA  
 (ONE PATIENT)

DATE OF ISOLATION	ORGANISM(S)
11/11/80	<i>Staphylococcus aureus</i>
11/18/80	<i>Streptococcus faecium</i>
11/19/80	<i>Streptococcus faecium</i> * <i>Streptococcus faecium</i> var. <i>casseliflavus</i>
11/21/80	<i>Streptococcus faecium</i> *
11/24/80	<i>Staphylococcus epidermidis</i> (2) <sup>a</sup> <i>Streptococcus faecalis</i> <i>Streptococcus faecium</i> * <i>Streptococcus faecium</i>
12/18/80	<i>Staphylococcus aureus</i>

\* Indicates species which gave same reaction pattern for all tests. All other isolates were intraspecifically different.

<sup>a</sup> Indicates two different strains isolated.

## LIMITATIONS OF THE INVESTIGATION

The scope of the investigation was limited in that access to the patients was available only to burn unit personnel. Since there was no control over any aspect concerning the patients and their bacteria previous to receiving the isolates, the investigation had many unknown and uncontrollable variables. For example, when sub-eschar clysis was performed, and isolates received, there was no way of ascertaining whether the bacteria cultured were from inside the wound itself, or on the skin (leading edge of the wound). Ideally, a sampling of the skin flora on unburned skin, and nasal passages, of the patients should have been done to determine which species of bacteria (particularly the species of *Staphylococcus*) were truly indigenous.

## CHAPTER IV

### SUGGESTIONS FOR FURTHER RESEARCH

Gram-positive bacterial species were investigated because the current literature pertains, for the most part, to the problems associated with the gram-negative species in burn wound infections. Also, a study of this nature had not been previously undertaken.

It was apparent throughout the course of this investigation that certain refinements might have been desirable. Monitoring the isolates for a longer time period (e.g., six months to one year) might have aided in detection of both a nosocomial bacterium and in finding stable patterns of bacterial succession. Including gram-negative strains also found in burn wounds (e.g., *Pseudomonas aeruginosa*) would also have been of value for succession studies. The *S. aureus* strains, in the future, should be subjected to more extensive tests for phenotyping. In cases where particular bacteria were implicated (or suspected) of being nosocomially transmitted, phage typing of the bacteria would be of value. Agarose gel electrophoresis of plasmid DNA would also be invaluable in fingerprinting the isolates for similarity.

## CHAPTER V

### SUMMARY

Species of *Streptococcus* and *Staphylococcus* from hospitalized patients at the OBC were monitored. All bacterial strains were characterized by biochemical tests and susceptibility to various antimicrobial agents, including antibiotics.

There was no evidence of increasing resistance to silver due to selective pressure from the use of Silvadene® ointment. There was no evidence of a nosocomial bacterium present in the OBC according to the data collected. Bacterial succession studies yielded no distinctive pattern among the patients. It appeared as if patients entered and left the burn unit with their indigenous flora.

The data presented in this investigation also showed that while multiple antibiotic-resistant bacteria do colonize burn victims, appropriate and timely antimicrobial therapy can spare the patient of bacteremia.

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