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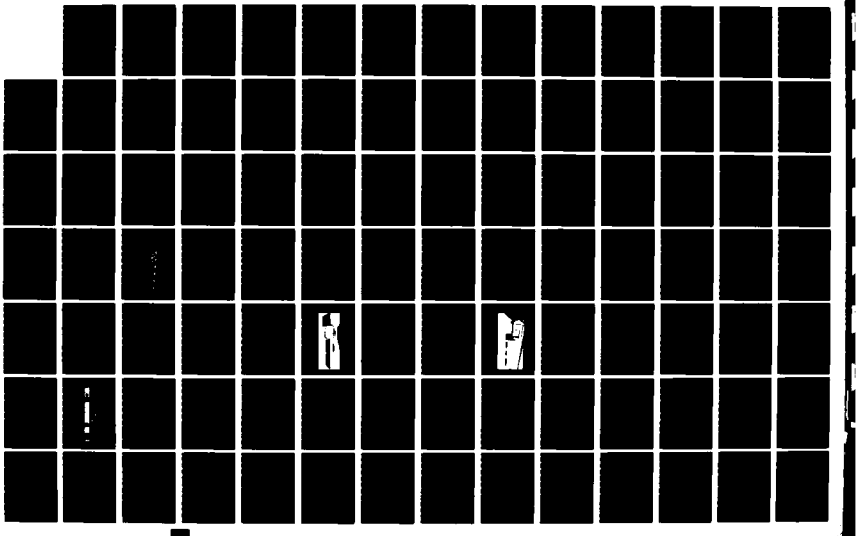

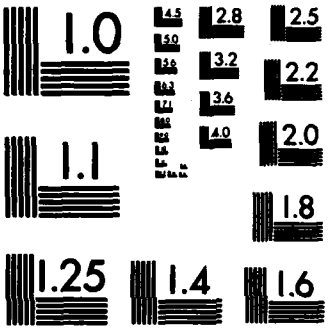

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PATHOPHYSIOLOGIC AND TAXONOMIC PROPERTIES OF COAGULASE-NEGATIVE MICROCOCCACEAE

A

THESIS

Presented to the Faculty of

The University of Texas Graduate School of Biomedical Sciences

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in Partial Fulfillment

of the Requirements

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By

Roy Joseph Almeida, M.S.

San Antonio, Texas

May, 1983

PATHOPHYSIOLOGIC AND TAXONOMIC PROPERTIES OF COAGULASE-NEGATIVE MICROCOCCACEAE

Roy Joseph Almeida

APPROVED:

Supervising Professor

March 17, 1983
Date

APPROVED:

A. J. Guarino, Ph.D.
Dean

DEDICATION

To my parents, Mary and John, for their continuing support and constant encouragement.

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I wish to sincerely thank each member of my supervising committee for their guidance and constructive criticisms. Dr. James Jorgensen, as my supervising professor, has been extremely generous in terms of the time and resources he has devoted to my research. Dr. Marvin Forland's expertise was especially helpful during my review of medical records. Dr. Virginia Thomas generously provided me with bacterial isolates as well as sound advice. Dr. John Alderete supported me with materials and information needed to begin tissue culture studies.

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PATHOPHYSIOLOGIC AND TAXONOMIC PROPERTIES OF COAGULASE-NEGATIVE *MICROCOCCACEAE*

Roy Joseph Almeida, M.S.

The University of Texas Graduate School of Biomedical Sciences at San Antonio

Supervising Professor: James H. Jorgensen, Ph.D.

Modern medical techniques have significantly increased the risk of infection by coagulase-negative staphylococci (C-NS). As many as 11 C-NS species have been implicated in a variety of infections occurring both in healthy and compromised patients. One C-NS species, *Staphylococcus saprophyticus*, has been reported as the second most common cause of urinary tract infections (UTIs) in young women in Europe. The purpose of this study is to determine if *S. saprophyticus* infections occur in South Texas, if speciation of C-NS aids in recognition of such infections, and what practical methods are available for their identification. Additionally, *in vitro* studies were conducted to obtain a better understanding of virulence factors possibly involved in *S. saprophyticus* UTIs.

Classification of C-NS species isolated from 289 Bexar County Hospital District clinical specimens showed that the majority of human C-NS species were present and that S. saprophyticus was a prominent pathogen in urinary infections. A patient chart review of "clinically significant" S. epidermidis and S. saprophyticus urine isolates revealed that S. saprophyticus was isolated predominantly from young outpatient females. Speciation of C-NS was especially useful in recognizing the presence of a UTI, by documenting that a C-NS was S. saprophyticus.

Antimicrobic susceptibility testing of S. epidermidis and S. saprophyticus urine isolates showed that S. saprophyticus isolates were generally more susceptible to a wider range of antimicrobics than S. epidermidis isolates. Nitrofurantoin and cephalothin appeared to be the most effective antimicrobic agents tested against these two species.

Testing C-NS isolates for susceptibility to the antibiotic novobiocin appears to be an excellent and cost-effective presumptive test for S. saprophyticus in human urinary specimens. Mueller-Hinton agar may be conveniently used in lieu of P agar to determine novobiocin susceptibility of C-NS. A group of 254 C-NS isolates were tested in parallel for novobiocin susceptibility on both media. Zones of inhibition of 16 mm or less around a 5 ug novobiocin disc on Mueller-Hinton agar indicated novobiocin resistance, as demonstrated by S. saprophyticus.

Special novobiocin elution discs were prepared for rapid, same-day testing of C-NS in the Abbott MS-2 Microbiology System (Abbott Laboratories, Diagnostics Division, Irving, TX). The MS-2 System correctly classified 91.5% of 82 S. epidermidis and S. saprophyticus isolates as either susceptible or resistant to novobiocin in an average time of only 5.8 hours.

The Vitek AutoMicrobic System Gram-Positive Identification (GPI) Card (Vitek Systems, Inc., Hazelwood, MO) was evaluated for precise species identification of a group of 150 C-NS isolates. Identifications obtained using the GPI Card were compared to reference identifications derived from 14 conventional biochemical tests (Kloos and Schleifer, 1975). The Auto-Microbic System correctly identified 59.3% (89/150) of the test isolates. The greatest accuracy was achieved with isolates of S. epidermidis (82.6%). S. hominis isolates were least often correctly identified (26.7%).

A group of 300 C-NS clinical isolates were tested in parallel using the API STAPH-IDENT System (Analytab Products, Plainview, NY) and Kloos and Schleifer's simplified scheme for identification of Staphylococcus species (1975). Use of the STAPH-IDENT System allowed correct classification of 92.7% (278/300) of the study isolates. However, 25.7% (77/300) of isolates required a supplemental test for final species-level identification (most often novobiocin susceptibility).

In vitro studies were conducted in order to elucidate the virulence of S. saprophyticus in human UTIs. These studies compared S. saprophyticus growth rate and tissue culture adherence characteristics with those of S. epidermidis and selected Gram-negative urinary pathogens. The growth rate in urine does not appear to be an important contributing factor in S. saprophyticus UTIs. Tissue culture adherence experiments proved that HeLa and Vero cells can be used for demonstrating C-NS adherence rates. These experiments showed that S. saprophyticus adhered significantly better than S. epidermidis to tissue cells.

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I. INTRODUCTION

A. General Background

Historically, clinical microbiology laboratories have relied almost exclusively on the coagulase test to differentiate Staphylococcus aureus (coagulase-positive and considered a frank pathogen) from coagulase-negative staphylococci (C-NS), which have been regarded as contaminants or only occasionally as opportunistic pathogens. However, state-of-the-art medical techniques such as immunosuppressive therapy, insertion of catheters, and extensive use of prostheses have significantly increased the risk of infection by C-NS normally found as part of the human body flora. C-NS have been frequently implicated as the etiological agents of prosthetic valve endocarditis (Speller and Mitchell, 1973), infections of cerebrospinal fluid shunts (Holt, 1971), infections of vascular and joint prostheses (Liekweg and Greenfield, 1977 and Wilson et al., 1973), infections of intravenous catheters (Collins et al., 1978), peritonitis in patients receiving continuous peritoneal dialysis (Rubin et al., 1980), subacute bacterial endocarditis (Kaye, 1976), urinary tract infections (Pead et al., 1977), eye infections (Valenton et al., 1973), ear infections (Feigin et al., 1973), and wound infections (Nord et al., 1976). Most clinical laboratories routinely report coagulase-negative Micrococcaceae as "Staphylococcus epidermidis" or as "coagulase-negative staphylococci" despite the fact that at least 11 distinct C-NS species have been isolated from human skin by Kloos (1982 - see Table 1). Kloos and Schleifer (1975) have developed a simplified scheme

TABLE 1

COAGULASE-NEGATIVE HUMAN *STAPHYLOCOCCUS* SPECIES

Species Group	Common Pathogen	Questionable or Uncommon Pathogen	Undetermined or Rare Pathogen
S. epidermidis	*S. epidermidis*	*S. haemolyticus*	*S. capitis*
		S. hominis	
		S. warneri	
S. auricularis			*S. auricularis*
S. saprophyticus	*S. saprophyticus*	*S. cohnii*	*S. xylosus*
S. simulans		*S. simulans*	
S. sciuri			*S. sciuri*

for routine identification of human Staphylococcus species. This conventional scheme, modified slightly by Kloos in 1982, is depicted in Tables 2 & 3. The relatively recent upsurge in C-NS infections coupled with the fact that resistance to particular antibiotics varies widely among different C-NS isolates (Wilkinson et al., 1980; Price and Fluornoy, 1982; and Marsik and Brake, 1982), prompted Gemmell to remark appropriately in 1980, "No longer can the coagulase-negative staphylococci be simply regarded as belonging to Staphylococcus albus (i.e., coagulase-negative Micrococcaceae) or even regarded as Staphylococcus epidermidis or Staphylococcus saprophyticus." Therefore, it now appears that speciation of C-NS is necessary under certain circumstances.

B. Staphylococcus saprophyticus as a Urinary Tract Pathogen

Pereira (1962) in Portugal was one of the first investigators to report C-NS strains as agents of urinary tract infections (UTI's). He concluded that the percentage of urinary infections in his country due to coagulase-positive and coagulase-negative Micrococcaceae was about 9%, with Staphylococcus aureus accounting for slightly more (5.1%) UTI's than coagulase-negative (3.9%) strains. He noted that "In recent years, many patients with urinary infection and intense pyuria have been observed, and in all of them a pure growth of a strain of Staphylococcus albus with particular biological characteristics was isolated." He remarked that in forty particular cases he studied the subjects were almost all women. The bacterial colonies were pigmented either white or yellow. All the coagulase-negative

TABLE 2

TYPICAL CHARACTERISTICS OF COAGULASE-NEGATIVE HUMAN *STAPHYLOCOCCUS* SPECIES

Species	Colony Size	Pigment	Anaerobic Growth	Nitrate Reduction	Alkaline Phosphatase	Arginine Utilization	Urease	Hemolysis	Novobiocin Resistance
S. epidermidis	S/M	-	+	+/±	+	+	+	-/±	-
S. hominis	S/M	+/-	-/±	+/±	-	-/±	+	-/±	-
S. haemolyticus	M/L	-/+	±/+	+/-	-	+	-	+/±	-
S. warneri	S/M	+/-	+	-/±	-	-/+	+	-/±	-
S. capitis	S	-	+/±	+/-	-	+/-	-	-/±	-
S. auricularis	VS	-	±/-	±/-	-	+/-	-	-	-
S. saprophyticus	M/L	+/-	+/±	-	-	-	+	-	+
S. cohnii	M/L	-/+	±/+	-	-/±	-	-/±	-/±	+
S. xylosus	M/L	+/-	+/±	+/-	+/-	-	+	-/±	+
S. simulans	L	-	+	+	±/-	+	+	±/-	-
S. sciuri	L	+/-	±/+	+	+/±	-	-	-	+

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TABLE 3

BIOCHEMICAL ACTIVITY OF COAGULASE-NEGATIVE HUMAN *STAPHYLOCOCCUS* SPECIES: CARBOHYDRATES

Species	Maltose	Trehalose	Mannitol	Xylose	Sucrose	Xylitol	Mannose
S. epidermidis	+	-	-	-	+	-	+/±
S. hominis	+	+/-	-/+	-	+	-	-/+
S. haemolyticus	+	+	+/-	-	+	-	-
S. warneri	+/±	+	+/-	-	+	-	-
S. capitis	-	-	+	-	+/-	-	+/±
S. auricularis	+/-	+/±	-	-	-/+	-	-/+
S. saprophyticus	+	+/-	+/-	-	+	+/-	-
S. cohnii	±/+	+	+/±	-	-	-/±	+/±
S. xylosus	+/±	+	+/±	+	+	-/±	+/±
S. simulans	-/±	+/-	+/±	-	+	-	+/-
S. sciuri	±/-	+/-	+	-/±	+	-	+/±

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strains studied were resistant to the antibiotic novobiocin and sensitive to all other antibacterial agents tested. He also noted that the cure of clinical symptoms always coincided with the disappearance of the coagulase-negative strain.

A year later Baird-Parker (1963), of the Unilever Research Laboratory in England, developed a classification system for micrococci and staphylococci based on physiological and biochemical tests. Staphylococci were distinguished by their ability to form acid from glucose anaerobically. Six subgroups were recognized within the genus Staphylococcus and seven within the genus Micrococcus. Four years later, Roberts (1967), also working in England, performed suprapubic aspirations of bladder urine from 40 pregnant women who had $> 10^4$ bacteria/ml of Gram-positive, catalase-positive coagulase-negative cocci in their midstream urine specimens. Of the 20 aspirates that were positive for such cocci, 14 (70%) contained micrococci and six (30%) contained staphylococci; of the remaining 20 patients having sterile aspirates, 17 (85%) had staphylococci in their midstream urine samples while only three (15%) had micrococci. Roberts concluded that some factor in the urinary tract was acting selectively in favor of micrococci, particularly those of subgroup 3 of the Baird-Parker classification.

In 1968 another Englishman, Mitchell, classified 147 strains of coagulase-negative Micrococcaceae isolated in pure growth from urines containing $\geq 10^5$ bacteria/ml. He demonstrated that strains of micrococci subgroup 3 were resistant to novobiocin and that these strains

generally were isolated from young females with acute cystitis or pyelonephritis. In contrast, novobiocin-sensitive strains of coagulase-negative staphylococci originated from patients with more chronic, anatomic abnormalities of the urinary tract.

Maskell (1974), using novobiocin resistance as being synonymous with group 3 micrococci, showed that 7% of urines sent to the Public Health Laboratory in Portsmouth, England, with a pure growth of $> 10^4$ bacteria/ml were due to coagulase-negative Micrococcaceae. Pyuria was significantly more common in the novobiocin-resistant (micrococcus subgroup 3) than in the novobiocin-sensitive infections. The novobiocin resistant infections occurred almost exclusively in sexually active women between the ages of 16 and 25 years.

In 1974 the novobiocin-resistant subgroup 3 micrococcus was reclassified as a staphylococcus on the basis of its DNA base ratio and its sensitivity to lysostaphin (Buchanan and Gibbons). It was named Staphylococcus saprophyticus, presumably because it was commonly isolated from the surface of animal carcasses (saprophytic = growing on dead tissues).

Sellin et al. (1975) at England's University of Bristol Medical School conducted a prospective study in young women and concluded that the novobiocin-resistant S. saprophyticus was the most common cause of UTI after Escherichia coli. However, these staphylococci were only rarely found among the normal flora of the genitourinary tract of young women, although other staphylococci and micrococci were commonly present. They hypothesized that the infecting staphylococci might be "selectively pathogenic in the urinary tract."

Gillespie et al. at the Bristol Royal Infirmary (1978) studied UTI's in women attending a clinic for sexually-transmitted diseases. Of 103 women under the age of 26 years, lactose-fermenting Gram-negative bacilli caused 63% of the UTI's, S. saprophyticus caused 28% and Proteus 9%. Of 38 women over 26 years, S. saprophyticus caused only 5% of all UTI's. These investigators found no evidence that the staphylococci were sexually transmitted nor were they related to sexual promiscuity.

Wallmark et al. in Sweden also reported on the frequency of S. saprophyticus as the cause of bacteriuria in 1978. Of 787 consecutive female outpatients with $\geq 10^5$ bacteria/ml, 173 (22%) yielded S. saprophyticus, predominantly in pure culture and in high counts. In the age group 16-25 years, this organism had a frequency of 42%. However, in hospitalized women and in men with bacteriuria, S. saprophyticus was a rare finding (0.9% and 0.5%, respectively). These authors also pointed out that colony counts lower than the commonly-expected 10^5 bacteria/ml may be significant in S. saprophyticus UTI's and that recurrent infections were common. Shortly afterwards, another Swedish group, Hovelius et al. (1979), isolated S. saprophyticus significantly more often from men with symptoms of urethritis than from a normal (asymptomatic) control group. Therefore, it appears that S. saprophyticus may also be a prominent cause of non-gonococcal urethritis.

When compared with the extensive European literature, reports concerning S. saprophyticus UTI's occurring in the United States

have been sparse. Williams et al. (1976) in Minnesota concluded that C-NS were infrequent urinary isolates (0.4% of all cultures) in their population and that S. epidermidis predominated over S. saprophyticus in urine containing > 10^4 C-NS/ml.

More recently, Jordan et al. (1980) at the University of Florida reported that S. saprophyticus should be recognized as an important urinary tract pathogen of young female outpatients in the United States. The Florida study's findings were essentially similar to those expressed by European investigators, in that S. saprophyticus was the second most common cause of UTI among young, female outpatients, but was only rarely found as a cause of UTI in hospitalized patients or as a contaminant of urine cultures in either group. Most cases presented as acute cystitis, but frank pyelonephritis and UTI in pregnant females were observed. S. saprophyticus was not commonly found in the indigenous flora of the rectum, vagina, or periurethral area. Jordan's group also made the observation that in contrast to S. epidermidis, which has a low pathogenic potential, S. saprophyticus is not an opportunistic pathogen of the urinary tract. Rather the organism is a primary pathogen and in that respect is comparable to E. coli or other enteric pathogens.

Kloos's conventional scheme for identification of C-NS (1982) is cumbersome for routine clinical laboratory use because of the large number of tests needed, use of highly specialized media, and the long incubation times involved (i.e., five days may be required for anaerobic growth testing and measurement of colony diameter while negative car-

bohydrate reactions are incubated for three days). A great deal of interest has arisen recently concerning automated and manual "same day" testing, as evidenced by the fact that three International Symposia on Rapid Methods and Automation in Microbiology have been held since 1973. Dr. Matsen of the University of Utah School of Medicine presented a paper at the most recent symposium (1981) and addressed the question of whether a patient benefitted from rapid results. During his presentation, Dr. Matsen discussed a list of ten special patient circumstances in which he felt that overall patient care was probably enhanced by early reporting of results. Since four of these special circumstances often involve C-NS infections (endocarditis, immunocompromised patients, patients with prostetic devices and patients with indwelling urinary catheters), one can appreciate that there is an urgent need to develop rapid methods for identification and antibiotic susceptibility testing of C-NS.

C. Research Plan

The aforementioned two American studies do not seem to be in agreement regarding the frequency of isolation of S. saprophyticus by clinical laboratories. Therefore, a study of the incidence of S. saprophyticus isolated from diagnostic specimens in a major medical center in South Texas would assist in defining the role of these species as pathogens in our region and clarify the likelihood of recovery of these species from different body sites.

A major question to be resolved is whether speciation of C-NS assists in defining infection versus colonization or contamination.

For example, if definitive identification of S. saprophyticus from a urinary or genital source strongly suggests that an infection exists, while isolation of S. epidermidis indicates superficial contamination of a specimen, valuable insight can be gained from accurate speciation. It is also important to determine if differences exist in antibiotic susceptibilities between S. saprophyticus and S. epidermidis. It may be important for clinicians to be aware of the likely antibiotic susceptibility of S. saprophyticus in selection of drugs for empiric therapy. Knowledge of the likely susceptibility of S. saprophyticus would also be helpful to a physician in the interim between isolation of S. saprophyticus and completion of in vitro susceptibility testing.

In order to elucidate the virulence of S. saprophyticus in human UTI's, in vitro studies were conducted. These studies compared S. saprophyticus growth rate and tissue culture adherence characteristics with those of S. epidermidis and selected Gram-negative urinary pathogens.

If infections caused by S. saprophyticus occur frequently in a particular region, and if speciation of C-NS is helpful in determining clinical significance, it must then be possible for clinical laboratories to easily and reliably recognize these species. This study will directly compare Kloos's simplified scheme for routine identification of human Staphylococcus species (1982) against recently-described abbreviated methods to include:

1. Determination of novobiocin-susceptibility alone. Several authors feel that identification of

S. saprophyticus may be made by demonstrating resistance to novobiocin alone (Meers et al., 1975; Wallmark et al., 1978; Shrestha et al., 1979; Anderson et al., 1981; Marrie et al., 1982).

However, other investigators feel that novobiocin resistance alone can not be relied on to differentiate S. saprophyticus from other C-NS (Williams et al., 1976; Hovelius and Mardh, 1977; Pead et al., 1977).

2. Vitek AutoMicrobic System Gram-Positive Identification Card: This product is designed to be used in the Automated Vitek Instrument for the identification of clinically-significant streptococci, staphylococci and a selected group of Gram-positive bacilli.
3. API STAPH-IDENT System: This commercial product was released for clinical use in December, 1981. This miniaturized manual system is said to readily identify both coagulase-positive and negative staphylococci.

Therefore, the last phase of this investigation evaluated the accuracy and cost-effectiveness of both of these commercial products for routine identification of C-NS in clinical specimens.

Finally, my research plan may be outlined in terms of the following objectives:

Specific Research Objectives

1. To determine the distribution of human C-NS species present in clinical specimens submitted to Medical Center Hospital.
2. To determine if S. saprophyticus infections occur frequently in South Texas and to define the spectrum of disease produced by this species.
3. To determine if speciation of the C-NS significantly assists in separating infection from colonization or specimen contamination.
4. To determine whether growth rate or tissue adherence characteristics can account for the virulence of S. saprophyticus in UTI's.
5. To evaluate rapid methods by which clinical laboratories may readily and accurately identify coagulase-negative members of the family Micrococcaceae.

II. MATERIALS AND METHODS

A. Research Objective #1

All catalase-positive, coagulase-negative Gram-positive aerobic cocci isolated over a six month period from urines, genital cultures, blood cultures, pulmonary secretions, cerebrospinal fluid, and exudate samples received in the Medical Center Hospital Microbial Pathology Laboratory were tested as follows:

1. Micrococci were first differentiated from staphylococci by testing the ability of strains to produce acid aerobically from glycerol (1%) in the presence of 0.4 μg of erythromycin per ml of purple agar (Difco Laboratories, Detroit, MI). After incubation at 35°C for 48 hours, those strains producing acid aerobically from glycerol (manifested by yellow indicator color extending out from the culture streak into the surrounding medium) were classified as staphylococci while those strains inhibited by the low level of erythromycin and unable to produce acid from glycerol were classified as micrococci (Schleifer and Kloos, 1975a) and were not tested further. American Type Culture Collection (ATCC) strains of S. epidermidis (14990) and Micrococcus luteus (E4698) were run as positive and negative controls, respectively, each time a new batch of purple agar with glycerol and erythromycin was prepared.
2. Strains classified as staphylococci were then speciated using tests contained in Kloos and Schleifer's simplified

scheme for routine identification of human Staphylococcus species (1975) to include:

a. Aerobic acid production from carbohydrates (sucrose, trehalose, mannitol, xylitol, lactose, maltose, ribose, xylose, and mannose): carbohydrate agars were prepared by adding filter-sterilized carbohydrate stock solution to an autoclave-sterilized purple agar base (Difco) medium to achieve a final 1% carbohydrate concentration. As many as eight strains were streaked radially on 100 x 15 mm four compartment plastic Petri dishes (Falcon, Oxnard, CA), two strains streaked per quadrant. Cultures were incubated aerobically at 35°C and examined at 24, 48, and 72 hours. A moderate to strong yellow indicator color extending out from the culture streak into the surrounding medium was interpreted as a positive (+) reaction. The absence of yellow indicator color under or around the culture streak within 72 hours was considered a negative (-) reaction. Quality control strains of staphylococci were used to monitor the performance of each batch of carbohydrate agar.

b. Phosphatase: Alkaline phosphatase activity was determined by a modification of the method of Pennock and Huddy (1967) using a 0.005 M solution of phenolphthalein monophosphate (sodium salt) instead of a 0.01 M solution of disodium phenylphosphate, in 0.01 M citric acid/sodium

citrate buffer (pH 5.8). Sterile, disposable plastic tubes (12 x 75 mm) containing 0.5 ml of the above buffer solution were inoculated to a density visually comparable to a McFarland #4 opacity standard (approximately 10^9 bacteria/ml). After incubation at 35°C for four hours, the reaction was stopped by adding 0.5 ml of 0.5 N sodium hydroxide and 0.5 ml of 0.5 M sodium bicarbonate. The final color was developed by adding 0.5 ml of 4-amino-antipyrine solution (0.6 g/100 ml) and 0.5 ml of potassium ferricyanide solution (2.4 g/100 ml). Color reactions were classified as follows:

+ : development of a moderate to intense red color;

- : color remained yellow;

Development of a pink color was interpreted as a weak positive (±) reaction. ATTC strains of S. epidermidis (14990) and S. saprophyticus (15305) were run as positive and negative controls, respectively, each time phosphatase testing was performed.

c. Novobiocin susceptibility testing: Four-five well-isolated colonies of each isolate grown overnight on plates of Trypticase soy agar with 5% sheep blood (BBL, Cockeysville, MD) were inoculated into 5 ml of tryptic soy broth (Difco). The broth cultures were incubated at 35°C for 2-5 hours, until a slightly visible turbidity developed. The turbidity of the broth cultures was then adjusted with sterile

broth to obtain a density visually comparable to that of a 0.5 McFarland opacity standard. A sterile cotton swab was dipped into the standardized suspension and pressed and rotated against the upper inside of the tube to express excess broth. The swab was then streaked evenly in three directions over the entire surface of a 100 x 15 mm plastic Petri dish containing 25 ml of P agar prepared as follows (Vera and Power, 1980): 10 g peptone, 5 g yeast extract, 5 g sodium chloride, 1 g glucose, and 15 g agar were mixed into 1 ℓ of distilled water. The mixture was adjusted with 0.5 N sodium hydroxide to a pH of 7.5 before autoclaving at 121^{o}C for 15 minutes. After cooling to 55^{o}C in a waterbath, the P agar was dispensed aseptically into sterile Petri dishes. Three to five minutes after swabbing of the plate to obtain confluent growth, a 5 ug novobiocin disc (BBL) was applied. The plate was then inverted and placed in an incubator at 35^{o}C. After 16-18 hours incubation, the plates were examined and the diameter of the zone of complete inhibition was measured to the nearest whole millimeter using vernier calipers. Isolates having a zone diameter greater than 16 mm were classified as being susceptible to 1.6 μg/ml of novobiocin while those with a diameter of 16 mm or less were considered resistant. The use of a 16 mm break point is equivalent to Kloos's criteria of classifying strains as resistant if inhibition

is not greater than 5 mm from the edge of a 6 mm disc. ATTC strains of S. epidermidis (14990) and S. saprophyticus (15305) were run as susceptible (-) and resistant (+) controls, respectively, on new lots of agar or novobiocin discs.

d. Lysostaphin susceptibility: The susceptibility of staphylococcal strains to 50 µg of lysostaphin (Sigma Chemical Co., St. Louis, MO) per ml of P agar was determined. A loopful of a saline suspension of organism adjusted to a density visually comparable with a 0.5 McFarland opacity standard was inoculated onto a 24 hour dried, P agar plate (60 x 15 mm) containing 50 µg of lysostaphin per ml of P agar and a control P agar plate without lysostaphin. Both plates contained 10 ml of P agar. Cultures were incubated at 35°C for 24 hours and then examined for growth. A strain demonstrating growth on the lysostaphin-containing plate was reported as being resistant to 50 ug/ml of lysostaphin while no growth was interpreted as sensitivity to lysostaphin. ATCC strains of S. aureus (25923) and S. epidermidis (14990) were inoculated onto each P agar plate as susceptible (-) and resistant (+) controls, respectively.

e. Anaerobic growth in thioglycolate medium: Tubes (16 x 150 mm) containing 8 ml of steamed and cooled (55°C) thioglycolate semisolid medium (Brewer's fluid thioglyco-

late medium plus 0.3% agar) (Evans and Kloos, 1972) were inoculated with 0.1 ml of a 1:100 dilution of a saline bacterial suspension with a density visually comparable to that of a 0.5 McFarland opacity standard. Tubes were incubated at 35°C for five days at which time growth characteristics were noted (for example, dense uniform growth throughout the medium, gradient from dense to light down the tube, large or small individual colonies, or absence of visible growth). An ATCC strain of S. epidermidis (14990) was used as a positive control and an uninoculated tube of semisolid thioglycolate as a negative control with each batch of tests run.

f. Colony diameter: This parameter was determined after incubation of strains on P agar at 35°C for three days and room temperature for an additional two days. Colony diameter was measured in millimeters and classified as follows:

Large, > 6 mm

Medium, 5-6 mm

Small, 2-4 mm

Very small, < 2 mm

g. Hemolysis: The ability of strains to hemolyze blood cells on Trypticase soy agar with 5% sheep blood (BBL) was observed after incubation at 35°C. Culture streaks were made by lightly inoculating a 1 cm line on the

surface of a blood agar plate with a loopful of cells. Up to eight streaks could be radially inoculated on each 100 x 15 mm plate. Hemolysis was characterized after 24, 48, and 72 hours of culture incubation and was interpreted according to the following scheme:

+ : strong, hemolysis extending $\geq$ 1.5 mm out from the culture streak within 48-72 hours.

± : moderate to weak, hemolysis extending trace to < 1.5 mm out from the culture streak within 72 hours.

- : no clearly detectable hemolysis within 72 hours.

h. Pigment: Strains were classified as yellow pigment producers (+) or non-producers (-) after five day's incubation at 35°C on P agar (Kloos et al., 1974).

i. Urease: Production of urease (Kloos, 1982) was also tested by streaking selected strains on urea agar slants (Difco). Slants were incubated at 35°C for 48 hours and strains were classified as urease positive (+) if a red color developed or urease negative (-) if there was no change in color.

B. Research Objective #2

The number of "clinically significant" S. saprophyticus strains isolated in section A were compared with the number of "clinically significant" S. epidermidis strains isolated during a similar period. Cultures were considered to be "clinically significant" when the following criteria were met.

1. Blood cultures - growth of C-NS present in multiple cultures (Kloos and Smith, 1980).
2. Cerebrospinal fluid - any growth of C-NS (Kloos and Smith, 1980).
3. Exudates - any growth of C-NS.
4. Genital cultures - any growth of S. saprophyticus (Wallmark et al., 1978; Sellin et al., 1975; Bollgren et al., 1981).
5. Pulmonary secretions - any growth of C-NS from trans-tracheal aspirates, bronchoscopies, wedge aspirates, biopsies, or percutaneous needle aspirates (Kloos and Smith, 1980).
6. Urine cultures - single species, greater than 10,000 organisms per ml of urine (Williams et al., 1976; Jordan et al. 1980; Bollgren et al., 1981).

"Clinically significant" S. saprophyticus strains were then tabulated by specimen source (i.e., blood, urine, etc.).

C. Research Objective #3

1. A patient chart review of selected S. epidermidis and S. saprophyticus clinical urine isolates was conducted to determine if these organisms were the probable cause of an infection. Chart reviews were conducted on outpatients (OP's) or inpatients (IP's) whose urines contained a pure culture of S. epidermidis or S. saprophyticus with greater than 10,000 organisms per ml of urine. Details from each chart review were recorded using a records' review worksheet (See Appendix A).

2. A retrospective study of 46 urine isolates previously identified as "S. epidermidis" (i.e., coagulase-negative Micrococcaceae) was conducted. These specimens, collected from patients with well-documented bacteriuria ($\geq$ 100,000 bacteria/ml), had previously been tested by Thomas et al., (1974) for the property of being antibody-coated bacteria. The isolates were then stored at -80°C in Todd-Hewitt broth. Upon thawing, the bacteria were streaked for isolation on Trypticase soy agar with 5% sheep blood (BBL). The plates were incubated overnight at 35°C and observed for growth. The tests described in Section A of Materials and Methods were then performed in order to determine which Staphylococcus species were represented by these isolates.
3. Antibiotic susceptibility testing by the method of Bauer et al., (1966) was performed on urine isolates of S. epidermidis and S. saprophyticus when these organisms were present in pure culture with greater than 10,000 organisms per ml of urine. This testing was performed to determine if significant differences in susceptibility patterns exist between these two organisms and if antibiotics used for empiric therapy of presumed UTI or non-gonococcal urethritis are likely to be effective against S. saprophyticus. Four-five well-isolated similar colonies growing on blood agar plates were transferred by an inoculating loop into 5 ml of tryptic soy broth (Difco). The broth was incubated for 2-5 hours

at 35°C until a slightly visible turbidity appeared. The turbidity of the broth culture was then adjusted with sterile broth to obtain a density visually comparable to that of a 0.5 McFarland opacity standard. Within 15 minutes after adjusting the turbidity of the inoculum, a sterile swab was dipped into the diluted inoculum and rotated firmly several times against the upper inside wall of the tube to express excess fluid. Next, the entire surface of a 150 x 15 mm Petri dish containing 60 ml of Mueller Hinton agar (BBL) was streaked three times, rotating the plate 60° between streakings to obtain even inoculation. The plate was then held at room temperature for 5-15 minutes to allow surface moisture to be absorbed. Antimicrobial discs (BBL) were applied by using a Sensi-Disc (BBL) dispenser to place 11 discs on each plate aseptically. Plates were then inverted and incubated at 35°C for 16-18 hours. The diameter of the zone of complete inhibition around each antimicrobial disc was then measured to the nearest whole millimeter using vernier calipers. Each organism was then classified as being resistant, intermediate, or susceptible to a particular antibiotic based on the zone diameter interpretive standards published by the National Committee for Clinical Laboratory Standards (NCCLS, 1979). Each time susceptibility tests were performed, control limits for zones of inhibition with S. aureus ATCC 25923 were checked to insure the correct performance of the entire procedure.

D. Research Objective #4

1. Growth Rate Studies. The Abbott MS-2 Microbiology System (Abbott Laboratories, Dallas, TX) was used to determine the mean generation times (i.e., doubling times) of C-NS and other urinary pathogens in pooled filter-sterilized human urine and in eugon broth.

   a. Growth Media. Urine for growth rate studies was obtained from clinical specimens received in the Medical Center Hospital Microbial Pathology Laboratory. Specimens were first visually inspected and turbid samples discarded. Prior to pooling, specimens were cultured for growth and tested for the presence of antibiotics. A 0.001 ml calibrated inoculating loop (American Scientific Products, McGaw Park, IL) was used to streak each specimen onto a Trypticase soy agar plate with 5% sheep blood (BBL). After 35^{o}C incubation overnight, the plates were examined for growth. Those urine specimens showing growth were discarded. Specimens demonstrating no growth (< 1,000 cfu/ml) were then tested for the presence of antibiotics by a bioassay using Bacillus subtilis (ATCC 6633). Heart infusion agar (Difco) was prepared according to manufacturer's instructions and autoclaved in 25 x 150 mm glass screw-top tubes which contained 25 ml of agar per tube. The appropriate number of tubes were then placed in a 55^{o}C waterbath (Lab-Line Instruments, Inc., Melrose

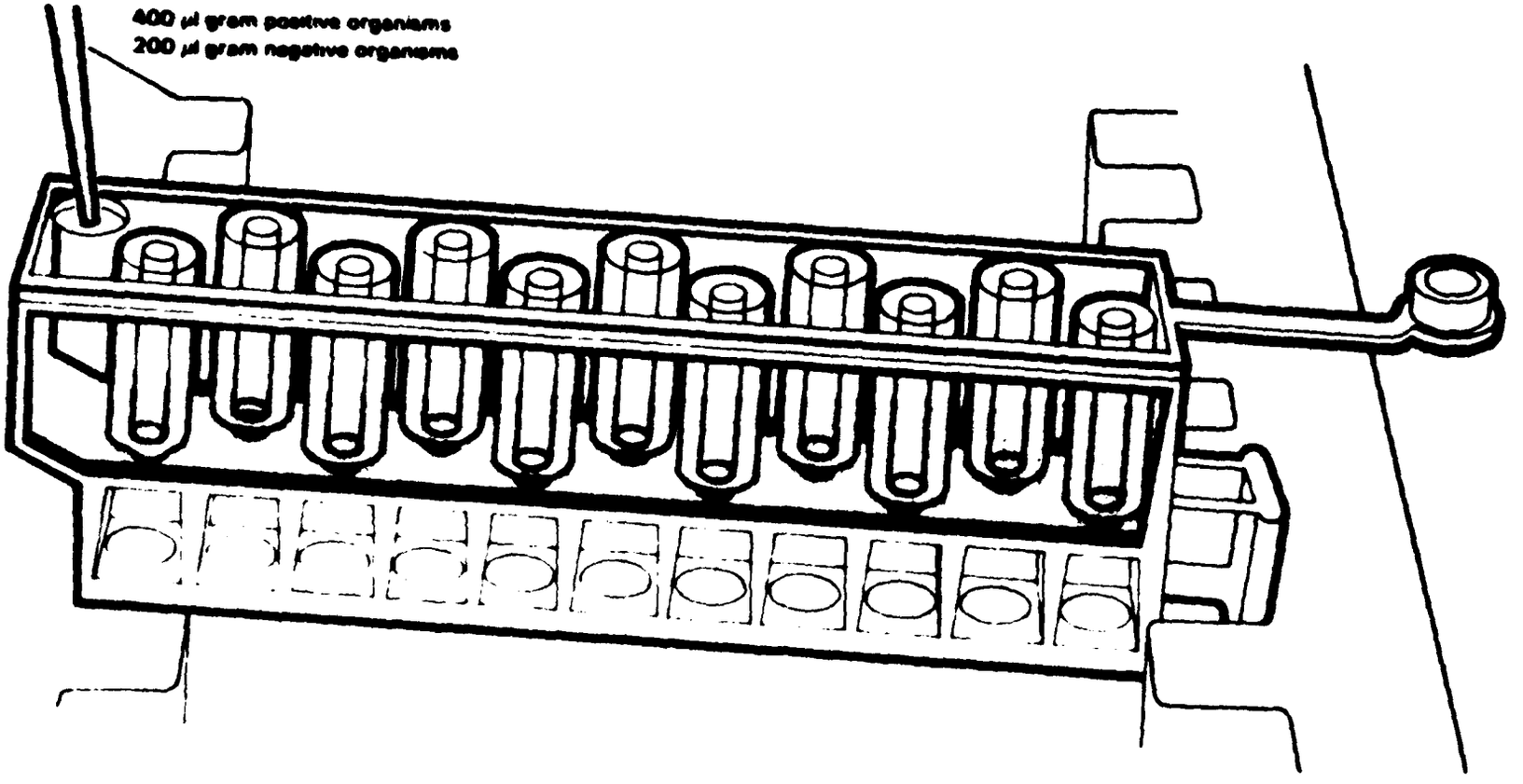
Park, IL). After the tubes had cooled, 250 µl of Bacillus subtilis suspension (Difco) was pipetted into each tube. Tube contents were then mixed so that the spores were evenly distributed throughout the agar and poured into sterile 150 x 15 mm plastic Petri dishes resting on a level surface. The agar-spore mixture was then immediately distributed evenly over the dish bottom by rolling and tilting the dish. The Petri dish cover was partially replaced, allowing steam to escape while the agar gelled. After about 20 minutes, a 6 mm diameter cork borer was used to cut the appropriate number of wells needed in the solidified agar (one well per urine specimen) and the wells were labeled by specimen number. A 5 ml plastic serological pipet (Falcon) connected to a vacuum line was used to aspirate the agar plugs. Twenty µl of each well-mixed urine sample was pipetted into each well. The bioassay plates were incubated at 35°C for 6 hours. The presence of any zones of inhibition, indicative of antibiotic activity, caused the urine specimens to be discarded. The remaining culture-negative, bioassay-negative urine specimens were pooled, centrifuged at 1500 g for 5 minutes to remove filter-blocking mucus (Anderson et al., 1976), and then filter-sterilized through a 0.45 micron Nalgene filter unit (Nalge Co., Rochester, NY). The pH of the pooled, filter-

sterilized urine, as determined by a Zeromatic SS-3 pH Meter (Beckman Instruments Inc., Fullerton, CA) was 6.0 at 25°C. The pooled, filter-sterilized urine was kept refrigerated overnight at 4°C and then allowed to reach room temperature prior to use. Eugon broth (Difco) was prepared and autoclaved according to manufacturer's instructions. The final pH of the broth, determined as above, was 7.0 at 25°C. The broth was kept refrigerated at 4°C prior to use. For certain growth rate studies, eugon broth was adjusted to the same pH as pooled human urine by adding 0.5 N HCl to eugon broth until a pH of 6.0 was attained.

b. Bacteria. All bacterial strains used in growth rate stuies were clinical urine isolates obtained from the Medical Center Hospital Microbial Pathology Laboratory. C-NS were identified by Kloos and Schleifer's simplified scheme for routine identification of human Staphylococcus species (1975). Members of the family Enterobacteriaceae were identified by the MICRO-ID System (MICRO-ID Identification Manual, 1981, General Diagnostics, Division of Warner-Lambert Co., Morris Plains, NJ). Enterococci were identified by colonial appearance, Gram stain, hydrolysis of bile esculin, and growth in 6.5% NaCl broth (Facklam, 1980). Isolates to be tested were streaked onto Trypticase soy agar plates with 5% sheep blood (BBL) and incubated overnight at 35°C prior to testing.

c. Determination of Mean Generation Time. A sterile inoculating needle was touched to four-five well-isolated morphologically-identical colonies on each blood plate and inoculated into 16 x 150 mm screw-top glass test tubes containing 4 ml of 0.85% sterile saline solution. Each tube was vortexed to yield a uniform cell suspension and the turbidity was adjusted to a density visually comparable to that of a 0.5 McFarland opacity standard. The MS-2 Disc Loader/Sealer was then used to seal two MS-2 Transfer Cuvette Cartridges (without antibiotic discs) for each isolate to be tested. The top surfaces of the sealed cartridges were then labeled (two cartridges per isolate number). One cartridge of each duplicate set was filled with 15 ml of pooled, filter-sterilized human urine. The second cartridge was filled with 15 ml of eugon broth. The upper growth chamber of each Transfer Cuvette Cartridge was then inoculated with the appropriate amount of prepared bacterial saline suspension (Figure 1). For Gram-negative isolates, 200 ul of the prepared saline cell suspension was pipetted into one each urine and broth-containing Transfer Cuvette Cartridges. For Gram-positive isolates, 400 ul of the prepared saline cell suspension was pipetted into one each urine and broth-containing Transfer Cuvette Cartridges. The fill port of each Transfer Cuvette

Figure 1. Inoculation of Abbott MS-2 Transfer Cuvette Cartridge


400 µl gram positive organisms
200 µl gram negative organisms

Cartridge was closed by breaking off the attached plug and firmly pressing it into position. The MS-2 System was then programmed to perform susceptibility testing and each Transfer Cuvette Cartridge was inserted into the MS-2 Analysis Module. After insertion of the last Transfer Cuvette Cartridge, the MS-2 was instructed to print landmark values for all cartridges under analysis. Thus, the system would print the following messages for each cartridge being incubated:

1) Time of initial light transmission reading.
2) Time of transfer of bacteria and medium from upper to lower cuvette growth chamber.
3) Time of first doubling of cultures in lower growth chamber.
4) Mean generation time (MGT) in minutes.

2. Tissue Culture Adherence Studies

a. Bacterial Strains. The bacterial isolates used in hemagglutination and adherence studies were S. saprophyticus and S. epidermidis strains obtained from the Medical Center Hospital Microbial Pathology Laboratory and from Dr. Virginia Thomas of the Department of Microbiology (see Materials and Methods, para C.2.). Additionally, S. saprophyticus ATCC 15305 was also tested for hemagglutination and adherence. All of the above strains were isolated from urine. For hemagglutination and

adherence testing bacteria were streaked onto Trypticase soy agar plates with 5% sheep blood (BBL) and incubated at 35°C overnight. Unless otherwise indicated, those bacteria to be tested for adherence were then transferred into 5 ml of tryptic soy broth (Difco) and were grown without shaking at 35°C for 20 hours. Bacteria were harvested by centrifugation (2500 rpm) and the sediment was washed twice with phosphate-buffered saline (PBS-pH 7.3). The sediment was then resuspended in PBS in 13 x 100 mm glass screw-top tubes and the turbidity of the bacterial suspension was adjusted to read 200 Klett units (approximately 1 x 10^9 bacteria/ml) on a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Co., Inc., NY).

b. Hemagglutination Studies. Bacteria were tested for the ability to hemagglutinate human and sheep erythrocytes. Human blood (O positive) was collected in an EDTA Vacutainer tube (Becton-Dickinson and Co., Rutherford, NJ). The tube was centrifuged, the plasma removed and the human red blood cells (HRBCs) washed three times with saline. Sheep red blood cells (SRBCs) preserved in modified Alsever's solution (Colorado Serum Company Laboratories, Denver, CO) were also washed three times with saline. One percent suspensions of HRBCs and SRBCs were made using Hanks Balanced Salts Solution (Difco) as

the diluent. Cell suspensions were stored refrigerated at 4°C prior to use. Using a Pasteur pipette, 3 drops of each 1% RBC suspension were placed in 12 x 75 mm glass tubes. Next, a wooden applicator stick was used to make a dense bacterial suspension in each tube. The rack of tubes was placed in a pan of ice on a Tek Tator Variable Speed Serological Rotator (American Scientific Products) set at 40 rpm for 15 minutes. After rotation, the tubes were observed microscopically at 35X for hemagglutination which was graded as:

+ : positive, medium to large-sized aggregates of RBCs.

- : negative, no hemagglutination.

± : weak positive, small clumps of agglutinated cells in a sea of free cells (mixed fields).

After initial observation, the tubes were stored at 4°C overnight and observed for hemagglutination the following day. Each time hemagglutination tests were performed, the following controls were run:

1) HRBC cell control: contained only 1% HRBC suspension.

2) SRBC cell control: contained only 1% SRBC suspension.

3) HRBC and SRBC positive controls: contained 1% HRBC suspension and 1% SRBC suspension, respect-

ively, and E. coli 6260 (obtained from Dr. Thomas, Department of Microbiology) which demonstrated strong hemagglutination with both cell suspensions.

4) HRBC and SRBC negative controls: contained 1% HRBC suspension and 1% SRBC suspension, respectively, and E. coli 6318 (obtained from Dr. Thomas, Department of Microbiology) which demonstrated no hemagglutination with both cell suspensions.

c. Tissue Culture Cells. HeLa (human cervical epitheloid carcinoma), Vero (African green monkey kidney) and MDCK (Madin-Darby canine kidney) cells were the three cell lines used in adherence studies (ATCC Catalogue of Strains II, 1981).

d. Tissue Culture Medium and Reagents. All three cell lines were grown in Basal Medium Eagle (BME) prepared in the following manner:

1) One packet of BME Autoclavable Powder Media (Flow Laboratories, Inc., McLean, VA) was added to 970 ml deionized water while stirring slowly to completely dissolve powder.

2) The solution was autoclaved on liquid cycle at 121^{o}C (15 psi) for 15 minutes.

3) After cooling, the pH of the BME was adjusted to 7.2 by adding approximately 30 ml of sterile 5.6% $NaHCO_3$ solution.

4) Next, 100 ml of membrane-filtered, cell culture grade fetal bovine serum (KC Biological, Inc., Lenexa, KS) was added.

5) Then, 10 ml of sterile 200 mM L-glutamine solution (Flow) was added.

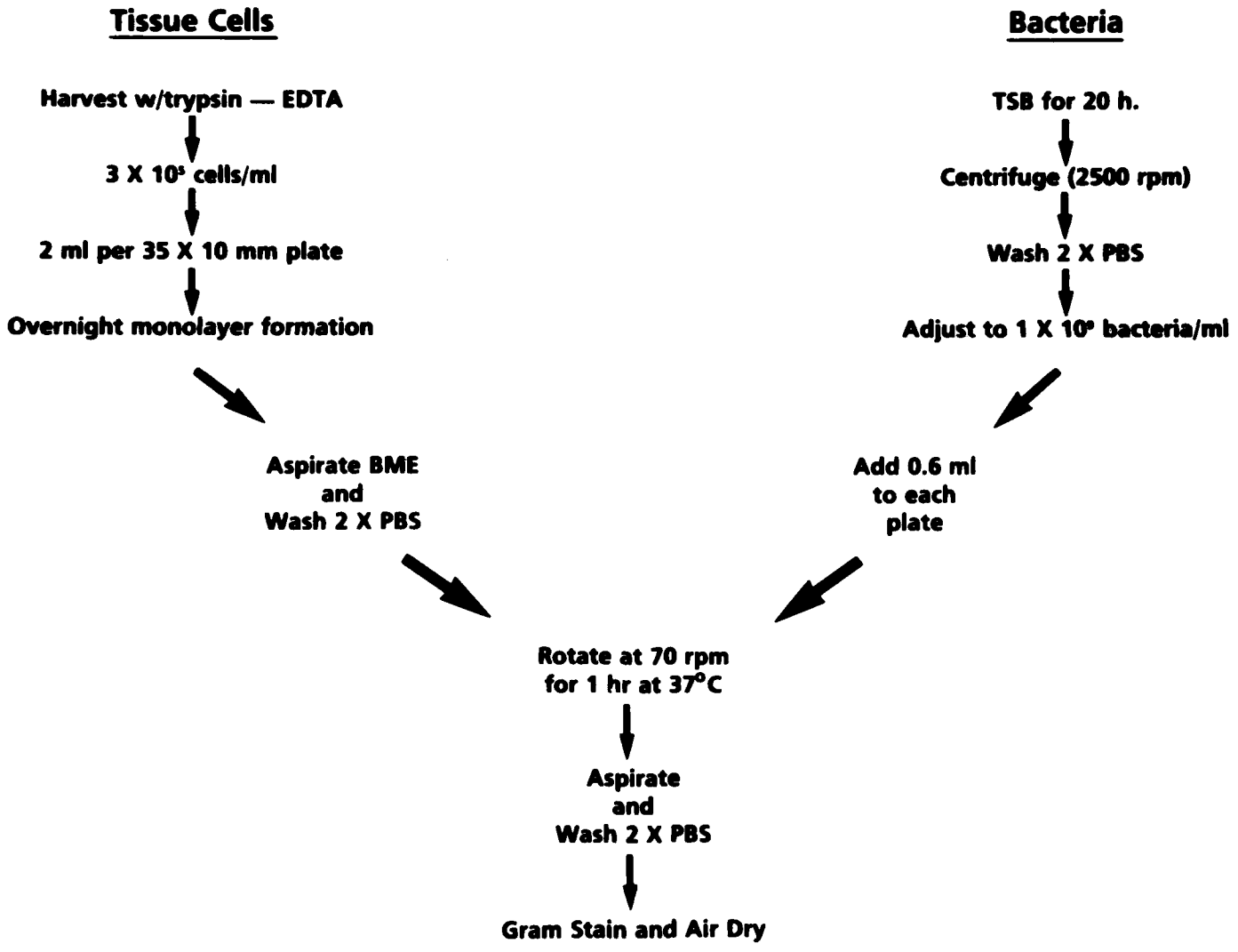
6) Finally, 20 ml of sterile Penicillin-Streptomycin (5,000 units-5,000 ug/ml) in normal saline (Flow) was added. The final BME formulation was stored at $4^{o}C$ prior to use.

Tissue culture cells were incubated at $35^{o}C$ under 5% CO_2 in 150 cm^2 plastic tissue culture flasks (Corning Glass Works, Corning, NY) containing 30 ml of BME prepared as above. The fluid medium was replaced with fresh BME every 24 hours until the cells were confluent at which time they were harvested by using a trypsin-EDTA solution (Parker, 1961) to remove the cells from the flask surface. The trypsin-EDTA solution was prepared as follows: 8 g NaCl, 0.4 g KCl, 0.58 g $NaHCO_3$, 1 g dextrose, 0.5 g trypsin (1:250) and 0.2 g EDTA were mixed into deionized water to make a final volume of 1000 ml. The solution was then filter-sterilized using a 0.22 nm pore size filter (Nalge Co.) and stored at $-20^{o}C$ prior to use.

e. Adherence Assay (see Figure 2). When the cell monolayer reached confluency, the BME was poured off into a waste beaker. The cell monolayer was then washed 2 X with 10 ml sterile PBS to remove any serum residue. Trypsinization of the monolayer was performed by adding 1.5 ml of trypsin-EDTA solution, sloshing the solution vigorously over the monolayer for approximately 30 seconds and then pouring off the solution into a waste beaker. The tissue culture flask was then kept at room temperature and tapped briskly every few minutes to see if the monolayer cells were becoming detached from the flask surface. When significant dislodgement was observed, 10 ml of fresh BME was added to the flask to inactivate the trypsin (NOTE: MDCK cells were very resistant to trypsinization and usually required two consecutive trypsinization treatments to dislodge the cells). The 10 ml of cell-BME suspension were then mixed thoroughly by flushing the suspension repeatedly with a 10 ml serological pipet (Falcon), being sure to direct the exiting stream against the flask side to aid cell dislodgement. A small amount of cell-BME mixture was then removed using a sterile 9" Pasteur pipet (American Scientific Products). Four drops of the cell suspension were added to an equal amount of 0.4% trypan blue stain in normal saline (Grand Island Biological Co., Grand Island, NY). The stain-cell suspension mixture was vortexed, aspirated into a Pasteur

Figure 2. Cell Culture Adherence Assay Procedure

ADHERENCE ASSAY



35-4

pipet and a drop was used to charge a hemacytometer (American Optical Corp., Buffalo, NY). After determining the cell concentration, the appropriate amount of fresh BME was added to the flask to achieve a concentration of 3×10^5 HeLa or Vero cells/ml (5×10^5 MDCK cells/ml). A 2 ml aliquot of the appropriate cell suspension was placed into labeled 35 x 10 mm tissue culture dishes (Corning), one dish for each bacterial strain to be tested for adherence. An additional dish received 2 ml and was used as a negative control. The dishes were incubated at 35^oC under 5% CO_2 to allow monolayer formation to occur overnight. The following day the dishes were viewed microscopically to ensure that they all contained complete monolayers. The fluid medium was then aspirated and the monolayers washed gently 2 X with PBS. Next, 0.6 ml of the appropriate 1×10^9 bacteria/ml PBS suspension (see Materials and Methods, para. D.2.a.) was added to each plate. The negative control plate received 0.6 ml of sterile PBS. Unless otherwise stated, the plates were then placed on a Tek Tator Variable Speed Serological Rotator (American Scientific Products) set at 70 rpm for one hour in a 37^oC walk-in incubator. Every 15 minutes during the incubation, the plates were tilted manually to ensure that the bacterial suspension was kept in contact with the entire monolayer. After the 60 minute incubation

period, the fluid was aspirated from the plates and they were rinsed gently 2 X with PBS. The monolayers were stained using a modified Gram stain as follows:

1) Flood plate with Gram crystal violet (Difco) and let stand for 30 seconds.
2) Pour off stain and rinse in running water.
3) Add Gram iodine (Difco) and let stand for 30 seconds.
4) Pour off stain and rinse in running water.
5) Add 95% ethanol and decolorize until solvent flows colorlessly from the plate.
6) Pour off solvent and rinse in running water.
7) Counterstain with Gram safranin (Difco) for 15 seconds.
8) Pour off stain and rinse in running water.

The tissue culture dishes were then inverted without lids and allowed to drain and air dry on a paper towel. When dry, the monolayers were observed for bacteria by using the oil objective (1000 X) of a light microscope (American Optical). First, 100 contiguous monclayer cells were observed and the number of cells with one or more bacteria attached (%) was recorded. Secondly, 40 contiguous monolayer cells were viewed and the number of bacteria attached to each tissue culture cell was computed as well as the standard deviation and range of values.

f. Statistical Methods. The mean and standard deviation of the number of bacteria adhering per cell were computed to provide an indication of central tendencies. The range was determined to demonstrate the difference between the largest and smallest values in a given bacterial strain. Differences in the ability of different bacterial strains to adhere to tissue culture cells were analyzed using the Mann-Whitney U Test for two independent groups (Mendenhall, 1979). Statistical analyses were performed using a Texas Instruments TI-55 programmable calculator.

E. Research Objective #5

1. Determination of Novobiocin Susceptibility.

a. All clinical staphylococcal isolates classified by Kloos and Schleifer's simplified scheme (see Materials and Methods, para. A and para C.2.) were categorized into one of two groups: novobiocin-susceptible strains or novobiocin-resistant strains. The species present in each of these two groups were then analyzed.

b. The use of Mueller-Hinton agar in lieu of P agar to determine novobiocin susceptibility of C-NS was evaluated. Two hundred and fifty-four C-NS isolates selected from the collection mentioned in the previous paragraph were included in this study. They included: S. epidermidis (111), S. hominis (50), S. saprophyticus (31), S. simulans (27), S. haemolyticus (23), S. warneri

(10), and S. capitis (2). Four-five well-isolated colonies of each isolate grown overnight at 35°C on plates of Trypticase soy agar with 5% sheep blood (BBL) were inoculated into 16 x 150 mm screw-top glass tubes containing 5 ml of tryptic soy broth (Difco). The broth cultures were incubated at 35°C for 2-5 hours, until a slightly visible turbidity appeared. The turbidity of the broth cultures was adjusted with sterile broth to obtain a density visually comparable to that of a 0.5 McFarland opacity standard. This inoculum suspension was simultaneously inoculated onto both P agar (see Materials and Methods, para A.2.c.) plates and Mueller-Hinton agar (Difco) plates (both in 100 x 15 mm plastic plates, prepared to yield an agar bed of 3-4 mm depth). Three to five minutes after swabbing of the plates to obtain confluent growth, a 5 ug novobiocin disc (BBL) was applied to each plate. The plates were inverted and placed in an incubator at 35°C. After 16-18 hours incubation, the plates were examined and the diameter of the zone of complete inhibition was measured to the nearest whole millimeter using vernier calipers.

c. A group of 82 C-NS isolates were then used to determine if the Abbott MS-2 Microbiology System (Abbott Laboratories) could be employed to provide a rapid, "same-day" indication of novobiocin resistance of C-NS. These were urinary isolates which had been speciated according to Kloos and

Schleifer's simplified scheme (1975) including being tested for novobiocin resistance by conventional means. Sixty-two of these were S. saprophyticus (novobiocin-resistant) and twenty were S. epidermidis (novobiocin-susceptible). Novobiocin elution discs for use in the MS-2 were prepared as follows: Novobiocin (Sigma Chemical Co.) was carefully weighed and added to sterile water to achieve a concentration of 68 µg/ml. This solution was filter-sterilized using a 0.22 nm pore size filter (Nalge Co.) and 20 µl of the 68 µg/ml solution was applied to 6 mm diameter absorbent paper discs (Schleicher and Schuell, Inc., Keene, NH). Discs were allowed to dry at room temperature for 4-6 hours and were then stored at 4°C in a dessicator until used. Novobiocin elution discs were manually inserted into MS-2 Transfer Cuvette Cartridges while other antibiotics to be tested were loaded using the MS-2 Disc Loader/Sealer. The specially prepared elution discs provided a 1.6 ug/ml novobiocin concentration in the lower reaction chamber when the transfer of 0.85 ml of inoculated MS-2 broth occurred during the test. This concentration was used because the novobiocin-resistant human C-NS species (S. saprophyticus, S. cohnii, and S. xylosus) are generally resistant to concentrations of novobiocin up to 1.6 ug/ml of medium (Kloos and Smith, 1980). A pilot study was carried out

to determine a means for interpreting novobiocin susceptibility tests in the MS-2. For this purpose, two isolates of S. epidermidis (ES0451 and EE0470) and two of S. saprophyticus (SE1912 and SE2160) were grown overnight on Trypticase soy agar with 5% sheep blood (BBL). A sterile inoculating needle was touched to four-five well-isolated morphologically identical colonies on each plate and inoculated into 16 x 150 mm screw-top glass test tubes containing 4 ml of 0.85% sterile saline solution. Each tube was vigorously mixed to yield a uniform cell suspension and the turbidity was adjusted to a density between a 0.5 and a #1 McFarland opacity standard. The MS-2 Disc Loader/Sealer was used to load and seal the following antimicrobial discs into each of four MS-2 Transfer Cuvette Cartridges:

1) Novobiocin (1.4 μg)
2) Penicillin (1 unit)
3) Methicillin (5 μg)
4) Clindamycin (0.5 μg)
5) Gentamicin (4 μg)
6) Erythromycin (3 μg)
7) Nitrofurantoin (30 μg)
8) Ampicillin (2.5 μg)
9) Cephalothin (10 μg)
10) Trimethoprim-sulfamethoxazole (25 μg)

A 400 µl aliquot of the prepared saline suspension of each organism was pipetted into a Transfer Cuvette Cartridge containing 15 ml of MS-2 Culture Medium. Cartridges were then inserted into a MS-2 Analysis Module and the Research System version of the MS-2 (Figure 3), which includes graphics capabilities (CRT and hard-copy printer), was used for the determinations. The MS-2 was instructed to transfer the bacteria-medium mixture from the upper growth chamber to the lower reaction chambers of the transfer cartridges when the change in optical density reached 0.008 (as is routinely done using the clinical system programming for susceptibility testing). The cartridges were incubated and monitored overnight in the analysis modules before the growth curves were examined manually. After analyzing the results of the pilot study performed using the MS-2 Research System, the MS-2's clinical system programming was then asked to interpret results of novobiocin testing by instructing the program that an amikacin disc was occupying position #1. This was done since programming for interpretation of novobiocin was not included in the MS-2's antimicrobial battery routine. Thus, the system was instructed to interpret the growth kinetics in position #1, containing novobiocin, as if an amikacin disc had been placed there. Twenty isolates of S. epidermidis

Figure 3. Abbott MS-2 Research System

and sixty-two isolates of S. saprophyticus were tested in this manner.

2. Evaluation of the Vitek AutoMicrobic System (AMS) Gram-Positive Identification (GPI) Card

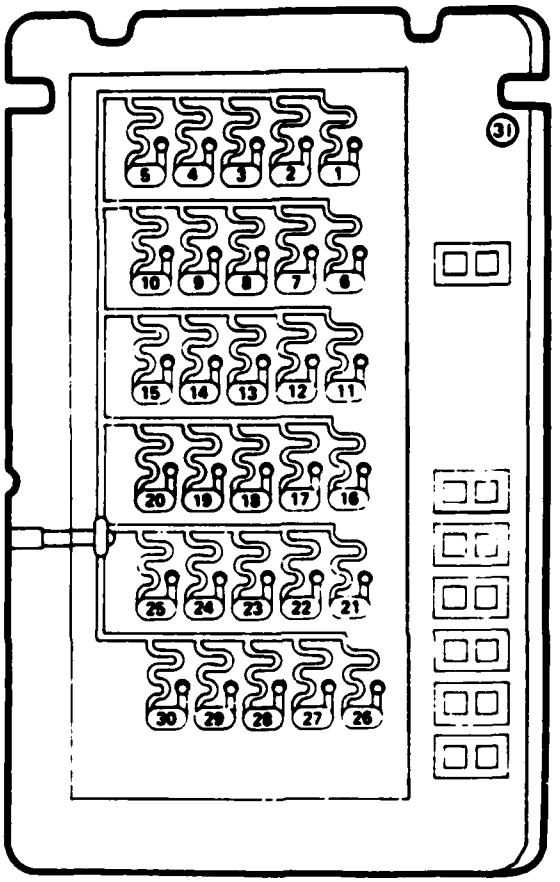
   a. GPI Card. The GPI Card is designed to be used in conjunction with the automated Vitek instrument (AMS), previously described by Aldridge et al. (1977) and shown in Figure 4. The card is said to allow species-level identification of S. aureus, S. epidermidis, S. saprophyticus, and S. hominis. Testing of other species of staphylococci should yield the response "Staphylococcus species," only (Ruoff et al., 1982). The GPI Card itself is a clear plastic card containing thirty wells in which test reactions occur (Figure 5).

   b. Bacteria. One hundred and fifty C-NS isolates which had been classified by Kloos's simplified scheme (1982) were tested with the GPI Card. The species and the number of isolates included in the study group were: S. saprophyticus (62), S. epidermidis (23), S. hominis (15), S. haemolyticus (15), S. simulans (15), S. warneri (15), and S. capitis (5). Additionally, ATCC strains of Staphylococcus epidermidis (12228) and Streptococcus faecalis (29212) were used as quality control organisms in accordance with the manufacturer's recommendations for checking the performance of the GPI Card. These isolates were first

Figure 4. Vitek AutoMicrobic System (AMS)

Figure 5. Vitek Gram-Positive Identification (GPI) Card

GRAM-POSITIVE ID CARD



WELL NUMBERS

1. POSITIVE CONTROL
2. BACITRACIN
3. OPTOCHIN
4. HEMICELLULASE
5. 5% NaCl
6. 10% BILE
7. 40% BILE
8. ESCULIN
9. ARGININE NEGATIVE CONTROL
10. ARGININE
11. UREA
12. TETRAZOLIUM RED
13. CARBOHYDRATE NEGATIVE CONTROL
14. GLUCOSE
15. LACTOSE
16. MANNITOL
17. RAFFINOSE
18. SALICIN
19. SORBITOL
20. SUCROSE
21. TREHALOSE
22. ARABINOSE
23. PYRUVATE
24. PULLULAN
25. INULIN
26. MELIBIOSE
27. MELEZITOSE
28. CELLOBIOSE
29. RIBOSE
30. POTASSIUM THIOCYANATE
31. CODE FOR CATALASE REACTION

streaked onto Trypticase soy agar with 5% sheep blood (BBL) and incubated at 35°C for 18-24 hours prior to testing.

c. Procedure (Vitek Product Insert for GPI Card, 1982, Vitek Systems, Inc., Hazelwood, MO).

1) The GPI Card was removed from its foil package and a black fine-tip marker (Sanford Corp., Bellwood, IL) was used to label the card with the appropriate sample number which would be visually interpreted by the instrument's optical scanner.

2) If the catalase test was positive (all staphylococcal isolates) the circular depression in the upper right corner of the card was blackened with the fine-tip marker. No mark in the depression indicated a catalase-negative reaction (as demonstrated by the ATCC quality control strain of Streptococcus faecalis).

3) Next, the AMS Diluent Dispenser Module was used to dispense 1.8 ml of sterile .45% saline into sterile 12 x 75 mm test tubes (one test tube per sample to be tested) placed in the tube holder section of AMS Filling Stands.

4) A sterile cotton swab was used to pick several morphologically similar colonies from a blood agar plate. The swab was then swirled in the

saline to achieve a bacterial suspension turbidity equivalent to that of a 0.5 McFarland opacity standard.

5) The short end of an AMS Transfer Tube was aseptically inserted into the port on the side of the GPI Card.

6) The mated Card/Transfer Tube unit was placed onto the appropriate AMS Filling Stand, with the long part of the Transfer Tube inserted into the test tube.

7) The Filling Stands were inserted into a Filling Rack (10 stands per rack) and the rack was placed into the Filling Module where card inoculation occurred.

8) The GPI Card/Filling Stand units were removed from the Filling Module and inserted into the Sealer Module which severed the Transfer Tube and sealed the card.

9) The sealed cards were placed into a Reader/Incubator tray which was inserted into the Reader/Incubator Module of the Vitek instrument.

10) The GPI Card biochemical results were then analyzed and stored automatically by the AMS programmed computer. At the completion of the incubation cycle (4-13 hours, depending on growth

rate), the Vitek's Data Terminal printed a status report for each card in the Reader/ Incubator.

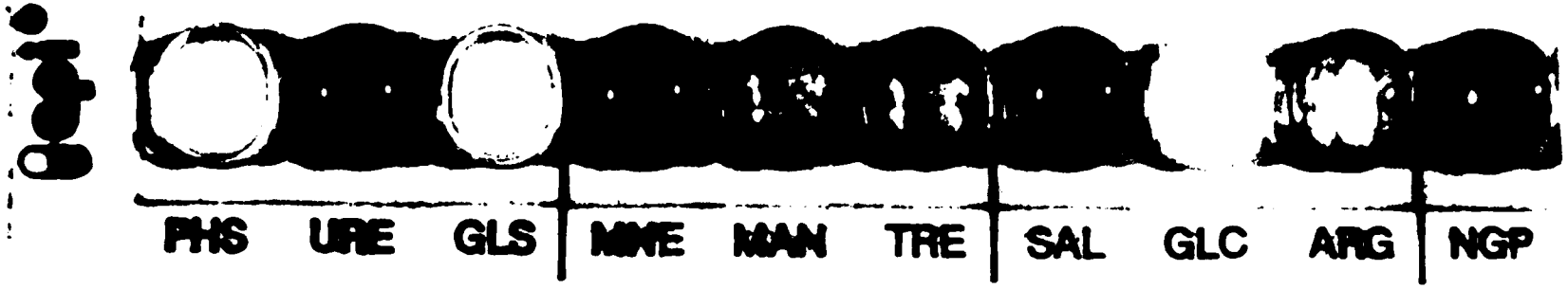
3. Evaluation of the API STAPH-IDENT System

   a. STAPH-IDENT Strip. The STAPH-IDENT strip, previously described by Kloos and Wolfshohl (1982) and shown in Figure 6, is a rapid manual method which employs ten tests (Table 4). The strip is said to allow species-level identification of the taxa listed in Table 5.

   b. Bacteria. Three hundred C-NS strains which had been classified by Kloos's simplified scheme (1982) were tested with the API STAPH-IDENT miniaturized biochemical test system. The species and the number of isolates included in this study group were: S. epidermidis (123), S. saprophyticus (64), S. hominis (48), S. simulans (22), S. haemolyticus (18), S. warneri (17), and S. capitis (8). Additionally, ATCC strains of S. epidermidis (14990), S. simulans (27851), S. sciuri (29060), and S. aureus (25923) were tested, in accordance with the manufacturer's recommendations, to insure that the API strip was performing properly. All isolates to be tested were first streaked onto Trypticase soy agar with 5% sheep blood (BBL) and incubated at 35°C for 18-24 hours.

   c. Procedure (API Product Insert for STAPH-IDENT System, 1981, Analytab Products, Plainview, NY).

Figure 6. API STAPH-IDENT System



STAPH-IDENT™

TABLE 4

API STAPH-IDENT SYSTEM TESTS

ABBREVIATION	TEST
PHS	Phosphatase
URE	Urea Utilization
GLS	Beta-Glucosidase
MNE	Mannose Utilization
MAN	Mannitol Utilization
TRE	Trehalose Utilization
SAL	Salicin Utilization
GLC	Beta-Glucuronidase
ARG	Arginine Utilization
NGP	Beta-Galactosidase

TABLE 5

TAXA IN THE API STAPH-IDENT SYSTEM DATA BASE

Human Species

S. aureus	S. haemolyticus
S. epidermidis	S. xylosus
S. saprophyticus	S. warneri
S. hominis	S. capitis
S. simulans	S. cohnii
S. sciuri	

Animal Species

S. hyicus

S. intermedius

1) Incubation trays were labeled with the appropriate isolate number and filled with 5 ml of tap water.
2) The API strips were removed from their sealed envelopes and one strip was placed in each tray.
3) A sterile cotton swab was used to pick several morphologically similar colonies from a blood agar plate. The swab was then swirled in 5 ml of API saline (0.85%) so that the final turbidity was equivalent to that of a McFarland #3 turbidity standard.
4) A 5 ml Pasteur pipette was used to dispense three drops of the adjusted bacterial suspension into each of the ten strip microcupules containing lyophilized substrates.
5) After inoculation, a plastic lid was placed on each tray and the strips were incubated for five hours at 35°C in a non-CO_2 incubator.
6) After 5 hours of incubation, the strips were removed from the incubator and a bottle of .35% Fast Blue BB Salt solution in methoxyethanol was removed from the refrigerator and allowed to come to room temperature before use.
7) The results for all tests with the exception of B-galactosidase (NGP) were recorded on API report sheets. Two drops of the .35% Fast Blue

BB Salt solution was then added to the NGP microcupule and 30 seconds was allowed for color development. After the 30 second waiting period, the NGP results were recorded.

8) After all results were recorded, a four digit profile code number was derived. The ten biochemical tests were divided into four groups as follows:

PHS	MNE	SAL	NGP
URE	MAN	GLC	
GLS	TRE	ARG	

Only positive reactions were assigned a numerical value. The value was dependent upon the location within the group, e.g.:

A value of ONE for the first biochemical in each group (for example, PHS, MNE, SAL, and NGP).

A value of TWO for the second biochemical in each group (for example, URE, MAN, and GLC).

A value of FOUR for the third biochemical in each group (for example, GLS, TRE, and ARG).

A four digit profile number was obtained by totaling the values of each of the groups. This number was located in API's STAPH-IDENT Profile Register (provided as a package insert with the STAPH-IDENT strips) to obtain an isolate identification. Strictly for the purpose of this study, API also provided a computer printout

which represented all possible STAPH-IDENT profiles, a first, second, and third identification choice for each profile number, an "estimated frequency of occurrence" for each choice, and an identification comment. This printout provided information which ordinarily would be available by telephone inquiry to API using a toll-free telephone number.

9) When a discrepancy arose between an isolate's classification by Kloos's simplified scheme (1982) and its identification by the API STAPH-IDENT System, the isolate was sent to Dr. Wesley Kloos at North Carolina State University for further testing and final arbitration.

III. RESULTS

A. Incidence of C-NS Species from Patients in South Texas

Kloos's scheme was used to classify 289 Bexar County Hospital District outpatient and inpatient clinical isolates which resulted in the body site distribution of C-NS species shown in Table 6. Seven of the ten human C-NS species were isolated from clinical samples. The isolated species included: S. epidermidis, S. haemolyticus, S. hominis, S. warneri, S. capitis, S. saprophyticus, and S. simulans. S. auricularis, S. cohnii, and S. xylosus were not isolated in this group of 289 cultures. Every C-NS species listed in Table 6 was isolated from a variety of sources except for S. saprophyticus which was found mainly in urine with two isolates recovered from blood cultures.

B. Clinical Significance of C-NS Isolates Encountered in the Study Group

After applying the criteria discussed in Materials and Methods to the S. saprophyticus isolates, 97.1% (34/35) of the S. saprophyticus urine isolates were considered to be "clinically significant," i.e., they contained greater than 10,000 organisms per ml of urine. Parameters used to determine the presence of UTI also included: physician's diagnosis, and typical symptoms including dysuria, urgency, frequency, burning, and pyuria. The two S. saprophyticus blood culture isolates were not considered to be "clinically significant" because they were isolated only from single sets of blood cultures. When the same criteria were applied to the S. epidermidis urine isolates, 40.4% (19/47) were classified as "clinically significant." Sixty-four percent of all C-NS

TABLE 6

DISTRIBUTION OF C-NS SPECIES BY BODY SITE FROM PATIENTS SEEN IN THE BEXAR COUNTY HOSPITAL DISTRICT

Species	Blood	Exudate	Genital	Urine	Other	Total
S. epidermidis	62	23	8	47	16	156
S. haemolyticus	0	4	4	4	3	15
S. hominis	19	5	0	9	6	39
S. warneri	6	4	4	1	1	16
S. capitis	5	2	0	0	1	8
S. saprophyticus	2	0	0	35	0	37
S. simulans	8	3	2	5	0	18
					GRAND TOTAL	289

urine specimens with greater than 100,000 bacteria/ml yielded S. saprophyticus while 27% yielded S. epidermidis.

C. Significance of Species-Level Identification of C-NS Isolated from Urine

1. Fourteen records were located from patients with greater than 10,000 S. epidermidis per ml of urine. Twelve of these records contained some evidence of C-NS UTI in addition to colony count and seven patients had a previous history of UTI. The average age of this population was 41 years. Seventy-five percent of these patients were hospitalized and 75% were females. Two of the females were pregnant and one was a diabetic.

Table 7 contains data on all of the S. epidermidis UTI cases, several of which may have acquired nosocomial infections. Four of the cases shown involved the use of catheters while most of the remaining patients suffered from conditions which interfered with the normal excretion of urine.

Twenty-nine records were located from patients with greater than 10,000 S. saprophyticus per ml of urine. Twenty-eight of these records showed evidence of C-NS UTI in addition to colony count and 12 patients had a previous history of UTI. The average age of this population was 25 years. Only one patient (4%) was hospitalized and 96% were females. Twenty-six percent of the female sub-population were pregnant and four patients were diabetics.

TABLE 7

PROMINENT CHARACTERISTICS OF *S. EPIDERMIDIS* UTI CASES

Patient	Status	Age	Sex	cfu/ml	Predisposing Factors
C.H.	IP	18	F	5×10^4	Catheterized - postpartum
M.O.	IP	32	F	$> 10^5$	Catheterized
S.S.	IP	33	F	5×10^4	Catheterized - end stage renal disease - Previous UTI's
M.S.	IP	44	F	5×10^4	Catheterized - diabetic with pyelonephritis - Previous UTI's
N.T.	IP	5 mo.	F	5×10^4	Anatomic abnormality - Grade IV reflux - Previous UTI's
T.B.	IP	70	M	$> 10^5$	Urinary retention secondary to prostate hypertrophy - Previous UTI's
S.M.	IP	81	M	$> 10^5$	Urinary retention secondary to prostate hypertrophy - Previous UTI's
H.L.	IP	78	M	$> 10^5$	Obstructive bladder tumor - Previous UTI's
D.W.	IP	18	F	5×10^4	Bladder tumor
H.P.	OP	75	F	$> 10^5$	Previous UTI's
M.A.	OP	22	F	$> 10^5$	Pregnant
M.R.	OP	25	F	$> 10^5$	None observed

Table 8 contains details about all of the S. saprophyticus UTI cases, the majority of patients presenting with high urine colony counts. Seven cases involved pregnancies and eight patients apparently had no predisposing factors.

2. Table 9 shows the species-level identifications of 46 urine isolates collected in 1974-77 from bacteriuric patients and previously identified as S. epidermidis (i.e., coagulase-negative Micrococcaceae). S. epidermidis was isolated more frequently than S. saprophyticus in this study. All of the 19 S. epidermidis patients and ten of the 11 S. saprophyticus patients were outpatient females. Eight of the S. epidermidis (42%) and nine of the S. saprophyticus outpatient females (90%) were attending pre-natal care clinics. Diabetic patients accounted for seven of the S. epidermidis patients (37%) and one of the S. saprophyticus (10%) female outpatients. Direct immunofluorescence testing of these isolates by Thomas et al. (1974) indicated that one patient from whom S. saprophyticus was isolated had an infection of the upper urinary tract (fluorescent antibody positive) while the remainder suffered from lower UTIs (fluorescent antibody negative).

3. Table 10 contains the Bauer-Kirby disc diffusion susceptibility test results on S. epidermidis and S. saprophyticus urine isolates. S. saprophyticus isolates were generally more susceptible to a wider range of antimicrobics than

TABLE 8

PROMINENT CHARACTERISTICS OF *S. SAPROPHYTICUS* UTI CASES

Patient	Status	Age	Sex	cfu/ml	Predisposing Factors
N.G.	OP	15	F	$> 10^5$	Pregnant
M.G.	OP	17	F	$> 10^5$	Pregnant with recurrent pyelonephritis
M.Y.	OP	17	F	$> 10^5$	Pregnant
M.B.	OP	18	F	$> 10^5$	Pregnant
I.C.	OP	19	F	$> 10^5$	Pregnant
R.L.	OP	21	F	$> 10^5$	Pregnant
J.N.	OP	21	F	$> 10^5$	Previous UTI's
A.R.	OP	22	F	5×10^4	Previous UTI's
D.K.	OP	22	F	$> 10^5$	Previous UTI's
M.P.	OP	22	F	5×10^4	Previous pyelonephritis
T.R.	OP	22	F	$> 10^5$	Previous UTI's
R.I.	OP	23	F	$> 10^5$	Previous UTI's
V.H.	OP	24	F	5×10^4	Previous UTI's
M.C.	OP	26	F	$> 10^5$	Previous UTI's
E.Y.	OP	27	F	$> 10^5$	Previous pyelonephritis
C.G.	OP	36	F	$> 10^5$	Previous UTI's
M.I.	OP	16	F	5×10^4	None observed
A.F.	OP	17	F	$> 10^5$	None observed
G.B.	OP	18	F	$> 10^5$	None observed
D.R.	OP	19	F	$> 10^5$	None observed
C.G.	OP	20	F	$> 10^5$	None observed
R.G.	OP	23	F	$> 10^5$	None observed
M.O.	OP	38	F	$> 10^5$	None observed
D.K.	OP	22	F	$> 10^5$	Diabetic
G.O.	OP	41	F	$> 10^5$	Diabetic and pregnant Previous UTI's
M.G.	OP	51	F	$> 10^5$	Diabetic
M.C.	OP	53	F	$> 10^5$	Diabetic
D.R.	IP	27	M	5×10^4	None observed

TABLE 9

SUMMARY OF 46 PATIENTS EXAMINED FOR ANTIBODY-COATED BACTERIA IN URINE CULTURES CONTAINING > 10^5 C-NS/ML

Species Present	No. Patients	Percent of Total
S. epidermidis	19	41%
S. saprophyticus	11	24%
S. hominis	6	13%
S. simulans	5	11%
S. haemolyticus	4	9%
S. warneri	1	2%

TABLE 10

ANTIMICROBIC SUSCEPTIBILITY TEST RESULTS ON SELECTED

S. EPIDERMIDIS AND *S. SAPROPHYTICUS* ISOLATES

Antimicrobic	% Susceptible	
	S. epidermidis (n = 19)	*S. saprophyticus* (n = 46)
Penicillin	5	37
Oxacillin	53	32
Ampicillin	6	68
Tetracycline	68	83
Nitrofurantoin	100	100
Sulfa-trimethoprim	68	98
Gentamicin	58	100
Clindamycin	63	98
Cephalothin	100	100
Nalidixic Acid	0	0
Sulfathiazole	92	98

S. epidermidis isolates. The exceptions to this generalization were:

Oxacillin - the percentage of S. epidermidis susceptible isolates was greater than the proportion of S. saprophyticus isolates which were susceptible.

Nitrofurantoin and cephalothin - 100% of both species were susceptible.

Nalidixic acid - 100% of both species were resistant.

The same isolates which underwent antimicrobic susceptibility testing were also checked for beta-lactamase production. The presence or absence of these enzymes was determined by using a rapid (one hour) beta-lactamase detection procedure which incorporates a chromogenic cephalosporin substrate (BBL Cefinase discs). Ninety-five percent of the S. epidermidis and seventy percent of the S. saprophyticus isolates demonstrated beta-lactamase activity.

D. Growth Rates and Tissue Adherence Properties of C-NS Species from Human Urine

1. Growth Rate Studies: The results of the experiments performed using the Abbott MS-2 Microbiology System are contained in Table 11. The Gram-negative organisms demonstrated shorter mean generation times in urine than the Gram-positive pathogens tested. The mean generation time of the S. saprophyticus isolates tested (47 minutes) was slightly longer than that of the S. epidermidis isolates tested (43 minutes). Genera-

TABLE 11

RESULTS OF GROWTH RATE STUDIES OF SELECTED URINARY PATHOGENS

IN HUMAN URINE AND BACTERIOLOGIC MEDIUM

Organism	No. Strains Tested	Mean Generation Time (min.)		
		Urine (pH = 6)	Eugon broth (pH = 7)	Eugon broth (pH = 6)
E. coli	3	25	19	20
Klebsiella	3	24	19	20
P. mirabilis	3	25	22	22
Enterococcus	3	35	23	28
S. epidermidis	3	43	34	36
S. saprophyticus	3	47	29	33

times in eugon broth were shorter than those in urine. When eugon broth was adjusted to the same pH as pooled urine (Table 11), there appeared to be little or no effect on generation times for Gram-negative organisms, while generation times of Gram-positive organisms increased slightly.

2. Tissue Culture Adherence Studies

   a. Hemagglutination Activity: Twenty-two S. epidermidis and 62 S. saprophyticus urine isolates were tested for the ability to hemagglutinate human and sheep erythrocytes. None of the S. epidermidis strains demonstrated hemagglutination with either species of red blood cells, and none of the S. saprophyticus isolates hemagglutinated human erythrocytes. However, six S. saprophyticus isolates (ATCC 15305, E2160, M1793, E2064, E1716, and P890) hemagglutinated sheep erythrocytes.

   b. Cell Culture Adherence Assays: The ability of four S. epidermidis and four S. saprophyticus urine isolates to adhere to HeLa, Vero, and MDCK cultured cells was studied. The results of these studies are shown in Tables 12-14. An obvious difference existed between the number of cells to which S. epidermidis and S. saprophyticus had adhered. The mean numbers of bacteria adherent to individual cells in culture further support the concept that a difference exists in adherence rates between these two species of C-NS. By application of the Mann-Whitney U Test for two

TABLE 12

HeLa CELL ADHERENCE OF *S. SAPROPHYTICUS* AND *S. EPIDERMIDIS*

Isolate	No. Cells to Which Bacteria Had Adhered/100 cells	No. of Bacteria Adhered/Cell (40 cell count)		
		Mean	Range	S.D.
Negative Control	0	0		
S. epidermidis G1450	6	0.02	0-1	0.16
S. epidermidis S451	12	0.05	0-1	0.22
S. epidermidis E2008	8	0.13	0-2	0.46
S. epidermidis S923	14	0.30	0-3	0.79
S. saprophyticus ATCC 15305	72	6.4	0-38	8.5
S. saprophyticus P890	53	2.1	0-7	1.9
S. saprophyticus G303	46	1.5	0-10	2.4
S. saprophyticus E296	98	8.1	1-23	6.0
S. saprophyticus M1468	85	3.7	0-13	3.3

$p < .008$

(Mann-Whitney U Test)

TABLE 13

VERO CELL ADHERENCE OF *S. SAPROPHYTICUS* AND *S. EPIDERMIDIS*

Isolate	No. Cells to Which Bacteria Had Adhered/100 Cells	No. of Bacteria Adhered/Cell (40 Cell Count)		
		Mean	Range	S.D.
Negative Control	0	0		
S. epidermidis G1450	2	0.10	0-2	0.38
S. epidermidis S451	4	0.10	0-2	0.44
S. epidermidis E2008	4	0.05	0-1	0.22
S. epidermidis S923	10	0.25	0-3	0.78
S. saprophyticus ATCC 15303	76	2.4	0-11	2.4
S. saprophyticus P890	84	2.2	0-12	2.2
S. saprophyticus G303	80	2.4	0-25	2.1
S. saprophyticus E296	58	1.7	0-9	1.8
S. saprophyticus M1468	84	2.4	0-10	2.1

$p < .008$

(Mann-Whitney U Test)

TABLE 14

MDCK CELL ADHERENCE OF *S. SAPROPHYTICUS* AND *S. EPIDERMIDIS*

Isolate	No. Cells to Which Bacteria Had Adhered/100 Cells	No. of Bacteria Adhered/Cell (40 Cell Count)		
		Mean	Range	S.D.
Negative Control	0	0		
S. epidermidis G1450	4	0.03	0-1	0.16
S. epidermidis S451	9	0.28	0-2	0.68
S. epidermidis E2008	4	0.08	0-1	0.27
S. epidermidis S923	0	0		
S. saprophyticus ATCC 15305	59	3.9	0-24	6.1
S. saprophyticus P890	53	0.50	0-18	1.7
S. saprophyticus G303	37	0.65	0-6	0.81
S. saprophyticus E296	28	0.43	0-3	0.81
S. saprophyticus M1468	36	0.33	0-5	0.89

$p < .008$

(Mann-Whitney U Test)

independent groups to these data, one finds that S. saprophyticus isolates adhered in significantly greater numbers ($p < .008$) to all three cell lines than did S. epidermidis isolates. There appeared to be no correlation between an isolate's ability to hemagglutinate erythrocytes and its ability to adhere to tissue culture cells. HeLa and Vero cells worked equally well for demonstrating adherence rates. The MDCK cells, conversely, were very resistant to trypsinization, tended to form small clumps in solution, and occasionally disintegrated when stained. Therefore, use of this cell line was not emphasized.

1) Influence of pH on adherence. When two S. saprophyticus isolates were resuspended in tubes of PBS which were adjusted to pH values ranging from 5-9, and the bacterial suspensions were used in an adherence assay with HeLa cells, the results in Table 15 were obtained. The two suspensions with the lowest pH values (pH 5 and 6) caused the monolayer to dissociate from the tissue culture dish after the 60 minute incubation of cells and bacteria at 37°C. Maximal adherence of S. saprophyticus ATCC 15305 was obtained at pH 8 while S. saprophyticus E296 displayed optimal adherence at pH 7.

TABLE 15

INFLUENCE OF pH ON ADHERENCE OF S. SAPROPHYTICUS ISOLATES ATCC 15305 AND E296 TO HeLa CELLS

pH of Phosphate Buffered Saline	No. Cells to Which Bacteria Had Adhered/100 Cells	No. of Bacteria Adhered/Cell (40 Cell Count)		
		Mean	Range	S.D.
	ATCC 15305			
5	Monolayer disrupted			
6	Monolayer disrupted			
7	78	2.0	0-15	2.7
8	85	4.4	0-14	4.4
9	74	3.8	0-45	7.3
	E296			
5	Monolayer disrupted			
6	Monolayer disrupted			
7	98	8.1	1-23	6.0
8	72	2.8	0-12	2.6
9	76	3.9	0-18	4.2

2) Influence of incubation temperature on adherence. The results of using three different incubation temperatures in adherence assays involving two S. saprophyticus isolates and HeLa cells are shown in Table 16. Maximal adherence for both isolates was observed at 37°C.

3) Influence of incubation time on adherence. When different incubation periods were used in adherence assays involving two S. saprophyticus isolates and HeLa cells, the results in Table 17 were obtained. Increasing the incubation time past one hour caused a decrease in adherence rates in S. saprophyticus ATCC 15305 and did not significantly increase adherence rates in S. saprophyticus E296.

4) Influence of bacterial culture medium on adherence. When two S. saprophyticus isolates were grown at 37°C for 20 hours on sheep blood agar plates or in BME without antibiotics and then used directly in HeLa cell adherence assays instead of being grown up in tryptic soy broth (Materials and Methods, para. D.2.a.), the results in Table 18 were observed. Adherence rates obtained with both isolates when either of the modifications were applied were lower than rates obtained when the original procedure

TABLE 16

INFLUENCE OF INCUBATION TEMPERATURE ON ADHERENCE OF S. SAPROPHYTICUS ISOLATES ATCC 15305 AND E296 TO HeLa CELLS

Incubation Temperature (°C)	No. Cells to Which Bacteria Had Adhered/100 Cells	No. of Bacteria Adhered/Cell (40 Cell Count)		
		Mean	Range	S.D.
	ATCC 15305			
4	67	1.9	0-5	1.9
25	85	4.5	0-18	4.3
37	94	5.1	0-15	4.1
	E296			
4	44	1.1	0-3	1.1
25	69	1.8	0-10	2.0
37	98	8.1	1-23	6.0

TABLE 17

INFLUENCE OF INCUBATION TIME ON ADHERENCE OF S. SAPROPHYTICUS ISOLATES ATCC 15305 AND E296 TO HeLa CELLS

Incubation Time (37°C)	No. Cells to Which Bacteria Had Adhered/100 Cells	No. of Bacteria Adhered/Cell (40 Cell Count)		
		Mean	Range	S.D.
	ATCC 15305			
1 h.	94	5.1	0-15	4.1
2 h.	93	5.0	0-15	3.6
3 h.	67	1.9	0-20	3.3
4 h.	70	1.6	0-6	1.4
	E296			
1 h.	98	8.1	1-23	6.0
2 h.	95	3.0	0-9	2.4
3 h.	94	5.2	0-16	4.2
4 h.	99	6.0	0-25	6.0

TABLE 18

INFLUENCE OF BACTERIAL CULTURE MEDIUM USED FOR PREPARATION OF BACTERIAL INOCULUM ON ADHERENCE OF S. SAPROPHYTICUS ISOLATES ATCC 15305 AND E296 TO HeLa CELLS

Medium	No. Cells to Which Bacteria Had Adhered/100 Cells	No. of Bacteria Adhered/Cell (40 Cell count)		
		Mean	Range	S.D.
	ATCC 15305			
TSB*	72	6.4	0-38	8.5
SBAP**	68	3.4	0-11	5.9
BME***	12	0.18	0-2	0.5
	E296			
TSB*	98	8.1	1-23	6.0
SBAP**	91	4.8	0-18	4.1
BME***	71	1.2	0-7	1.8

*TSB: Tryptic soy broth (normal adherence procedure)

**SBAP: Sheep blood agar plates

***BME: Basal Medium Eagle without antibiotics

was followed. The adherence rates were markedly lower when BME, a chemically-defined medium, was used as a bacterial culture medium, but only slightly lower when sheep blood agar, a complex medium like tryptic soy broth, was used as the final bacteriologic growth medium.

E. Practical Means for Species-Level Identification of C-NS

1. Determination of Novobiocin Susceptibility

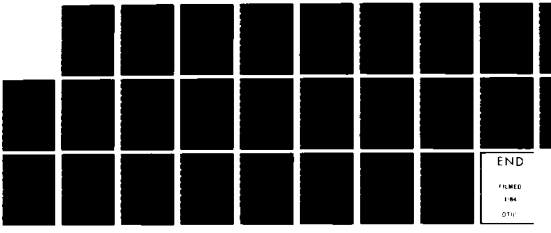
a. The 289 C-NS cultures from the Medical Center Hospital were categorized into 37 novobiocin-resistant isolates (all S. saprophyticus) and 252 novobiocin-susceptible isolates comprised of the following species: S. epidermidis (156), S. hominis (39), S. simulans (18), S. warneri (16), S. haemolyticus (15), and S. capitis (8).

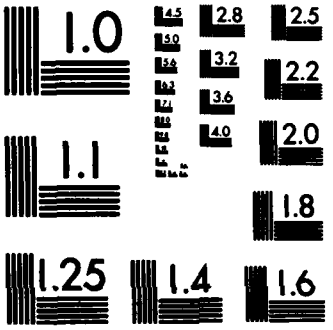
The 46 urine isolates previously collected from bacteriuric patients from 1974-77 were categorized into 11 novobiocin-resistant isolates (all S. saprophyticus) and 35 novobiocin-susceptible isolates consisting of the following species: S. epidermidis (19), S. hominis (6), S. simulans (5), S. haemolyticus (4), and S. warneri (1).

b. In the study involving the testing of 254 C-NS isolates in parallel for novobiocin susceptibility on P agar and Mueller-Hinton agar, a zone diameter of 16 mm appeared to represent a breakpoint between novobiocin-susceptible and -resistant C-NS (Table 19). Thus, isolates having a zone diameter greater than 16 mm were considered suscep-

AD-A135 368 PATHOPHYSIOLOGIC AND TAXONOMIC PROPERTIES OF COAGULASE-NEGATIVE MICROCOCCACEAE(U) AIR FORCE INST OF TECH WRIGHT-PATTERSON AFB OH R J ALMEIDA MAY 83 2/2

UNCLASSIFIED AFIT/CI/NR-83-65T F/G 6/13 NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TABLE 19

MEAN NOVOBIOCIN ZONE DIAMETERS OF C-NS ON P AGAR AND MUELLER-HINTON AGAR

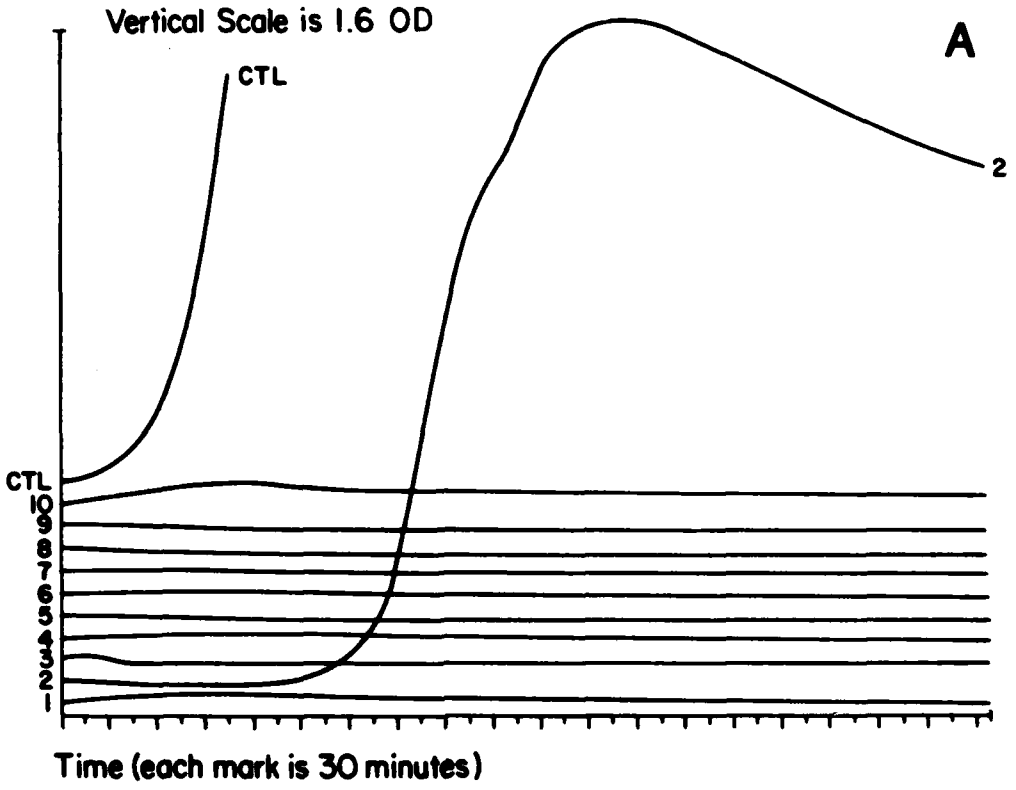
Bacteria	Zone Diameter[a]	
	P Agar	Mueller-Hinton Agar
Novobiocin-susceptible staphylococci (> 16 mm)	28.6 ± 4.1	29.0 ± 3.6
Novobiocin-resistant staphylococci (≤ 16 mm)	7.5 ± 3.0	10.1 ± 1.9

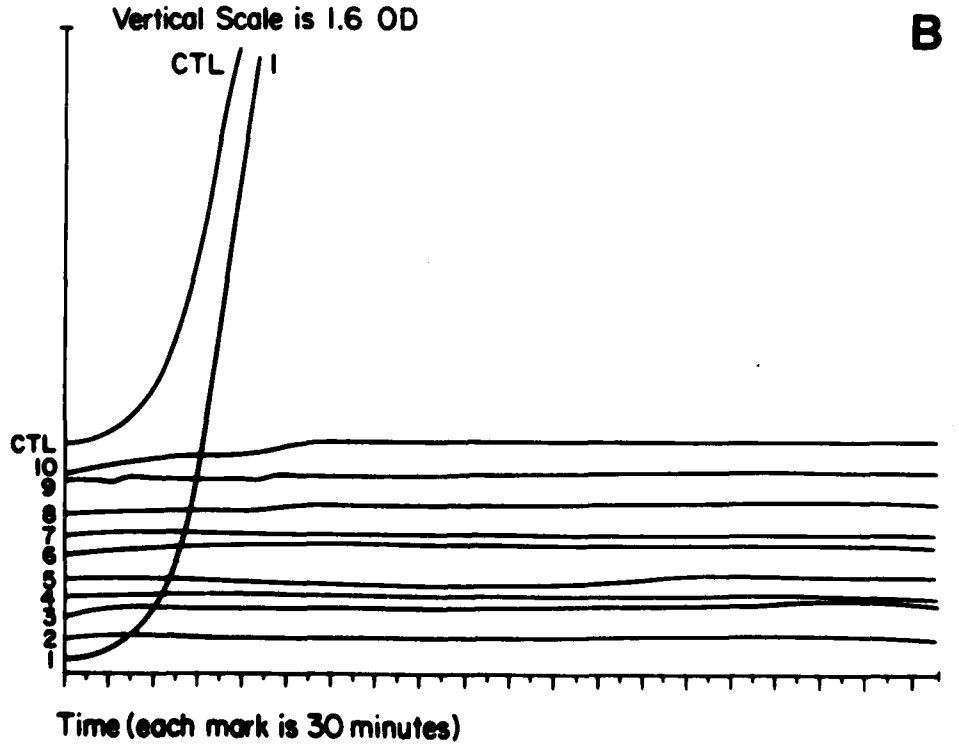
[a]Mean zone size ± 1 standard deviation.

tible (S. epidermidis, S. simulans, S. haemolyticus, S. hominis, S. warneri, and S. capitis) while those with a diameter of 16 mm or less were considered resistant (S. saprophyticus). A total of 222 isolates were susceptible to novobiocin on both P agar and Mueller-Hinton agar by the same breakpoint criteria, whereas 31 isolates (all S. saprophyticus) were novobiocin-resistant on both media, for a 99.6% agreement. Only one isolate (S. simulans) was classified as susceptible to novobiocin on P agar (19 mm) but resistant on Mueller-Hinton agar (16 mm). The correlation coefficient of zone diameters as determined on both media was 0.93.

c. The growth curves of two C-NS isolates used in the pilot study to determine a means for interpreting Abbott MS-2 novobiocin susceptibility tests are shown in Figure 7. S. epidermidis EE0470 showed uninhibited growth in the control position of the cuvette and in position #2 (penicillin disc). Growth was inhibited, however, in position #1 (novobiocin disc). S. saprophyticus SE1912 showed growth in the control position and in position #1 (novobiocin disc). The results were identical to results of novobiocin susceptibility testing of these strains by Kloos and Schleifer's simplified method. They also were identical to the Bauer-Kirby disc diffusion test results for the nine other antimicrobial agents included in the MS-2 system.

Figure 7. Abbott MS-2 System Growth Curves of S. epidermidis EE0470 (Fig. 7A) and S. saprophyticus SE1912 (Fig. 7B) Tested Against Ten Antimicrobial Agents. CTL = growth control; position 10 = trimethoprim-sulfamethoxazole (25 μg); 9 = cephalothin (10 μg); 8 = ampicillin (2.5 μg); 7 = nitrofurantoin (30 μg); 6 = erythromycin (3 μg); 5 = gentamicin (4 μg); 4 = clindamycin (0.5 μg); 3 = methicillin (5 μg); 2 = penicillin (1 unit); 1 = novobiocin (1.4 μg). OD = optical density.


Vertical Scale is 1.6 OD
A
CTL
2
CTL
10
9
8
7
6
5
4
3
2
1
Time (each mark is 30 minutes)


Vertical Scale is 1.6 OD
B
CTL
1
CTL
10
9
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7
6
5
4
3
2
1
Time (each mark is 30 minutes)

Of the 20 S. epidermidis isolates tested using the Abbott MS-2's clinical system programming, 18 were interpreted by the MS-2 as being susceptible to novobiocin when programmed interpretive criteria for amikacin were used. The remaining two isolates were reported as having an intermediate reaction to novobiocin (amikacin). Of the 62 S. saprophyticus isolates tested, 57 were interpreted by the MS-2 as being resistant to novobiocin (amikacin). Two isolates were interpreted as having an intermediate reaction, and three isolates failed to grow adequately for testing in the MS-2 medium. Therefore, in comparison to Kloos and Schleifer's simplified method for determining novobiocin resistance, the MS-2 system was able to correctly classify 90% and 92%, respectively, of S. epidermidis and S. saprophyticus strains with amikacin susceptibility criteria. Although the program for interpretation of amikacin kinetics was used in these experiments, close inspection of Figures 7A and 7B suggests that the kinetics of inhibition by novobiocin with the isolates were similar to those of all of the other antimicrobial agents tested. Thus, similar results probably could have been obtained with interpretive programming for any of the other drugs.

The average time of incubation necessary to produce results for both the S. epidermidis and S. saprophyticus strains was 5.8 hours (range: 4.8 to 6.1 hours).

2. Table 20 compares the C-NS species identifications obtained by Kloos and Schleifer's conventional scheme and the Vitek AutoMicrobic System GPI Card. For the purpose of this study, an AMS identification probability of 80% or greater was considered an acceptable identification. The GPI Card demonstrated its greatest accuracy with S. epidermidis isolates (82.6% correct identifications), and was least able to identify S. hominis isolates (26.7% correct). The overall agreement between GPI Card identifications and those using the scheme of Kloos and Schleifer was only 59.3%. For those 50 isolates tested which the GPI Card was expected to classify only as "Staphylococcus species" (S. haemolyticus, S. simulans, S. warneri, and S. capitis), an acceptable identification was obtained only 40% of the time. The average incubation time needed for a correct identification of C-NS isolates in this study was 10.7 hours. The AMS did not recommend that supplemental testing be performed to clarify or improve the identification accuracy on any isolates tested.

TABLE 20

C-NS IDENTIFICATIONS OBTAINED BY CONVENTIONAL METHODS

AND BY USE OF THE VITEK AMS GPI CARD

	No. Isolates Tested	No. Isolates Correct by GPI Card	% Correct for GPI Card	Average Elapsed Time
S. saprophyticus	62	46	74.2%	9.2 h.
S. epidermidis	23	19	82.6%	13.0 h.
S. hominis	15	4	26.7%	10.5 h.
Staphylococcus spp.	50	20	40.0%	11.6 h.
Overall	150	89	59.3%	10.7 h.

3. The accuracy of the API STAPH-IDENT System identifications for each species as compared to classification by Kloos and Schleifer's simplified scheme is shown in Table 21. Use of the API strip allowed correct classification of between 71% and 100% of all species tested. This resulted in an overall agreement of 92.7% (278/300) when compared with the conventional classification scheme. However, Table 22 shows that while 63% of the isolates were identified correctly by the STAPH-IDENT strip alone, 25.7% (77/300) of the isolates required supplemental testing, 4% (12/300) were considered to be only partly correct due to an identification comment of "good likelihood, but low selectivity identification," and 7.3% (22/300) were incorrectly identified. The species incorrectly identified by STAPH-IDENT consisted of 14 S. hominis, seven S. epidermidis (six of these were phosphatase-negative by both API and conventional methods), and one S. saprophyticus isolate. Table 23 provides a tabulation of the supplemental tests required for a correct identification of 77 of the isolates. Novbiocin susceptibility testing was the supplemental procedure most often required (80.5% of all supplemental tests), although xylose acidification and coagulase tests were required for correct identification of some isolates.

TABLE 21

COMPARISON OF API STAPH-IDENT RESULTS WITH IDENTIFICATIONS BASED ON THE KLOOS AND SCHLEIFER SCHEME

C-NS Species	No. Isolates	No. Correct by API	% Correct by API
S. epidermidis	123	116	94%
S. hominis	48	34	71%
S. haemolyticus	18	18	100%
S. warneri	17	17	100%
S. capitis	8	8	100%
S. saprophyticus	64	63	98%
S. simulans	22	22	100%
Total: 300 Isolates		Overall Agreement:	92.7%

TABLE 22

ANALYSIS OF API STAPH-IDENT IDENTIFICATION RESULTS

Outcome	No. Isolates	% of Total	Cumulative % of Total
Correct ID (Strip Alone)	189	63%	63%
Correct ID (W/Supplemental Tests)[a]	77	25.7%	88.7%
Partly Correct ID (GLLS)[b]	12	4%	92.7%
Incorrect ID	22	7.3%	

Total: 300 Isolates

[a]See text and Table 23 for supplemental tests required

[b]GLLS - good likelihood, but low selectivity, see text for explanation

TABLE 23

SUPPLEMENTAL TESTS REQUIRED FOR CORRECT IDENTIFICATIONS

USING THE API STAPH-IDENT SYSTEM

Supplemental Test	No. Times Required[a]	% of Supplemental Tests
Novobiocin	62	80.5%
Xylose	12	15.6%
Coagulase	3	3.9%
TOTAL:	77	

[a]Never more than one supplemental test required/isolate

Table 24 is a tabulation of the STAPH-IDENT Analytical Profile Index (computer printout) comments associated with the C-NS identifications. Sixty-five percent of the profiles had an associated comment of either "excellent," "very good," "good," or "acceptable." However, 35% (105/300) of the identifications were considered to be good likelihood, but low selectivity identifications (GLLS). The presence of a GLLS identification comment indicated that there was no significant difference between the likelihood of species listed as the first, second, or sometimes third choice for a given profile number in the Analytical Profile Index. Two C-NS species were associated with a disproportionate number of GLLS comments; eighty-seven percent of all S. hominis identifications and 38% of all S. warneri identifications had GLLS profiles.

Table 25 shows the number of isolates which had four digit profile numbers which were listed in the STAPH-IDENT Profile Register and those which were not. The Register is a one page list of profile numbers and corresponding identifications which is included in each STAPH-IDENT kit. Although all of the 300 C-NS isolates in this study had profile numbers which were listed in API's expanded computer

TABLE 24

TABULATION OF API STAPH-IDENT ANALYTICAL PROFILE INDEX COMMENTS

Profile Index Comment	No. of Isolates	% of Isolates	Cumulative % of Total
Excellent ID	123	41%	41%
Very Good ID	2	0.7%	41.7%
Good ID	8	2.7%	44.4%
Acceptable ID	62	20.6%	65%
GLLS[a]	105	35%	

Total: 300 Isolates

[a]GLLS - good likelihood, but low selectivity

TABLE 25

RELIABILITY OF API STAPH-IDENT PROFILE REGISTER

Status	No. of Isolates	% of Total	% Correct Responses[a]
Profile Listed in Register	258	86%	89.2%
Profile Not Listed in Register[b]	42	14%	10.8%
TOTAL:	300		

[a]Correct responses include: Correct ID's by strip alone, Correct ID's W/Supplemental Tests, and Partly Correct ID's (GLLS).

[b]Required Call to API Customer Service Department.

data base (Analytical Profile Index), 14% of the profile numbers were not included in the Profile Register. Thus, 42 of the study isolates would have required telephone contact with API to obtain an identification.

IV. DISCUSSION AND SUMMARY

The coagulase-negative staphylococci (C-NS) have been the object of increasing scrutiny in recent years. Formerly regarded as non-pathogens, this group of organisms has been implicated in a variety of infections occurring both in healthy and compromised patients (Gemmell, 1980). One C-NS species, S. saprophyticus, has been reported as the second most common cause of urinary tract infections (UTIs) in young European women. However, U. S. studies concerning the frequency of isolation of S. saprophyticus by clinical laboratories have not been in agreement. My research was designed to determine if S. saprophyticus infections occur in South Texas, if speciation of C-NS aids in detection of such infections, and what practical methods are available for this purpose. Furthermore, in vitro studies were conducted in an attempt to better understand the virulence factors involved in S. saprophyticus UTIs.

Some difficulties were initially experienced in using Kloos and Schleifer's simplified scheme (1975), particularly in the identification of phosphatase-negative S. epidermidis isolates which were often mis-classified as S. hominis. However at Kloos's suggestion, anaerobic growth in thioglycolate was successfully used to differentiate these two species.

Classification of C-NS species isolated from Bexar County Hospital District patients demonstrated that the majority of human C-NS species were present in South Texas and that S. saprophyticus was a prominent pathogen in urinary infections. Nearly twice as many "clinically

significant" S. saprophyticus urine isolates (34) were encountered during the same period that 19 "clinically significant" S. epidermidis isolates were encountered.

The data resulting from the patient chart review of "clinically significant" S. epidermidis and S. saprophyticus urine isolates are in close agreement with European studies (Gillespie et al., 1978 and Wallmark et al., 1978) in which S. saprophyticus was isolated predominately from young outpatient females. This species was usually found in high counts ($\geq 10^5$ bacteria/ml) and in pure culture. However, as Wallmark previously noted, a high count is not always present. Five of our S. saprophyticus UTI cases had colony counts lower than the expected 10^5 bacteria/ml, although greater than 10^4/ml of urine.

The S. epidermidis cases encountered in this study conformed to Mitchell's findings (1968) that UTIs attributed to manipulation or pathological abnormalities of the urinary tract were often caused by novobiocin-susceptible C-NS strains.

Speciation of C-NS was especially useful in recognizing the presence of a UTI, by documenting that a C-NS was S. saprophyticus. For example, 97% of S. saprophyticus urine isolates as compared to only 40% of S. epidermidis urine isolates were considered to be "clinically significant," i.e., they contained greater than 10,000 organisms/ml of urine. This finding supports the observation of Jordan et al. (1980) that S. saprophyticus is a primary pathogen of the urinary tract and is rarely found as a contaminant of urine cultures.

The results of the retrospective study of 46 previous C-NS urine isolates demonstrated that S. saprophyticus was present in South Texas

at least eight years ago and possibly longer. The data from this phase of the study agree both with my more recent chart review findings and the European studies in demonstrating that the target population consists of primarily outpatient females (91% of all S. saprophyticus UTIs in the retrospective study occurred in OP females.

Antimicrobic susceptibility testing of S. epidermidis and S. saprophyticus urine isolates suggests that there is a significant difference in susceptibility patterns between these two species, with S. epidermidis tending to be resistant to a wider spectrum of antimicrobial agents than S. saprophyticus. Two previous studies (Wallmark et al., 1978 and Nicolle and Harding, 1982) have also reported that all of their S. saprophyticus isolates were nalidixic acid resistant. Of the agents commonly used for acute urinary tract infection, nitrofurantoin and cephalothin appeared to be the most effective, while nalidixic acid was the least effective against S. epidermidis and S. saprophyticus urine isolates.

The results of the growth rate studies indicate that growth rate in itself does not appear to be an important contributing factor in S. saprophyticus UTIs, since the mean generation time for this organism in urine was nearly twice that of the Gram-negative pathogens tested. These results are similar to the urine growth rate studies conducted by Anderson et al. (1976) in which the enhanced virulence of subgroup 3 micrococci over other Micrococcaceae in the urinary tract of young women did not appear to be due to rapid growth in midstream urine. Since S. saprophyticus also did not demonstrate a

faster growth rate than S. epidermidis in the current study, it does not appear to be the explanation for the greater frequency of S. saprophyticus as a cause of UTI.

Tissue culture adherence experiments proved that HeLa and Vero cells can be used successfully for demonstrating adherence rates in C-NS. Use of these cell lines minimizes the day-to-day experimental variations observed in adherence tests with exfoliated cells and thus allows for more reproducible experiments. My results were in agreement with those of Colleen et al. (1979) and Mardh et al. (1979), both of whom used exfoliated cells, in showing that S. saprophyticus adhered significantly better than S. epidermidis to tissue cells.

Conditions for optimal S. saprophyticus adherence included: growth of test organisms in tryptic soy broth at 37°C for 20 hours, suspension of bacteria in physiological saline, and a bacteria-tissue cell incubation period of one hour at 37°C. These results suggest that the adherence properties of S. saprophyticus may well be a key contributing factor to its ability to cause UTIs.

Testing C-NS isolates for novobiocin-susceptibility appears to be an excellent and cost-effective screening method for detection of S. saprophyticus in human urinary specimens. In the group of 335 C-NS isolates which were tested for susceptibility to novobiocin, all 48 S. saprophyticus isolates encountered were resistant to novobiocin, while all other species isolated were novobiocin-susceptible. Although S. cohnii and S. xylosus are reportedly resistant to novobiocin, these species are rarely encountered in human clinical specimens (none were

isolated in my six month study). These results agree with those of Marrie et al. (1982) in finding that resistance to the 5 ug novobiocin disc has a high positive predictive accuracy (100% in my study vs. 93% in Marrie's) as a presumptive test for S. saprophyticus.

Results obtained from novobiocin susceptibility testing of C-NS on P agar and Mueller-Hinton agar indicated a high degree of agreement. The use of a 16 mm breakpoint is equivalent to Kloos and Schleifer's criteria (1975) of classifying C-NS strains as resistant if inhibition is not greater than 5 mm from the edge of a 6 mm disc. Thus, Mueller-Hinton agar is a suitable alternative medium for determining the susceptibility of C-NS to novobiocin. Based on these data, I propose using Mueller-Hinton agar, a 5 ug novobiocin disc, and a breakpoint of 16 mm to determine novobiocin susceptibility of C-NS. Unlike P agar, Mueller-Hinton agar is easily prepared, commercially available, and in current use in many clinical laboratories for antimicrobial susceptibility testing. Thus, a single Mueller-Hinton agar plate may be used concurrently for testing the susceptibility to novobiocin and other antimicrobics.

There was also a high degree of agreement between results obtained from novobiocin susceptibility testing by Kloos and Schleifer's simplified method (1975) and those obtained with the automated Abbott MS-2 instrument. Although novobiocin discs and MS-2 programming for their interpretation are not currently available from Abbott Laboratories, the MS-2 system can be used to determine novobiocin resistance in clinical isolates by interpretation with another antibiotic's algorithm.

Amikacin was chosen for this purpose, although the novobiocin curves closely resembled those of the other antibiotics included in this testing. Even greater accuracy could be achieved if novobiocin kinetic data were employed to develop a unique interpretive program, as was done by Abbott Laboratories for the currently available antimicrobial agents. Thus, it is possible to identify Gram-positive, catalase-positive, coagulase-negative, novobiocin-resistant urinary tract staphylococcal isolates presumptively as S. saprophyticus by rapid, same-day testing in the Abbott MS-2 system.

The AutoMicrobic System GPI Card demonstrated the highest percentage of correct identifications on the two most common C-NS pathogens, S. epidermidis (82.6%) and S. saprophyticus (74.2%). Addition of a novobiocin susceptibility test to the card or performance of this procedure as a supplemental test would probably improve identification of S. saprophyticus. Although the overall agreement rate of the GPI Card with conventional identification (59.3% for all C-NS species) was lower than that obtained in the study conducted by Rouff et al. (1982), both studies have found difficulty in correctly identifying the unspecified "Staphylococcus species" group with the GPI Card. For example, 18 of the 50 isolates (36%) in the "Staphylococcus species" group in my study were given incorrect species-level identifications while Ruoff's group remarked that 21 of 37 of their C-NS isolates (57%) in this same group were given incorrect definitive species identifications. Thus, while use of the GPI Card in the AMS was very convenient, the accuracy of identifications in this study and that of

Ruoff et al. (1982) were not encouraging. Further refinement of the computer software used for these identifications, or addition of tests such as novobiocin susceptibility might improve the accuracy sufficiently for routine clinical laboratory use.

The rapid, manual STAPH-IDENT System was found to be extremely convenient to use. Inoculation and interpretation of reactions required a minimum of time and effort. Biochemical reactions, including those involving the chromogenic substrates (alkaline phosphatase, B-glucosidase, B-glucuronidase, and B-galactosidase), were generally clear-cut and easy to interpret. The five hour incubation period was seen as an obvious asset over the three-five day total incubation required for certain of the conventional tests used in this study.

The STAPH-IDENT System was able to achieve a high rate of correct identifications, when compared to conventional methods on the two most common human C-NS pathogens, S. epidermidis (94%) and S. saprophyticus (98%). Additionally, two other C-NS species tested (S. haemolyticus and S. simulans) yielded 100% correct identification rates by use of the STAPH-IDENT strip alone. The overall agreement between identifications derived from STAPH-IDENT and conventional methods in my study and that of Kloos and Wolfshohl (1982) was greater than 90%.

Micrococci were very rarely (<1/300) isolated from human clinical specimens during the isolate collection phase of this study. However, it should be noted that the STAPH-IDENT data base does not presently include micrococci, and that a Micrococcus species could be erroneously identified as a C-NS species. Thus, for complete accuracy, it might be necessary to perform a preliminary test such as acid from

glycerol in the presence of erythromycin to effectively exclude Micrococcus species.

This study was in agreement with that of Kloos and Wolfshohl (1982) in finding that addition of a novobiocin susceptibility test to the strip or performance of this procedure as a supplemental test could improve the accuracy of C-NS identifications. In addition to a novobiocin susceptibility test, performance of a rapid coagulase and xylose utilization test in conjunction with STAPH-IDENT would significantly reduce the relatively high percentage (35%) of GLLS identifications (Table 24).

The majority of profile numbers encountered in this study were listed in the one-page Profile Register (Table 25). The profiles included in the Profile Register are those most commonly encountered and which have tne associated comments of either "excellent," "very good," "good," or "acceptable" identifications. In a few instances, GLLS codes are included in the Profile Register and the first and second identification choices are listed. There were 10 of 42 profiles encountered in this study which were not listed on the Profile Register, but which yielded a correct identification using the STAPH-IDENT strip alone and which had a comment of "acceptable identification" or better in the more complete Analytical Profile Index. Thus, there are certain profiles which should be added to the existing Profile Register.

Occasionally, the Profile Register did not correspond exactly to the expanded Analytical Profile Index, e.g. Profile number 2040. The Profile Register indicated that S. saprophyticus could be different-

iated from the second choice, S. hominis by novobiocin susceptibility testing. However, the expanded data base listed a GLLS comment and a first identification choice of S. saprophyticus, second choice of S. epidermidis, and third choice of S. hominis with corresponding "frequencies of occurrence" of 1/13, 1/18, and 1/24 respectively. Thus it would seem preferable to make available a more complete Profile Index for routine use than is provided by the current single page listing of common profiles.

Much work remains to be done in order to reach a complete understanding of the role of C-NS in human disease. However, my research findings may be summarized as follows:

1. Staphylococcus saprophyticus is isolated from clinical specimens in South Texas and its presence in urine is strongly suggestive of urinary tract infection.
2. Speciation of C-NS urine isolates or, as a minimum, novobiocin susceptibility testing of these isolates significantly assists in separating infection from colonization or specimen contamination.
3. Clinical laboratories must be selective in their choice of practical methods for identification of C-NS species.
4. Tissue culture adherence assays confirm that S. saprophyticus urine isolates adhere in significantly greater numbers than do S. epidermidis urine isolates.

APPENDIX A

RECORDS REVIEW WORKSHEET

I. Patient Data:

- A. Name
- B. G. No.
- C. Age
- D. Sex
- E. OP/IP (Circle one)

II. Specimen Data:

- A. Collection Date
- B. Source/Method of Collection
- C. Culture No.

III. Lab Findings:

- A. Urinalysis
  - 1. cfu/ml:
  - 2. pH:
  - 3. pyuria ($\geq$ 5 WBC's/hpf):
  - 4. hematuria (> 5 RBC's/hpf):
  - 5. proteinuria (> trace):
- B. Other lab tests (hematology, radiology)

IV. Physical Examination: (see Table 8.1, Asscher, 1980)

Records Review Worksheet
Page 2

V. History: (previous UTI's, instrumentation, pregnancy, kidney stones, diabetes, anatomical abnormalities, invasive procedures, etc.)

VI. Physician's Diagnosis: (If UTI, do symptoms or test results allow level diagnosis?)

a. Lower UTI-frequency, dysuria, hematuria, burning, itching, and painful urination.

b. Upper UTI-tenderness over kidneys, flank pain, fever, chills, and leukocytosis.

VII. Outcome (Treatment and Followup):

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VITA

Roy Joseph Almeida, son of Mary and John Almeida, was born in Newark, New Jersey, on March 23, 1948. He graduated from Seton Hall Preparatory School and entered the U. S. Air Force Academy in 1966. Upon graduation from the academy in 1970, he received his Bachelor of Science Degree in Life Sciences and a commission as a Second Lieutenant. He married Margarida G. Almeida in 1974, in Lisbon, Portugal. They have one child, Michelle, born February 26, 1980. After serving in various managerial and administrative positions, he was selected in 1977 to attend the Clinical Laboratory Internship Program at the School of Medical Technology, Wilford Hall USAF Medical Center, Lackland Air Force Base, Texas. From 1978 to 1980 he served as the Chief of Laboratory Services, USAF Hospital, Patrick Air Force Base, Florida. In 1980, he received his Master of Science Degree in Systems Management from the Florida Institute of Technology. He was admitted to the Graduate School of Biomedical Sciences of The University of Texas Health Science Center at San Antonio in January of 1981.

His abstracts* and journal publications include:

*Almeida, R. J. and J. H. Jorgensen. 1981. Incidence of unusual enteric pathogens in South Texas. Texas Branch of the American Society for Microbiology Meeting, San Antonio, TX.

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