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Skin infections among beach users and staphylococci in Hawaii marine waters

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SKIN INFECTIONS AMONG BEACH USERS AND STAPHYLOCOCCI IN HAWAII MARINE WATERS

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ABSTRACT

The purpose of this study was to recover staphylococcus bacteria from marine recreational waters off Oahu, Hawaii and to determine if these organisms bear any relation to staphylococcal infections in humans.

The first element of the study was to develop a reliable and feasible method to recover staphylococci, especially S. aureus, from marine recreational waters and to determine the concentrations of these bacteria at selected beaches. The findings show that TGA and VJ supplemented with 0.005% sodium azide used in the membrane filtration technique were the most reliable and selective media for the recovery of staphylococci from marine waters. The significant correlation between total staphylococci and S. aureus indicated that they were consistently present together. The concentrations of total staphylococci from marine waters were also significantly correlated with the number of beach users suggesting that humans are the source of these bacteria. In classifying the beaches ("Low Staph" defined as less than 100 total staphylococci or 10 S. aureus per 100 ml), it was found that the "High Staph" sites were the popular, heavily-used beaches and the "Low Staph" were the less-used sites.

The second element of the study was to determine the association between staphylococcal skin infections and

seawater exposure. The data were collected retrospectively through telephone interviews of study subjects including 53 cases of staphylococcal skin infections and 53 non-infected controls. A significant association was found between the infections and marine water contact. The odds ratio indicated that those who developed staphylococcal skin infections were almost 4 times more likely to have had a history of seawater contact than those without the infections. There were no significant differences between cases and controls in their frequencies of exposure to seawater, nor in their duration of seawater contact. No significant difference was found between the two groups in their visits to "High Staph" and "Low Staph" sites. The similar characteristics of S. aureus isolates from marine waters and from skin specimens of the cases provides strong evidence that they were of the same source and that marine waters are a vehicle of transmission of staphylococcal skin infections.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PART ONE

INTRODUCTION

CHAPTER I

LITERATURE REVIEW

A. Staphylococcus bacteria and staphylococcal infections

Staphylococci are gram-positive cocci that occur singly, in pairs, short chains, and grape-like clusters (Kloos and Jorgensen, 1985). They are catalase-positive and can be divided into two groups on the basis of coagulase production (an enzyme that causes blood plasma to clot). Twenty-three species of staphylococci have been identified, two are coagulase-positive and twenty-one are coagulase-negative (Pfaller and Herwaldt, 1988). Of these twenty-three species, only three are clinically significant: S. aureus, S. epidermidis, and S. saprophyticus and all of them are most commonly associated with human infections (Kloos and Jorgensen, 1985; Cohen, 1986).

Most staphylococcus species are common inhabitants of skin, anterior nares, and mucous membranes. Most coagulase-negative staphylococci are found on the skin and frequently in the nasopharynx. The coagulase-positive S. aureus is commonly found in the anterior nares and occasionally on the skin. Approximately 30 percent of normal adults are nasal carriers of S. aureus (Melish, 1981; Novick, 1990).

Skin infections caused by S. aureus are the most common human staphylococcal infections (Kloos and Jorgensen, 1985). The first type of the localized infection primarily occur around the hair follicle and cause minor trauma or juvenile acne (Waldvogel, 1985). Some clinical terms included in this category are :

1) Folliculitis. This is the most benign infection and is defined as a pyoderma involving the hair follicle and its immediate surroundings. It is clinically characterized by a series of raised, often painful reddish lesions with an indurated basis, each of them being centered on a hair follicle.

2) Furuncles and Carbuncles. Furuncles (boils) represent the extension of the infectious process involving the hair follicle. They are defined as a deep-seated infection in and around the hair follicle located on the hairy areas of the body such as face, neck, axillae, and buttocks. Carbuncles are more serious deep-seated infections of several hair follicles, resulting from the spreading of the infectious process into subcutaneous tissue and usually located at the base of the neck.

3) Impetigo. This is a very superficial staphylococcal skin infection affecting mostly children, usually on exposed areas of the body, i.e. on the face and the legs. The disease starts as a red macule, which evolves

into a vesicle containing cloudy fluid based on an area of erythema.

4) Wound Infections. Staphylococcal infection is the major postsurgical infectious complication since incision favors the local spread of the organism. Staphylococcal postsurgical infections are characterized by the progressive appearance of edema, erythema, and pain around the surgical incision two or more days after surgery.

The second type of localized infection is involved with diffused skin rash in which local symptoms and signs are overshadowed by the elaboration of potent exotoxins. One disease entity involves mostly neonates and infants, and is characterized by a diffuse rash, scalding, and exfoliation. It is referred to as either the staphylococcal scalded skin syndrome (SSSS) or Ritter's disease when it occurs in neonates. The other disease entity occurs in young menstruating women and is called the toxic shock syndrome (TSS). It is characterized by diffuse rash with hypotension and shock.

Although most coagulase-negative species are non-pathogens, S. epidermidis is by far the most frequent cause of infection from intravascular catheters and prostheses (Norvick, 1990). S. epidermidis is also the major pathogen involved in nosocomial bacteremia (Stillman et al., 1987; Martin et al., 1989) and shunt infections (Keucher and Mealey, 1979; Schoenbaum et al., 1975). Another coagulase-

negative species, S. saprophyticus is the most common cause of urinary tract infections in young women (Latham et al., 1983; Marrie et al., 1982).

Staphylococci may be transmitted by multiple routes including contact with infected persons, contact with asymptomatic carriers, air-borne spread, and through contaminated objects. Of these, the person with a staphylococcal lesion appears to be particularly important in the spread of staphylococci. Persons with open draining lesions disseminate organisms into their environment and to others by direct contact. Staphylococci may also be spread by asymptomatic carriers who have staphylococci in one or more body sites, including the nose, skin, hair, nails, axillae, as well as their own clothing. From these areas, carriers may transmit staphylococci to other persons or inanimate sources. Cuts or abrasions of the skin provide a portal of entry to the pathogen with subsequent local or possibly generalized infection.

Melish (1981) noted that staphylococci are wide spread in the environment and can be cultured from clothing, carpets, and virtually all environmental surfaces. Environmental staphylococci may serve as an important reservior of contact diseases.

B. Staphylococcus Bacteria in Recreational Waters

Staphylococci, including many pathogenic strains of S. aureus, are a major bacterial contaminant of recreational waters with a high bather density (Klapes and Vesley, 1986). The abundance of these organisms on the skin and in the body cavities allows them to be easily shed into water by bathers. The major significance of S. aureus and other staphylococci in recreational waters is that they might infect breaks, cuts, and scratches on the skin, or infect the mucous membranes of the nose, ears, and eyes of bathers (Evans, 1977). There is a general consensus that swimmers have a greater occurence of contact and gastrointestinal diseases than non-swimmers, and these swimmers have a higher incidence of contact diseases than gastrointestinal diseases (Stengren and Starzyk, 1984).

Staphylococci have a great resistance to sunlight drying and salinity; and they are well adapted to survival outside human hosts (Evans, 1977). As a result, staphylococci have been suggested as an indicator of pollution in swimming pools and other recreational waters (Favero et al., 1964; Evans, 1977; Ortiz et al., 1979; Favero, 1985). Recent studies outside the U.S. have also made similar suggestions. In Italy, Tosti and Volterra (1988) suggested that the best indicators of the hygienic condition of water in a swimming pool are staphylococci. A

study of coastal water in Israel (Yoshpe-Purer and Golderman, 1987) recommended that monitoring of S. aureus as a supplementary indicator in populated beaches would add valuable information on the sanitary quality of the seawater. Alonso et al. (1989) in their study of marine recreational waters in Spain, concluded that total staphylococci is a suitable indicator in evaluating marine water quality. The results of freshwater studies in Canada (Seyfried et al., 1985a) showed that staphylococci were the most consistent indicators for predicting total morbidity rates (gastrointestinal as well as infections of the skin, ear, and respiratory tract) among swimmers. These results strongly support the earlier assertion of previous investigators that staphylococci are reliable indicators of recreational water quality. The recognition that recreational waters serve as vehicles for non-fecal borne diseases has resulted in a number of studies analyzing swimming pools for non-fecal bacteria such as S. aureus. Relatively few studies have addressed the concentrations of these bacteria in natural recreational waters.

The major obstacle to the use of staphylococci as indicators of potential health hazard is the lack of a highly selective and quantitative method for their recovery from water (Evans, 1977; Stengren and Starzyk, 1984; Klapes, 1983; Klapes and Vesley, 1986; Borrego et al., 1987). Investigators have used a variety of selective media,

including Staphylococcus 110 medium (S110), Chapman-Stone agar, Mannitol Salt agar (MSA), Tellurite Glycine agar (TGA), Vogel-Johnson agar (VJ), Baird-Parker medium (BP), or modifications of these standard formulations for enumerating total staphylococci or S. aureus. The consensus is that none of these media are either sufficiently selective for staphylococcal species or adequately differential for S. aureus (Evans, 1977; Klapes, 1983). The above mentioned media were normally used to recover staphylococci or S. aureus from foods and milk (Rayman et al., 1978; Moore and Nelson, 1962; Smuckler and Appleman, 1964). In later years, researchers have put more effort into developing for a better medium and method for recovery and enumeration of S. aureus in swimming pools. Alico and Dragonjac (1986) reported that in disinfected pool water, the most selective and reliable medium for S. aureus was VJ medium supplemented with 0.5% pyruvate. To simplify the method to identify the presumptive colonies recovered on selective media (S110, MSA, and VJ supplemented with 1% pyruvate) as S. aureus, Klapes and Vesley (1986) developed the simplified thermonuclease (STN) test.

Although a number of studies of staphylococci in water have been published, most were aimed at the recovery of these bacteria from fresh swimming pool water. Among the 23 reported studies of staphylococci in recreational waters, 13 were on fresh swimming pools, 5 were on freshwater streams

and lakes, and 5 were on seawater. In freshwater studies, the media used to recover staphylococci were VJ (Ortiz, 1977; Ortiz et al., 1979), VJ supplemented with 0.5% pyruvate (Seyfried et al., 1985b.), MSA+0.005% sodium azide (Stengren and Starzyk, 1984), S110 (LeChavellier, 1980), and BP+0.005% sodium azide (Lebaron and Baleux, 1988). The media reported in seawater studies included S110 (Gunn et al., 1982), Borrego-Florido-Romeo-O, or BFR-O (Borrego et al., 1988) and Single-step-staphylococcus-selective agar or 4-S agar (Mintzer-Morgenstern and Katzenelson, 1982).

C. Epidemiological Approach in the Study of Swimming-Associated Illnesses.

An epidemiological study is the only way to determine whether illnesses are associated with swimming and caused by a specific etiological agent. Two approaches to test these hypotheses are experimental and observational. The experimental approach provides the basic model for investigations in which the impact of varying some well-controlled factors are being studied in experiments. In the observational approach, the occurrence of disease is observed in different groups of people on the basis of some experience or exposure. Assignment into groups on the basis of exposure to a factor is not under the control of the investigator (Mausner and Kramer, 1985). Cochran (1965)

stated that a claim of proof of cause and effect must specify the mechanism by which the effect is produced, virtually requiring experimentation. In contrast, most observational studies end with an opinion or judgment about causality, not a claim of proof.

There are two major analytic methods for observational studies of etiology, retrospective and prospective. The purpose of both kinds of studies is to produce a valid estimate of a hypothesized cause-effect relationship between a suspected risk factor and a disease. In retrospective studies, diseased and nondiseased groups (cases and controls) are selected and compared for presence or absence of an antecedent factor. Such a study is retrospective because it compares the two groups with regard to the presence of some element in their past experience. The term "case-control method" is usually applied to this kind of study to indicate the way the study group is assembled. A prospective study starts with a group of people (a cohort), all who are free of a given disease, but who vary in exposure to a suspected risk factor. The cohort is followed over time to compare the rate of disease which develops in relation to exposure to the factor. This type of study is also known as "the cohort method" (Mausner and Kramer, 1985).

While the experimental method is universally acknowledged to be the most powerful explanatory method, it

is often not available to the medical investigator because of ethical or logistic considerations. As a consequence, the choice of a research strategy often is limted to the cohort or the case-control approach. The case-control method is often the research strategy of choice, particularly when initiating an exploratory study of disease etiology or investigating a rare disease (Mantel and Haenszel, 1959; Sartwell, 1974). It is especially useful for the study of rare diseases in which a cohort study will be inefficient because much effort must be devoted to follow-up of individuals who remain free of the study disease.

Several epidemiological studies of swimming-associated illnesses have been conducted in the past in which both retrospective and prospective approaches have been employed. Calderon and Mood (1981) conducted two studies of otitis externa. One was a prospective study comparing boy scouts who swam in a freshwater lake with boy scouts who swam in a chlorinated swimming pool at several camps. Three percent of over 800 study subjects reported ear complaints in the week following camp, but none had otitis externa confirmed by a physician. The other study was a retrospective study conducted at Yale University comparing 29 cases with 29 controls who were matched by age and sex. In this study, swimming and length of time spent swimming were among a few factors found to be positively associated with cases of

otitis externa. In addition, Springer et al. (1985) carried out a case-control study to assess the association between otitis externa and the type of water (freshwater lakes and rivers, chlorinated pools, or the ocean) to which swimmers are exposed. Otitis externa was found to be positively associated with the amount of swimming during the proceeding week and also was associated more with swimming in freshwater as compared with swimming in the ocean or a swimming pool. D'Alessio et al. (1980) conducted both case-control and cohort studies to determine if swimming activities increase the risk of acquiring enteroviral infection in children. The results of the case-control study showed a statistically significant higher rate of swimming activities among children who had the infection as compared to the well controls. The cohort study results concluded that children who swam consistently reported more illnesses of viral etiology than those who refrained from swimming activities.

In addition, the case-control method has been used to investigate outbreaks of several diseases such as the outbreak of Legionnaire's Disease in Philadelphia (Fraser and McDade, 1979), Giardiasis in Colorado (Wright et al., 1977), and staphylococcal infections among rafting guides in Tennessee (Decker et al., 1986).

In short, on reviewing the advantages of the case-control method for exploratory studies of etiology and

comparing it to the logistical, financial, and temporal demands of the cohort method, it was determined that the case-control method was preferable for this study. Therefore, the case-control method was chosen as the epidemiologic study approach to determine association of staphylococcal skin infections with bathing in marine recreational waters.

CHAPTER II

THE PROPOSED STUDY

A. Statement of the Problem

The possible health effects from recreational water use has been a long standing public health concern in many countries. Epidemiological studies regarding health risks to bathers and studies of the microbiological quality of recreational waters have been undertaken since the early 1950's. While gastrointestinal disease results were of major interest in most studies (Cabelli, 1978; Cabelli et al., 1982; Fattal et al., 1986; Brown et al., 1987), many studies have shown a higher incidence of contact diseases (eye, ear, skin, and respiratory tract infections) than intestinal illnesses among swimmers in both fresh and marine waters (Stevenson, 1953; Mujeriego et al., 1982; Foulon et al., 1983; Seyfried et al., 1985a). Gastrointestinal illnesses are caused by enteric pathogens which multilply in the intestinal tract of humans and animals and their main route of transmission is ingestion. However, infections acquired from recreational water use are not limited to gastrointestinal diseases but extend to skin, eye, ear, and respiratory infections as well. Microbial pathogens responsible for these diseases are not transmitted to humans

by ingestion but by direct contact of pathogens in the water to the site (eye, ear, nose, skin) of infection.

The pathogens responsible for these swimming associated non-fecal borne diseases are primarily bacteria such as Pseudomonas aeruginosa, Vibrio alginolyticus, and Staphylococcus aureus. The source of P. aeruginosa is in the environment (soil, water, plant) and it can also be recovered from human waste (Rhame, 1979). V. alginolyticus is a marine bacterium whose normal habitat is esturine and marine waters. S. aureus is a known pathogen found in nasal membranes, hair follicles, skin, and wounds of humans and warm-blooded animals (Baird-Parker, 1974). S. aureus and other staphylococci are constantly shed from the skin, mouth, nose, and throat of bathers into recreational waters. The stability of S. aureus in bathing waters increases their potential health hazard as a water-borne agent to infect cuts, scratches, and abrasions on the skin and mucous membranes of the nose, ears, and eyes of bathers.

Seyfried et al. (1985a), in their study conducted in Ontario, reported that the incidence of swimming-associated diseases resulted in more respiratory, eye, ear, and skin infections than gastrointestinal illnesses. Seyfried et al. (1985a) also concluded that among the various bacterial indicators measured, the concentrations of total staphylococci bacteria in recreational waters correlated best with all morbidities associated with swimming. In a

review of that study, Favero (1985) concurred that recreational waters are a more significant vehicle for non-fecal borne diseases (eye, ear, and skin infections) than fecal borne diseases (gastroenteritis) and urged the U.S. EPA to seriously consider monitoring recreational waters for non-fecal borne bacterial pathogens such as staphylococci. Shuval (1986) also reviewed the study of Seyfried et al. and concluded that beaches in Ontario may have been heavily contaminated with staphylococcus bacteria from the bodies of bathers, and thus, these organisms may indeed be a possible indicator of body contact pathogens of non-fecal origin. In other studies, staphylococci have been recommended as an index of bather pollution in swimming pools (Favero et al., 1964; Robinton and Mood, 1966), in fresh recreational waters (Ortiz, 1977), and in seawater (Yoshpe-Purer and Golderman, 1987; Alonso et al., 1989).

In Hawaii, the use of beaches for recreational purposes is very popular among the local population and tourists. Infections associated with marine activities, including otitis externa, wound infections, gastroenteritis and miscellaneous soft tissue infections, have been reported to be commonplace in Hawaii (Chang and Pien, 1986). The first documented water-related skin infections occurred in 1949 when swimmers'itch or locally known as "Pearl Harbor itch" was reported in sea bathers in West Loch of Pearl Harbor and certain sections of the Ala Wai Canal (Arnold and Bonnet,

1950). The causative agent of the condition was not identified at the time. One year later, a marine larval schistosome capable of producing dermatitis in human volunteers was found in infected marine snails (Chu, 1952). However, this Hawaiian marine schistosome was not believed to be responsible for the previous swimmers' itch cases.

A number of studies have been undertaken in Hawaii to identify microorganisms which may cause superficial skin infections. Pien et al. (1977) recovered V. alginolyticus in eight superficial infections associated with swimming related accidents and in one of the cases S. aureus was listed as an associated bacteria with this vibrio biotype. Fujioka and Kling (1985) reported that V. alginolyticus is the predominant vibrio in the marine waters of Hawaii. In a later study by Pien et al. (1983), S. aureus was cited as the most common bacterial isolate associated with marine injuries. Sims et al. (1983) reported that S. aureus is one of many other human pathogenic species cultured from open marine wounds (e.g. coral cuts) as well as from seawater and marine life.

The degree to which skin infections are a concern to beach users in Hawaii is not easily documented because many affect tourists who may not receive medical attention until they return home. Minor skin infections are also self-treated. Additionally, there are no methods available to readily recover the etiological agents (staphylococci) from

waters and, thus, there are no water quality regulations for these bacteria. Among adults, canoe paddlers often experience some degree of staphylococcus infections but they rarely seek medical treatment (Wyatt, 1987). Dr. Jeremy Lam of Kapiolani Hospital in Honolulu observed frequent skin infections in patients with a recent past history of swimming at southern beaches on Oahu (Lam, 1986). In September, 1986, Lam wrote a letter to the Hawaii State Department of Health stating that in the past 5 years many patients with skin infections had been swimming during the week in which their infections began. These patients had Ritter's syndrome or staphylococcus infections. In response to this report by Lam, the State Department of Health organized an ad-hoc committee on staphylococcus in water to investigate the levels of staphylococcus bacteria in recreational waters and to develop further means to relate these levels to skin infections (Lawhead, 1987).

A newspaper article (Honolulu Star Bulletin/ Advertiser, November 16, 1987) detailed the above mentioned chain of events and reported the ongoing and possible future actions regarding skin infections among beach users in Hawaii. This article prompted a local dermatologist (Leong, 1987) to respond in support of the hypothesis that staphylococcal infections were associated with marine water exposures via swimming at south shore beaches on Oahu. Thus, there were at least two local physicians on Oahu who

agreed that staphylococcal infections were occurring in association with swimming at certain beaches in Hawaii.

Only few studies have provided any information regarding the occurrence of staphylococcus bacteria in natural waters (Ortiz, 1977; Gunn and Colwell, 1983; Seyfried et al., 1985b; Fattal et al., 1986; Yoshpe-Purer and Golderman, 1987; Alonso et al., 1989). To date, no epidemiological research has been done to determine if an association exists between marine recreational water use in the open environment and staphylococcal infections.

B. Study Goals and Objectives

The goals of this study were:

1. To determine whether staphylococcus bacteria, especially S. aureus, are consistently present in marine recreational waters of Hawaii and can be readily recovered from these waters.
2. To address the possibility that staphylococcal infections of humans could be transmitted via marine recreational waters.
3. To determine the relationship of indicator bacteria and other water quality parameters to the concentrations of staphylococcus bacteria in marine recreational waters.

4. To determine whether an association exists between the incidence of staphylococcal skin infection and recreational use of marine waters.

To achieve the goals of this study, the following specific objectives were addressed:

1. Develop a method to reliably and feasibly recover and enumerate total staphylococcus and S. aureus from various recreational water sites primarily on Oahu.
2. Identify the beach sites with high and low densities of staphylococcus bacteria.
3. Determine the survivability and replicative potential of staphylococcus bacteria in natural marine recreational waters as well as factors which affect the stability of staphylococcus under natural field conditions.
4. Determine the possible sources of staphylococcus bacteria in marine recreational waters.
5. Determine the concentrations of other bacteria (vibrio, pseudomonas) and phosphate in association with water tested for concentrations of staphylococcus bacteria.
6. Determine whether there is any association with waters with higher levels of staphylococcus bacteria and reported incidences of staphylococcal infections in humans.

PART TWO

WATER QUALITY AND EPIDEMIOLOGICAL STUDIES

CHAPTER III

QUALITATIVE ASSESSMENT OF STAPHYLOCOCCUS BACTERIA IN RECREATIONAL WATERS (PHASE 1)

A. Objectives

The main objective of Phase 1 was to evaluate all commercially available methods and media for the qualitative recovery and enumeration of staphylococcus bacteria from marine recreational water samples. The selected media and methods were subsequently used for an environmental survey of staphylococcus bacteria. This survey was conducted to evaluate the media selectivity in recovering staphylococcus bacteria, to determine their relationship to indicator bacteria and to observe their survivability and persistence in marine recreational waters. The results of this preliminary study were presented at the American Public Health Association 117th Annual Meeting, Chicago, Ill (October, 1989).

B. Materials and Methods

1. Sample collection and assay

Water samples were collected in sterile polyethylene containers (Nalgene) and immediately stored in an ice-chest. All water samples were transported to the laboratory and processed within 4-6 hours. Appropriate dilutions of the samples were filtered through a 0.45 μm membrane filter (Gelman GN-6) using the membrane filtration technique. The filter was placed on the selective media for staphylococci, incubated and target colonies counted as described in the next section.

Sand samples were collected in sterile Nalgene bottles and bird feces in sterile vials, immediately stored in the ice-chest and delivered to the laboratory for analysis within 4-6 hours. The fecal samples were weighed and diluted 1:100 in sterile phosphate buffer solution (PBS). The sand samples were weighed and eluted 2 times with an equal volume of sterile PBS, appropriate dilutions of the eluent were then membrane filtered (Oshiro, 1989).

2. Media

The regular monitoring of staphylococci in recreational waters has been hindered because of the lack of selective and reliable media for its recovery (Klapes, 1983). The following commercially available media were evaluated for

the reliability and specificity for recovering staphylococci from marine recreational waters.

2.1 Mannitol Salt Agar (MSA) and Staphylococci 110 medium (S110), obtained from Difco, are the two most commonly used recovery media for staphylococcus bacteria from human clinical samples and from swimming pools. MSA is a selective medium for the isolation of pathogenic staphylococci. S110, because of its high concentration of sodium chloride, is a selective culture medium for the isolation of pathogenic strains of staphylococci (Difco, 1984). Growth of most bacteria other than staphylococci is inhibited by the high salt concentration (7.5%). These two media were first used in the assay of staphylococcus bacteria from freshwater streams and marine recreational water samples.

2.2 Tellurite Glycine agar (TGA) and Vogel Johnson (VJ) media, manufactured by Difco, are commonly used to recover staphylococcus bacteria from clinical and environmental samples. These two media use tellurite as a chemical inhibitor of other bacteria and allows staphylococcus bacteria to form distinct black colonies which are easy to discern. TGA was devised by Zebovitz, Evans, and Niven for quantitative detection of coagulase-positive staphylococci from foods and other sources, such as skin, mucous membrane, feces, air and soil (Zebovitz et al.,1955). VJ agar permits early detection of

coagulase-positive and mannitol-positive colonies of S. aureus (Vogel and Johnson, 1960). Vogel and Johnson modified the TGA formula of Zebovitz, Evans and Niven by increasing the mannitol content and adding phenol red for a pH indicator and also doubling the amount of tellurite solution. VJ was reported to be selective for staphylococcal species recovered from swimming pool waters (Klapes, 1983; Covert and Scarpino, 1987).

3. Biochemical tests

All agar media methods recover a target colony which is presumptively identified as a given bacterium (e.g. staphylococci). However, these colonies must be further tested, usually by several biochemical tests to identify them as to genus and species.

Figure 3.1 delineates the steps for the characterization and identification of staphylococci and S. aureus. All bacterial isolates were first characterized by gram stain and the catalase test using 3% hydrogen peroxide. Gram-negative bacteria were identified as the non-staphylococcus group. Gram-positive cocci which appeared in grape-like clusters and gave a positive catalase reaction were further examined by lysostaphin sensitivity.

Lysostaphin sensitivity is the test to differentiate between staphylococci and micrococci, the genus most closely related to staphylococci. The staphylococci are susceptible

to lysostaphin while the micrococci produce negative reaction. The tube test involved adding 0.2 ml of lysostaphin solution into the suspension of organism prepared in 0.2 ml of sterile saline. The suspension was incubated at 35 °C for 2 hours. Clearing of the suspension indicates susceptibility to lysostaphin (Janda, 1986).

The coagulase test is a test method to detect the coagulase enzyme (an enzyme capable of clotting plasma) produced by S. aureus. There are two types of coagulase, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as clumping factor, remains attached to the cell wall of the organism. The coagulase tube test is the most frequently used method because of its accuracy and its ability to detect both bound and free coagulase. It is the most widely used test for the differentiation of the coagulase-positive and pathogenic S. aureus from the coagulase-negative and non-pathogenic species (Difco, 1986). The test was performed by inoculating 0.5 ml of the overnight broth culture of the test organism to the tube of 0.5 ml rehydrated plasma (Bacto Coagulase Plasma obtained from Difco). The tube was mixed gently and incubated in a water bath at 37 °C. Formation of clot was examined after 4 hours. If no clot was formed, incubation was continued until 24 hours. Clot formation was considered to be a positive test of S. aureus.

Latex agglutination slide test is a rapid and reliable agglutination test procedure for the rapid differentiation of S. aureus from coagulase-negative staphylococci (Baker et al., 1985). This test is used for the detection of coagulase (clumping factor) and protein A from primary cultures suspected to be S. aureus. The Bacto Staph Latex Test kit used in this study was performed by mixing 4-5 colonies from suspected staphylococcal culture with one drop of the Bacto Staph Latex Reagent on the test slide. The slide was rotated by hand and the agglutination of coagulase (clumping factor) and protein A with yellow latex particles was formed. Staphylococcal colonies containing clumping factor and/or protein A, when mixed with yellow latex reagent, agglutinated within 45 seconds into clumps (Difco, 1986).

The STAPHase System is used for the identification of S. aureus. STAPHase consists of a macrocupule (wells) containing vacuum dried rabbit plasma with ethylene-diamine-tetra-acetic acid (EDTA). Contents of the macrocupules are rehydrated with sterile distilled water and inoculated. Organisms which produce the enzyme coagulase will either agglutinate (within 1 minute) or cause gelation of the plasma when incubated at 35-37 °C for up to five hours (API Analytab Products, 1984).

4. Sampling sites

In Phase 1 of the study TGA and VJ media were used to recover staphylococcus bacteria at 15 marine water beaches (Ala Moana, Magic Island, Kuhio Beach, Hanauma Bay, Sandy Beach, Waimanalo, Kailua, Ewa Beach, Haleiwa, Hauula, Kahaluu, Kahana Bay, Makaha, Nanakuli, and Waimea Bay), 6 freshwater streams (Kaelepulu, Kahaluu, Kahana, Kaupuni, Nanakuli, Waimea), and one brackish water canal (Ala Wai).

C. Results and Discussion

1. Evaluation of MSA and S110 media to recover staphylococci from recreational waters

Presently, there are no identified highly selective and quantitative methods to recover and enumerate staphylococcus bacteria from recreational waters. In this study, S110 and MSA media were initially employed to analyze the fresh and marine recreational water samples using the membrane filtration technique with incubation for 48 hours at 37 °C. The colonies which grew on these two media were not well isolated and produced mucoid-like substance on the surface. When tested by gram-stain test, most of these colonies were identified as gram-positive rod-shaped bacteria indicating that they were non-staphylococcus. Based on these results it was concluded that these two media were not selective in recovering staphylococci from natural environmental waters

of Hawaii. In this regard, S110 medium used with membrane filtration has previously been reported not to be sufficiently selective for the recovery of staphylococci (Alico and Palenchar, 1975).

2. Evaluation of TGA and VJ media to recover staphylococci from recreational waters

Recreational waters were then analyzed by TGA and VJ. The results demonstrated that these two media were superior to MSA and S110 in recovering staphylococcus bacteria from recreational waters in Hawaii. Both TGA and VJ use tellurite, an inhibitor of other bacteria, and allow staphylococci to form distinct balck colonies.

In order to determine the media selectivity of TGA and VJ, they were used to analyze 22 marine water samples for staphylococcus bacteria (Appendix A). The results show that the concentrations of total staphylococci recovered on TGA and VJ were not significantly different ($t = 1.20$, $p > 0.20$). Similarly, the levels of S. aureus obtained from water samples by using the two media were not significantly different ($t = 0.00$, $p > 0.99$). Thus, the use of both media was continued at this point in the study.

The analysis of results given in Table 3.1 and 3.2 showed variable percentages (10% to 100%) in the recovery of total staphylococci indicating that non-staphylococcus bacteria in marine waters were also forming target colonies

similar to staphylococci on these two media. In two water samples, Hanauma Bay (right) and Waimanalo samples, the media were excellent as 100% of the target colonies were staphylococcus bacteria. However, in other recreational water samples the percentages of total staphylococcus ranged from 10% to 75%. When freshwater and brackish water samples were analyzed (Table 3.3), TGA and VJ were ineffective because the non-staphylococcus bacteria in these waters form colonies similar to that of staphylococci. It can be seen that in this table the percentages of total stapylococci ranged from 3.3% to 20%. Thus, TGA and VJ are unreliable as media to recover staphylococci from freshwater streams and from brackish water containing freshwater. These two media can be relied upon to recover staphylococci from marine waters as a qualitative method but not as a quantitative method.

Oshiro (1989) pointed out that marine sites receiving stream waters were found to have noticeably higher quantities of indicator bacteria than those sites where no streams exist. In order to investigate whether freshwater streams could contribute to staphylococci densities in marine waters, 21 samples from Ala Wai Canal and 37 samples obtained from 6 freshwater streams were analyzed. The results of the analyses are presented in Table 3.3. Very low levels of S. aureus ranging from < 2 to 3.7 CFU/100 ml were recovered from these samples. Total staphylococci

levels recovered from these water samples were also relatively low with the highest recovered from Waimea Stream (44 CFU/100 ml).

It was observed that when TGA and VJ media were used to analyze fresh or brackish water most of the bacteria growing on the two media were confirmed to be non-staphylococcus bacteria. This may account for the very low percentage (3.3% - 20%) of staphylococcus bacteria recovered from freshwater streams and brackish water. The results of the assessment showed that both TGA and VJ were not suitable when fresh and brackish water were analyzed, because many non-staphylococcus bacteria present in these waters also grew as black colonies. Accordingly, the significance of fresh and brackish waters regarding their contribution to staphylococci loads in marine water beaches remains to be determined.

3. Use of TGA and VJ to recover staphylococci from marine recreational waters of Oahu

To assess the specificity of TGA and VJ media and to compare the expected concentrations of staphylococcus bacteria in marine waters throughout Oahu, water samples from 15 beaches were assayed for staphylococci and S. aureus. The results summarized in Table 3.1 show that S. aureus could be recovered most of the time at the most popularly-used beaches located on the south shore and the

windward side of the island. The S. aureus recovery rate ranged from 6% to 100% of the presumptive colonies. The mean CFU/100 ml of S. aureus ranged from < 2 to 144 with the highest recovered from Waimanalo Beach. The percent recovery for total staphylococci was in the low to moderate range; although 100% of the presumptive colonies were recovered from the samples collected from Hanauma Bay (on the right, facing the ocean) and Waimanalo Beach. However, this was based on only a single sample examination. The mean concentrations of total staphylococci analyzed from these most popularly-used beaches were between 16 and 181.2 CFU/100 ml.

S. aureus was observed at a very low level in water samples taken from the beaches on the north shore and on the leeward side of the island, < 2 CFU/100 ml from most beach water samples (Table 3.2). However, the range of total staphylococci recovered in these samples was from 2 to 425.0 CFU/100 ml with the highest mean value observed in the Kahana sample. The total staphylococci percent recovery was between 10% and 70% of the presumptive colonies.

It can be seen that the concentrations of staphylococcus bacteria recovered from the heavily used beaches were much higher than those of the less popularly used beaches. One possible explanation may be bather pollution. The low to moderate percent recovery of staphylococcus bacteria from marine recreational waters

indicated that the TGA and VJ media resulted in reliable qualitative assessment for the presence of these bacteria.

4. Evaluating biochemical and cultural characterization for staphylococci identification

In clinical microbiology, the coagulase test is the single most reliable test to identify staphylococcus bacteria as a pathogenic S. aureus. As a first test for the presence of S. aureus in water, three hundred and five target black colonies (gram-positive cocci and catalase positive) recovered on TGA and VJ were randomly selected to test for coagulase reaction. The results showed that 20% (61/305) of the tested colonies yielded a positive (4+) result. To confirm that they were true S. aureus isolates, these 61 coagulase-positive isolates are further tested by four biochemical tests (DNase test, lysostaphin sensitivity, latex agglutination, and STAPHase) which were used for identification of S. aureus as well as growth on staphylococci selective media: Baird-Parker agar (BP), MSA, and Columbia CNA agar (CNA).

DNase Test agar is recommended for determining deoxyribonuclease (DNase) activity of microorganisms, particularly staphylococci. A close correlation has previously been shown between DNase activity and coagulase production of S. aureus (Difco, 1984). In this study,

ninety-three percent (57/61) of the coagulase-positive isolates gave a positive reaction to the DNase Test agar indicating that they were confirmed to be S. aureus.

Lysostaphin sensitivity is the test to differentiate staphylococci from micrococci. The staphylococci are sensitive to lysostaphin while the micrococci are resistant (Janda, 1986). Thus, coagulase-positive S. aureus was expected to be susceptible to this test. The results given in Table 3.4 showed that 93% (4/61) of the isolates examined in this study were sensitive to lysostaphin indicating that they were true S. aureus.

Seven percent (4/61) of the coagulase-positive presumptive S. aureus isolates could not be confirmed by DNase or lysostaphin sensitivity test. Since these isolates were negative for the two tests, they were probably not S. aureus. In this regard, the coagulation of plasma by organisms other than S. aureus has previously been reported (Bayliss and Hall, 1965; Young and Leitner, 1964).

The latex agglutination and STAPHase tests are other rapid tests used for the confirmation of S. aureus. Ninety-two percent (56/61) of the coagulase-positive isolates were confirmed by latex agglutination test and 96 percent (44/46) were confirmed to be S. aureus by STAPHase, respectively.

BP agar has been reported to be successful for the isolation of S. aureus from foods (Rayman et al., 1978). Coagulase-positive staphylococci formed black colonies

surrounded by clear zone. In this study, 84% (51/61) of the isolates examined grew on this medium with this description of colony morphology. It can be concluded that the colony morphology on BP medium correlates moderately with the identification of the isolates as coagulase-positive staphylococci.

MSA is a selective medium for recovering pathogenic staphylococci from mixed bacterial populations (Koneman et al., 1988). Most strains of S. aureus can ferment mannitol and form acid producing colonies with a yellow halo. The results (Table 3.4) showed that 95% (58/61) of the coagulase-positive isolates produced the described characteristics. Thus, the colony characteristics on MSA highly correlated with other biochemical test results of coagulase-positive S. aureus.

CNA medium is a selective medium for gram-positive cocci. The 100% growth of the coagulase-positive isolates on this medium indicate that they belonged to the staphylococci group.

The results of all the confirmative biochemical tests are summarized in Table 3.4 and show a high percentage of positive reactions obtained from the biochemical tests performed on the coagulase-positive (4+) cultures. Thus, all biochemical tests results showed good correlations with the coagulase tube test.

Up to 69 cultures that reacted negatively to the coagulase test were randomly and similarly tested by the selected biochemical tests. It was found that, except for growth on CNA, very few positive results (from 0% to 30%) were obtained from most tests (Table 3.5). The 94% growth on CNA medium was not surprising because CNA is used for the detection of gram-positive cocci.

Based on these analyses it can be concluded that the coagulase tube test used to identify S. aureus yielded consistent results with other biochemical tests. The coagulase tube test was thus used routinely throughout this study.

5. Samples analyzed concurrently with DOH

Since there are no standard methods to recover and enumerate staphylococcus bacteria from recreational waters, it was useful to compare the methods used in the study with those of another laboratory. The analysis of nine marine water samples (split samples) for total staphylococci and S. aureus was conducted concurrently with the Hawaii State Department of Health (DOH) Laboratory. Membrane filtration using VJ medium was the technique employed in the recovery of the organisms.

The results in Table 3.6 show that S. aureus levels were in a similar range for most samples. Two samples which yielded considerable levels of S. aureus were obtained from

Hanauma Bay (464 and 276 CFU/100 ml) and from Ala Moana Beach (27 and 35 CFU/100 ml). Although the results obtained from the DOH laboratory had slightly higher levels of total staphylococci in most samples, the differences were not substantial with the exception of the single Public Bath sample. The similarity of the results supported the use of the methods for recovering and enumerating staphylococcus bacteria from recreational waters.

6. Relationship between staphylococci and indicator bacteria in marine recreational waters

The monitoring of recreational water quality is routinely done for fecal indicator bacteria. To compare the staphylococci levels to that of indicator bacteria, marine water and sand samples were examined concurrently with Oshiro's study (Oshiro, 1989) for various indicator bacteria. Twenty-nine marine water samples taken from a total of 12 beaches were analyzed for total staphylococci using TGA medium. The results summarized in Table 3.7 show that the levels of total staphylococci analyzed from marine water samples were higher than levels of the indicator bacteria studied (Fecal coliform, Enterococci, and E. coli). The beaches were classified in Oshiro's study based on the bacterial recreational standard of 35 enterococci/100 ml as "A" = Excellent (0-3.5 enterococci/100 ml), "B" = Good (3.6-35 enterococci/100 ml), "C" = Substandard (36-350

enterococci/100 ml), and "D" = Poor (greater than 350 enterococci/100 ml). Seven of the 12 beaches (Ewa, Haleiwa, Hauula, Makaha, Nanakuli, Sandy Beach, and Waimanalo) examined in this present study were classified as "A" group by Oshiro. These 7 beaches had a range of total staphylococci between 0 and 144 CFU/100 ml. Four beaches (Hanauma Bay, Kailua, Waikiki, and Waimea) were classified as "B" group by Oshiro and had concentrations of total staphylococci ranging from 0 to 792 CFU/100 ml. Only one beach (Kahana) with a total staphylococci level ranging between 144 and 1000 CFU/100 ml was in the "C" class. The results in Table 3.7 show that total staphylococci levels were generally much higher than indicator bacteria levels in all three classifications specified by Oshiro. The assessment of total staphylococci levels in this comparative analysis could be made qualitatively that the highest ranges were in the "C" class, the moderate were in "B", and the lowest in "A" category.

7. Analysis of beach sand

Staphylococci were reported to be dominant in beach sand and sediment in a study of beaches at Biscayne Bay, Fla (Buck, 1976). Concentrations as high as 500 staphylococci per gram were recovered from beach sand samples in that study. In addition, high levels of fecal indicator bacteria (10^5 CFU per 100 gram of sand) have been recovered from sand

samples of some beaches around Oahu (Oshiro, 1989). In this present study, 15 sand samples obtained from 8 sites (Table 3.8) were analyzed on TGA for presumptive staphylococci concurrently with Oshiro's study for the analysis of indicator bacteria (fecal coliforms, E. coli, and enterococci). Additional sand samples obtained from 8 beaches and sediment samples from 2 beaches (Table 3.9) were analyzed on TGA and VJ for staphylococci and S. aureus to determine whether beach sands on the island of Oahu harbor staphylococci and may possibly be a source of these bacteria in recreational waters.

The results in Table 3.8 show that in general presumptive staphylococci were recovered in higher quantities than the three indicator bacteria analyzed. A sand sample obtained from Waimea yielded the highest levels of all bacteria examined. The concentration of presumptive staphylococci in this sample was 1.25×10^5 CFU/100 gm which was in the comparable range to that of the indicator bacteria. Presumptive staphylococci level recovered from the Hanauma sand sample was relatively high (91080 CFU/100 gm) compared with that of enterococci density (440 CFU/100 gm). The presumptive colonies of staphylococci recovered from these sand samples were not tested further for confirmation.

Table 3.9 summarizes the results of beach sand and sediment samples analyzed on TGA and VJ. Low concentrations

of total staphylococci (ranging from < 2 to 14.0 CFU/100 gm) were recovered from sand samples obtained from Ewa Beach, Kahana Beach, Kailua Beach Park, and Makaha Beach. These beaches, except for Kailua Beach Park, are not heavily used for recreation. Sand samples with high total staphylococci levels ranging from 285.8 to 3.0×10^4 CFU/100 gm were obtained from popularly used beaches such as Hanauma Bay, Sandy Beach, Waimea Beach, and Waikiki Beach. S. aureus was observed at very low levels (between < 2 and 16 CFU/100 gm) in sand samples obtained from Ewa Beach, Kahana Beach, Sandy beach.

It can be seen from the moderate percent recovery of staphylococci (10% to 70%) from beach sand that many non-staphylococcus bacteria still grew on TGA and VJ media. In this regard, Oshiro (1989) pointed out that high densities of indicator bacteria in sand were due to soil. This could be the case for false positive staphylococci in sand because bacillus which is abundant in soil also formed black colonies similar to that of staphylococci. The methodology to recover staphylococcus bacteria, especially S. aureus, from sand samples needs to be improved and made more selective.

The samples of sand sediment from the sand under water at Kahala Beach and Sans Souci Beach Park yielded moderate levels (between 22 and 48 CFU/100 gm) of total staphylococci. Buck (1976), in the study in Florida, also

reported very low levels of staphylococci recovered from sediment samples (between < 1 to < 10 CFU per gram of sediment). In this regard, Oshiro (1989) found that indicator bacteria density recovered from sand washed by water and underneath the water was 2 - 3 logs lower than that obtained from sand samples collected further inland. It was concluded in that study that the water had a washing effect, cleaning the sand of its attached bacteria and that 20% of the bacteria from the sand could be released into the water.

It has been well documented that the main sources of staphylococcus bacteria are various parts of the human body such as the skin, wounds, nasal membranes, and hair follicles (Bergey's Manual, 1974) and the nares of warm-blooded animals (Evans et al.,1983; Hajek, 1976). At most beaches, the sandy area is the place most occupied by humans and animals, especially birds. Thus, the beach sand may serve as a source for staphylococcal contamination of man. The presence of staphylococcus bacteria, especially S. aureus on the sand is relevant because sand is an abrasive agent and can easily act as a means of inoculating bacteria onto broken skin layers to initiate an infection.

8. Analysis of birds' feces

During this study, there was public concern regarding the contribution of birds' waste to the water quality of Hanauma Bay. The intestinal contents of 12 pigeons from Hanauma Bay were analyzed for fecal coliforms (FC), fecal streptococcus (FS), Enterococci, E. coli, Clostridium perfringens (CP), S. aureus, and total staphylococci. The analysis was completed by the Water Resources Research Center Laboratory. Total staphylococci was analyzed by using VJ medium with membrane filtration method.

In general, the results (Table 3.10) show heavy growth of FC, E. coli, FS, and Enterococci in the intestinal content of nearly all the birds tested. There was no recovery of CP, S. aureus, and total staphylococci in any of the tested pigeons' intestinal content.

At some beaches, the presence of birds on beach sand was obvious; and they were suspected to be one of the sources of staphylococcus bacteria in sand and water. The results above showed that birds' fecal material examined in this study did not have recoverable quantities of either S. aureus or other staphylococcal species. This finding was in agreement with a report presented at the Hanauma Bay Ad Hoc Committee Meeting, where it was stated that staphylococcus bacteria was not frequently recovered from birds' feces at the Hawaii State Animal Quarantine Laboratory (Sawa, 1989). The examination of an animal source for staphylococcus

bacteria in this current study was limited to only pigeons' intestinal contents.

There is a lack of information in the literature regarding the analysis of bird feces for staphylococcus bacteria. However, it has previously been shown that coagulase-positive staphylococci may be isolated from the anterior nares of pigeons, dogs, minks, and horses (Hajek, 1976). The skin and nasopharynx of healthy birds have been found to harbor S. aureus (Devriese et al., 1975). The canine and feline species were reported to have high carrier rates of coagulase-positive staphyloccocci on the skin as well as in the nose (Morrison et al., 1961). Courter and Galton (1962) reported that common sites of staphylococcal infections in dogs were the skin, ear canal and inside of the ear flap. Some of these animals such as birds, cats, and dogs are frequently present at most beaches. They may possibly account for the high levels of staphylococcus bacteria in beach sand. These animals have been reported as major contributors of indicator bacteria to fresh and marine recreational waters in Hawaii (Oshiro, 1989).

9. Persistence and multiplication of staphylococci in marine waters

Several experiments were carried out to determine whether staphylococci could multiply in seawater. The first experiment entailed leaving water samples in sampling

bottles at room temperature for 4 days and measuring the levels of presumptive staphylococci (black shiny colonies on VJ agar) every 24 hours. As shown in Table 3.11, the levels of presumptive staphylococci dropped slightly on DAY 2 and DAY 3 for all samples. The next experiment on presumptive staphylococci entailed adding nutrients (0.05% peptone) to seawater samples following the measurement of presumptive staphylococci on the first day (DAY 0) and measuring these samples at room temperature on two additional days (Table 3.12). In most samples, the levels of presumptive staphylococci slightly increased on DAY 1, then dropped on DAY 2. The results obtained from these two experiments on presumptive staphylococci were not sufficient to give any clear information regarding the probable multiplication of the organism in seawater. The third experiment involved seeding S. aureus into filter-sterilized seawater (0.1 ml of stock culture/1 litre of seawater). The seeded seawater sample was analyzed on DAY 0 for the background levels of S. aureus. The sample was then divided into two portions. To one, 0.05% peptone was added; to the other, no peptone was added. Measurements of the organism were conducted in the two portions of the sample every 24 hours for four additional days. The results shown in Table 3.13 demonstrate that without added peptone, the levels of S. aureus dropped on DAY 2, DAY 3, and DAY 4. With the addition of peptone, the S. aureus levels were stable

through DAY 4, indicating that the organism could survive in the presence of nutrients.

The depletion of organic nutrients was probably one of the reasons for the decrease of presumptive staphylococci density in the first experiment. When a nutrient (0.05% peptone) was added into seawater in the second experiment, growth of staphylococci was observed by an increase in its density on the first day. The drop off on DAY 2 probably resulted from their inability to obtain organic nutrients where there was intense competition with other bacteria. In sterile seawater, without peptone, the S. aureus population declined which was probably a result of an absence of nutrients. Whereas in the presence of peptone, the population density of S. aureus was stabilized. Evans (1977) noted that S. aureus was unlikely to multiply in most waters, and it required a considerable array of organic nutrients. S. aureus has previously been reported to be susceptible to starvation (Sinclair and Alexander, 1984), and a decrease in numbers of this pathogen in seawater has also been observed (Saz et al, 1963). However, from these experiments it can only be concluded that S. aureus could survive in the presence of nutrients but probably not replicate. Additional research is needed to further study the persistence and survival of staphylococcal organisms in the environment. There are many factors involved in the natural ecosystem which need to be taken into account.

D. Summary

The qualitative assessment of staphylococcus bacteria in recreational waters was a crucial step in the selection of media and methods for the subsequent sampling and analysis in this study. This assessment involved the selection of specialized recovery media and biochemical tests for identification of staphylococci and S. aureus. The results were compared with those obtained from currently accepted laboratory analysis. At this time TGA and VJ media were used in the testing of recreational water samples around Oahu. This testing revealed staphylococcus levels higher than indicator bacteria for all samples collected and led to the investigation of several possible staphylococcal sources in the environment in the next phase of the study. Of particular significance for marine recreational waters was the confirmation of the persistence of staphylococci in marine waters. However, the use of TGA and VJ still needed improvement due to the presence of many non-staphylococcus bacteria in recreational waters which formed colonies similar to those of staphylococci on the media.

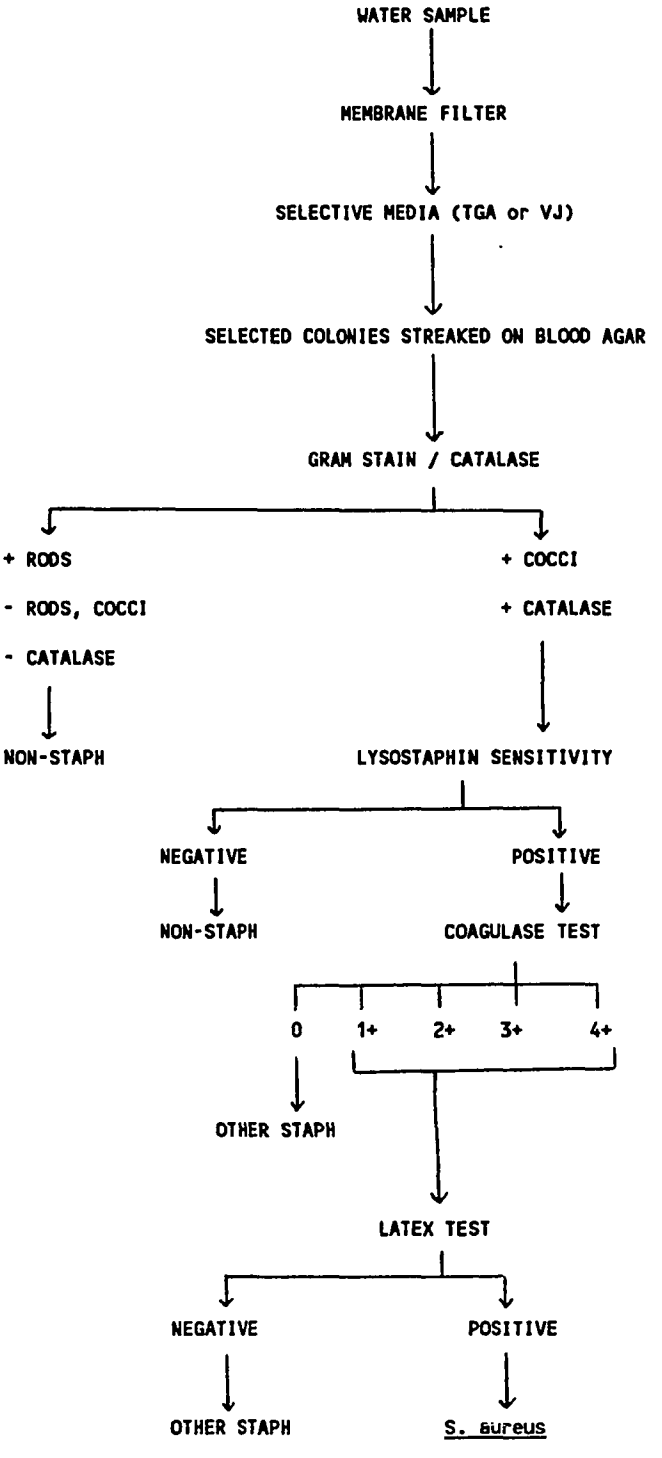

Figure 3.1 Procedure for Recovery of Staphylococcus Bacteria from Marine Recreational Waters

Table 3.1 The Concentrations of Staphylococcus Bacteria (CFU/100 ml) Recovered from the South Shore and Windward Side of the Island of Oahu

Site	No. of Sample (n)	Media	Total Staphylococci	S. aureus
Ala Moana Beach	(4)	TGA	72.5 (75%)	15.9 (20%)
	(5)	VJ	128.2 (68%)	35.6 (6%)
Magic Island	(1)	TGA	16 (30%)	< 2
	(1)	VJ	69 (67%)	< 2
Kuhio Beach	(7)	TGA	131.4 (67%)	106.7(17%)
	(5)	VJ	109.2 (51%)	77.6 (21%)
Hanauma Bay (middle)	(12)	TGA	181.2 (56%)	39.4 (13%)
	(8)	VJ	112.7 (67.2%)	52.9 (37%)
Hanauma Bay (right)	(1)	TGA	28 (100%)	8 (30%)
	(1)	VJ	16 (100%)	< 2
Hanauma Bay (left)	(1)	TGA	22 (50%)	< 2
	(3)	VJ	18 (70%)	5 (40%)
Sandy Beach	(5)	TGA	21.8 (68.6%)	38.6 (40%)
	(4)	VJ	34.4 (72%)	14.7 (42%)
Waimanalo Beach	(1)	TGA	144 (100%)	144 (100%)
	(1)	VJ	88 (80%)	11 (10%)
Kailua Beach	(4)	TGA	46.5 (60%)	23.5 (30%)
	(4)	VJ	33.7 (42.5%)	3.0 (8%)

Table 3.2 The Concentrations of Staphylococcus Bacteria (CFU/100 ml) Recovered from the North Shore and Leeward Coast of the Island of Oahu

Site	No. of Sample (n)	Media	Total Staphylococci	S. aureus
Ewa Beach	(3)	TGA	5.1 (37%)	< 2
	(1)	VJ	2 (25%)	< 2
Haleiwa Beach Park	(3)	TGA	40.5 (19%)	< 2
	(1)	VJ	14 (50%)	< 2
Hauula Beach Park	(2)	TGA	145.7 (50%)	< 2
	(1)	VJ	26 (50%)	< 2
Kahaluu Beach Park	(1)	TGA	3 (10%)	< 2
	(1)	VJ	8 (50%)	< 2
Kahana Bay	(3)	TGA	425.0 (27%)	< 2
	(1)	VJ	232 (10%)	< 2
Makaha Beach	(3)	TGA	32.8 (57.6%)	< 2
		VJ	ND	ND[a]
Nanakuli Beach Park	(3)	TGA	55.5 (70%)	< 2
	(1)	VJ	61 (60%)	< 2
Waimea Bay	(3)	TGA	14.4 (40%)	< 2
		VJ	ND	ND

[a]ND = NOT DONE

Table 3.3 The Concentrations of Staphylococcus Bacteria (CFU/100 ml) Recovered from Freshwater Streams and Brackish Water

Site	No. of Sample (n)	Media	Total Staphylococci	S. aureus
Ala Wai Canal	(3)	TGA	4.6 (6.7%)	3.7(3.3%)
(McCully Bridge)	(3)	VJ	< 2	< 2
Ala Wai Canal	(3)	TGA	10.3 (6.7%)	2.2(3.3%)
(Ala Moana Bridge)	(3)	VJ	< 2	< 2
Kaelepulu Str.[a]	(3)	TGA	2.4 (3.3%)	< 2
	(5)	VJ	2.9 (8%)	< 2
Kahaluu Str.	(1)	TGA	1 (10%)	< 2
	(1)	VJ	2 (20%)	< 2
Kahana Str.	(1)	TGA	22 (10%)	< 2
	(1)	VJ	< 2	< 2
Kaupuni Str.	(1)	TGA	< 2	< 2
	(1)	VJ	< 2	< 2
Nanakuli Str.	(1)	TGA	< 2	< 2
	(1)	VJ	< 2	< 2
Waimea Str.	(1)	TGA	< 2	< 2
	(1)	VJ	44 (10%)	< 2

[a]Str. = Stream

Table 3.4 Results of Biochemical Tests Performed on Coagulase Positive (4+) Cultures

	DNase	BP	CNA	MSA	Latex	Lyso[a]	STAPHase
Positive	57	51	61	58	56	57	44
Negative	4	10	0	3	5	4	2
Not Done	0	0	0	0	0	0	25
Total	61	61	61	61	61	61	61
% Positive	93	84	100	95	92	93	96

[a]Lysostaphin Sensitivity

Table 3.5 Results of Selected Biochemical Tests Performed on Randomly Chosen Coagulase Negative Cultures

	DNase	BP	CNA	MSA	Latex	Lyso[a]
Positive	6	1	44	14	0	7
Negative	63	45	2	32	29	40
Not Done	0	1	1	1	0	0
Total	69	47	47	47	29	47
% Positive	9	2	94	30	0	15

[a]Lysostaphin Sensitivity

Table 3.6 Water Samples Analyzed Concurrently With the Hawaii State Department of Health

Date	Site	Total Staphylococci		S. aureus		
		[a](a)	(b)	(c)	(d)	(e)
05/25/88	Waikiki 1	112	136	< 4	14	< 3
	Waikiki 2	284	124	< 4	< 2	< 3
06/13/88	Hanauma	68	32	< 4	3	4
06/27/88	Hanauma	696	644	464	276	23
	Ala Moana[b]	460	348	27	35	< 3
	Magic Isl.	96	104	< 4	< 2	< 3
	Ala Moana[c]	296	384	< 4	< 2	< 3
	Gray Beach	444	360	26	< 2	4
	Public Bath	492	28	< 4	< 2	< 3

[a] (a)(c) = DOH membrane filtration results (cfu/100 ml)
(b)(d) = UH membrane filtration results (cfu/100 ml)
(e) = DOH results of Most Probable Number (MPN/100 ml)
[b]Sample taken from the far east side of the beach
[c]Sample taken from the far west side of the beach

Table 3.7 Concentrations of Fecal Coliforms (FC), *Escherichia coli* (EC), Enterococci (EN), and Total Staphylococci from Marine Recreational Waters

DATE	Sampling Site	FC	EC	EN	Total Staph
06/04/88	Ewa	0	0	0	3
07/20/88		4	0	2	3
08/10/88		5	0	0	15
06/15/88	Haleiwa	0	0	1	21
07/18/88		1	0	0	78
08/08/88		0	1	1	0
06/15/88	Hauula	0	0	0	15
06/14/88	Makaha	0	0	0	32
07/20/88		0	2	4	110
08/10/88		0	0	0	10
06/14/88	Nanakuli	0	0	0	99
07/20/88		0	0	0	72
08/10/88		0	0	0	24
07/19/88	Sandy Beach	1	1	0	81
08/09/88		0	0	0	5
08/09/88	Waimanalo	2	0	1	144
01/13/88	Hanauma Bay	103	1	52	50
07/19/88		6	8	8	409
08/09/88		11	7	7	792
07/19/88	Kailua	0	1	0	61
06/13/88	Waikiki	2	1322	0	432
07/19/88		0	1	0	54
08/09/88		4	0	0	102
06/15/88	Waimea	0	0	0	0
07/18/88		2	1	0	26
08/08/88		40	39	8	8
06/15/88	Kahana	88	15	1	1000
07/18/88		400	68	8	533
08/08/88		720	280	>129	144

Table 3.8 Concentrations of Fecal Coliforms (FC), *Escherichia coli* (EC), Enterococci (EN), and Presumptive Staphylococci from Beach Sand Samples

DATE	Sampling Site	FC	EC	EN	Presumptive Staphylococci
06/14/88	Ewa	16	8	<8	96
07/20/88		<2	<2	<8	0
08/10/88		<4	<4	<4	0
06/13/88	Hanauma Bay	80	<40	440	91080
06/15/88	Kahana	<80	<80	<80	712
07/18/88		600	<40	<40	0
06/13/88	Kailua	<40	<40	<40	31
07/19/88		16	2	<2	0
06/14/88	Makaha	<2	<2	<2	33
07/20/88		<2	2	4	0
08/10/88		<2	<2	<2	83
06/13/88	Sandy Beach	<50	<50	<50	21
07/19/88	Waikiki	4	<2	2	296
06/15/88	Waimea	16	<4	<4	27
07/18/88		$>2.9 \times 10^6$	$>1.2 \times 10^6$	$>1.1 \times 10^6$	1.25×10^5

Table 3.9 The Concentrations of Staphylococcus Bacteria (CFU/100 gm) from Beach Sand Samples on the Island of Oahu

Site	No. of Sample (n)	Media	Total Staphylococci	S. aureus
Sand Samples:				
Ewa Beach	(3)	TGA	4.6 (10%)	4 (6.7%)
	(1)	VJ	4 (10%)	< 2
Hanauma Bay	(3)	TGA	$3.0x10^4$ (30%)	< 2
	(1)	VJ	$3.3x10^3$ (70%)	< 2
Kahana Bay	(3)	TGA	8.9 (30%)	7.8 (23%)
	(1)	VJ	< 2	< 2
Kailua Beach Park	(2)	TGA	5.6 (16.5%)	< 2
	(1)	VJ	< 2	< 2
Makaha Beach Park	(3)	TGA	14.0 (13.3%)	< 2
	(1)	VJ	< 2	< 2
Sandy Beach	(3)	TGA	716.4 (40%)	< 2
	(1)	VJ	40 (67%)	7 (11%)
Waimea Bay	(3)	TGA	$3.7x10^3$ (40%)	< 2
		VJ	ND[a]	ND
Waikiki Beach	(2)	TGA	285.8 (20%)	< 2
		VJ	ND	ND
Sediment Samples:				
Kahala Beach	(1)	TGA	22 (40%)	< 2
	(1)	VJ	40 (60%)	< 2
Sans Souci Beach Park	(1)	TGA	48 (30%)	16 (10%)
	(1)	VJ	32 (40%)	8 (10%)

[a]ND = NOT DONE

Table 3.10 Indicator Bacteria Levels of Pigeon Intestinal Content (CFU/gram weight)

Pigeon Number	FC	*E. coli*	FS	Ent	CP	*S. aureus*	Total Staph
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	9200	10000	2000	4800	Neg	Neg	Neg
3	400	1200	400	Neg	Neg	Neg	Neg
4	400	Neg	Neg	Neg	Neg	Neg	Neg
5	17200	21600	17200	1480000	Neg	Neg	Neg
6	9200	10000	Neg	Neg	Neg	Neg	Neg
7	15600	27600	4000	7600	Neg	Neg	Neg
8	1520000	840000	2000	400	Neg	Neg	Neg
9	9200	Neg	Pos	Neg	Neg	Neg	Neg
10	4000	2800	Neg	400	Neg	Neg	Neg
11	12400	13600	15200	13600	Neg	Neg	Neg
12	1200	2000	800	4000	Neg	Neg	Neg

FC = Fecal coliform
FS = Fecal Streptococcus
Ent= Enterococci
CP = *Clostridium perfringens*
Neg = Negative

Table 3.11 Observation of Presumptive Staphylococci in Water Samples Left at Room Temperature

Samples	CFU/100 ml of black shiny colonies on VJ agar DAY 0	DAY 1	DAY 2	DAY 3
Hanauma Bay:				
Right[a]	46	46	22	32
Middle	32	12	8	12
Left[a]	84	20	4	2
Ala Wai Canal:				
Diamond Head	148	178	88	64
McCully Bridge	136	96	91	17
Ala Moana Bridge	26	17	11	12

[a]Facing the Ocean

Table 3.12 Experiment on Multiplication of Presumptive Staphylococci With 0.05% Peptone Added To Seawater Samples

Samples	CFU/100 ml of black shiny colonies on VJ agar DAY 0 (no peptone)	DAY 1 (0.05% peptone)	DAY 2 (0.05% peptone)
Ala Moana	66	11	12
Waikiki	73	129	104
Hanauma Bay	564	612	281
Kuhio Beach	144	201	72

Table 3.13 Multiplication Test for S. aureus (CFU/100 ml) in Filter-Sterilized Seawater on VJ agar

Time	Without Peptone	With 0.05% Peptone
DAY 0	1.2×10^5	1.2×10^5
DAY 1	1.3×10^5	2.9×10^5
DAY 2	4.0×10^3	3.0×10^5
DAY 3	< 400	1.1×10^5
DAY 4	< 400	3.0×10^5

CHAPTER IV

QUANTITATIVE ASSESSMENT OF STAPHYLOCOCCUS BACTERIA IN RECREATIONAL WATERS (PHASE 2)

A. Objectives

The objectives of this phase were to develop a better medium with high selectivity to reliably and quantitatively recover staphylococcus bacteria from marine waters, to classify the beaches examined for these bacteria into "High Staph" and "Low Staph" sites, and to determine their possible sources in the environment. The characteristics of S. aureus isolates from marine waters and from clinical skin cultures were to be analyzed.

B. Materials and Methods

1. Media

TGA and VJ incorporated with 0.005% sodium azide (TGA+AZ and VJ+AZ) were employed as selective media for staphylococci recovery from marine recreational waters in Phase 2 of the study. It has been reported that sodium azide at concentrations of 0.0049% or 0.005% can suppress the growth of bacillus without affecting the number of

staphylococci (Smuckler and Appleman, 1964; Stengren and Starzyk, 1984; Borrego et al., 1987). Other media used for comparison with these two media were: mTG supplemented with 0.005% sodium azide (mTG+AZ), Baird-Parker with 0.005% sodium azide (BP+AZ), and Borrego, Florido, and Romeo (BFR-0). A modification of TGA (mTG) was done by using TGA as a basal medium incorporated with colistin (15 mg/l) and nalidixic acid (10 mg/l). Colistin (polymyxin) has been used in selective media for gram-positive bacteria because of its activity against gram-negative bacteria (Kucer and Bennett, 1979). Nalidixic acid was reported by Ellner et al. (1966) to suppress growth of Proteus, Klebsiella, and Pseudomonas species.

Baird-Parker (BP) medium permits the detection and isolation of coagulase-positive staphylococci after 24 hours incubation at 37 °C. The tellurite and egg yolk components differentiate the coagulase-positive staphylococci from the coagulase-negative staphylococci by the formation of black, shiny, convex colonies surrounded by a clear zone. BFR-0 was prepared according to the published method which was originally formulated for the selective enumeration of S. aureus in water (Borrego et al., 1987). The medium contains glycine and sodium pyruvate as growth stimulators of S. aureus. Potassium thiocyanate (25 gm/l), sodium chloride (100 gm/l), and sodium azide (0.049 gm/l) were included in

the medium as selective agents. All of the commercially available media were obtained from Difco Laboratories.

2. Biotyping based on antibiotic sensitivity and phage typing

Epidemiologically, it is important to differentiate various strains of staphylococci into types or groups, especially when an outbreak of staphylococcal infections occurs in order to detect the source of the outbreak. A variety of techniques have been used for this, including antibiotic susceptibility patterns and bacteriophage typing. The emergence of a staphylococcal strain with a unique sensitivity pattern can be a valuable signal to the clinical microbiologist and can provide a marker with which similar strains can be detected. Bacteriophage typing, on the other hand, is the most established system for epidemiological typing (Kloos and Smith, 1980). It is a technique that further characterizes or biotypes certain strains of bacteria after they have been identified by genus and species. S. aureus is the species of stapylococcus most commonly typed, as it is the most commonly encountered pathogen in the genus (Kloos and Smith, 1980).

In this study, antibiotic sensitivities to penicillin, ampicillin, methicillin, tetracycline, vancomycin, erythromycin, streptomycin, and cefamandole were performed using the Kirby-Bauer disk diffusion technique on

Mueller-Hinton agar. Phage typing was completed by the Hawaii State Department of Health. The antibiotic patterns and phage type of S. aureus recovered from marine recreational waters were compared with those of the S. aureus obtained from clinical skin culture to determine whether they were of the same strain.

3. Identification of coagulase-negative staphylococci

The STAPHTrac technique was applied for the identification of some of the coagulase-negative staphylococci recovered from the water samples in Phase 2 of the study. The STAPHTrac test strip holding 20 cupules of biochemical tests was inoculated with a pure culture and was then incubated for 24 hours at 37 °C. The test results were transformed into a numerical profile. The bacterial strain was identified by referring to the Profile List (API Analytab Product, 1986).

4. Statistical methods

The data of staphylococcus bacteria and S. aureus recovered from all samples were analyzed using the Statistical Analysis System (SAS, 1985) statistical package on an IBM mainframe computer. Descriptive statistics were determined for staphylococci concentrations in recreational waters from each sampling site. The statistical significance of differences between media was tested with

the Student's t-test. This test was applied in the comparison of different media for recovering staphylococci from marine waters. Relationships between the levels of staphylococci in marine waters and other variables (the number of beach users, sampling sites, and months of sample collection) were analyzed using the simple and multiple regression techniques.

5. Sampling sites

During Phase 2 of the study, the analyses of 195 samples of marine recreational water taken from 27 sampling sites (Table 4.1) around the island of Oahu were conducted. Thirteen sampling sites were located in the southern section of the island, 4 on the northern part, 5 on the east and 5 on the western side of the island (Figure 4.1). The water samples were analyzed for total staphylococci and S. aureus to determine their background levels at each site. The sampling sites were classified into "High Staph" and "Low Staph" beaches based on the levels of total staphylococci and S. aureus analyzed in this phase of the study. Concentrations of less than 100 staphylococci/100 ml or 10 S. aureus/100 ml were used as cut-off points for defining "Low Staph" beaches. This classification of the beaches was employed in the subsequent epidemiological analysis (Phase 3) to determine the relationship between the case subjects

and the control group and their exposure to staphylococci in marine waters.

C. Results and Discussion

1. Comparative study of various media

The use of TGA and VJ in Phase 1 of the study was found to be unsatisfactory in the quantitative enumeration of staphylococci from marine waters. Selective media preventing staphylococci from being overgrown needed to be developed by the modification of commercially available media. Several selective agents and inhibiting agents have been reported to be successful when incorporated into some media in recovering staphylococcus bacteria from food and water. The 4-S medium was used for the isolation and recovery of coagulase-positive staphylococci from food (Mintzer-Morgenstern and Katzenelson, 1982) and seawater (Yoshpe-Purer and Golderman, 1987). S110+AZ was reported to be useful in suppressing the growth of bacillus cells without affecting the staphylococcal growth in the isolation of staphylococcus bacteria from meat pot pies (Smuckler and Appleman, 1964).

Sodium azide (0.0049% or 0.005%) has been reported to suppress the growth of gram-positive rods without affecting the numbers of staphylococci in food and in water (Smuckler and Appleman, 1964; Borrego, et al., 1987; Stengren and

Starzyk, 1984). To assess the effectiveness of sodium azide, 0.005% sodium azide was incorporated into several media reported to be successful in the recovery of staphylococcus bacteria from food and water in previous studies. Marine water samples and water samples from the Ala Wai Canal (brackish water) were tested for comparison of the following media:

(1) TGA

(2) TGA + Sodium azide (AZ)

(3) mTG (TGA + Colistin + Nalidixic acid)

(4) mTG+AZ

(5) VJ

(6) VJ+AZ

(7) 4-S or Single-step-staphylococcus-selective agar (Mintzer-Morgenstern and Katzenelson, 1982)

(8) 4-S+AZ

(9) S110+AZ (Smuckler and Appleman, 1964)

(10) S110 + tellurite

(11) S110 + AZ + tellurite

The results summarized in Table 4.2 show the comparative recovery of staphylococcal colonies from the various media (1) to (6) based on the analysis of 6 samples each of marine waters and brackish water (Appendix B). It can be seen that percent recovery of S. aureus and total staphylococci is consistently higher when media TGA, mTG, and VJ are combined with sodium azide, a suppressor of other

bacteria. TGA+AZ and VJ+AZ were chosen to analyze the subsequent marine water samples in this phase owing to their relatively high percent recovery (83% and 90% for total staphylococci and 27% and 31% for S. aureus). Media (7) - (11) were not satisfactory due to their very low percent recovery of staphylococcus organisms and too much growth of other bacteria. In the Ala Wai samples, although a very low percent recovery of S. aureus was obtained, the percent recovery of total staphylococci was considerably higher when the non-staphylococcus suppressor, sodium azide, was added to the three media. When supplemented with sodium azide and tellurite, 4-S and S110 media were not successful in the assay of the brackish water samples from the Ala Wai Canal.

It can be seen that the addition of 0.005% sodium azide enhanced the recovery of staphylococcus bacteria from marine waters in most cases. The use of 4-S and S110 media with and without the addition of sodium azide and tellurite, however, was not successful in the analyses of marine and brackish waters. The use of TGA, mTG, and VJ supplemented with sodium azide in the assay of brackish water looked promising. The percent recovery of total staphylococci was increased from 0 to 48, 26 to 48, and 0 to 33, for TGA, mTG, and VJ respectively. Future research is needed to determine the usefulness of these media in recovering staphylococcus bacteria from brackish and fresh waters. The main objective

of this study was to analyze marine waters for staphylococci.

Attempts were made to compare TGA+AZ and VJ+AZ with two other media, BFR-0 and Baird-Parker added with sodium azide (BP+AZ). BFR-0 had 0.0049% sodium azide in its contents and was reported to recover more than 75% of staphylococci from recreational waters in a study conducted in Spain (Borrego, et al., 1987). Baird-Parker agar supplemented with 0.005% sodium azide was used for recovery of S. aureus in waters in a French study (Lebaron and Baleux, 1988). When used concurrently with TGA+AZ and VJ+AZ in the assay of total staphylococci in marine waters (Appendix C), the recovery rate on BFR-0 was significantly lower than that of the other two media ($t = 5.57$, $p < 0.005$). Similarly, the percent recovery of total staphylococci on BP+AZ (Appendix D) was lower than that obtained from TGA+AZ and VJ+AZ ($t = 2.49$, $p < 0.05$).

2. Comparative selectivity of VJ+AZ and TGA+AZ in recovering staphylococci from Hawaii marine waters

The two most reliable media determined from this study (TGA+AZ and VJ+AZ) were employed simultaneously to analyze marine water samples in order to determine the media selectivity.

2.1 Analyzed by VJ+AZ medium

The analysis of marine water samples using VJ+AZ medium is given in Table 4.3 and 4.4. The 27 sampling sites were classified into "Low Staph" and "High Staph" sites by using a maximum allowable concentration of less than 100 staphylococci/100 ml (Table 4.3) and less than 10 S. aureus/100 ml (Table 4.4) for defining "Low Staph". The data of total staphylococci and S. aureus in these two tables are presented in ascending order. The maximum allowable concentration of less than 100 staphylococci/100 ml was suggested by Favero et al. (1964) as indicators of pollution in swimming pools. When the maximum allowable concentration of 100 staphylococci/100 ml was applied to this study, there were 19 sites in the "Low Staph" group and 8 sites in the "High Staph" group. The "Low Staph" category had mean values ranging from 1.6 to 90.0 staphylococci/100 ml. All but one of the 8 sampling sites in the "High Staph" group were located in the south shore of Oahu. The mean concentrations of total staphylococci in this group ranged from 122.2 to 689.9 CFU/100 ml with the highest value recovered from Hanauma Bay (middle of the bay) sample.

Table 4.4 presents the same information as in Table 4.3 but uses a different cut-off point, 10 S. aureus/100 ml for defining "Low Staph". The "Low Staph" sites consisted of 18 beaches with the mean concentrations of S. aureus ranging from < 2 to 9.0 CFU/100 ml. There were 9 sampling sites in

the "High Staph" group with the mean values of S. aureus ranging from 19.4 to 92.9 CFU/100 ml. It should be noted that by using the S. aureus cut-off point, the numbers of sampling sites in both categories changed very slightly from those presented in Table 4.3. The changes are that the Waimanalo and Kailua sites are now in the "High Staph" group whereas the Sans Souci site is in the "Low Staph" group.

At present, there are no national water standards for staphylococci. Using the proposed standard of 100 staphylococci/100 ml as the maximum allowable concentration, it can be seen that 8 of the sites had levels of total staphylococci far exceeding this number. These sites (Table 4.3) consisted of Ala Moana Beach, Waimea Beach, Hanauma Bay in the middle area and on the left side of the bay, and the four sites in Waikiki area (WKK1 = Behind the Police Station, WKK2 = Kuhio Beach, WKK3 = Queen's Surf Beach, WKK4 = Sans Souci Beach). Ala Moana Beach is located in central Honolulu and provides a vast recreational area for locals and tourists. The beach is usually moderately populated. Its shallow water and wave barriers make the place ideal for young children who are usually present at the beach. Waimea Beach is the only site in the "High Staph" group which is located on the north shore. It is one of the most popular surfing resorts on the island. At the eastern end of the beach, there is a shallow area where the Waimea River

empties out into the ocean; and many young children are usually seen in this area.

Hanauma Bay's daily crowds of visitors may be the major factor accounting for the high concentrations of staphylococci in its waters. On the left and right side of the bay there are coral reefs and sharp rocks, so most swimming takes place in the middle area of the bay. On the left (facing the ocean) of the bay is the fish-feeding area. The fish-feeding activities introduce a lot of nutrients into the aquatic environment and may consequently have a great impact on the bacterial quality of the water.

The four sampling sites in the Waikiki area were all classified as in the "High Staph" category. Large numbers of beach users were always observed at these sites. The area behind the police station was always crowded with sunbathers, swimmers, and surfers. The Kuhio Beach site, because of its wave barriers, served almost as a pool for children. Queen's Surf Beach and Sans Souci Beach are located in proximity of a big park and residential area, and many local families and children were seen at these two beaches.

Table 4.3 also shows that S. aureus levels ran parallel to total staphylococci levels. By using the range of S. aureus to determine the cut-off point, as shown in Table 4.4, the sites in the "High Staph" category were almost the same as those given in Table 4.3. The two additional sites

for the "High Staph" group in Table 4.4, Waimanalo and Kailua Beach, are located on the windward side of the island and are the most widely used in that area. Kailua Beach has Kaelepulu stream opening into the ocean at the middle of the beach. The beach and the stream were often closed to the public due to contamination in the stream.

Most of the beaches in the "Low Staph" group are located in the less popular areas and very few people were present at the beach during sampling time. It may be of interest that Magic Island and Hanauma Bay (right side of the bay) are in this group. It was observed that very few individuals were present at Magic Island although it is located at the end of Ala Moana Beach Park. Water samples were collected from a reservoir-like body of water located at the end of Magic Island. It was not an open beach compared with Ala Moana Beach. Hanauma Bay on the right side, as mentioned earlier, is full of sharp rocks and coral reefs. This area is not usually used for any recreational activities.

Although there is no predetermined S. aureus level to separate sites into high and low categories, Table 4.4 shows that there is a natural break in data at less than 9 and greater than 19 CFU/100 ml. It can be seen that whether 100 total staphylococci/100 ml or 10 S. aureus/100 ml are used as cut-off points, there are only slight differences between sampling sites in the "High Staph" and "Low Staph"

categories. Of particular interest was that the staphylococcal counts at the beaches were consistent with the findings in the Phase 1 of this study (Table 3.1 and Table 3.2). The conclusions drawn from Table 4.3 and Table 4.4 were that the sampling sites with high levels of staphylococcus bacteria were located in the popular recreational areas and were always heavily used, and low levels of the organisms were observed at the less popularly used sites. The fact that high numbers of total staphylococci and S. aureus were consistently found at the sampling sites with heavy bathing loads supports the conclusion that the levels of staphylococcus bacteria are strongly associated with swimmer density. These results are in agreement with the findings of other investigators. Ortiz et al (1979), in their study in freshwater ponds, found that S. aureus concentrations were positively correlated with the number of swimmers using the ponds. Dufour (1990) also found that staphylococci levels were related to bathing density. In a seawater study by Fattal et al. (1986), it was reported that staphylococci density was associated with swimmer density.

2.2 Analyzed by TGA+AZ medium

A total of 143 marine water samples from the same 27 sampling sites was analyzed by using TGA+AZ medium. The range and mean concentrations in CFU/100 ml of total staphylococci and S. aureus are presented in Table 4.5. It

can be seen that the pattern of high and low levels of total staphylococci and S. aureus was consistent with those analyzed on VJ+AZ. The mean levels of total staphylococci ranged between 4.9 and 527.9 CFU/100 ml and S. aureus levels ranged from < 2 to 121.0 CFU/100 ml. It is of interest that the Hanauma Bay (middle area) samples yielded the highest concentrations of total staphylococci on both VJ+AZ and TGA+AZ and the highest level of S. aureus on TGA+AZ.

Table 4.6 shows the mean values (derived from the data shown in Appendix E) of total staphylococci and S. aureus analyzed for the comparative selectivity of TGA+AZ and VJ+AZ. Although the two media were not significantly different in enumerating S. aureus ($t = 1.24$, $p = 0.2155$), the mean concentrations of total staphylococci recovered from VJ+AZ were significantly higher ($t = 2.28$, $p = 0.0244$). Thus, the analytic part of this study is hereafter based on the values of total staphylococci and S. aureus using VJ+AZ medium.

It can be concluded that compared to the media previously used in other studies, TGA+AZ and VJ+AZ were the most successful in recovering staphylococcus bacteria including S. aureus in Hawaii marine waters. It was also noted that the concentrations of these organisms determined by the two media were consistently in comparable ranges. By using the t-test analysis, it was found that there was no significant difference between the mean values of S. aureus

recovered from the two media but the mean levels of total staphylococci obtained from VJ+AZ were significantly higher than those obtained from TGA+AZ. Therefore, only the results obtained from the VJ+AZ medium were used subsequently in the statistical analysis.

3. Statistical analysis

The analysis in this section is based on the results of total staphylococci and S. aureus recovered on VJ+AZ medium. The correlation coefficient was used as a measure of association between two continuous variables that estimates the direction and strength of linear relationship (Bohrnstedt and Knoke, 1988). The variables TOTAL (levels of total staphylococci), AUREUS (S. aureus levels), and USER (the number of beach users) were analyzed by PROC CORR of the SAS program (SAS, 1985). Results in Table 4.7 show that the levels of total staphylococci were significantly correlated with that of S. aureus ($r = 0.59$, $p = 0.0001$) and with the number of beach users ($r = 0.44$, $p = 0.0001$).

To determine the relationships between the variation of total staphylococci levels (entered in the model as TOTALRT or the square root form of TOTAL) and three other variables, MONTH (the months when water samples were collected), SITE (sampling sites), and USER, regression analyses were performed using PROC GLM of the SAS program. First, square root of total staphylococci was regressed on each of the

three variables separately. It was found that the months had some effect on the variation of total staphylococci levels with a borderline significance ($F = 1.877$, $p = 0.053$), whereas sampling sites and the number of beach users both had a very highly significant effect on total staphylococci variation ($F = 2.61$, $p = 0.0002$ and $F = 7.517$, $p = 0.0001$, respectively). The highly significant effects of sampling sites and the number of beach users on total staphylococci variation led to another step of the analysis, that is to determine the effect of one variable while controlling the other. The results in Table 4.8 show that after controlling for the effect of the number of beach users, the association between sampling sites and square root of total staphylococci was not significant ($F = 1.10$, $p = 0.35$). But after controlling for the effect of sampling sites, the relationship between the number of beach users and square root of total staphylococci was still highly significant ($F = 12.23$, $p = 0.0006$).

The results of regressing square root of total staphylococci on all three variables are given in Table 4.9. After adjusting for months and the number of beach users, the effect of sampling sites on the variation of total staphylococci levels was not significant ($F = 1.10$, $p = 0.36$). After controlling for sampling sites and the number of beach users, the association between months and square root of total staphylococci was of borderline significance

(F = 1.85, p = 0.059). The relationship between the number of beach users and the square root of total staphylococci was still highly significant after controlling for the effect of months and sampling sites (F = 10.50, p = 0.0016).

The strong correlation between the concentrations of total staphylococci and S. aureus levels indicated that they were consistently present together. In other words, total staphylococci was an indicator of the presence of S. aureus. In order to determine the seasonal variation of staphylococcus bacteria in marine waters, the months of sample collections were analyzed in the regression analysis. The results at both the bivariate and multivariate levels revealed the borderline significant effect of months. Future study with larger sample size is needed to address the importance of seasonal variation of staphylococcus bacteria in the marine recreational waters. In the bivariate analysis, the location of the beaches (sampling sites) and the number of beach users showed a highly significant effect on total staphylococci variation. After controlling for the effects of other variables, the effect of sampling sites was no longer significant whereas the number of beach users still showed a highly significant effect. It can also be stated that the number of beach users is a more valid indicator of the staphylococci concentrations in recreational waters than the beach locations.

4. Possible sources of staphylococcus bacteria in marine waters

In Phase 2 of the study, the new improved selective media, TGA+AZ and VJ+AZ, were used in the analysis of fresh and brackish water samples, sand samples, and sewage samples for possible sources of staphylococcus bacteria.

4.1 Fresh, brackish water, and sand samples

In this section, 19 samples of freshwater stream samples obtained from Kaelepulu Stream, 9 brackish water samples from Ala Wai Canal, 1 sand and 1 sediment samples from Hanauma Bay were analyzed for total staphylococci and S. aureus. The results given in Table 4.10 show that the percent recovery of total staphylococci in the samples taken from Ala Wai Canal and Kaelepulu Stream were higher than those recovered on these media without the addition of sodium azide (Table 3.3). Up to 46% of presumptive colonies (8.7 CFU/100 ml) of total staphylococci were recovered on TGA+AZ from Ala Wai Canal and 45% (19.7 CFU/100 ml) on VJ+AZ were obtained from Kaelepulu Stream, compared to 6.7% obtained on TGA in the former samples and 8% on VJ in the latter (Table 3.3). However, very low levels of S. aureus (< 2 CFU/100 ml) were recovered from these fresh and brackish waters. Further studies should be conducted on the use of TGA+AZ and VJ+AZ for the recovery and enumeration of staphylococci in fresh and brackish waters.

Two sand samples obtained from Hanauma Bay, one from beach sand and the other from underwater sediment, were analyzed by using VJ+AZ medium. The results (Table 4.10) show the percent recovery of total staphylococci from presumptive colonies were 70% and 80%. These percentages were higher than the results shown in Table 3.9 when TGA and VJ media were used. It should be noted that the total staphylococcus levels recovered from beach sand (5.3×10^5 CFU/100 gm) were appreciably higher than that obtained from sand under the water(768 CFU/100 gm).

The results presented in this section were based on only few samples. However, the use of VJ+AZ which recovered 70% and 80% of total staphylococci from the Hanauma Bay sand samples appears promising and needs further investigation. The results obtained thus far indicated that beach sand may be a source of staphylococcus bacteria.

4.2 Sewage

The possibility of beach water contaminated by sewage was also examined. Four samples of primary treated sewage taken after the primary clarifier were obtained from three wastewater treatment plants (Honouliuli, Sand Island, and Kailua) for the enumeration of staphylococci using VJ+AZ medium to suppress the growth of non-staphylococcus bacteria.

The results of the analyses showed that no staphylococcal colonies were recovered on the medium in two

of the four samples. The gram stain results of selected colonies indicated that they were gram-positive rods. Very low percentages of total staphylococci were recovered from the other two sewage samples, 30% (960 CFU/100 ml) of presumptive colonies from the Sand Island sample and 50% (2000 CFU/100 ml) from the Honouliuli sample. These staphylococcal colonies were lysostaphin sensitive but reacted negative to coagulase test indicating that they were coagulase-negative staphylococci. These numbers were calculated from the low percentages of presumptive colonies and had to be based on the numbers of total staphylococci and from non-staphylococcus bacteria which grew on the medium. This indicates that only small populations of staphylococcus bacteria were present in sewage. In this regard, Sinclair and Alexander (1984) reported that S. aureus density declined markedly (less than 10 cells/ml on day 3) in sewage because of its susceptibility to starvation in such an environment. It is stated in Bergey's Manual (1974) that the primary source of coagulase-positive staphylococci in beach water was usually not from sewage contamination but the bathers themselves who harbor the bacteria in several parts of the body. Staphylococcus bacteria recovered from sewage could not be confirmed as S. aureus.

5. Relationship of other water quality parameters to staphylococci densities in recreational waters

The results presented in Table 4.11 are of 19 marine water samples and one freshwater sample (Kaelepulu Stream) tested for salinity, turbidity, pH, phosphate, staphylococci, vibrios, pseudomonas, enterococci, E. coli, and C. perfringens. Staphylococci were examined on two media, TGA+AZ and VJ+AZ. The presumptive counts of staphylococcus bacteria in the two media were similar for most samples. The Hanauma Bay samples (W135 - W137) were taken from the far left and far right side of the bay (facing the ocean), and the middle area. The highest counts of presumptive staphylococci were recovered from the middle area sample where most of the swimming takes place. Vibrios were recovered in the range of between 120 and 7200 CFU/100 ml. The highest count was observed in the water sample taken from Kailua beach (W165). Pseudomonas Isolation Agar was used to recover pseudomonas bacteria from all water samples. The range of pseudomonas counts was between 240 and 24,800 CFU/100 ml with the highest, again, recovered from the Kailua sample (W165). The levels of enterococci ranged from 0 to 88 CFU/100 ml with the highest count obtained from the Waimea sample (W168). The E. coli concentrations in marine water samples ranged from 0 to 30 CFU/100 ml, whereas the Kaelepulu stream sample contained 1304 CFU/100 ml. C. perfringens were recovered in very low

levels in all samples with the highest count of 8 CFU/100 ml in the Kaelepulu sample.

The recovery of staphylococci from the 3 Hanauma Bay samples indicated that the middle area was the most contaminated. The staphylococci density obtained from the middle area was about 100 times greater than those recovered from the left and right areas. The staphylococci levels in these three Hanauma Bay areas seemed to parallel that of vibrios and pseudomonas but not other indicator bacteria or the physical and chemical measurements.

The results of the 2 Haleiwa samples (W162 & W167) are also of interest. The staphylococci density recovered from sample W162 was about 10 times greater than that of sample W167. The high concentrations of staphylococci in sample W162 were consistent with the levels of vibrios, pseudomonas, enterococci, and E. coli. On the other hand, the levels of these bacteria were low in sample W167. The concentrations of clostridium in all water samples examined were very low indicating the absence of sewage contamination.

All bacterial levels recovered from the two Waimea samples (W163 & W168) were high. Again, the high counts of bacteria did not particularly reflect any of the physical or chemical results. Seven of the 19 marine water samples contained enterococci levels higher than the present Hawaii State Water Quality Standard of 7 enterococci/100 ml.

The level of enterococci in the freshwater Kaelepulu Stream sample was very high (64 CFU/100 ml) compared to this standard. At this time the recommended standard for fresh recreational water in Hawaii is either 126 E. coli or 33 enterococci per 100 ml.

In general, the assessment of this set of marine and freshwater samples showed that staphylococci levels paralelled those of other bacteria. The physical and chemical measurements i.e., salinity, turbidity, pH, and phosphate, were not useful in indicating levels of microbial contamination of recreational waters.

6. Persistence of staphylococcus bacteria in marine recreational waters

In order to examine the persistence of staphylococci in a marine beach, an attempt was made to observe the levels of total staphylococci over a 24 hour period at Kuhio Beach (Waikiki). This site was chosen based on its convenience for sample collection. Samples were collected every 2 hours beginning at 3 p.m. on the first day and ending at 3 p.m. on the second day. The number of individuals present at the beach and in the water was also estimated. Thirteen samples taken from the same spot were analyzed on VJ+AZ medium for total staphylococci. A subset of the cultures were tested for gram stain, catalase test, and lysostaphin sensitivity for the assessment of total staphylococci. The data

summarized in Appendix F are depicted in the graph presented in Figure 4.2. The results show that the levels of the organisms started decreasing after 7 p.m. and increased again at 9 a.m. the next morning. The peaks were observed at 5 p.m. (1840 CFU/100 ml) and 7 p.m. (1720 CFU/100 ml) on the first day and at 11 a.m. (1568 CFU/100 ml) and 1 p.m. on the second day (1728 CFU/100 ml). The lowest level (60 CFU/100 ml) was obtained at 5 a.m. in the morning indicating that total staphylococci could be recovered from this site throughout the 24 hour period. Interestingly, the fluctuation in staphylococci levels paralleled the number of the people present at the beach and in the water.

As shown in Figure 4.2, the variation of staphylococci density corresponded well with the number of beach users. The peaks of staphylococci concentrations were observed in the afternoon and a considerable number of individuals were present at the beach and in the water between 11 a.m. and 5 p.m. In this regard, Ortiz and his colleagues found that S. aureus levels in freshwater ponds declined at night and increased again the next day after swimming resumed. They concluded that the swimmers were the source of pollution to the ponds (Ortiz et al., 1979).

7. Antibiotic sensitivity patterns of S. aureus

The antibiotic sensitivities to vancomycin, cefamandole, streptomycin, tetracycline, erythromycin, methicillin, penicillin, and ampicillin were performed on 679 isolates of S. aureus recovered from marine waters and 52 isolates from clinical skin cultures. Most of the S. aureus isolates recovered from both sources showed susceptibility patterns for the first six antibiotics (Table 4.12). The range of sensitivities to these six antibiotics for S. aureus from marine waters was from 90.7% to 99% and from 76.9% to 100% for the isolates from skin cultures. Most of the S. aureus isolates recovered from marine waters were resistant to penicillin (90%) and ampicillin (79%). Similarly, about 94% of the isolates recovered from clinical skin cultures were resistant to penicillin and 92% were resistant to ampicillin.

Antibiotic sensitivity results of s. aureus showed similar susceptibility patterns on isolates from marine waters and from clinical skin cultures. It was observed that most of S. aureus isolates obtained from both sources were sensitive to vancomycin, cefamandole, streptomycin, tetracycline, erythromycin, and methicillin; and they were highly resistant to penicillin and ampicillin. Molavi and LeFrock (1984) noted that strains of penicillin-resistant S. aureus were encountered with increasing frequencies, particularly among isolates obtained from hospital-acquired

infections. It is of interest that, in this current study, sensitivity to methicillin was observed at a very high percentage (92% for isolates from marine waters and 96% for those obtained from human skin specimens). This is in contrast to the findings of Molavi and LeFrock (1984) that methicillin-resistant strains of S. aureus have been occurring increasingly among the hospital isolates. However, Ohana et al. (1989) found that the incidence of methicillin-resistant S. aureus in community-acquired skin infections was as low as 1% and the resistance to ampicillin of these isolates was 98%. In a recent study in Hawaii, Palmer (1989) also found that S. aureus isolated from dolphins and dolphins handlers were resistant to penicillin and ampicillin but were sensitive to methicillin as well as several other antibiotics. The importance of the findings in this present study is that isolates of S. aureus obtained from clinical skin cultures and from marine recreational waters were consistant in their antibiotic sensitivity patterns indicating that they had a common source.

8. Phage typing (Biotype)

Bacteriophage (or phage) typing is a technique that further characterizes or biotypes certain strains of bacteria after they have been identified by genus and species, especially for epidemiological studies. This is analogous to "fingerprinting" or establishing individual

differences among strains. Phage typing consists of subjecting a pure culture of a bacterial strain to a battery of bacterial viruses, or phages, to determine which of the phages will lyse or destroy the bacteria (Report, 1975). S. aureus is often phage typed for epidemiological purposes or for an investigation of disease in man (Parker, 1972).

The Subcommittee on the Phage Typing of Staphylococci of the International Committee on Systematic Bacteriology (Report, 1975) designated an international set of typing phages for S. aureus consisting of 23 phages, grouped as follows:

Group I - 29, 52, 52A, 79, 80

Group II - 3A, 3C, 55, 71

Group III - 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85

Not allocated - 81, 94, 95, 96

Cultures were reported non-typable (NT) when no lysis of the bacterial culture occurred at 100 times the concentrations of the routine test dilution (100 x RTD) of the phages used. The groups generally do not correlate highly with other characteristics of the strains, although certain types of infection do correlate moderately well with the phage groups. For example, S. aureus strains causing food poisoning are almost always group III and those producing exfoliative or epidermolytic toxin which causes bullous impetigo are most often group II. The results of S.

aureus phage typing in this study completed by DOH were reported following the above guidelines.

Phage typing was performed on 48 S. aureus isolates from clinical skin cultures and 259 isolates from marine waters (Table 4.13). Fifty-six percent of the skin isolates of S. aureus and 22% of the water isolates were lysed by phage group II. The non-typable isolates comprised 27% of the S. aureus strains from skin cultures and 30% of those recovered from marine waters. Reactions to phage group III were observed in 15% of the skin isolates and 29% of those obtained from marine waters.

The phage group distributions of S. aureus isolated from human skin culture specimens and from marine waters were very similar. Of particular interest was the fact that the isolates from both sources clustered among phage group II and III, and NT. S. aureus strains lysed by phages group II and III have previously been found to be associated with human skin infections (Dillon, 1968; Mobacken et al, 1975; Anthony et al, 1967). The non-typable group accounted for about one-third of S. aureus isolates recovered from both sources. The importance of this finding is that there is a consistency of percentage occurrence for this non-typable phage group as well as in the known phage types responsible for human infections. The above findings strongly support the hypothesis that these organisms were from the same

source and that marine waters were vehicles of transmission of staphylococcal skin infections.

9. Identification of coagulase-negative staphylococci by STAPHTrac test

STAPHTrac is a test for the speciation of coagulase-negative staphylococci (API, 1986). Table 4.14 lists the various groups of the 100 isolates of staphylococci recovered from marine waters. It can be seen that most of the isolates tested were identified as S. hominis 1 (23%) and S. warneri (23%). S. hominis 2, as well as S. aureus, each comprised 13% of the isolates tested. These results confirmed that staphylococcus bacteria recovered from marine waters truly belonged to the staphylococci genus and could be identified to a species level.

Coagulase-negative staphylococci are known to be pathogens in man and animals (Mitchell, 1968; Devriese and De Keyser, 1980). The ability of S. epidermidis, one species of coagulase-negative staphylococci, to cause a variety of human infections has been well documented (Bentley, 1979; Marsik and Parisi, 1973; Patrick, 1990). In this current study, S. epidermidis was not found in the examination of 100 isolates recovered from marine waters. However, coagulase-negative staphylococci have been isolated from human infections in other studies. Nord et al. (1975) listed the following staphylococci isolated from human

urinary tract infections and wound infections: S. saprophyticus, S. haemolyticus, S. hominis, S. cohnii, S. warneri, and S. simulans. Adegoke (1986) reported that S. xylosus, S. sciuri, S. warneri, and S. cohnii were the dominant coagulase-negative staphylococci isolated from man with boil and wound infections. S. saprophyticus was reported to be the common species of coagulase-negative staphylococci that caused lower and upper urinary tract infections (Latham et al., 1983; Marrie et al., 1982). Therefore, the recovery of these coagulase-negative staphylococcus bacteria in marine waters indicated that they could be potential health hazards to bathers. The water quality monitoring for staphylococcus bacteria should include the coagulase-negative species of these organisms.

D. Summary

The extensive qualitative work in Phase 1 provided sufficient data for initial media selection and a refined method for staphylococcus bacteria identification. This was a prerequisite to the quantitative assessment of total staphylococci and S. aureus concentrations in recreational waters. TGA+AZ and VJ+AZ media gave higher recoveries of staphylococcus from recreational waters than other media examined in this study. These two media were therefore used in the subsequent analysis of beach waters. Extensive

monitoring of marine recreational waters on Oahu beaches was undertaken to classify them according to levels of staphylococcus contamination. The significant findings of this phase of the study were that total staphylococcus levels were highly correlated to that of S. aureus and to the number of beach users. The use of two maximum allowable concentrations, 100 staphylococci/100 ml and 10 S. aureus per 100 ml, in classifying beaches yielded almost indentical results. The characterizations of S. aureus isolates from marine recreational waters and from clinical skin specimens provided strong evidence that they were of the same source.

Table 4.1 Marine Recreational Water Sites Analyzed for Staphylococcus Bacteria in Phase 2

1. Ala Moana	15. Maili
2. Bellows	16. Makaha
3. Ewa	17. Nanakuli
4. Haleiwa	18. Punaluu
5. Hanauma Bay Mid[a]	19. Sand Island
6. Hanauma Bay Lft[b]	20. Sandy Beach
7. Hanauma Bay Rt[c]	21. Turtle Bay
8. Ilikai	22. Waikiki 1[d]
9. Kaaawa	23. Waikiki 2[e]
10. Kahana	24. Waikiki 3[f]
11. Kailua	25. Waikiki 4[g]
12. Kualoa	26 Waimanalo
13. Lanikai	27. Waimea
14. Magic Island	

[a,b,c]Area of Hanauma Bay when facing the ocean: middle, left, and right

[d]Waikiki 1 = Behind the police station
[e]Waikiki 2 = Queen's Surf Beach
[f]Waikiki 3 = Kuhio Beach
[g]Waikiki 4 = Sans Souci Beach

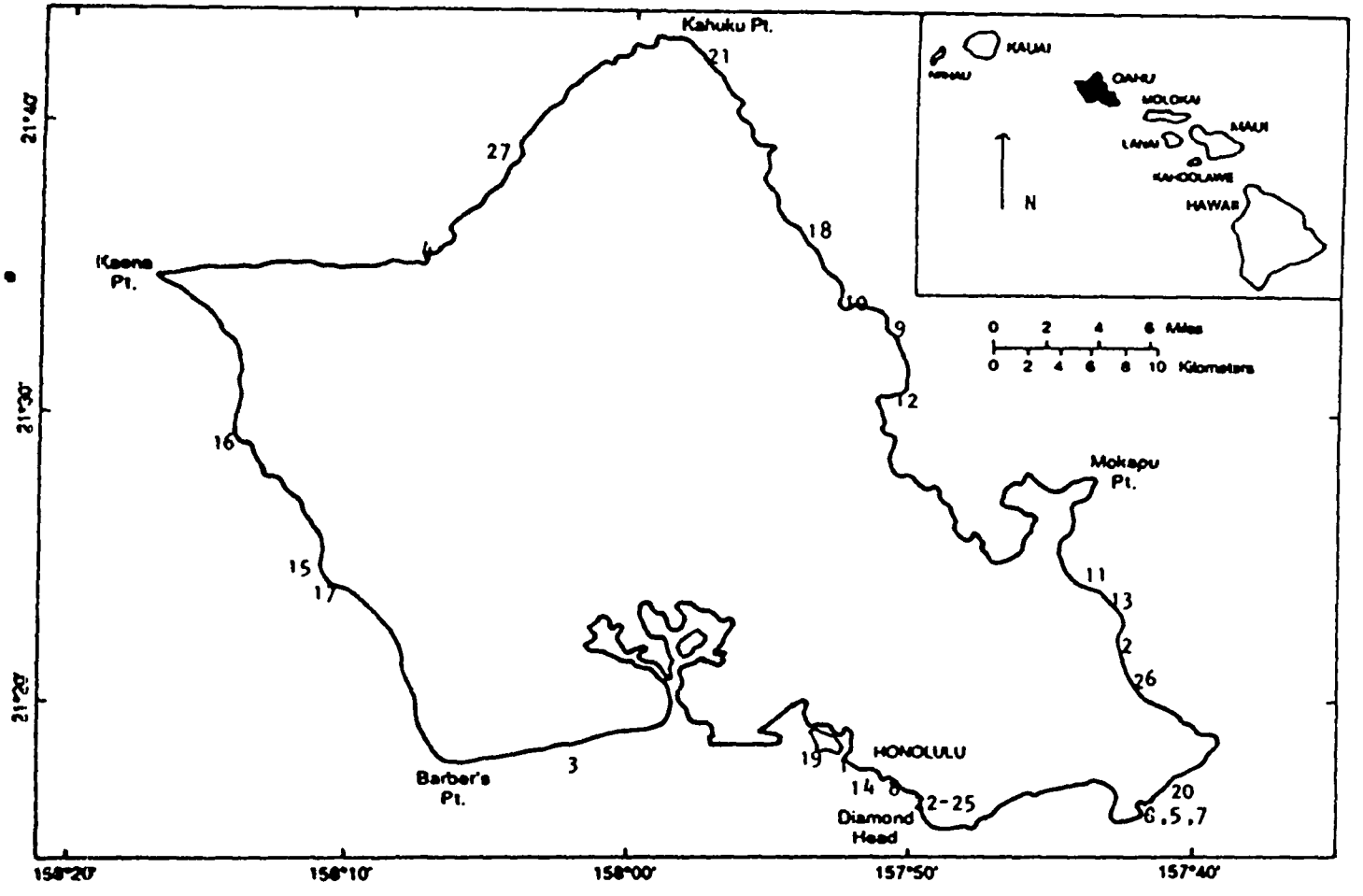

Figure 4.1 Locations of Marine Recreational Water Sites Analyzed for Staphylococcus Bacteria in Phase 2 as Listed in Table 4.1

Table 4.2 Percent Recovery of S. aureus and Total Staphylococci on Various Media

	TGA	TGA+AZ	mTG	mTG+AZ	VJ	VJ+AZ
Marine water:						
S. aureus	28	27	20	28	15	31
Total Staphylococci	65	83	45	73	50	90
Brackish water: (Ala Wai Canal)						
S. aureus	0	5	5	0	0	0
Total Staphylococci	0	48	26	48	0	33

Table 4.3 Geometric Mean Concentrations (CFU/100 ml) of Staphylococci Recovered from Selected Marine Water Sites on Oahu on VJ+AZ Medium; "Low Staph" Defined as Less Than 100 Staphylococci/100 ml.

Sampling Sites	Total Staphylococci	Range	S. aureus	Range
"Low Staph" Sites:				
Maili (3)[a]	1.6	1 - 2	< 2	< 2
Kahana (3)	12.9	< 2 - 47	< 2	< 2
Ewa (6)	14.0	4 - 272	< 2	< 2
Punaluu (3)	16.3	5 - 72	< 2	< 2
Bellows (3)	26.0	5 - 76	4.3	< 2 - 41
Haleiwa (6)	33.2	4 - 2304	2.0	< 2 - 5
Kualoa (4)	36.2	10 - 340	< 2	< 2
Sand Island (4)	36.8	< 2 - 230	5.1	< 2 - 66
Magic Island (4)	41.9	3 - 416	2.7	< 2 - 52
Lanikai (3)	58.0	< 2 - 120	< 2	< 2
Hanauma RT[b] (2)	64.6	6 - 196	< 2	< 2
Turtle Bay (3)	64.8	9 - 1044	< 2	< 2
Nanakuli (3)	68.0	3 - 588	< 2	< 2
Makaha (4)	71.7	24 - 213	5.3	< 2 - 14
Ilikai (4)	74.6	10 - 544	< 2	< 2
Waimanalo (9)	76.5	20 - 598	22.3	< 2 - 72
Sandy Beach (7)	84.2	20 - 608	9.0	< 2 - 30
Kailua (15)	87.7	< 2 - 1869	19.4	< 2 -234
Kaaawa (3)	90.0	50 - 162	2.4	< 2 - 13
"High Staph" Sites:				
Ala Moana (17)	122.2	8 - 436	70.0	< 2 - 257
Wkk1 PS[c] (5)	130.8	42 - 479	56.9	< 2 - 217
Wkk3 QS[d] (6)	156.2	14 - 45	22.3	< 2 - 137
Waimea (10)	159.7	< 2 - 2200	92.9	< 2 -2200
Wkk2 KH[e] (7)	227.2	61 - 2124	77.5	< 2 - 472
Wkk4 NAT[f] (4)	341.7	118 - 2268	< 2	< 2
Hanauma LFT[g] (2)	602.4	252 - 1440	32.6	14 - 76
Hanauma MID[h] (16)	689.7	88 - 2880	51.7	< 2 -2600

[a] Number of samples
[b, g, h] Area of Hanauma Bay when facing the ocean: right, left, and middle
[c] Waikiki 1 = Behind the police station
[d] Waikiki 3 = Queen's Surf Beach
[e] Waikiki 2 = Kuhio Beach
[f] Waikiki 4 = Sans Souci Beach

Table 4.4 Geometric Mean Concentrations (CFU/100 ml) of Staphylococci Recovered from Selected Marine Water Sites on Oahu on VJ+AZ Medium; "Low Staph" Defined as Less Than 10 S. aureus/100 ml.

Sampling Sites	Total Staphylococci	Range	S. aureus	Range
"Low Staph" Sites:				
Maili (3)[a]	1.6	1 - 2	< 2	< 2
Kahana (3)	12.9	< 2 - 47	< 2	< 2
Ewa (6)	14.0	4 - 272	< 2	< 2
Punaluu (3)	16.3	5 - 72	< 2	< 2
Bellows (3	26.0	5 - 76	4.3	< 2 - 41
Haleiwa (6)	33.2	4 - 2304	2.0	< 2 - 5
Kualoa (4)	36.2	10 - 340	< 2	< 2
Sand Island (4)	36.8	< 2 - 230	5.1	< 2 - 66
Magic Island (4)	41.9	3 - 416	2.7	< 2 - 52
Lanikai (3)	58.0	< 2 - 120	< 2	< 2
Hanauma RT[b] (2)	64.6	6 - 196	< 2	< 2
Turtle Bay (3)	64.8	9 - 1044	< 2	< 2
Nanakuli (3)	68.0	3 - 588	< 2	< 2
Makaha (4)	71.7	24 - 213	5.3	< 2 - 14
Ilikai (4)	74.6	10 - 544	< 2	< 2
Sandy Beach (7)	84.2	20 - 608	9.0	< 2 - 30
Kaaawa (3)	90.0	50 - 162	2.4	< 2 - 13
Wkk4 NAT[f] (4)	341.7	118 - 2268	< 2	< 2
"High Staph" Sites:				
Waimanalo (9)	76.5	20 - 598	22.3	< 2 - 72
Kailua (15)	87.7	< 2 - 1869	19.4	< 2 - 234
Ala Moana (17)	122.2	8 - 436	70.0	< 2 - 257
Wkk1 PS[c] (5)	130.8	42 - 479	56.9	< 2 - 217
Wkk3 QS[d] (6)	156.2	14 - 45	22.3	< 2 - 137
Waimea (10)	159.7	< 2 - 2200	92.9	< 2 -2200
Wkk2 KH[e] (7)	227.2	61 - 2124	77.5	< 2 - 472
Hanauma LFT[g](2)	602.4	252 - 1440	32.6	14 - 76
Hanauma MID[h](16)	689.7	88 - 2880	51.7	< 2 -2600

[a] Number of samples
[b, g, h] Area of Hanauma Bay when facing the ocean: right, left, and middle
[c] Waikiki 1 = Behind the police station
[d] Waikiki 3 = Queen's Surf Beach
[e] Waikiki 2 = Kuhio Beach
[f] Waikiki 4 = Sans Souci Beach

Table 4.5 Geometric Mean Concentrations (CFU/100 ml) of Staphylococci Recovered from Selected Marine Water Sites on Oahu on TGA+AZ Medium

Sampling Sites	Total Staphylococci	Range	S. aureus	Range
Maili (3)[a]	4.9	< 2 - 30	< 2	< 2
Kahana (3)	14.1	< 2 - 56	3.3	< 2 - 37
Ewa (6)	19.7	2 - 260	3.5	< 2 - 6
Punaluu (3)	18.5	< 2 - 132	< 2	< 2
Bellows (3	15.7	4 - 39	2.9	< 2 - 25
Haleiwa (6)	23.6	2 - 160	4.8	< 2 - 16
Kualoa (4)	38.9	8 - 209	< 2	< 2
Sand Island (4)	36.8	0 - 238	2.6	< 2 - 48
Magic Island (4)	43.9	5 - 509	4.0	< 2 - 127
Lanikai (3)	72.9	12 - 2016	< 2	< 2
Hanauma RT[b] (2)	60.8	13 - 284	< 2	< 2
Turtle Bay (3)	39.3	9 - 612	5.9	< 2 - 68
Nanakuli (3)	42.4	< 2 - 230	< 2	< 2
Makaha (4)	83.7	20 - 350	18.3	< 2 - 70
Ilikai (4)	38.9	5 - 912	< 2	< 2
Waimanalo (9)	61.6	< 2 - 472	56.2	< 2 - 236
Sandy Beach (5)	52.4	< 2 - 250	6.0	< 2 - 9
Kailua (14)	80.3	< 2 - 1834	39.3	< 2 - 688
Kaaawa (3)	68.2	31 - 197	2.6	< 2 - 13
Ala Moana (14)	108.3	25 - 524	70.8	< 2 - 216
Wkk1 PS[c] (3)	258.0	108 - 572	82.9	36 - 278
Wkk3 QS[d] (6)	105.4	10 - 384	26.9	< 2 - 74
Waimea (10)	230.8	< 2 - 1640	85.9	< 2 - 294
Wkk2 KH[e] (6)	280.7	128 - 1360	46.3	<2 - 248
Wkk4 NAT[f] (4)	263.6	79 - 1792	6.3	<2 - 133
Hanauma LFT[g] (2)	524.2	198 - 1388	33.8	< 2 - 88
Hanauma MID[h] (12)	527.9	59 - 2400	121.0	<2- 2160

[a] Number of samples
[b, g, h] Area of Hanauma Bay when facing the ocean: right, left, and middle
[c] Waikiki 1 = Behind the police station
[d] Waikiki 3 = Queen's Surf Beach
[e] Waikiki 2 = Kuhio Beach
[f] Waikiki 4 = Sans Souci Beach

Table 4.6 Comparison Between the Two Most Reliable Media in Recovering Staphylococcus Bacteria Recovered from Marine Waters (N = 141)

	TGA+AZ	VJ+AZ	t-value	p
Total Staphylococci	275.8[a]	329.3[a]	2.28	0.0244
S. aureus	61.8[a]	68.6[a]	1.24	0.2155

[a] Mean concentrations in CFU/100 ml

Table 4.7 Correlations Coefficient Between TOTAL[a], AUREUS[b], and USER[c] (N = 155)

	TOTAL	AUREUS	USER
TOTAL	1.0000 (0.0000)[d]	0.5931 (0.0001)	0.4370 (0.0001)
AUREUS	0.5931 (0.0001)	1.0000 (0.0000)	0.4376 (0.0001)
USER	0.4370 (0.0001)	0.4376 (0.0001)	1.0000 (0.0000)

[a]The levels of total staphylococci
[b]The levels of S. aureus
[c]The number of beach users
[d]p-value

Table 4.8 Results of Regression Analysis With TOTALRT as the Dependent Variable

R^2 = 0.4039, N = 155

Source	df	F	p
SITE	26	1.10	0.3511
USER	1	12.23	0.0006

Table 4.9 Regression Analysis Results When TOTALRT Was Regressed on the Three Variables

R^2 = 0.4854, N = 155

Source	df	F	p
SITE	26	1.10	0.3564
MONTH	10	1.85	0.0592
USER	1	10.50	0.0016

Table 4.10 The Concentrations of Staphylococcus Bacteria Recovered from Freshwater Streams, Brackish Water (CFU/100 ml), and Sand Samples (CFU/100 gm)

Site	No. of Sample (n)	Media	Total Staphylococci	S. aureus
Ala Wai Canal	(4)	TGA+AZ	8.7 (46%)	< 2
	(5)	VJ+AZ	7.8 (30%)	< 2
Kaelepulu Stream	(7)	TGA+AZ	10.0(36%)	< 2
	(12)	VJ+AZ	19.7(45%)	< 2
Sand (Hanauma Bay)	(1)	VJ+AZ	$5.3x10^5$ (70%)	< 2
Sand (Hanauma Bay) sediment	(1)	VJ+AZ	768 (80%)	< 2

Table 4.11 Results of Indicator Bacteria and Other Water Quality Parameters in Water Samples Around Oahu

Sample #	Samples	CFU/100 ml							Salinity (ppt)	Turbidity (NTU)	pH	Phosphate (mg/l)
		TGA+AZ	VJ+AZ	Vibrios	Pseudo-monas	Entero-cocci	E. coli	Clostri-dium				
W133	Kaelepulu	22	8	3000	6800	64	1304	8	23	3.4	ND	0.068
W134	Kailua	12	24	1000	1160	2	16	3	35	14	ND	0.002
W135	Hanauma(lft)	< 2	< 2	1240	680	0	0	0	35	0.6	ND	Not detectable
W136	Hanauma(mid)	468	880	3360	5200	5	5	0	35	3.4	ND	.029
W137	Hanauma(rt)	4	8	800	2440	3	3	0	35	1.0	ND	.039
W160	Ewa	260	272	3600	2920	2	13	1	34	7	7.2	0.077
W161	Makaha	144	304	1560	2480	3	4	< 2	35	2.5	7.12	0.267
W162	Haleiwa	280	2880	2200	14200	20	9	< 2	36	8.0	8.89	0.078
W163	Waimea	888	1092	2160	6800	31	27	< 2	37	4.3	7.15	0.009
W164	Kualoa	24	32	2760	16400	2	23	< 2	35	3.3	7.15	0.056
W165	Kailua	760	420	7200	24800	39	27	6	36	4	8.84	0.052
W166	Makaha	< 2	< 2	2080	3480	2	1	0	35	2.5	7.65	0.023
W167	Haleiwa	20	8	480	1640	0	0	0	35	1.5	8.07	0.004
W168	Waimea	1640	2200	520	3400	88	30	1	35	2.7	8.05	Not detectable
W169	Kualoa	12	18	1080	1960	0	0	0	34	3.0	8.11	Not detectable
W170	Kailua	8	12	1240	240	9	0	0	36	4	8.10	0.002
W171	Waimanalo	8	44	640	1000	0	0	0	36	3	8.39	0.227
W172	Hanauma	66	88	600	1680	13	1	0	35	1.5	8.13	Not detectable
W173	Ala Moana	68	64	800	960	0	5	0	36	1.7	8.05	Not detectable
W174	Kuhio BP	680	652	120	1000	6	13	0	36	2	8.17	Not detectable

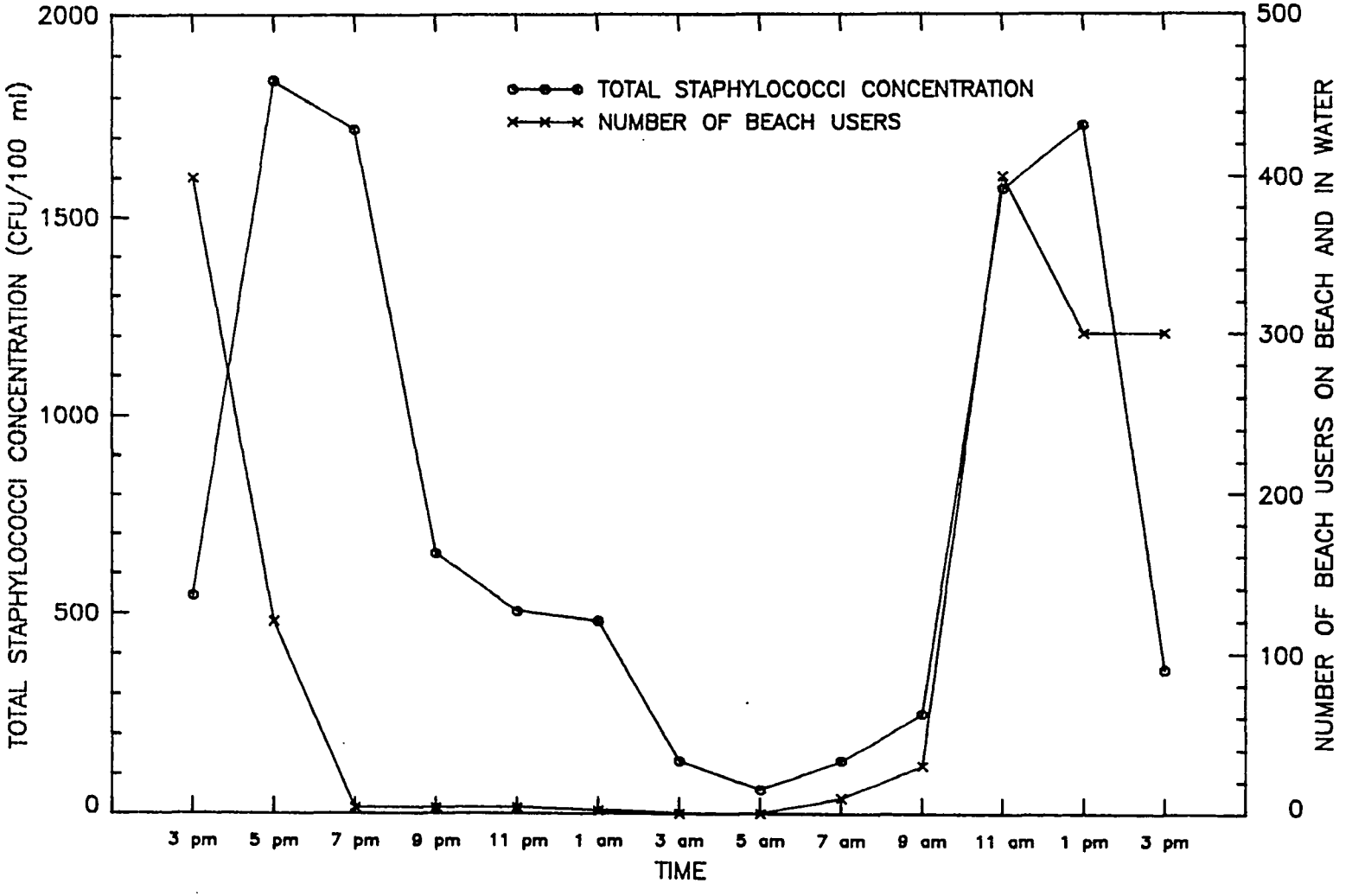

Figure 4.2 Twenty-four Hour Observation of Total Staphylococci and the Number of Beach Users

Table 4.12 Percentage Distribution of Antibiotic Sensitivities of S. aureus Recovered from Marine Recreational Waters[a] and from Clinical Skin Cultures[b]

Antibiotics/ Culture Sources	Resistant (%)	Intermediate (%)	Sensitive (%)
Vancomycin			
Marine waters	0.9	0.1	99
Skin cultures	0	0	100
Cefamandole			
Marine waters	0.7	0.3	99
Skin cultures	3.8	0	96.2
Streptomycin			
Marine waters	0.3	0.3	96.4
Skin cultures	3.8	0	96.2
Tetracycline			
Marine waters	5.2	3.1	91.7
Skin cultures	1.9	1.9	96.2
Erythromycin			
Marine waters	8.5	0.8	90.7
Skin cultures	23.1	0	76.9
Methicillin			
Marine waters	1.8	6.0	92.2
Skin cultures	0	3.8	96.2
Penicillin			
Marine waters	80.9	0.8	18.3
Skin cultures	94.2	0	5.8
Ampicillin			
Marine waters	79.4	2.0	18.6
Skin cultures	92.3	3.8	3.8

[a]N = 679, [b]N = 52

Table 4.13 Phage Typing of S. aureus Isolated from Clinical Skin Cultures and from Marine Waters

Phage Types	-------Sources of S. aureus------ Skin Cultures	Marine Waters
Group I	0	10 (4%)
Group II	27 (56%)	58 (22%)
Group III	7 (15%)	75 (29%)
Nontypable (NT)	13 (27%)	78 (30%)
Not allocated	0	18 (7%)
Mixed	1 (2%)	20 (8%)
Total	48 (100%)	259 (100%)

Table 4.14 Identification of Staphylococcus Species Recovered from Marine Recreational Waters Using STAPHTrac Test

Identification	Frequency (in percent)
S. hominis 2	13
S. aureus	13
S. xylosus 1	2
S. cohnii	7
S. saprophyticus	3
S. hominis 1	23
S. simulans	5
S. warneri	23
S. xylosus 1/2	1
S. xylosus 2	5
S. haemolyticus 3	2
S. hyicus hyicus	1
S. sciuri	1
S. haemolyticus	1
Total	100

CHAPTER V

ASSOCIATION BETWEEN STAPHYLOCOCCAL SKIN INFECTIONS AND SEAWATER CONTACT: AN EPIDEMIOLOGICAL APPROACH (PHASE 3)

A. Objectives

The objectives of Phase 3 of this study were to by epidemiological design, determine the relationship between staphylococcal skin infections and seawater exposure, determine the association between visits to "High Staph" versus "Low Staph" beaches among study subjects with a history of seawater exposure, and determine the relationship of staphylococcal skin infections to other possible risk factors.

B. Materials and Methods

1. Research design

The retrospective approach was the selected method of investigation for this study. In this type of study, diseased and non-diseased groups (cases and controls) were compared for the presence and absence of suspected risk factors. It compares the two groups retrospectively with regard to the presence of certain elements in their past

experience. The term "case-control method" is usually applied to this kind of research to indicate the way the study group is assembled (Mausner and Kramer, 1985).

The dependent variable was staphylococcal skin infections (physician's diagnosis with or without laboratory verification). The main independent variable was exposure to marine water. The diseased (cases) and non-diseased (controls) groups were obtained from 5 pediatric offices. Skin culture swabs of the cases were processed in the laboratory for the confirmation of the causative bacterial agent, S. aureus. Information regarding past marine water contact was obtained from the cases and controls by phone interview. The study subjects consisted of 53 cases and 53 controls with ages ranging from 4 months to 16 years. The cases and controls were compared as to their histories of marine water contact within the preceding 10 days of the onset of symptoms or visit to a physician. Comparison was also made between groups regarding the sites where they had been in contact with marine water. The odds ratio was the measure of association used to compare the two groups. The data from these study subjects were obtained between May 1 and December 20, 1989.

Analyses of total staphylococci and S. aureus in the marine recreational water samples from the sites visited by the cases and controls and some selected sites around the island of Oahu were conducted. When the water samples were

collected, the approximate number of individuals on the beach and in the water were also recorded. The relationship between the number of beach users during the sampling time and the concentrations of total staphylococci in marine water was determined. The levels of total staphylococci were used to classify the studied beaches as "High Staph" or "Low Staph" sites. At present, there are no national water standards for staphylococci. Therefore, the maximum allowable of 100 staphylococci per 100 ml of water was used for the cut-off point as suggested by Favero et al. (1964).

Contingency tables were constructed for categorical variables that were dichotomous (yes or no) and chi-squares with p-values were obtained. The logistic regression was used to analyze the association between exposure to marine waters and the status of study subjects (case or control). The odds ratios and their 95% confidence intervals were calculated using standard statistical techniques. This measure of association was used to test the association between staphylococcal skin infections and the past exposure to marine water. It was also applied to the association between skin infections and exposure to the varying levels of staphylococci in marine waters (high or low) among study subjects who had been in contact with marine waters.

2. Recruitment of study subjects

During the study period of May 1 - Dec 20, 1989, 53 cases of staphylococcal skin infections and an equal number of controls were recruited from 5 pediatric offices in Honolulu. The disease definition under this study is as follows:

Bullous impetigo or impetigo contaginosa is a superficial infection of the skin caused by S. aureus coagulase-positive;

Clinical Criteria for Identification:

- blister-like lesion with clear or cloudy fluid
- yellowish crusting around lesion
- erythema or redness of lesion greater than 2 mm in diameter
- peripheral spreading
- burn-like (cigarette burn) in appearance
- scaling skin either dry or weeping
- present more than 24 hours
- present in one or more lesions
- generally circular or oval in shape (Lam, 1989).

Study subjects were of both genders and of ages between 4 months and 16 years. All newly developed cases, not chronic or existing cases, occurring in the participating medical offices during the study period were invited to participate in the study. Meetings with the medical staff at all pediatric offices were conducted for clarification of the

study protocol and the subject selection procedure. The selection procedure suggested by Schlesselman (1982) was used, i.e., a procedure for the selection of a sample assuring each individual an equal chance of appearing in the study. This method is called the unbiased ascertainment of eligible cases and controls, meaning that the identification of a case and control does not depend on an individual's exposure status in regard to the study factor (marine water contact). All eligible cases arising within a defined time frame were recruited. Thus, each case and control, whether exposed to marine water or not, would have had an equal chance of selection. Another important suggestion of Schlesselman (1982), the eligibility criteria, was also applied in the selection procedure. Criteria for exclusion of certain individuals from the study were applied equally to potential cases and controls to avoid biasing the estimate of the odds ratio. Individuals with the conditions listed below were not selected to be study subjects:

- psoriasis and skin diseases of other kinds
- frequent staph infections (more than 5 times within the past year)
- water-associated diseases such as otitis externa (swimmers' ear) and swimmers' itch
- presence of conditions which prevent water contact including sutures and fractures

After consent was obtained from cases (or their parents), clinical skin culture swabs were taken from the infected area by medical personnel using a sterile dry swab from a "Culturette" (Marion Scientific). The skin culture swabs were kept at 4 °C for no longer than 48 hours before being processed in the laboratory. They were then plated directly onto blood agar plate (BAP), mannitol salt agar (MSA), and Baird-Parker agar (BP) and incubated at 37 °C for 24 hours. Isolates on BAP were characterized for confirmation of S. aureus by gram stain, catalase test, coagulase test, latex agglutination test, antibiotic sensitivity, and phage typing.

Phone interviews of the cases or their parents were subsequently conducted. The controls for the study consisted of patients who visited the medical offices with conditions other than skin infections such as physical check-ups, colds, stomach aches, eye irritations, and sore throats. The controls were of the same gender as the cases, of similar age (± 5 years of cases), and visited doctors within the same week as cases. Phone interviews of the controls or their parents were conducted following their consent to participate in the study. A questionnaire (Appendix H.) developed for the phone interview of cases and controls contained the basic information regarding age, sex, the presence or absence of seawater contact during the preceding 10 days, beach locations where contact with

seawater occurred, and the average time actually spent in the water during each water contact.

3. Sample size of study subjects

The sample size of 56 cases and 56 controls was initially calculated based on the formula suggested by Schlesselman (1974) to obtain a sample size (n) per group. The number of study subjects in a case-control study depends on the specification of the values given below. The selected parameters for the values chosen for this study were:

1) The desired level of significance, $\alpha = .05$

2) The desired study power, $1 - \beta = 90\%$ ($\beta = .10$)

3) The relative risk (R) that the required sample size is expected to detect = 3.7, and

4) An estimate of the expected rate of exposure among controls (p_0) = 0.24. This value was obtained by telephone interviews of 25 randomly selected households to determine whether any household member had been exposed to marine water within the 10 day period. Ten days is the incubation period of staphylococcal skin infections.

The calculated number of cases and controls was originally scheduled to be obtained between May 1 and September 31, 1989 and, due to unforeseen delays, later extended to December 20, 1989. There were 53 cases identified at all of the participating pediatric offices

within this time period. Changing the sample size from 56 to 53 per group slightly reduced the study power from 90 to 88.2 percent.

4. Evaluation of methods to identify S. aureus from clinical skin samples

In order to test the method on clinical specimens, nineteen human skin culture swabs were provided by a pediatrician's office. The culture swabs were taken from patients with a diagnosis of presumptive staphylococcal skin infection. Several biochemical tests were performed on the cultures in an attempt to identify the etiological agent. Three selective media were used for the initial isolation: BAP, TGA, and MSA. Following the observation of typical S. aureus characteristics, isolates from BAP were then tested for gram stain, catalase, coagulase, and latex agglutination tests. As shown in Table 5.1, 16 of the 19 cultures (88.9%) that grew on the selective media produced positive reaction to all of the tests performed, confirming the presence of S. aureus. Three culture swabs showed no growth on the selective media. Thus, the media and methodology were determined to be satisfactory for the identification of the etiological organism of interest from clinical samples.

C. Results and Discussion

1. Characteristics of the study subjects

Data on 53 cases of staphylococcal skin infections and 53 matched controls were collected from 5 pediatric offices in Honolulu. The majority of the cases were obtained from Dr. Jeremy Lam's Pediatric Office (49%) and from Kaiser Outpatient Pediatric Clinic (39%). The cases consisted of 31 males and 22 females. The average age of the cases was 4.5 years from a range of 4 months to 16 years of age. The majority (83%) of the cases were under the age of 7.

Figure 5.1 shows the distribution of the staphylococcal skin infections cases obtained during the study period (the data are summarized in Appendix G). It is noticeable that the highest number of cases, 19 of 53 cases (36%) was obtained in August. One possible explanation may be that it was during summertime so the children had more time for recreational activities at the beach. The hot summer weather may also explain the high number of children spending time in the water. However, since the sources of study subjects were limited to only five pediatric offices in Honolulu, the cases' distribution could not be representative of all staphylococcal skin infections occurring at the time of the study.

The number of persons per room in a household as defined by the number of people in the household divided by

the number of rooms in the household (excluding bathrooms and kitchen), has been used as a proxy measurement for socioeconomic status (SES). A person/room ratio of less than 1.0 was considered a high SES; a ratio between 1.0 and 1.4 was an intermediate SES; and a ratio of greater than 1.4 was considered a low SES (Calderon and Mood, 1981). This information is given for cases and controls in Table 5.2. It indicates that the majority of cases and controls were of an intermediate socioeconomic status, and the two groups were not significantly different in this regard (Chi-square = 2.782, p = 0.249). Low SES, overcrowding, and unhygienic conditions have been previously identified as risk factors for children's superficial skin infections (Dillon, 1968; Masawe, et al., 1975; Montgomery, 1985). Since the case group and the controls in this study were similar in SES, this was not likely to play an important role in skin infection cases.

Schlesselman (1982) suggested that individuals selected as controls should be free of the study disease and be similar to the cases in regard to past potential for exposure during the time period of risk under consideration. A control group for this study was selected from individuals who did not have skin infections and who visited the medical offices for such things as physical check-ups, upper respiratory tract infections, stomach aches, and eye

irritations. The controls were matched with the cases by age (± 5 years) and sex.

2. Etiological agents of staphylococcal skin infections

The 53 skin culture swabs were plated onto BAP, BP, and MSA. Forty-seven of the 53 grew on these three media mostly with typical characteristics of S. aureus; i.e., beta-haemolytic pattern on BAP, black colonies with surrounding clear zone on BP, and positive reactions on MSA. These 47 cultures were tested further for confirmation as S. aureus (Table 5.3). Grape-like clusters of gram positive cocci and positive catalase reaction was observed in 97.9% (46/47) of the cultures tested. When these 46 isolates were tested by coagulase tube test, 91.3% (42/46) yielded positive (4+) reaction. Forty-one of the 42 (97.7%) coagulase-positive isolates were confirmed positive by the latex agglutination test. Four of the 46 isolates (8.7%) gave negative reaction to coagulase tube test.

3. Seawater exposure

General information on seawater exposure is shown in Table 5.4. Wading and swimming were the most common aquatic activities among the cases and controls. A total seawater exposure time of less than 3 hours, during the 10-day period, was experienced by the majority of cases and

controls. There was no significant difference among the two groups in this regard (Fisher's exact probability = 0.53). Most study subjects reported having contacted seawater only once during the 10-day period. The Fisher's exact probability of 0.11 indicated no significant difference among the cases and controls in their frequencies of seawater contact.

As shown in Table 5.5, cases reported having a past seawater contact significantly more often than controls (Chi-square = 9.66, p = 0.001). The odds ratio was calculated to be 3.78 with the 95% confidence interval (C.I.) between 1.57 and 9.19. This odds ratio indicates that those who have developed a staphylococcal skin infections are 3.78 times more likely to have had a history of seawater contact in the preceding 10 days than those without the infections.

A logistic regression analysis was used to assess the relationship between the presence of staphylococcal skin infections (dependent variable) and age, sex, and any seawater exposure (independent variables). The findings (Table 5.6) show the significant relationship between any seawater exposure and the presence of staphylococcal skin infections (Chi-square = 10.15, p = 0.0014), after controlling for the effects of age and sex. When adding other independent variables into the model (Table 5.7), seawater exposure was the only variable that indicated a

significant association with staphylococcal skin infections (Chi-square = 11.41, p = 0.0007).

The beaches visited by the study subjects within the previous 10 days are listed in Table 5.8. The beaches were classified into "High staph" and "Low staph" beaches using the maximum allowable of less than 100 staphylococci/100 ml as the cut-off point (Table 4.7). It can be seen in Table 5.9 that the proportion of those who visited "High staph" beaches versus "Low staph" beaches was not significantly different among the cases and controls (Chi-square = 1.53, p = 0.2157).

4. Relationship of age and sex to staphylococcal skin infections

The case subjects of this study were between 4 months and 16 years old with a mean age of 4.5 years. The majority (83%) of the group was under the age of 7. Young children are at increased risk for bacterial skin infections from skin scrapes and abrasions due to the tenderness of their skin. They have a greater chance of acquiring infections while engaged in recreational activities in waters containing the etiological organisms. The age distribution of cases in the present study corresponded with that reported in previous studies. Dillon (1968) reported that subjects with staphylococcal impetigo lesions in Alabama ranged in age from 7 months to 17 years old with a mean of

4.8 years. In Montgomery's study of infected skin lesions in children of Papua New Guinea, the ages ranged from 2 months to 12 years old (Montgomery, 1985). S. aureus has commonly been reported as one of the main organisms isolated from skin infections in preschool and school-age children (Dillon, 1968; Masawe, et al, 1975; Montgomery, 1985). Among adults, community-based skin infections due to S. aureus are relatively uncommon. Outbreaks of these infections in adults have occured almost exclusively in institutions such as hospitals and nursing homes (Ruben and Norden, 1982), but have also been noted among members of football teams and among river rafting guides (Pollard, 1967; Seidenfeld and Martin, 1983; Decker et al., 1986).

The case subjects in this study demonstrated a female to male ratio of 1 to 1.4. There is, however, no known inherent predisposition to staphylococcal skin infections related to sex difference. The higher male representation may be explained by the greater number of young risk-taking males found in beach activities. The sex ratio in the current study was similar to that of the two previously cited studies (Dillon, 1968 and Montgomery, 1985) on skin infections. The ratio of female to male subjects in Dillon's study was 1 to 1.2 and 1 to 1.6 in Montgomery's study.

5. Seawater contact duration, frequency, and sites

This study was to address the possible relationship between staphylococcal skin infections and exposure to marine waters. The results of the analyses showed a strong association between a history of seawater contact and the presence of staphylococcal skin infections. The odds ratio indicated that individuals with staphylococcal skin infections were almost 4 times more likely to have had a history of seawater contact than those without the infections.

Among the 106 study subjects (53 in each group), a subgroup of 35 cases and 18 controls with a history of marine water exposure was analyzed. A majority of the cases and controls spent a total time of less than 3 hours in seawater in the previous 10 days (Table 5.4). There was no statistically significant difference between these two groups with respect to amount of time spent in seawater. This is a clear difference from other swimming-associated diseases, such as otitis externa, where Calderon and Mood (1982) found that cases spent significantly more time swimming than controls. In this study, no significant difference was found in the frequency of seawater contact between cases and controls (Table 5.4). Most of them had been exposed to seawater only one time during the 10-day period.

Cases and controls were not significantly different regarding their visits to the recreational sites classified as "High Staph" and "Low Staph" beaches. However, the proportion of those who visited "High Staph" beaches versus "Low Staph" beaches was higher among cases than controls (odds ratio = 2.5), but the association was not significant ($p > 0.20$; 95% C.I. for the odds ratio = 0.7 to 9.7). The data are given in Table 5.9. Although the C.I. was large, the p value of 0.21 suggested that visiting the "High Staph" beaches could be a possible risk factor. Future research needs to be carried out with a larger sample size to further explore this association. It is of interest that Ala Moana Beach was reported to be visited by 16 of the 35 cases (Table 5.8). Although the subjects of this study were not representative of Oahu residents, the relatively high frequency of cases' visits to this particular beach deserves further investigation. Perhaps the shallow and calm water at this beach made it attractive for use by children.

6. Relationship of staphylococcal skin infections and other possible risk factors

The association between marine water contact and staphylococcal skin infections remained very strong even after controlling for the effect of age and sex. This relationship was investigated further by including some additional variables in the logistic regression model.

These included swimming pool water contact, freshwater contact, having any household members with skin infections, and sharing clothing or towels with another person during the 10-day period prior to the clinical visit.

The presence of staphylococci and S. aureus in swimming pool water has been well documented in other studies (Favero et al., 1964; Klapes, 1983; Havelaar and During, 1985; Alico and Dragonjac, 1986; Klapes and Vesley, 1986; Mates and Schaffer, 1986; Tosti and Volterra, 1988). Robinton and Mood (1966), in their swimming pool study, found that staphylococci were shed in large numbers by swimmers and S. aureus was consistently present. Therefore, an exposure to these organisms in swimming pool water could be a possible factor for skin infections.

Staphylococcus bacteria has been recovered in freshwater ponds (Ortiz, 1977; Calderon and Mood, 1982). Moreover, total staphylococci levels in freshwater lakes have been correlated with total illnesses, eye and skin infections (Seyfried et al., 1985a). The concentrations of staphylococci were also found to be related to bathing density in a study conducted at a freshwater pond (Dufour, 1990). Infections might have been developed through contacts of infected individuals within the same household.

Multiple cases of staphylococcal skin lesions in families were reported to be common (Dillon, 1968). Decker and his co-workers in their case-control study of

staphylococcal skin infections among river guides found that persons who had had infected roommates were at significantly greater risk of acquiring infections than those without infected roommates. In that study, they suggested that sharing of towels or clothing be discouraged (Decker et al., 1986). In the present study, it was found that none of the above additional variables showed any significant relationship with staphylococcal skin infections.

Seawater exposure was the only variable that was significantly associated with the staphylococcal skin infections. There have been studies of various kinds of illnesses found to be swimming-associated or water-related. In a series of prospective studies, Cabelli and his associates found that the rate of gastrointestinal symptoms was significantly higher among swimmers relative to non-swimmers (Cabelli et al., 1975; Cabelli et al., 1979; Cabelli et al., 1982). In studies conducted by Fattal and his colleagues, significant differences were found between swimmers and non-swimmers in the incidence of enteric symptoms, respiratory symptoms, ear infections, and "sick" symptoms, but not for skin symptoms (Fattal et al., 1986; Fattal et al., 1987). The findings of the studies in Canada showed that swimmers had higher rates of morbidity than a control group of non-swimmers. Types of illnesses included respiratory infections, gastrointestinal infections, ear,

and skin symptoms (Seyfried et al., 1985a). Other investigators also reported significantly higher rates of diseases such as Swimmers' Ear (Calderon and Mood, 1981), Shigellosis (Rosenberg et al., 1976), Norwalk gastrointestinal illness (Baron et al., 1982), and enteroviral disease (D'Alessio et al., 1980) among swimmers when compared to non-swimmers.

The relationship between skin symptoms and recreational water use has been examined previously in only very few studies. The main finding of the present study was the strong association between marine water contact and staphylococcal skin infections, suggesting that individuals who have been exposed to marine waters have a greater chance than non-exposed persons in contracting staphylococcal skin infections with water serving as the transmission medium.

D. Summary

The data analysis in this part of the study was based on 53 cases of staphylococcal skin infections and 53 non-infected control individuals. The results showed that persons with staphylococcal skin infections were almost four times more likely to have had a history of seawater contact than the non-infected controls. No significant difference was found between the two groups in their visits to "High Staph" versus "Low Staph" beaches. The cases and their

control group were not significantly different regarding the duration of seawater contact and frequencies of visits to the recreational marine waters. The characterizations of S. aureus isolates obtained from the infected cases along with those obtained from seawater were found to be very similar. These findings added strong support to the conclusion that marine recreational waters serve as transmission media for staphylococcal skin infections.

Table 5.1 Preliminary Results of S. aureus Tests on 19 Clinical Skin Cultures

	Growth on BAP TGA MSA	+Cocci, +Cat[a]	4+Coag[b]	Latex
No. Positive	16	16	16	16
Percentage	88.9	88.9	88.9	88.9

[a] Gram positive cocci and positive reaction to catalase test
[b] Coagulase result of 4+ positive

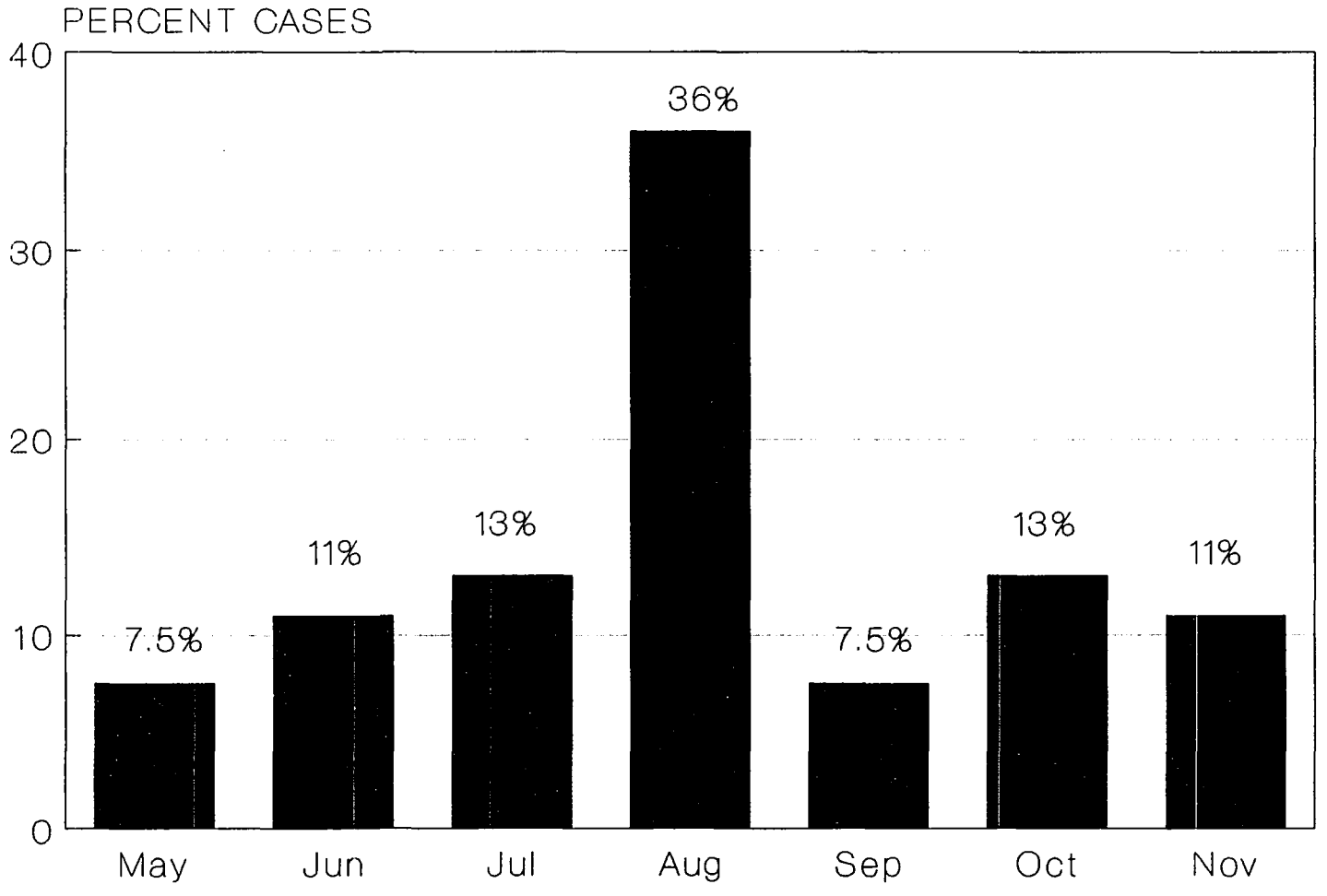

Figure 5.1 Seasonal Distribution of Cases

Table 5.2 The Number of Persons/Room Ratio for All Study Subjects

Persons/Room	Cases	Controls	Total
< 1	6	7	13
1 - 1.4	28	34	62
> 1.4	19	12	31
Total	53	53	106

Chi-square = 2.782
p = 0.249

Table 5.3 Confirmed Tests for S. aureus Recovered from Clinical Skin Cultures

Growth on BAP, BP, MSA	+Cocci,+Cat.[a]	Coag. negative	4+ Coag.[b]	Latex+
47/53	46/47 (97.9%)	4/46 (8.7%)	42/46 (91.3%)	41/42 (97.6%)

[a]Gram positive cocci and positive reaction to catalase test
[b]Coagulase result of 4+ positive

Table 5.4 History of Seawater Exposure by Activities, Duration, and Frequency During the 10-Day Period for All Study Subjects

	Cases	Controls
1) History of any seawater exposure	35	18
2) Activitites:		
Wading	16	5
Swimming	17	7
Carried by parents	2	4
Boogie board	0	2
3) Time spent in seawater[a]:		
Less than 3 hours	26	14
3 hours or more	9	4
4) Frequency of seawater contact[b]:		
One time	27	17
More than one time	8	1

[a] Fisher's exact probability = 0.53
[b] Fisher's exact probability = 0.11

Table 5.5 Any Seawater Contact Among Cases and Controls During the 10-Day Period

		Cases	Controls	Total
Seawater contact	Yes	35	18	53
	No	18	35	53
	Total	53	53	106

Chi-square = 9.66, p = 0.001
Odds Ratio = 3.78
95% Confidence Interval for odds ratio between 1.57 and 9.19

Table 5.6 Logistic Regression Analysis Demonstrating the Association Between Age, Sex, and Presence or Absence of Seawater Exposure With the Occurrence of a Staphylococcal Skin Infection

Source	df	Chi-square	p
Intercept	1	5.88	0.0153
Age	1	0.70	0.4014
Sex	1	0.05	0.8188
Seawater[a]	1	10.15	0.0014

[a] Seawater exposure during the 10-day period

Table 5.7 Logistic Regression Analysis Demonstrating the Association Between Additional Independent Variables and the Presence of Staphylococcal Skin Infection

Source	df	Chi-square	p
Intercept	1	4.13	0.0421
Age	1	0.84	0.3600
Sex	1	0.04	0.8387
Seawater exposure	1	11.41	0.0007
Pool[a]	1	0.19	0.6656
Fresh[b]	1	0.62	0.4319
Infect[c]	1	1.26	0.2610
Share[d]	1	0.07	0.7844

During the 10-day period:
[a]Any swimming pool water contact
[b]Any fresh water contact
[c]Any other household members having skin infections
[d]Sharing clothing or towels with another person

Table 5.8 List of the Beaches Visited by Cases and Controls During the 10-Day Period

Beaches	Cases	Controls	Total
"Low staph" Beaches:			
Punaluu	1	0	1
Bellows	0	1	1
Haleiwa	0	2	2
Kualoa	2	0	2
Sand Island	1	1	2
Lanikai	2	0	2
Turtle Bay	1	0	1
Nanakuli	0	1	1
Ilikai	1	1	2
Waimanalo	1	3	4
Kailua	1	0	1
"High staph" Beaches:			
Ala Moana	16	2	18
WKK3 (Queen's Surf)	4	3	7
Waimea	2	0	2
WKK2 (Kuhio Beach)	1	2	3
WKK4 (Sans Souci)	1	2	3
Hanauma Bay	1	0	1
Total	35	18	53

Table 5.9 The Number of Study Subjects Exposed to Seawater Categorized as "High Staph" and "Low Staph" Beaches

"High Staph" is defined as ≥ 100 staphylococci/100 ml.
"Low Staph" is defined as < 100 staphylococci/100 ml.

	Cases	Controls	Total
"High staph"	25	9	34
Beaches visited			
"Low staph"	10	9	19
Total	35	18	53

Chi-square = 1.53, p = 0.2157
Odds ratio = 2.5 (95% C.I. = 0.66, 9.66)

PART THREE

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER VI

SUMMARY AND CONCLUSIONS

A. Summary

This study had two main components, the recovery and enumeration of staphylococcus bacteria from recreational waters (Phase 1 and Phase 2) and the investigation of an association between staphylococcal skin infections with seawater exposure (Phase 3).

Phase 1 of the first component focused on the assessment of recovery media and methods for the identification of staphylococcus bacteria, especially the pathogen S. aureus. TGA and VJ media were used to recover total staphylococci and S. aureus from marine and fresh waters and from sand samples. These two media were effective in recovering S. aureus from marine waters but due to the interference of non-staphylococcus growth, the results were qualitative rather than quantitative. It was therefore concluded that TGA and VJ media were qualitatively effective in recovering the bacteria of interest from recreational waters, but that improvements in these media were needed. Following the evaluation of the media selectivity, TGA and VJ were used in the survey of recreational waters around Oahu to determine the levels of

staphylococci and indicator bacteria, and to carry out experiments on the potential for staphylococci multiplication in marine waters.

In Phase 2, TGA+AZ and VJ+AZ were determined to be the best and most reliable media for quantitatively recovering staphylococcus bacteria from marine recreational waters. The two media were then subsequently used for the routine analysis of staphylococcus bacteria in marine waters. Based on the quantitative analyses of these organisms, the beaches examined around Oahu were classified into "High Staph" and "Low Staph" sites. The quantitative assessment of staphylococci in marine recreational waters also found them to be highly correlated to the levels of S. aureus and to the number of beach users. The S. aureus isolates recovered from marine waters and clinical skin cultures were shown to have similar characteristics based on the coagulase reaction, antibiotic sensitivities and phage types.

The second component (Phase 3) of the study was to determine the association between staphylococcal skin infections and seawater exposure. Statistical analysis revealed a strong association between skin infections and marine water contact. There were no significant differences between cases and controls in their frequencies of exposure to seawater, nor in their duration of seawater contact. No significant difference was found between the two groups in their visits to "High Staph" and "Low Staph" sites. The

conclusion that marine recreational waters serve as vehicles for staphylococcal skin infections was strongly supported by both the statistical analysis of the epidemiological data and the bacteriological examination of the etiological agents.

However, one of the limitations of this case-control study is that the findings may not be generalizable to the total population. In this study, the sample population was children seen mainly at two primary care settings in Honolulu. The findings of this study may be generalizable to pediatric patients at these two sites but cannot be assumed to generalize to all children in Honolulu, Oahu, or the State of Hawaii.

These findings should not be generalized to adults without further study as it was previously noted that children's skin is much different from adult skin, placing the former at higher risk for injury and subsequent bacterial infection.

B. Conclusions

1. The addition of 0.005% sodium azide to TGA and VJ (TGA+AZ and VJ+AZ) was found to enhance the recovery of total staphylococci and S. aureus from marine waters.

2. There is a strong correlation between total staphylococci and S. aureus indicating that they were

consistently present together. Thus, total staphylococci is an indicator of the presence of S. aureus.

3. There was a significant correlation ($r = 0.44$, $p = 0.0001$) between the concentrations of total staphylococci obtained from marine waters and the number of beach users. This finding suggests that humans are the source of these bacteria.

4. In classifying the beaches as containing high or low concentrations of staphylococcus bacteria, it was found that the cut-off criteria established in this study (10 S. aureus per 100 ml) and the proposed standard (100 Staphylococci per 100 ml) yielded the same results.

5. There was a significant relationship between a history of seawater exposure and the incidence of staphylococcal skin infections. The odds ratio indicated that individuals with staphylococcal skin infections were almost 4 times more likely to have had a history of seawater contact than those without infections.

6. S. aureus isolates recovered from marine waters and from clinical skin cultures had several identical characteristics indicating that the strains of S. aureus from both sources are similar. These results added strong support to the hypothesis that marine water is a vehicle for the transmission of staphylococcus bacteria.

CHAPTER VII

PROJECT RECOMMENDATIONS AND FUTURE STUDIES

A. Project Recommendations

The following recommendations are based on the findings of this study.

1. The media TGA and VJ supplemented with 0.005% sodium azide used with membrane filtration methods are recommended for the recovery of staphylococcus bacteria as well as S. aureus from marine recreational waters in Hawaii.

2. It is recommended that total staphylococci and S. aureus be used as microbial indicators of pollution for marine bathing waters in Hawaii. The regulatory standard should be stated as: "Based on a sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period), the geometric mean of staphylococci densities should not exceed 100 total staphylococci per 100 ml". If this number is exceeded, then S. aureus should be assayed for as a more definitive supplemental standard with a maximum allowable level of less than 10 S. aureus per 100 ml.

3. Physicians, especially pediatricians, should be made aware of the risk of water-associated staphylococcal skin infections and should educate their patients that the

infections may be transmitted through recreational waters as well as person to person, or self infection and are more probable in the waters where beaches are heavily used. Similar information may be included with materials explaining staphylococcal infections or recreational water-related risks for those adults with water-related occupations or hobbies.

4. Consideration should be given to the posting of warning signs to inform beach users of the infectious hazards when they go into marine waters with open cuts or sores. Families should be warned of the risk to children from cuts, scratch, and sand abrasion and possible infections from water transmission.

5. Bathers should be warned that when cuts from coral or sharp rock occur in the water, they should seek medical attention and inform physicians of their water-related activities.

B. Future Studies

Media and methods for the recovery of staphylococcus bacteria from freshwater streams still need improvement. The addition of sodium azide into TGA, VJ, and BP needs further study.

The recovery and enumeration of staphylococcus bacteria from sewage samples has not been sufficiently studied. More

selective media and appropriate dilutions of the samples for the membrane filtration technique should be further studied.

The seasonal variation of staphylococcus bacteria in the open environment has not been fully investigated. The 24-hour study of staphylococci at certain beaches needs to be repeated. Other factors that may affect bacterial activities in natural waters such as wave height, tidal effects, and water temperature should be included in the observations.

Studies of staphylococcus bacteria in marine recreational waters as well as its association with health effects need to be extended to other islands in Hawaii and in the Pacific region, as marine recreational activities are common in these locations.

Other important factors which should be examined include behaviors which may contribute to skin infections. These might include bathing with soap before and after swimming, kinds of activities in seawater (boogie boarding, surfing, diving, etc.), and children's level of play activity in the sand. A more careful examination of some of these behaviors would serve to strengthen and/or reveal possible confounders.

Study subjects for future studies should include various age groups, individuals involved in water-related activities or occupations, and should be drawn from multiple medical offices covering more locations. Other types of

research design such as the prospective study should be conducted to compare the results with this current study.

As the public health ramifications of this study are far-reaching, it is imperative that additional studies be done in order to replicate the findings and to further investigate other aspects of marine water exposure which may be correlated with an increased risk for recreational water-associated illnesses.

Appendix A Total staphylococci and S. aureus Concentrations (CFU/100 ml) Recovered on TGA and VJ from Selected Marine Water Sites on Oahu

Date	Sites	Total staphylococci		S. aureus	
		TGA	VJ	TGA	VJ
04/18/88	Ala Moana (Mid)	67	36	0	0
04/18/88	Ala Moana (East)	40	18	5	0
02/15/89	Ala Moana (East)	263	132	113	38
06/14/88	Ewa	3	2	0	0
06/15/88	Haleiwa	21	14	0	0
05/30/88	Hanauma Bay (Lft)	22	18	0	0
05/30/88	Hanauma Bay (Mid)	151	238	17	106
06/13/88	Hanauma Bay (Mid)	50	30	50	30
06/14/88	Hanauma Bay (Mid)	9	16	3	3
06/15/88	Hauula	15	26	0	0
10/21/88	Kahala	15	8	0	0
10/10/88	Kahaluu	3	8	0	0
10/21/88	Kailua	2	2	0	0
10/21/88	Makapuu	3	3	0	1
06/14/88	Nanakuli	99	61	0	0
06/27/88	Public Bath	7	20	0	0
04/12/89	Sandy Beach	2	8	0	0
05/30/88	Sandy Beach	108	60	32	36
05/25/88	WKK1	137	96	0	14
05/25/88	WKK2	168	62	0	0
03/05/89	WKK2	98	164	49	41
10/21/88	WKK4	1	2	0	0

Appendix B Percent Recovery of S. aureus and Total Staphylococci on Various Media from Selected Marine Water Sites and a Brackish Water Site

Date	Sites	TGA		TGA+AZ		mTG		mTG+AZ		VJ		VJ+AZ	
		SA[a]	TS[b]	SA	TS	SA	TS	SA	TS	SA	TS	SA	TS
Marine Waters:													
01/15/89	WKK3	20	70	17	83	20	100	20	80	0	33	30	90
02/15/89	Ala Moana	30	60	15	85	20	10	60	60	20	50	40	90
03/15/89	WKK2	40	30	40	80	20	40	20	60	10	30	20	100
03/29/89	Kailua	10	80	25	80	10	40	20	85	30	60	20	90
04/12/89	Waimea	40	80	40	80	20	50	20	90	20	80	20	90
05/14/89	Ala Moana	30	70	30	90	30	30	30	60	10	50	60	80
Brackish Water:													
01/15/89	Ala Wai	0	0	0	25	0	0	0	10	0	0	0	0
02/15/89		0	0	17	50	17	33	0	50	0	0	0	40
03/15/89		0	0	0	70	0	30	0	50	0	0	0	60
03/29/89		0	0	13	50	0	0	0	75	0	0	0	33
04/12/89		0	0	0	60	13	67	0	70	0	0	0	25
05/14/89		0	0	0	33	0	25	0	33	0	0	0	40

[a] SA = S. aureus
[b] TS = Total staphylococci

Appendix C Percent Recovery of Total Staphylococci on BFR-0, TGA+AZ, and VJ+AZ Media

Date	Sites	BFR-0	TGA+AZ	VJ+AZ
06/24/89	Ala Moana	50	80	100
06/28/89		50	80	90
07/15/89		25	90	80
07/24/89		0	70	80
06/24/89	Bellows	100	50	90
06/24/89	Ewa	0	17	10
06/24/89	Haleiwa	0	60	90
06/24/89	Hanauma Bay (Mid)	30	100	100
06/28/89		30	90	90
07/15/89		20	100	100
07/24/89		30	90	90
06/24/89	Kailua	70	50	60
06/28/89		20	80	80
07/27/89		0	70	70
06/24/89	Makaha	20	50	60
06/28/89	Sandy Beach	90	80	80
06/28/89	Waimanalo	70	100	90
06/24/89	Waimea Bay	100	80	80
06/28/89	Wkk1	50	100	90
06/24/89	WKK2	30	90	80
07/15/89		20	40	80
07/24/89	WKK3	0	100	100

Appendix D Percent Recovery of Total Staphylococci on BP+AZ, TGA+AZ, and VJ+AZ Media

Date	Sites	BP+AZ	TGA+AZ	VJ+AZ
07/31/89	Ala Moana	100	80	90
08/09/89		90	80	100
08/27/89		90	100	100
09/16/89		100	100	100
09/27/89		60	100	100
09/16/89	Hanauma Bay (Mid)	90	100	100
11/13/89		100	100	100
08/27/89	Kailua	75	100	100
08/27/89	Sand Island	100	100	80
09/27/89	WKK1	70	90	100
09/16/89	WKK2	90	100	90
08/09/89	WKK3	80	80	100
08/27/89		90	90	100

Appendix E Total Staphylococci and S. aureus Concentrations (CFU/100 ml) Recovered on TGA+AZ and VJ+AZ from Selected Marine Water Sites on Oahu

Date	Sites	Total Staphylococci		S. aureus	
		TGA+AZ	VJ+AZ	TGA+AZ	VJ+AZ
02/05/90	Ala Moana	54	64	0	0
02/15/89		123	157	70	98
05/14/89		76	60	76	54
05/29/89		108	140	108	140
06/11/89		83	113	21	32
06/24/89		253	360	190	216
06/28/89		93	180	0	20
07/15/89		126	291	70	218
07/24/89		25	61	0	0
07/31/89		80	297	20	33
08/09/89		176	288	44	115
08/27/89		308	428	216	257
09/16/89		524	436	104	0
09/27/89		40	33	0	0
05/14/90	Bellows	4	5	1	2
05/21/90		25	46	25	41
06/24/89		39	76	0	0
01/21/90	Ewa	260	272	0	0
05/14/89		12	10	0	0
05/29/89		38	14	0	0
06/11/89		14	4	2	0
06/24/89		2	5	0	0
07/24/89		18	10	6	0
01/21/90	Haleiwa	160	2304	0	0
02/05/90		20	8	0	0
05/14/89		20	14	7	5
05/29/89		14	26	0	0
06/24/89		96	50	16	0
08/09/89		2	4	1	1
02/05/90	Hanauma Bay	59	88	7	0
05/21/90	(Mid)	3160	3240	632	0
05/29/89		364	264	364	264
06/11/89		1692	2880	188	0
06/24/89		524	672	262	202
06/28/89		468	972	104	324
07/14/89		2400	2600	2160	2600
07/24/89		216	310	168	138
08/09/89		612	960	204	0
08/17/89		326	232	0	46

Appendix E Total Staphylococci and S. aureus Concentrations (CFU/100 ml) Recovered on TGA+AZ and VJ+AZ from Selected Marine Water Sites on Oahu

(Continued)

Date	Sites	Total Staphylococci		S. aureus	
		TGA+AZ	VJ+AZ	TGA+AZ	VJ+AZ
09/16/89	Hanauma Bay	344	644	172	64
11/13/89	(Mid)	468	880	94	176
08/17/89	Hanauma Bay	198	252	88	76
09/16/89	(Lft)	1388	1440	13	14
08/17/89	Hanauma Bay	13	6	0	0
09/16/89	(Rt)	284	696	0	0
05/02/90	Ilikai	14	10	0	0
05/14/90		5	23	0	0
05/21/90		36	248	0	0
07/15/89		912	544	0	0
05/14/90	Kaaawa	197	349	0	0
05/21/90		31	90	0	0
07/15/89		52	90	18	13
05/14/89	Kahana	0	0	0	0
05/14/90		50	47	0	0
05/21/90		56	46	37	0
01/21/90	Kailua	684	420	0	0
02/05/90		5	12	0	0
03/29/89		102	191	38	64
05/14/89		72	19	9	7
05/29/89		21	0	0	0
06/11/89		142	136	0	0
06/24/89		248	214	50	0
06/28/89		253	182	0	0
07/15/89		1834	1869	688	234
07/24/89		8	13	8	13
07/27/89		95	168	0	0
08/09/89		0	9	0	2
08/27/89		220	204	0	0
10/05/89		4	16	0	0
01/21/90	Kualoa	19	28	0	0
02/05/90		8	18	0	0
08/27/89		209	340	0	0
09/16/89		72	10	0	0
08/27/89	Lanikai	2016	120	0	0
09/16/89		16	28	0	0

Appendix E Total Staphylococci and S. aureus Concentrations (CFU/100 ml) Recovered on TGA+AZ and VJ+AZ from Selected Marine Water Sites on Oahu

(Continued)

Date	Sites	Total Staphylococci		S. aureus	
		TGA+AZ	VJ+AZ	TGA+AZ	VJ+AZ
09/27/89	Lanikai	12	0	0	0
05/02/90	Magic Island	52	62	0	0
05/14/90		5	3	2	0
08/27/89		509	416	127	52
09/16/89		28	40	0	0
05/14/89	Maili	2	2	0	0
05/14/90		30	2	0	0
05/21/90		2	1	0	0
01/21/90	Makaha	130	213	0	0
05/29/89		54	28	22	14
06/24/89		350	185	70	0
07/15/89		20	24	4	2
05/14/89	Nanakuli	230	588	0	0
06/11/89		166	178	0	0
08/09/89		2	3	0	0
05/14/90	Punaluu	0	5	0	0
05/21/90		48	72	5	0
06/11/89		132	12	0	0
05/02/90	Sand Island	0	0	0	0
05/14/90		35	42	0	0
05/21/90		238	230	48	66
08/27/89		220	83	0	10
05/29/89	Sandy Beach	27	20	9	0
06/28/89		250	368	0	0
07/24/89		56	90	0	0
08/09/89		20	30	4	9
05/14/90	Turtle Bay	9	9	0	0
07/24/89		11	29	3	0
09/16/89		612	1044	68	0
02/05/90	Waimanalo	8	35	0	0
05/14/89		22	20	0	0
05/29/89		84	96	60	72
06/11/89		42	44	14	22
06/28/89		472	598	236	0
07/15/89		224	100	32	0

Appendix E Total Staphylococci and S. aureus Concentrations (CFU/100 ml) Recovered on TGA+AZ and VJ+AZ from Selected Marine Water Sites on Oahu

(Continued)

Date	Sites	Total Staphylococci		S. aureus	
		TGA+AZ	VJ+AZ	TGA+AZ	VJ+AZ
07/24/89	Waimanalo	8	27	0	3
08/09/89		396	416	88	52
01/21/90	Waimea	710	1092	0	0
02/05/90		1640	2200	164	2200
05/14/89		0	1	0	1
05/29/89		150	362	113	271
06/11/89		910	614	0	0
06/24/89		40	64	10	0
07/15/89		1030	874	294	125
07/24/89		19	18	0	0
08/09/89		130	186	0	0
08/27/89		115	108	0	0
05/29/89	WKK1	278	217	278	217
06/28/89		572	479	57	53
09/27/89		108	46	36	0
02/05/90	WKK2	544	522	0	0
03/05/89		138	61	34	0
05/14/89		248	136	248	68
06/24/89		151	154	17	77
07/15/89		128	324	32	162
09/16/89		1360	2124	0	472
01/05/89	WKK3	10	14	2	2
06/11/89		156	364	52	0
07/24/89		36	84	0	8
08/09/89		294	456	74	137
08/27/89		216	376	48	113
09/16/89		384	198	38	0
05/02/90	WKK4	79	132	0	0
05/14/90		96	118	12	0
08/27/89		355	386	133	0
09/16/89		1792	2268	0	0

Appendix F Twenty-four hour Observation of Total Staphylococci and the Number of Beach Users

Time	Total Staph (CFU/100ml)	No. of individuals on the beach and in the water
3 pm	544	400
5 pm	1840	120
7 pm	1720	4
9 pm	648	4
11 pm	504	4
1 am	480	2
3 am	132	0
5 am	60	0
7 am	133	10
9 am	249	30
11 am	1568	400
1 pm	1728	300
3 pm	360	300

Appendix G Seasonal Distribution of Cases

Months	No. of cases	Percentage
May	4	7.5
June	6	11.3
July	7	13.2
August	19	36
September	4	7.5
October	7	13.2
November	6	11.3
Total	53	100.0

Appendix H Skin Infection Study Questionnaire

Sample No.:_____
Date:_____
Source:_____

Name:_____
(last) (first)
Age:_____YEARS

Sex: (Circle number)
1. MALE 2. FEMALE

Address:_____
Street Apt. City Zip

Phone No.:_____

FOR CASES ONLY:

1. When did you first notice the infection?
A. WITHIN THE PAST 3 DAYS. -----> IF THIS IS THE ANSWER, GO TO QUESTION A-1.

B. MORE THAN 3 DAYS, PLEASE SPECIFY WHEN _____
IF THIS IS THE ANSWER, GO TO QUESTION B-1.

A-1. Did you go into seawater within the last 10 days? (Circle the answer)
1.) YES ------> GO TO QUESTION 3.
2.) NO -------> STOP

B-1 Did you go into seawater at any time within 10 days before you noticed the infection? (Circle the answer)
1.) YES ------> GO TO QUESTION 3
2.) NO -------> STOP

FOR CONTROLS ONLY:
2. What was your reason for visiting the doctor? _____

3. Have you been in seawater within the last 10 days?
1.) YES ------> GO TO QUESTION 3
2.) NO ------> STOP

FOR CASES AND CONTROLS:

4. Which location(s) did you go into seawater?
ANSWER IN COLUMN a.

5. Please indicate the number of times you visited each location.
ANSWER IN COLUMN b.

6. Please indicate the date you went into seawater at each location.
ANSWER IN COLUMN c.

7. What activities did you perform in the water at each location?
ANSWER IN COLUMN d.

8. How long were you in the water each time?
ANSWER IN COLUMN e.

Beach location(s) visited (a)	No. of visits (b)	Date (c)	Activities performed (d)	Total time in water (e)

8. How many people live in your household? _____

9. How many rooms are there in the dwelling unit? (not including bathroom and kitchen) _____

10. Within the past 10 days, has anyone in your household had skin infection?
1.) YES 2.) NO

11. Within the past 10 days, have you shared towels or clothes with someone else?
1.) YES 2.) NO

Appendix I Documents on Cooperation, Consent, and Approval

June 15, 1989

Re: Cooperation with Staph Skin Infections Study

Dear Doctor:

I am a doctoral student in the School of Public Health at the University of Hawaii. In partial fulfillment of the requirements of my degree, I am conducting a Case-Control Study of Staphylococcus aureus infected persons on Oahu.

I am asking for your cooperation in this study since several pediatricians have indicated numerous staph infected cases among their patients. I have been working closely with Dr. Jeremy Lam, who endorses this research as seen in the enclosed letter, in establishing the case-control criteria for the study.

In order to access enough patients for this study, I am asking for your assistance in distributing short consent forms to your patients at admission and obtaining culture swab from the infected skin area. The "Culturette" swab will be provided by me. I will appreciate your providing patients' information regarding their age, gender, and medical conditions for classifying patients as cases or controls (see enclosed cases and controls criteria). Follow-up patients contacts (phone interview) for the identified cases and controls will be done directly by me.

This research is designed to determine the role played by one possible source of staph infections and I am confident that it will provide valuable information to you regarding the etiology of this problem in Hawaii. The study period will be from May 1, 1989 to September 30, 1989. Your time involved in distributing consent forms and taking swab from each patient will be less than five minutes.

I will come for explanation of the study in more detail at a later date. Your Prompt response will be greatly appreciated. If you have any questions, please feel free to call me at any time 948-8894.

Thank you very much for your time and interest in this research study.

Sincerely yours,

Naowarut Charoenca

CONSENT FORM FOR SKIN INFECTION STUDY

Naowarut Charoenca, Principal Investigator
University of Hawaii, School of Public Health
1960 East-West Rd., Honolulu, Hawaii 96822

This study is being conducted for the University of Hawaii School of Public Health. The purpose of the study is to determine if swimming in the ocean can cause skin infections. Some Honolulu physicians have noticed skin-infected individuals have been recent swimmers. Information provided by you will help in determining the source of these kinds of infections.

If you agree to participate in the study, a culture swab from the infected skin area will be taken in the doctor's office, and you will be interviewed by phone within the next few days. Some questions will be asked concerning your recreational activities in seawater within the past 10 days prior to the infection, beach locations which you have visited, and how long and how many times you have been in seawater within that period. The phone interview will take about 10 to 15 minutes of your time.

All of your responses will be strictly confidential, and you will not be personally identified in the reporting of the results. It is expected that all beach users will benefit from the knowledge gained from this study.

I have read and understand the above information. I freely agree to take part in this study, understanding that it involves: 1) a culture swab taken from my infected skin area done in the doctor's office; 2) a later phone interview for about 10 to 15 minutes concerning information about my previous activities in seawater. I understand that my participation is entirely voluntary and that I may refuse to answer any question if I choose, or may withdraw my consent to participate at any time without penalty or without any way affecting the health care I receive. If I have any questions about the study, I may contact Ms. Naowarut Charoenca at the University of Hawaii School of Public Health 948-8894.

Please sign **BOTH** copies if you are willing to participate.

_____ _____

Signature of Participant Date

DEPARTMENT OF HEALTH AND HUMAN SERVICES

PROTECTION OF HUMAN SUBJECTS ASSURANCE/CERTIFICATION/DECLARATION

☒ ORIGINAL ☐ FOLLOWUP ☒ EXEMPTION (previously undesignated)

☒ GRANT ☐ CONTRACT ☐ FELLOW ☐ OTHER

☒ New ☐ Competing continuation ☐ Noncompeting continuation ☐ Supplemental

APPLICATION IDENTIFICATION NO. *(if known)*

POLICY: A research activity involving human subjects that is not exempt from HHS regulations may not be funded unless an Institutional Review Board (IRB) has reviewed and approved the activity in accordance with Section 474 of the Public Health Service Act as implemented by Title 45, Part 46 of the Code of Federal Regulations (45 CFR 46–as revised). The applicant institution must submit certification of IRB approval to HHS unless the applicant institution has designated a specific exemption under Section 46.101(b) which applies to the proposed research activity. Institutions with an assurance of compliance on file with HHS which covers the proposed activity should submit certification of IRB review and approval with each application. (In exceptional cases, certification may be accepted up to 60 days after the receipt date for which the application is submitted.) In the case of institutions which do not have an assurance of compliance on file with HHS covering the proposed activity, certification of IRB review and approval must be submitted within 30 days of the receipt of a written request from HHS for certification.

1. TITLE OF APPLICATION OR ACTIVITY

SKIN INFECTIONS IN BEACH USERS AND STAPHYLOCOCCI IN HAWAII MARINE WATERS

2. PRINCIPAL INVESTIGATOR, PROGRAM DIRECTOR, OR FELLOW

Naowarut Charoenca

3. FOOD AND DRUG ADMINISTRATION REQUIRED INFORMATION *(see reverse side)*

4. HHS ASSURANCE STATUS

☒ This institution has an approved assurance of compliance on file with HHS which covers this activity.

M-1217 Assurance identification number 01 IRB identification number

☐ No assurance of compliance which applies to this activity has been established with HHS, but the applicant institution will provide written assurance of compliance and certification of IRB review and approval in accordance with 45 CFR 46 upon request.

5. CERTIFICATION OF IRB REVIEW OR DECLARATION OF EXEMPTION

☐ This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This certification fulfills, when applicable, requirements for certifying FDA status for each investigational new drug or device. *(See reverse side of this form.)*

_____ Date of IRB review and approval. *(If approval is pending, write "pending." Followup certification is required.)*
(month/day/year)

☐ Full Board Review ☐ Expedited Review

☐ This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by 45 CFR 46 will be reviewed and approved before they are initiated and that appropriate further certification *(Form HHS 596)* will be submitted.

☒ Human subjects are involved, but this activity qualifies for exemption under 46.101(b) in accordance with paragraph (3) & (5) *(insert paragraph number of exemption in 46.101(b), 1 through 5)*, but the institution did not designate that exemption on the application.

6. Each official signing below certifies that the information provided on this form is correct and that each institution assumes responsibility for assuring required future reviews, approvals, and submissions of certification.

APPLICANT INSTITUTION	COOPERATING INSTITUTION
NAME, ADDRESS, AND TELEPHONE NO. UNIVERSITY OF HAWAII CHS # 5124 2540 Maile Way Honolulu, Hawaii 96822 (808) 948-8658	NAME, ADDRESS, AND TELEPHONE NO.
NAME AND TITLE OF OFFICIAL *(print or type)* Paul Kakugawa, Assistant Director	NAME AND TITLE OF OFFICIAL *(print or type)*
SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)* April 17, 1989	SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)*

HHS 596 (Rev. 1/82)

(If additional space is needed, please use reverse side under "Notes.")

KAISER PERMANENTE

DATE: 27 July 1989

TO: Naowarut Charoenca
School of Public Health
1960 East West Road
Honolulu HI 96822

FROM: Amod Jain, M.D.
Chair, Research Committee

SUBJECT: TISSUE SAMPLE STUDY REQUEST -
STAPH SKIN INFECTION SURVEY

I am pleased to inform you that your project has been formally approved for the period June through September 1989 , by the following K-PMCP bodies:

Research Committee	:	16 June 1989
Executive Committee	:	27 July 1989

Your project has also been approved today by Ronald J. Mikolajczyk, Hospital Administrator.

Written reports must be submitted annually to the Research Committee and within 60 days after termination of the project. A request for continuance of this project must be submitted to the Research Committee at least 60 days prior to its termination date.

Your cooperation in this matter is appreciated.

MO/du(0416d-2)

c: Co-investigator, Dr. David Paperny

Kaiser Permanente
3288 Moanalua Road Honolulu, Hawaii 96819 (808) 834-5333

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