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Previous studies have indicated that staphylococci have potential for use as indicators of water quality in swimming pool and other recreational waters. However, these organisms are not yet included in the official guidelines for recreational water quality promulgated by health authorities. The purpose of this study is to determine water quality of swimming pools and spas using staphylococci as microbiological indicators.

On three occasions, between January and February 1988, water samples were collected from 14 public, indoor, chlorinated swimming pools and spas in Linn and Benton Counties. Any pool was considered unsanitary if *Staphylococcus aureus* was isolated and identified using the protocol outlined in the Standard Methods for the Examination of Water and Wastewater in accordance with the Oregon State Health Division guidelines. The temperature, water clarity, pH, free chlorine, and total alkalinity likewise were measured for a more effective evaluation of the bacteriological results.

Based on the above criteria, *Staphylococcus aureus* was isolated and identified in 33 percent of the swimming pools. The number of total coliforms isolated from these pools were not any higher than the other pools. *Staphylococcus aureus* was not recovered from water samples collected from the spas.

Staphylococcal and coliform densities increased with decreasing concentration of free chlorine, but the densities of both organisms increased with increasing bathing load. However, no statistical significance was noted from the correlations ($p > 0.05$).

The number of total staphylococci and total coliforms isolated from the surface microlayer using the Millipore membrane filter was higher than those obtained from the inlet and outlet sites. When these organisms were correlated, a significant result was observed for the surface microlayer ($r = 0.5836$, $p = .01423$), but not for the other two sampling sites (inlet and outlet). Thus, the use of the membrane filter is a more effective means of recovering these organisms.

Results of this study suggest that swimming pools that appear to be well-maintained could harbor pathogenic organisms such as *Staphylococcus aureus*. Furthermore, in comparison to coliforms, *Staphylococcus aureus* was found to be a more sensitive indicator of recreational water quality. Further investigations appear to be warranted to confirm these findings.

Staphylococci as Microbiological Indicators to Estimate the Quality of Swimming Pool Waters

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I. INTRODUCTION

The term "pollution" did not come to light until man settled in groups and lived together in close proximity with each other. Water is a prime necessity of life and its presence provides valuable means of man's existence. It is ironic, though, that what attracted man to settle before has been polluted by man himself.

The use of recreational waters differs with customs which further differ with geography, climate, population, and their standards of living (2). Hammer stated that:

> The many ways in which water promotes the economic and general well-being of the society are known as beneficial uses. All domestic, industrial, and agricultural wastes affect in some way the normal life of a river or lake and when the influence is sufficient to render the water unacceptable for its best usage, it is said to be polluted (18, p. 157).

Likewise, when microorganisms are introduced into swimming pool water by bathers to a degree that implies a direct danger of transmission of potential pathogens and destroys the hygienic quality of the water, pollution is said to have occurred.

Among the number of recreational waters around, the swimming pool is one of the most widely used facilities. It is a body of water of limited

size contained in a holding structure (2, 48). Its water quality has traditionally been regulated by chemical and bacteriological testing, which are greatly influenced by the operation and maintenance of the system.

The growing interests in physical fitness activities and hydrotherapy have significantly contributed to the dramatic increase in the number of spas, hot tubs, and whirlpools in the world today. These facilities are designed for recreational and therapeutic use and for physiological relaxation.

The water in swimming pools, hot tubs, spas, and whirlpools is generally treated (halogenated) potable water, although some swimming pools and spas are supplied by thermal springs or salt water. They may be operated either by "overflow-refill" or by "recirculating" type of water systems. "Overflow-refill" is one in which 5 to 15 percent of the pool volume is run to waste during each 24 hours and the deficit of water is replaced from the city mains. "Recirculating" is one in which the entire pool volume is fed back into the pool after chlorination and filtration, and no fresh water is added (1, 33).

In the United States, swimming pools are well utilized by people from all walks of life, especially during the summer months. Disinfectants are serving a special purpose to maintain water quality and set free the hazards of having a disease or infection brought about by swimming pool and spa water. Pools are disinfected for health, safety, and aesthetic purposes.

> It is in the interest of public health that the pool be disinfected to prevent the transmission of disease. It is in the interest of the swimmer's safety that the water be free of turbidity or color so that the bottom of the pool at its greatest depth is visible at all times. It is in the interest of aesthetics that the pool be immaculately clean. This means the absence of film, scum, leaves, surface debris, and aquatic growth such as algae (66, p. 466).

Although the water is for swimming only, it has been emphasized that it should be of drinking quality, nonirritating, and free of objectionable odor or tastes (66).

The popularity and consistent use of these recreational water facilities have indicated a deep concern about the risk of water pollution and disease transmission (11). There are instances when one or two parts of the sanitation system fall short of its goal, thus creating certain problems. Even if the pools are properly disinfected, not all organisms being shed by bathers are killed by disinfectants. There are those that are called "resistants." However, the spread of infection will at least be minimized if pool waters are constantly monitored for sanitation standards. Water quality depends primarily on the efficacy of disinfection and this process is measured by the use of microbiological indicators chosen on the basis of results from several studies, recommendations, and endorsements of researchers who vouch for the capacity of the organism to be a good index of pool sanitation.

Statement of the Problem

Swimming pools in Linn and Benton Counties are not being monitored microbiologically. Sanitarians are only using the physical (water clarity and temperature) and chemical (pH, alkalinity, and free chlorine residual) parameters to evaluate the quality of the water. While it may be true that the water could be considered safe for as long as those parameters are within the tolerance limit, there are some organisms that survive the usual normal water treatment.

Since bathers shed a lot of organisms while swimming, the water is subject to contamination and serves as a vehicle of infection. Not only are

pathogens introduced into the water, but also normal inhabitants in large numbers on a man's body that are capable of polluting the water. Some organisms, when exposed to free chlorine residuals, are not efficiently destroyed as they come in contact with the water. Several investigators have recommended the importance of doing bacteriological examination of pool water in exchange for chemical tests; while some others support the idea of performing both examinations at the same time.

The increasing number of diseases and infections caused by recreational waters has prompted health authorities to include in the maintenance regulation the bacteriological aspect of the water. However, this is only done when the sanitarians feel the need for it. Any discrepancies in the operating system or the presence of infection could easily be detected if a routine check were performed periodically by using effective bacteriological indicators.

Purpose and Objectives of the Study

The purpose of this study was to determine the quality of public swimming pool and spa waters in Linn and Benton Counties using staphylococci as microbiological indicators.

Following are the objectives of this study:

1. To isolate and enumerate total staphylococci and identify if any of the isolates is *S. aureus*.
2. To enumerate total coliforms from swimming pool and spa waters.
3. To determine if a correlation exists between staphylococcal density and the level of free chlorine residual.

4. To determine if a correlation exists between staphylococcal density and bathing load.

The physical, operational, and chemical data of the pools, which include water clarity, temperature, dimensions, water capacity, type of chlorination, type of filter, average bathing load/day, pH, total alkalinity, and chlorine content were measured for effective evaluation of the bacteriological results.

The capacity of the organisms to cause different kinds of illnesses is a characteristic of public health importance. They are the causative agents of many systemic diseases and skin infections, including boils, carbuncles, pustules, abcesses, and post-operative infections (20, 31).

Moreover, about 50 percent of the total staphylococci isolated and documented from several investigations were found to be S. *aureus*, which is a potential pathogen (13). The significance of S. *aureus* in swimming pools and spas concerns situations in which the bacterium might infect the eyes, ears, cuts, and scratches on the skin of bathers. This can lead to more serious problems if the infected person works in a restaurant and inadvertently contaminates foods with the organism. It is capable of producing a thermostable enterotoxin which is the second most common cause of food poisoning in the United States (20, 38, 39).

Because coliforms are still the indicators of choice of swimming pool and other recreational water quality (9, 36, 37), the presence of these organisms were also tested in accordance with *Standard Methods for the Examination of Water and Wastewater* (48).

Assumptions

1. Water samples collected from each sampling site were true representative samples.
2. Organisms were evenly distributed throughout the swimming pool and spa waters.

Definition of Terms

ALKALINITY - the amount of bicarbonate, carbonate, or hydroxide compound present in water solution (61).

AVERAGE BATHING LOAD - average number of people per day using the pool or spa from opening to closing time.

CHLORINE - is a chemical element of the halogen family that is commonly used as a pool disinfectant (62).

CHLORINE GAS - is 100 percent pure chlorine with a characteristic odor and greenish yellow color, which is about two and one-half times as heavy as air (61, 66).

DIATOMACEOUS EARTH FILTER - utilizes diatomaceous earth as the filter bed medium in a thin coating over filter septa or bags (61, personal communication with Dr. W. E. Sandine).

DISINFECTANT - a chemical substance used to kill microorganisms (20).

FILTER - a device that separates solid particles from water by recirculating it through a porous substance (61).

FREE RESIDUAL CHLORINE - is that portion of hypochlorous acid which remains in the water uncombined with ammonia. This is

in the form of chlorine that must be maintained for adequate disinfection (62).

GENERAL-USE PUBLIC SPA POOL - means any public spa pool other than limited-use public spa pool (37).

GENERAL-USE PUBLIC SWIMMING POOL - means any public swimming pool other than limited-use public swimming pool (36).

HOT TUB - a hydrotherapy spa constructed of wood as distinct from other spa units formed of plastics, concrete, metal, and other materials (61).

LIMITED-USE PUBLIC SPA POOL - means any public spa pool which is utilized by members, residents, and patrons of a residential housing facility, traveler's accommodation, athletic clubs (37).

LIMITED-USE PUBLIC SWIMMING POOL - means any public swimming pool operated in connection with a companion facility, such as residential housing facility, traveler's accommodation, and athletic clubs, the use of which is limited to residents, patrons, and members of the said facilities (36).

PEAK TIMES - the time of the day when the pools were most loaded with bathers.

pH - is the negative logarithm (to the base 10) of the concentration or activity of the hydrogen ions (personal communication with Dr. W. E. Sandine). It is also defined as the concentration of hydrogen ions in a solution (59, 62).

PUBLIC SPA AND HOT TUBS - any spa and/or hot tub, other than residential spa or hot tub, which is intended solely for bathing

and designed primarily to direct water or air-enriched water under pressure onto the bather's body with the intent of producing a relaxing or therapeutic effect (37, 61).

PUBLIC SWIMMING POOL - means an artificial structure, and its appurtenances, which contains halogenated water more than two feet deep for swimming or recreational bathing and which is used by any segment of the public (37).

PURE CULTURE - a culture that contains only one kind of microorganism (49).

RAPID PRESSURE SAND FILTER - a filter that uses a medium made of 20 inches of filter sand with an effective size of 0.4 to 0.5 μm and a uniformity coefficient of 1.75 underlaid with gravel or other effective support (61).

SAMPLING SITE - refers to one of the three areas (inlet, outlet or surface microlayer) in the pools where water samples were collected.

SODIUM DICHLORO-ISOCYANURATE - is a white powder that contains 60 percent available chlorine (62).

SODIUM HYPOCHLORITE - is a clear, slightly yellow liquid solution, which is about 12-15 percent available chlorine in commercial form (62).

SPA OR HYDROTHERAPY SPA - is a water facility designed for recreational and therapeutic use. This terminology includes whirlpools, hot tubs, and hot spas (61).

II. REVIEW OF LITERATURE

Background Information on the Importance of the Physical and Chemical Parameters of Swimming Pool and Spa Waters

The physical and chemical parameters used to determine the water quality of swimming pools and spas were measured with importance based on the following principles and background information.

Water Clarity

Water clarity is desired primarily "to eliminate safety hazards involved with turbid or unclear water" (12). Persons in distress must be distinguishable by the lifeguards or other people near the water. The present standards for clarity involve the ability to see a submerged disc which supposedly reflects the ability to see a submerged swimmer (28). Another consideration of clarity also involves aesthetics. Apparently, turbid water does not look attractive and appealing to any bather or swimmer. Naturally, when pool water is turbid, one would suspect that sanitation is not properly maintained.

Temperature

How the body reacts in water is affected by changes in temperature. It either brings comfort and satisfaction, or danger to individuals because the amount of activity in the water depends on it. In 1967, the Federal Water Pollution Control Administration stated that:

> temperature was a "catalyst", a depressant, an activator, a restrictor, a stimulator, a controller, a killer, one of the most

important and most influential water quality characteristics to life in water (42).

It is considered as an important physical parameter that regulates many of the beneficial uses of water. Temperature affects the aesthetic and sanitary qualities of the water as well as microorganisms present, especially pathogens. Coliforms dramatically lose viability with increasing temperatures (42). In pools and spas, elevated temperatures are health hazards and can cause bather discomfort. Low temperatures also give discomfort to the bather and decrease the effectiveness of chlorination (42, 61, 62).

pH

The pH level is a factor to be considered in chlorination. It affects chlorine concentration based on the equilibrium between hypochlorous acid and hypochlorite ion. It measures the acidity and alkalinity of water. It has been recommended (36, 37, 59, 61, 62) that a slightly alkaline pH (7.2-7.6) be maintained in pools and spas. Above this range, the efficiency of chlorine will decrease, and it can cause scaling, cloudy water or a clogged filter. Below this range, the water can corrode the pool surfaces and other support equipment, even though lower pH values potentiate chlorine destruction of microorganisms.

Maintaining the pH within this range would allow disinfectants to function reasonably well, prevent scale formation, and impede corrosion.

Alkalinity

This parameter measures the buffering capacity of the water, which is regulated by the pH level. Its importance lies in the fact that "it affects the amount of chemicals which need to be added to accomplish calculation, softening, and control of corrosion in the distribution system" (46).

Alkalinity helps to neutralize excess acid produced during chemical coagulation. Excessive alkalinity can cause irritation to bathers or swimmers.

Free Residual Chlorine

The terms "free chlorine," "free residual chlorine," and "free chlorine residual" are used interchangeably in this study and therefore refer to one and the same item.

Two kinds of acids are formed when chlorine is added to water: hypochlorous acid and hydrochloric acid. The latter is considered a useless by-product of chlorination, while the former can exist in either a molecular or ionized state and is referred to as "free" chlorine in swimming pool and spa water. The portion of hypochlorous acid which remains uncombined with ammonia is called "free residual chlorine," which must be maintained for excellent disinfection (61, 62, 66).

Purposes of Bacterial Indicators for Swimming Pool and Spa Sanitation

Over the past decades, surveys, investigations, and research have yielded ambiguous results with regard to the choice of indicator organism for determining the quality of swimming pool and spa waters. Authorities have established guidelines to assess water quality as a result of concern by health, medical, and environmental personnel that somehow swimming pool and spa water facilitates the spread of diseases and infections.

When a person goes swimming, microorganisms are washed off the skin and out of some body cavities and into the water, polluting it to some degree (44). Several investigators (5, 18, 42, 44, 61) have indicated that the normal and potentially infectious microbial flora of the human body have

been comprehensively studied so as to provide health officials the information needed to establish standards of bacterial quality of swimming pools and spas. Even though epidemiologic evidence linking diseases with swimming is not alarming, the possibility is always present (44).

The usual role fulfilled by bacterial indicators for swimming pool and spa water quality is quite different in many respects than the role of bacterial indicators for other uses of water (35).

Bacterial indicators for swimming pool sanitation have the primary purpose of evaluating the accuracy and adequacy of the chemical tests to regulate the chemical conditioning and disinfection of the water. A secondary purpose is to assist in the evaluation of the effectiveness of disinfection and filtration of the water and the operation of the system. A tertiary purpose that sometimes serves a dominant role is to provide additional information in any epidemiologic studies which maybe undertaken subsequent to a suspected outbreak of human disease associated with swimming pool and spa water (35, 44).

Characteristics of Indicator Bacteria

In Cabelli's "Introduction of Recreational Water Quality" (5), he points out that the best indicator is one in which its densities correlate best with health hazards associated with a given type of pollution source. Accordingly, the best indicator should possess the following characteristics:

1. It should be consistently and exclusively associated with the source of the pathogens.
2. It must be present in sufficient numbers to provide an accurate density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.
3. It should approach the resistance to disinfectants and environmental stress, including toxic materials, of the most resistant pathogen potentially present at significant levels in the source.

4. It should be quantifiable in recreational waters by reasonably facile and inexpensive methods with accuracy, precision, and specificity (5, p. 223).

A strong body of evidence gathered from several researchers have supported the capacity of staphylococci to be the best indicator to estimate the quality of pool water (1, 4, 7, 10, 14, 19, 35, 44, 45, 66). This choice is supported by the following reasons: 1) they are shed easily and freely and are derived from the mouth, nose, throat, and skin surfaces and therefore, indicate the potential presence of pathogens (2, 10, 14, 22, 26, 67); a review of literature (7, 14, 22, 40) revealed that more than 50 percent of the total staphylococci recovered were *S. aureus*, which is a potential pathogen; 2) they are more resistant to environmental stress such as sunlight, drying, salinity, and disinfectant (chlorine) treatment than the coliforms (2, 6, 7, 10, 14, 22, 23, 43, 67); and 3) they are easily isolated and culturable by the available modern techniques in microbiology with accuracy, precision, and specificity (1, 22, 23, 27, 29, 48).

Characteristics and Clinical Significance of Staphylococci

Members of the *Staphylococcus* genus are gram-positive cocci, about 0.5 to 1.5 μm in diameter, occurring singly, in pairs, tetrads, short chains, and irregular "grape-like" clusters. The organisms are nonmontile, nonsporeforming, unencapsulated, and usually give a positive reaction to catalase. Growth is more rapid and abundant under aerobic conditions, except for some strains of *S. aureus* and *S. saccarolyticus*, which is anaerobic.

This group of microorganisms is a member of the family *Micrococcaceae*, which includes the genera *Micrococcus*, *Staphylococcus* and *Planococcus*. The genus *Staphylococcus* currently is composed of 20 species (25, 27, 31). The species most commonly associated with human infections

are *S. aureus*, *S. epidermidis*, and *S. saccarolyticus*. The two species of veterinary interest are *S. intermedius* and *S. hyicus*. Some of the organisms may be isolated from a variety of animal products like meat, cheese, and milk and from environmental sources like soil, air, dust, and fomites. Furthermore, some species are found to be opportunistic pathogens of humans and/or animals (20, 25, 27).

The growing popularity of swimming pools and spas has been accompanied by increasingly frequent outbreaks of illnesses produced by several microorganisms. Some of these bacteria are normal inhabitants in some body areas, but if given the opportunity to colonize in other parts of the body, they can be pathogenic (Table 1).

Table 1. Disease Caused by Staphylococci That are Pathogenic to Humans.

Organism	Disease	Body Part Affected
1. *S. aureus*	Cellulitis	Skin
	Carbuncles	Skin
	Pustules, Boils	Skin
	Meningitis	Meningis
	Osteomyelitis	Bones
	Food Poisoning	Gastro-intestinal tract
	Toxic Shock Syndrome	Vagina with multi-system involvement
2. *S. epidermidis*	Bacteremia	Blood
	Infective Endocarditis	Heart
3. *S. saphrophyticus*	Urinary tract infection	Urinary tract/kidney
4. *S. saccharolyticus*	Urinary tract infection	Urinary tract/kidney

Skin infections caused by *S. aureus*, otherwise known as coagulase-positive staphylococci, are the most common staphylococci infections of man. Large populations of this organism are normally found in the nares of 40-50 percent of humans and carriers may be found in up to 50 percent of food-

handlers. The carrier state also is high among persons with skin infections (20).

The organism is also capable of producing a thermostable enterotoxin in foods, resulting in a high percentage of cases of food poisoning (25, 27, 30). A documented case by the Center for Disease Control in 1975 (38) occurred in an aircraft wherein 57 percent of the passengers and a crew had gastrointestinal illness characterized by diarrhea, vomiting, abdominal cramps, and nausea. Epidemiologic investigation revealed food poisoning that resulted from a ham prepared by a cook with a finger lesion from which S. *aureus* was cultured. An identical strain was recovered from the left-over ham. Several similar situations have occurred on a cruise ship (52), on another aircraft (39), in restaurants (53, 54, 55) and among persons eating foods from vending machines (51).

A case-control study (56) of white water rafting river guides following an outbreak of cellulitis, furunculosis or abcesses revealed that S. *aureus* was the causative agent. Investigators pointed out that constant exposure of the guides to river rafts and the associated trauma to their ankles and lower legs provided excellent means for infection to occur. The infectious strain was recovered not only from the wounds of the guides, but also from the rafts even after several hours of alternating water submersion and drying. This supports the fact that the organism is resistant to drying and sunlight exposure (20, 31).

Toxic shock syndrome, a community-acquired disease, has also been attributed to S. *aureus*. Although it is primarily a disease of menstruating women associated with the use of tampons and vaginal contraceptive sponges, a few cases also have been recognized in males. The toxin is associated

with fever, shock, and multisystem involvement (63). Toxic shock syndrome brought about by the use of a continuous subcutaneous insulin infusion pump (CSIIP) can lead to a serious disease and even death when proper asepsis is not observed and maintained (64). The organism has also caused considerable morbidity and mortality as a nosocomial pathogen. However, the development and use of antibiotics have provided successful therapy of *S. aureus* infections over the years.

At present, an increasing concern for infections caused by organisms that are endogenous to the host is seen. Some bacteria which were once considered saphrophytic, non-pathogenic or less pathogenic are now found capable of causing infections.

The human coagulase-negative species include *S. epidermidis*, *S. haemolyticus*, *S. warneri*, *S. capitis*, *S. saccharolyticus*, *S. auricularis*, *S. saphrophyticus*, *S. cohnii*, *S. xylosus*, and *S. simulans*. These organisms constitute a major component of man's normal flora and now have been documented as causative agents of any one of the following infections: bacteremia, infective endocarditis, osteomyelitis, genito-urinary tract infections, peritonitis associated with dialysis, infections of the ventriculoatrial or other cerebrospinal fluid shunts, intravenous catheters, and joint prostheses. Most of these infections are prominently caused by *S. epidermidis*. It has been learned that *S. saccarolyticus* is one of the most common causes of urinary tract infections in young, sexually active females. Other coagulase-positive staphylococcal species of veterinary importance are *S. intermedius*, which causes a variety of infections among dogs, and *S. hyicus*, which is associated with infections in pigs and cattle (38, 39).

Previous Studies on the Importance of Staphylococci as Indicators of Choice for Swimming Pool and Spa Water Quality

The usefulness of microbial organisms to determine the hygienic quality of swimming pool and spa waters has been debated for years. The capacity of coliforms as indicators of water quality in swimming pools and spas still remains in question. Results of several investigations revealed that coliforms are not the best indicators of pool water quality (40, 43, 65). They are eliminated by chlorine, like *Salmonella* and *Shigella* (14), and are not normally shed from bathers, nor do they survive a halogen environment (1, 7, 14, 23). The Public Health Laboratory Service Water Sub-Committee in the United Kingdom found out that in water with a free residual chlorine greater than 0.10 ppm, coliforms were seldom isolated from samples (35). The group recommended staphylococci and hemolytic streptococci as more valuable indicators of pollution since they are more resistant to chlorine.

Barrow (2) stipulated that the predominant organisms in swimming pools and spas may not be related to human excreta, with which coliforms are closely associated, but to the nose, mouth, and skin.

With the advancement of science and technology, new avenues have been explored that popularized the cocci as better indicators. Staphylococci have recently received much recognition as playing a more valuable role as indicators of water quality (3, 7, 10, 14, 35, 44). A study by Robinton and Mood (44), using five young physical education teachers, showed that the dominant types of organisms shed by bathers into the water were cocci. Present were members of the genera *Neisseria*, *Sarcina*, *Micrococcus*, and *Staphylococcus*. The last 70 samples out of 100 collected during the survey

were examined for the presence of *Staphylococcus* and all were found to be positive. Likewise, the 100 samples also were examined for coliforms and only 61 percent showed positive results, using the multiple dilution tube method, and only 71 percent when the membrane filter technique was used. To compare the efficiency of other indicators, the samples were also cultured to enumerate *Streptococcus salivarius* and fecal streptococci. They found out that only 33.7 percent and 55 percent of the samples were positive using, respectively, the multiple dilution and membrane filter techniques.

Favero et al. (14) strongly suggested that staphylococci are the best indicators since they are readily detected and more highly resistant to chlorine than coliforms, *E. coli* and *P. aeruginosa*. In addition to their being potential pathogens causing a variety of infections, they also indicate pollution from the mouth, nose, throat, and skin surfaces of bathers (14, 35). They proposed a standard of less than 100 staphylococci/100 ml of water (less than 1/ml).

The reasons pointed out by Evans (10) and Williams (67) paralleled with those of Favero and co-workers in considering S. *aureus* and other staphylococci to be better indicators of the hygienic quality of swimming pools.

Favero and Drake (13) in 1966 found that staphylococci and bacilli were predominant in chlorinated swimming pools and approximately 50 percent of the staphylococci isolated were S. *aureus*.

It was proposed by Keirn and Putnam (23) in 1968 and Evans (10) in 1977 that staphylococci and enterococci be included on the list of indicator organisms for swimming pools and also as organisms to test the efficacy of potential pool disinfection.

In 1973, Palmquist and Jankow (40) reported that on the basis of coliform counts and chlorine levels, some pools considered safe were causing a high incidence of otitis externa. They recommended that *S. aureus* and *Pseudomonas* should be included in the line-up of bacterial evaluation of swimming pools in addition to coliforms. Results of their study showed that 45.7 percent of the "unsafe" pool samples contained a chlorine level of 0.25 ppm. Furthermore, *Pseudomonas* genus and *S. aureus* counts were detected in 91.5 percent of the "unsafe" pools.

These microbes are excreted into the water by several ways. McLean (33) stressed that coughing, sneezing or blowing water out of the mouth are major pathways of water contamination by bathers. These have long been discouraged, but everybody knows that these actions still commonly occur. He also mentioned that inadequately halogenated pools provide an infection hazard for children.

A study of Crone and Tee (7) showed that staphylococci were found more frequently than coliforms, of which about 65 percent were *S. aureus*. It was concluded that recovery of staphylococci from a 100 ml water sample from a pool that has not been used for at least 10 hours indicates inadequate filtration and chlorination.

Ritter and Treece (43) found that *S. aureus*, *S. salivarius*, and the enterococci were 5 to 20 times more resistant to chlorine than the coliform bacteria and could easily be recovered from swimming pool water.

Experiments of Kloos and Musselwhite (26) revealed that *S. aureus* and *S. epidermidis* were dominant in the nose and that *S. epidermidis* inhabits the skin predominantly, hence both are easily introduced into the water.

An environmental model for swimming pool bacteriology (free available chlorine, pH, and swim load) was tested by Paul (41) in 1970. He

demonstrated its efficiency in predicting the maximum probable bacterial sensitivity in a water sample with 95 percent confidence in accuracy.

Use of an Appropriate Culture Medium for the Enumeration of Staphylococci

Perhaps the biggest problem that confronts investigators in the evaluation of either total staphylococci or S. *aureus* as potential indicators of swimming pool infection hazards is the lack of sufficient information on a recovery system, enumeration procedure, and medium that is selective, accurate, and reliable (10, 17, 35).

Earlier studies have utilized different kinds of culture media to either isolate total staphylococci or S. *aureus* from swimming pool and spa water samples. These include Chapman-Stone, Phenol Red Monnitol Salt, Vogel-Johnson (14), M-staphyococcus broth (3, 25) or Staph 110 (7, 29, 44).

Klapes (22) in 1983 compared Vogel-Johnson and Baird-Parker media to recover staphylococci from swimming pool waters using the membrane filter technique. From pools where water contained adequate free chlorine residual, Vogel-Johnson medium was found more selective than Baird-Parker. On the other hand, where water samples contained very low levels of chlorine (none was in the free form), staphylococci grew abundantly on both media with increased sensitivity for S. *aureus* detection. Vogel-Johnson agar proved to be a superior selective medium for S. *aureus*, compared to Chapman-Stone and Phenol Red Mannitol Salt agars (14).

A recent study by Alico and Dragonjac (1) showed that Vogel-Johnson medium supplemented with 0.5 percent pyruvate was selective, accurate, and reliable for growth of staphylococci. It recovered twice as many typical colonies than Vogel-Johnson agar, which was only 60 percent selective for S.

aureus. The well-defined black colonies on Vogel-Johnson with pyruvate proved to be approximately 80 percent *S. aureus*.

Relationship Between Natural Bathing Water Quality and Health

An attempt to determine the relationship between natural bathing water quality and health was carried out by Stevenson (58) who studied a great lake, an inland river, a fresh water recirculating pool, and tidal water. Results of this study provided valuable information as follows:

> Pool swimmers had an incidence for all types of illness of 13.8 per 1000 person-days. Eye, ear, nose, and throat ailments comprised 68 percent of this incidence; gastro-intestinal, 15 percent, and other illnesses, the balance (58, p. 535).

Furthermore, river swimmers in this same group study had 24 percent of the total incidence rate in gastrointestinal ailments; eye, ear, and respiratory illness were 53 percent of the total. It was concluded that for all bathing water studied, eye, ear, nose, and throat ailments represented more than half of the overall illness incidence, gastrointestinal up to one-fifth and skin irritations and other illnesses the balance.

Although identification of the causative agents was not done, assumptions could be made based on the findings of other researchers (2, 10, 14, 26, 67) that staphylococci may be the cause of a significant portion of the overall illness incidence.

According to an epidemiological study of Seyfried and co-workers (47), illness associated with swimming can be related to the microbiological quality of the water. Results indicated that swimmers had a higher illness incidence than non-swimmers and that infections of the eye, ear, nose, and throat represented more than half of the total illness incidence.

In another study by the same group of investigators (46), it was found that swimming-associated morbidity was closely related to total staphylococci, fecal coliforms, and fecal streptococci. A dose-response relationship was evident for staphylococci.

In light of the existing evidence, much has been said about the potential of staphylococci to be the indicator of choice to estimate the quality of swimming pool and spa waters.

III. METHODS AND PROCEDURES

Study Area and Location

Water samples were collected from 14 indoor, chlorinated, and licensed swimming pools and spas in Linn and Benton Counties between January 14, 1988 and February 22, 1988. These facilities were located in the cities of Corvallis, Philomath, Albany, Lebanon, and Sweet Home. Benton County is bounded in the north by Polk County, Lincoln County in the west, and the Willamette River on the east. Linn County is likewise bounded by the Willamette River on the west, Marion County to the north, and it covers up to the cross of the Cascade Mountains eastward. Both counties have Lane County as their southern boundary. A map of the study area is included in Appendix A. This investigation was limited to public swimming pools and spas that were operational throughout the year. The list of these facilities was obtained from the health departments of the two counties through the sanitarians in charge. The facilities were open from 5:30 a.m. to 9:30 p.m. and accommodated adequate numbers of bathers.

The facilities were placed in four groups (see Appendix D) based on their geographical location so that the time difference between sample collection and analysis did not exceed six hours, as recommended by *Standard Methods* (48).

An introductory letter (see Appendix B) was mailed to the pool directors about three weeks before sample collection to inform them about the

activity. After five days, it was followed up by telephone and an appointment was made to visit the facility. Verbal consent to participate in this study also was sought during the phone conversation.

Each swimming pool and spa was assigned a three-digit identification number, starting from 101 to 114 to mask the identity of the facilities during sample and data analyses.

A worksheet (see Appendix C) was specially designed to record all the data of this investigation.

Operational data about the facilities (items 1-11 on the worksheet) were gathered by interviewing the pool directors before samples were collected. At the same time the purpose of the study was explained to the staff in charge of the participating facilities.

A pilot study was done one month before the actual investigation started and the methodology for this project was tested in accordance with the protocol outlined in *Standard Methods* (48). Whatever weaknesses and points that were omitted in the process of writing the research proposal were identified and necessary changes were made after consultation with the committee members and other resource persons.

Sampling was done under normal operating conditions during peak times between 6:00 a.m. and 7:00 p.m.

Physical Measurements

Pool water was analyzed for pH, alkalinity, and free chlorine residual using the Bioguard Multitest Kit. The kit has five reagents, a sampling compartment with comparator charts, and a procedure to measure pH, alka-

linity, and free chlorine residual. The National Spa and Pool Institute recommends the use of this kit as a convenient means of measuring the above mentioned parameters.

An attempt was made to test the sensitivity, accuracy, precision, and reliability of this kit. Ten water samples were collected from nine facilities and submitted to Schaeffer's Recreation where they analyzed the samples for the same parameters using a computerized Bioguard Water Analyzer. Results were compared to the one obtained by the investigator at the pool side and the readings were identical. It was decided then that since both readings yielded identical results, measurements done using the kit at the pool side were reliable and sufficient.

Temperature measurement was done using a standard laboratory thermometer calibrated in degrees centigrade. The instrument was held in the water for at least five minutes before the reading was recorded.

Water clarity was measured using the standard 2-inch diameter clarity disc which has met the criteria set by the Oregon State Health Division for measuring water clarity in swimming pools and spas. The clarity was considered acceptable if the clarity disc, which was divided into alternate black and red quadrants, was clearly visible and the separate colors discernible through 15 feet (4.57 m) of water. Water clarity was considered unacceptable if the clarity disc was not clearly visible and the separate colors not discernible through 15 feet (4.57 m) of water (36, 37). These procedures were performed at pool side the day samples were collected. All readings were recorded on the worksheet provided for the purpose.

Sample Collection

Accurate and reliable results in water testing largely depend on proper sampling techniques. The sets of equipment used were prepared according to the procedures outlined in *Standard Methods* (48).

Glass sampling bottles of 500 ml and 1000 ml capacity were used to collect the samples for this study. The bottles were thoroughly cleansed with hot water and detergent recommended for cleaning laboratory glasswares. After a hot water rinse to remove all traces of residual washing compound, all bottles were finally rinsed with distilled water.

Aluminum foil sheets were used to wrap the bottles and caps before autoclaving. The purpose of doing this was to provide maximum shield against microbial contamination. The aluminum sheets covering the bottles were left until after the analysis was started.

Before autoclaving, sufficient sodium thiosulfate, a dechlorinating agent, to provide a concentration of about 100 mg/L in the sample, was added to the sampling bottles. A 10 percent solution was prepared by weighing 10 grams of the sodium thiosulfate crystals and dissolving in 100 ml of distilled water. For 500 and 1000 ml capacity bottles, 0.45 ml and 0.90 ml, respectively, of the 10 percent solution was pipetted into the sampling bottles to completely neutralize free chlorine residuals and to prevent continuation of bacteriocidal action during sample transit. The bottles were loosely capped before autoclaving at 121°C for 15 minutes at 15 psi pressure. Sterilization was done the day before sample collection.

The identification numbers of each swimming pool and spa were taped into the sampling bottles. The bottles were conveniently placed in a styrofoam cooler during transportation.

While samples were collected, the cap of the bottle with the aluminum sheet was removed as a unit. The desired sample volume was collected by holding the bottle near its base at an angle of 45 degrees. The bottle was plunged vertically into the water at elbow depth to fill, making sure that the dechlorinating agent was not washed out. The bottle was then immediately capped and put back in the cooler. Enough space (about 2.5 cm) was left in the sampling bottle to facilitate mixing of the sample prior to filtration.

Each facility had three sampling sites and a representative sample was collected from each of the three sites. The inlet site (referred to as sampling site 1) represented clean water that came directly from the chlorinator. The outlet site (referred to as sampling site 2) represented a sample of used water just before it went back to the chlorinator. Since most bacteria shed by bathers are in body oils, saliva, and mucus discharges, the surface microlayer of the water (referred to as sampling site 3) was also sampled. This was done by floating a Millipore membrane filter on the water surface for 30 seconds until the grids of the filter were visible. Excess water on the filter was allowed to drain and the filter was immediately placed on a previously prepared petri dish with M-Endo broth medium for coliforms and Vogel-Johnson agar medium for staphylococci using aseptic techniques.

Microbiological examination of the water was started promptly after collection to avoid unpredictable changes. However, in situations where transport to the laboratory required at most an hour, the samples were kept cool in a styrofoam cooler.

Bacteriological Procedures

Materials

The water used in the preparation of reagents, culture media, and for rinsing all glasswares was distilled and deionized obtained from the Microbiology Department of Oregon State University. This water has been tested and found free of traces of dissolved metals and bacteriocidal or inhibitory compounds. The term "distilled water," as used in this investigation, actually refers to distilled and deionized water.

All glassware items needed to carry out procedures were thoroughly cleansed, with a final rinse using distilled water, and air dried. Each of the glassware items and the filtration units were individually wrapped in aluminum foil sheets and autoclaved before sample collection was done.

All media, in dehydrated form, used in this study were purchased form Difco Laboratories, Detroit, Michigan. The media were prepared according to *Standard Methods* (48) and directions indicated by the manufacturer on the label attached to each bottle were followed. A triple beam balance standardized for accuracy and precision was used to weigh the desired amount of the media. A sterile graduated cylinder was used to measure the volume of distilled water needed for rehydration. The mixture, after manual shaking, was heated using an electric stove to completely dissolve all components. Procedures to prepare the media are discussed below.

M-Endo Broth MF (for enumeration of total coliforms). M-Endo broth is a formulation of the Millipore Filter Corporation for use in the membrane filter technique for detection and quantification of coliform organisms.

To rehydrate the medium, 48 grams of the powder were dissolved in 1000 ml of distilled water containing 20 ml of ethanol. The mixture was agitated by shaking to make sure the medium ingredients were thoroughly dissolved. It was heated to boiling, but was removed from heat at once and allowed to cool to room temperature. Prolonged exposure to heat or to an autoclave destroys the sugar components of the medium.

Sterile absorbent pads were placed on each sterile plastic petri dish and 1.8 to 2.0 ml of the dissolved medium, enough to saturate the absorbent pad, was transferred using a sterile pipette. All M-Endo broth medium was freshly prepared to ensure best results. The petri dishes were kept refrigerated until bacterial examination was started.

Vogel-Johnson Agar Medium (for enumeration of staphylococci). Vogel-Johnson agar medium is used for isolating staphylococci, especially coagulase-positive and mannitol fermenting strains.

To rehydrate, 60 grams of the medium were dissolved in 1000 ml of distilled water. The mixture was heated to boiling to completely dissolve the medium. It was sterilized in the autoclave at 121°C for 15 minutes at 15 psi pressure. While sterilization was going on, petri dishes (100 X 15 mm) were prepared for plating on a well-disinfected table. After autoclaving, the medium were allowed to cool to 45-50°C and 20 ml of 1 percent Bacto-Chapman Tellurite Solution were added. This reagent was responsible for the black color of the staphylococcal colonies. The medium was mixed using a sterile glass rod.

Using aseptic techniques, 10 to 15 ml of the medium were poured into each petri dish and allowed to solidify. Since Vogel-Johnson medium is agar based, dispensing the medium into petri dishes was done while the mixture was still warm, because agar solidifies about 38 to 40°C. The prepared

medium was stored in the refrigerator until further use. However, to avoid erroneous results brought about by contamination due to prolonged storage, no medium was used if it had been refrigerated longer than three days.

Bacto-Peptone Water (0.1%). Bacto-Peptone Water has been recommended by *Standard Methods* (48) for use in bacteriological analyses. A 0.1 percent solution was used as a rinse in this investigation. It was prepared by weighing 10 grams of peptone and allowing it to dissolve in 100 ml of distilled water to make a 10 percent solution. A 1:100 dilution of the 10 percent solution was made to produce a 0.1 percent concentration. Sterilization was done in the autoclave at 121°C for 15 minutes at 15 psi pressure. The solution was sparklingly clear and neutral in reaction. This was used as a rinse to reduce halogen injury to the organisms. The amount of the 0.1 percent peptone water used to wash off all water samples that adhered to the sides of the filter funnel was twice the volume of the filtered sample.

All of these products passed a performance test to indicate they were free of microorganisms that would interfere with their intended function.

Sample Analysis

The protocol outlined in the *Standard Methods for the Examination of Water and Wastewater* (48) was followed. Bacterial examinations were done at the Environmental Health Laboratory, Department of Health, Waldo Hall, Oregon State University. The laboratory is equipped to conduct water bacteriology. The major equipment available consisted of autoclave, incubators, stove, pH meter, colony counter, weighing scale, and glassware items.

One of the most important items of equipment used in this investigation was the filtration assembly. It is composed of an electric vacuum

pump, a PVC vacuum manifold, a funnel, a filter holder, and a clamp. Except for the vacuum pump, all components were cleansed, with a final rinse with distilled water, dried, wrapped in aluminum foil sheets, and autoclaved before use.

All membrane filters, absorbent pads, and petri dishes were purchased from Millipore Filter Corporation in Bedford, Massachusetts. Likewise, the filtration assembly was also manufactured by the same company.

The working area was disinfected with Liqui-nox, a recommended disinfectant, before sample filtration was started. A gas burner was used to sterilize the forceps used in the analysis.

Membrane Filter Technique. The use of the membrane filter technique has been approved, based on recommendations of the United States Environmental Protection Agency (48). It has been recognized by the United States Public Health Service as a reliable method for the detection of coliform organisms in water. Although the procedure for isolation of staphylococci in recreational waters has not yet been officially standardized (personal communication with Anita Highsmith of the Recreational Waters Committee for Standard Methods), most investigators are using the membrane filter technique for isolation of staphylococci. Its advantages over the multiple tube technique are:

a. Higher degree of reproducibility of results.
b. Large volumes of sample can be used for testing, so sensitivity is greater.
c. It yields definite results in a shorter span of time (4, 48).

One thousand milliliters of sample were filtered through a 0.45 μm hydrophilic grid membrane filter, observing strict asepsis to prevent contamination. Precision of manufacture was based on the assumption that

bacteria larger than 0.4 μm cannot pass through the filter (48). When all samples have been filtered, a 0.1 percent peptone water with twice the sample volume was used as a rinse to reduce halogen injury to the organisms. All bacteria present in the sample were retained directly on the filter surface.

Membrane Filter Technique for Total Staphylococci. The filtration units were assembled aseptically. A sterilized smooth-tipped forceps was used to pick up a sterile membrane filter from its package. The filter was placed on the base of the filter holder and centered on the support screen on the filter holder. Before the sample was filtered, it was agitated by shaking, using up and down motion. Since the funnel of the filtration assembly has only a 250-ml capacity, four 250-ml volumes were filtered at a time until a total volume of 1000 ml was filtered. When all the sample had passed through the filter, a rinse solution of 0.1 percent peptone water, twice the volume of the sample, was filtered to rinse all samples that adhered to the sides of the funnel. This solution helps to reduce halogen injury to the organisms. Meanwhile, the petri dish with Vogel-Johnson agar medium was labelled with the identification number, sample site, and date. Using a sterilized forceps, the filter with grid side up was placed on the nutrient surface of this labelled petri dish with Vogel-Johnson agar and incubated upside down in an incubator for 42-48 hours at 35 $\pm$ 0.5°C. The membrane filter which was used to sample the surface microlayer of the water was also incubated in the same manner. It has been a standard procedure in microbiology that petri dishes should be incubated upside-down to prevent water of condensation from accumulating in the media, which could lead to erroneous results. This procedure was done for samples collected from the inlet and the outlet sites of the facility.

The PVC vacuum manifold used in this analysis allowed three simultaneous filtrations, so samples for staphylococci and coliform analysis were processed at the same time. However, every time a sample was filtered, a new set of sterilized filtration units was used.

Membrane Filter Technique for Total Coliforms. The same preparation was done as in the preceding procedure. One thousand ml of water sample were filtered in the same manner as the samples for total staphylococci. After the desired sample volume had passed through the filter, 0.1 percent peptone water, twice the volume of the sample was used as a rinse solution. The petri dish was likewise labeled for proper identification. The membrane filter was transferred aseptically to a petri dish with M-Endo broth medium. This and the filter from the surface microlayer were incubated upside-down for 22-24 hours at 35 ± 0.5°C.

Reading of the Results. After the prescribed incubation period, colonies recovered for total staphylococci and total coliforms were counted using a Quebec colony counter. This counter provided satisfactory visibility and had grids to facilitate accurate counting of the colonies. The petri dish was placed directly under the magnifying lens of the counter.

Round, black, small to pinhead, well-defined, raised, and mucoid colonies on Vogel-Johnson agar were presumptive of staphylococci. Typical coliform colonies on M-Endo broth medium were pink to dark red, showing a golden green metallic sheen that varied from a small pinhead to complete coverage of the colony surface in the center, periphery, or over the entire surface.

All colonies exhibiting the above mentioned characteristics of staphylococci or coliforms were counted and recorded on the worksheet.

Isolation of Pure Cultures of Staphylococci. Black, round, well-defined, raised, mucoid, and small to pinhead colonies were typical of staphylococci on Vogel-Johnson agar. A pure culture of the same, which was used for the coagulase test, was isolated. Using a sterile inoculating loop, a typical staphylococci colony from the original Vogel-Johnson agar medium was picked and streaked to new Vogel-Johnson agar medium. It was incubated upside-down for 42-48 hours at 35 ± 0.5°C. The dish was examined after the prescribed incubation period and the same procedure was repeated once more. After this, a pure plate culture (plate with identical colonies) was believed to have been isolated.

Identification of *Staphylococcus aureus*. After a pure culture of staphylococci was isolated, identification as *S. aureus* was made based on the following criteria: macroscopic examination of the colonies on Vogel-Johnson agar medium, mannitol fermentation, catalase reaction, gram stain reaction, cell morphology and arrangements, and coagulase reaction. Each test is briefly outlined below.

Macroscopic examination involved examination by the naked eye of the morphology, color, size, and appearance of the colonies. Round, black, small to pinhead, well-defined, raised, and mucoid colonies were characteristic of staphylococci.

Mannitol is an alcohol that is fermented by *S. aureus* and a few other species of staphylococci. Vogel-Johnson agar medium has mannitol included in its formulation and fermentation of it by the organism was indicated by a change in the color of the culture medium from rose pink to yellow.

Catalase is an enzyme that is produced by all species of staphylococci. This enzyme catalyzes the decomposition of hydrogen peroxide to oxygen and water. To perform this test, 2-3 colonies of staphylococci were trans-

ferred to a glass slide and one drop of 3 percent hydrogen peroxide was added. Since staphylococci are known to produce catalase, after the addition of hydrogen peroxide, bubbles should be formed at the site where the colonies were smeared. This was considered a positive test.

A microscopic examination of the isolate was done by first doing a gram stain. A loopful of distilled water was placed on a glass slide and a thin smear of the colony was made. It was allowed to dry by air and heat fixed by passing the slide 4-5 times through a bunsen burner flame. After it had cooled off for a few minutes, staining was done.

Gram stain includes four kinds of reagents applied one after the other on the smear. Crystal violet, the initial stain, was applied for one minute and the excess stain was rinsed with distilled water. A mordant or a dye-fixing substance, iodine, was added next, and rinsed after one minute. Ethanol, which acted as a decolorizer, was applied for about 15 seconds then rinsed. The smear was finally counterstained with Safranin for 15-30 seconds and rinsed with distilled water. After the smear had dried, it was mounted on the microscope for further visualization of the cells. Focus was done by using the low and high power objectives and later switched to oil immersion objective after a drop of immersion oil was added to the smear. The oil acted as a medium for the oil immersion objective to magnify the cells. Necessary adjustments on the microscope were made to obtain a sharp focus.

A gram stain was positive if the cells under the microscope were stained blue, and negative if the cells were colored red or pink. All isolates in this investigation were gram positive.

Morphologically, bacterial cells could either be cocci or bacilli. Cocci appeared as round cells, while bacilli were rod-shaped. Staphylococci were considered gram positive cocci.

Further examination was done on how the cocci looked under the microscope. Staphylococcal cells were arranged in irregular "grape-like" clusters, tetrads, short chains of 3 or 4 cells, in pairs, and single cells. All of these features were observed in all parts of the smears. All results were recorded on the spaces provided for on the worksheets.

Coagulase is an enzyme-like protein that clots oxalated or citrated plasma in the presence of a factor contained in many sera. It is a definitive test for *Staphylococcus aureus* (57, 60) when done in conjunction with gram stain reaction, colony and cell morphology, catalase, and mannitol fermentation. In this investigation, coagulase activity was demonstrated using the API-Staphase III kit (50). The materials included were macrocupules (containing the reagent), incubation trays and lids, and a package insert. The procedure on the accompanying brochure was followed and is summarized as follows: Two drops of sterile distilled water were added to the macrocupule and the liquid was allowed to stand for at least one minute. Using a sterile wooden applicator stick, a confluent sweep of growth (from a pure plate culture) or five morphologically similar colonies was inoculated into the macrocupule. The inoculum was thoroughly emulsified into the contents of the Staphase III macrocupule and anytime within one minute agglutination could be observed. A positive agglutination test appeared as clumps that were not dissolved in fluids. A positive result was considered definitive and no further observation was required.

Since none of the tests in this investigation was positive for agglutination, incubation was necessary. This was done by adding approximately

two ml of sterile distilled water to the incubation tray to provide a humid atmosphere during incubation. The macrocupule was placed into the tray, covered with a lid, and incubated for five hours at 35-37°C. After the incubation period, the macrocupule was tilted back and forth to visualize any coagulation. A positive reaction was one in which the contents of the macrocupule did not flow when tilted back and forth. A negative reaction was one in which the mixture in the macrocupule flowed freely when moved. Coagulation was further verified by rubbing an applicator stick along the bottom of the macrocupule. After the test results were recorded, the incubation unit and the applicator sticks were autoclaved. All coagulase positive staphylococci were considered pathogenic for humans.

Positive and negative controls (pure cultures of *S. aureus* and *S. epidermidis*, respectively) were run simultaneously with the above mentioned procedure for a definite identification of *S. aureus*. These control isolates were obtained from the Stock Culture Collection of the Microbiology Department of Oregon State University.

Collection of water samples from the 14 swimming pools and spas was done at three different times. The same procedures, protocol, and materials were followed and used from the preparation part of the sample collection up to the identification portion of *S. aureus*.

IV. ANALYSIS OF DATA

Statistical Instruments

Results of the physical and chemical tests were compared against the standard values from the guidelines of the Oregon State Health Division for water quality of swimming pools (36) and spas (37). Detailed information of each criteria is found in Appendices E and F.

A sample of 1000 ml of water was collected from each of the inlet and outlet sites of all the pools. Since the usual way of expressing staphylococci and coliform levels in water is in terms of number of organisms/100 ml, the following formula was followed:

$$\frac{\text{Number of colonies counted}}{\text{sample size}} \times 100 = \text{Number of total org/100 ml}$$

where,

number of colonies = number of colonies counted for each organism

sample size = 1000 ml

100 = a constant.

This formula was used to compute the number of organisms/100 ml from the inlet site (sampling site 1) and outlet site (sampling site 2) of each pool. The organisms isolated from the surface microlayer (sampling site 3) were expressed as numbers of organisms/filter.

Pearson's correlation coefficient (r) was chosen as the appropriate measure to determine whether or not correlations existed between the free

chlorine and bathing load and staphylococcal and coliform densities. Pearson's correlation coefficient (r) is a statistical tool that summarizes the extent of correlation between two variables. It is a well-defined numerical index of the relationship of two variables with a wide range of application in data analyses (21).

Correlation coefficient values range from -1.00 to +1.00. An r=1 denotes a perfect positive correlation. The correlation of variables X and Y increases as the value of r increases. On the other hand, the reverse can also happen if the value is r=-1. The value of either one of the variables increases as the other decreases. This is otherwise known as negative correlation.

Another way of looking at the extent of relationship between two variables is to make a scattergram. This measure provides a pictorial representation of the data plotted correspondingly to the variables represented by the horizontal and vertical axes (21). Like Pearson's r, if high values of one variable are associated with high values of the other variable, a positive linear relationship is present.

Results

The operational data of the swimming pools and spas are presented in Tables 2 and 3 and the results of the physical and chemical tests are recorded and shown in Tables 4 and 5. Based on the Oregon Administrative Rules 333-60-200 and 333-62-165, the water clarity was acceptable for all 14 pools and the temperatures were within the standard allowable limit.

Free chlorine levels of 6 swimming pools (67%) were within the required range of 0.8-3.0 ppm. The maximum limit was exceeded by one pool,

Table 2. Operational Data of Swimming Pools.

Swim Pool Code	Dimensions in Feet[1]	Capacity in Gallons	Type of Chlorination	Type of Filter	Avg. Load Per Day[2]
101	75 X 35 X 9.5	160,000	Gas	Sand	190
104	164 X 51 X 12	300,000	Gas	Diatomaceous Earth	500
105	165 X 56 X 13	485,000	Gas	Diatomaceous Earth	500
106	40 X 22 X 4.5	20,000	Gas	Diatomaceous Earth	400
107	36 X 18 X 4	25,000	Sodium dichloro-isocyanurate	Sand	70
109	60 X 30 X 10.6	180,000	Gas	Diatomaceous Earth	250
110	75 X 45 X 13	250,000	Gas	Sand	200
111	75 X 30 X 10	122,000	Gas	Diatomaceous Earth	75
114	75 X 25 X 9	86,000	Sodium hypochlorite	Sand	170

[1] Length X width X deepest depth.
[2] Average number of bathers per day.

Table 3. Operational Data of Spas.

Spa Code	Dimensions in Feet[1]	Capacity in Gallons	Type of Chlorination	Type of Filter	Avg. Load Per Day[2]
102	12 X 9 X 3	1,200	Gas	Cartridge	85
103	8.5 X 8.5 X 3.5	560	Trichloro-isocyanurate	Cartridge	50
108	16 X 6 X 4.6	2,000	Sodium dichloro-isocynaurate	Sand	40
112	10 X 6 X 3.5	1,800	Sodium dichloro-isocynaurate	Cartridge	6
113	6 X 6 X 2.5	325	Sodium dichloro-isocynaurate	Cartridge	6

[1] Length X width X deepest depth.
[2] Average number of bathers per day.

Table 4. Chemical and Physical Data of Swimming Pool Water.

Swim Pool Code	Temp (°C)	pH	Free Chlor. Resid.[1] (ppm)	Total Alka-linity (ppm)	Water Clarity[2]
101	27	7.7	0.67	213	Acceptable
104	27	7.5	1.10	147	Acceptable
105	25	7.5	2.60	147	Acceptable
106	32	7.5	1.83	183	Acceptable
107	28	6.8	4.00	237	Acceptable
109	28	7.6	1.37	153	Acceptable
110	27	7.5	1.67	107	Acceptable
111	28	7.2	0.80	87	Acceptable
114	28	7.6	0.73	87	Acceptable

[1] Free chlorine residual.
[2] Water clarity is considered acceptable if a standard 2" clarity disc, divided into alternate black and red quadrants, is clearly visible and the separate colors discernible through 15 feet (4.57 m) of water.

Table 5. Chemical and Physical Data of Spa Water.

Spa Code	Temp (°C)	pH	Free Chlor. Resid.[1] (ppm)	Total Alka-linity (ppm)	Water Clarity[2]
102	38	7.7	2.37	137	Acceptable
103	38	8.0	2.93	143	Acceptable
108	40	7.3	3.50	223	Acceptable
112	32	7.6	1.83	100	Acceptable
113	28	7.6	0.73	87	Acceptable

[1] Free chlorine residual.
[2] Water clarity is considered acceptable if a standard 2" clarity disc, divided into alternate black and red quadrants, is clearly visible and the separate colors discernible through 15 feet (4.57 m) of water.

while a reading below 0.8 ppm was noted in two other pools. Total alkalinity was within 80-200 ppm for 7 pools and a reading above the upper limit was recorded in 2 of the facilities. Except for one, the pH were kept at an ideal range of 7.2-7.6 ppm (Table 4).

The free chlorine levels of all of the spas were within 1.5-5.0 ppm. Three spas had allowable pH readings (7.2-7.6), while 2 were off the maximum limit. Alkalinity was maintained between 80-175 ppm, as required, for all facilities, but one (Table 5).

Water samples were collected at three different times for the 13 swimming pools and spas. One facility was only sampled once due to a maintenance problem beyond the operator's control.

In spite of the numerous investigations that indicated the potential of staphylococci to be the best indicators of water quality in swimming pools and other recreational waters, there has been no allowable maximum number set for these organisms to serve as the basis for sanitary decisions. However, it was stated in the Oregon Administrative Rules that: "*Pseudomonas*

aeruginosa and other human pathogens shall not be present in any samples tested using Standard Methods for testing water and wastewater" (36, p. 21).

These criteria have been established for drinking water quality and the same should apply to swimming pools and spas since the presence of any pathogen in these facilities implies a risk of disease transmission (personal communication with Dr. W. H. Anderman).

Based on the above criteria, three out of nine swimming pools were considered unsanitary at three different times due to the recovery of the pathogen S. *aureus* from the water samples. None of the samples collected from the five spas were found to contain S. *aureus*.

Because coliforms are still the official indicators of choice for swimming pool water quality (9, 36, 37), analysis of the same was done.

Water samples were collected from all public, indoor and chlorinated pools of Philomath, Lebanon and Sweet Home. In the same manner, 71 and 86 percent of the pools in Corvallis and Albany, respectively, were included in the study. The number of organisms isolated and identified per city are presented in Table 6.

The number of total staphylococci and total coliforms/100 ml isolated from the inlet site (sampling site 1) and outlet site (sampling site 2) of each of the facilities were averaged from the three observations (Tables 7, 8, 9 & 10). Results of the paired t-test showed that there was no difference between the number of organisms recovered from the inlet and outlet sites of the pools. Since no difference was noted, the number of organisms from both sites were combined and Pearson's r and scattergram were done to determine the extent of the relationship between organism density and the variables free chlorine and bathing load.

Table 6. Total Staphylococci and Total Coliforms Indentified Per City.

City	Nr[1]	Percent Sampled	Number of Total Staphylococci in 1000 ml Water Sample (Minimum-Maximum) Site 1	Site 2	Site 3	Number of Total Coliforms in 1000 ml Water Sample (Minimum-Maximum) Site 1	Site 2	Site 3
Corvallis	7	71.4	0-3	0-2	0-63	0-12	0-18	0-2
Philomath	1	100.0	2-9	5-42	1-24	1-2	1-10	0-3
Albany	7	85.7	0-376	0-71	0-24	0-25	0-36	0-4
Lebanon	1	100.0	0	0-2	1-48	0	0	0-3
Sweet Home	1	100.0	1-2	0-11	2-45	0-1	0-2	0-8

[1] Number of pools.

Table 7. Total Staphylococci Isolated From Each Sampling Site of the Swimming Pools with the Respective Means During the Three Observation Periods.

Swim Pool Code	Total Staphylococci per 100 ml X_1[a]	Total Staphylococci per 100 ml X_2[a]	Total Staphylococci per filter X_3[a]	Mean Total Staphylococci per 100 ml $\bar{X}_1$[b]	Mean Total Staphylococci per 100 ml $\bar{X}_2$[b]	Mean Total Staphylococci per filter $\bar{X}_3$[b]	$\frac{\bar{X}_1+\bar{X}_2}{2}$
101	0.3	0.0	3				
	0.0	1.1	10				
	0.0	0.0	63	0.10	0.37	25.33	0.23
104	0.1	0.4	4				
	37.6	5.5	0				
	0.9	0.5	5	12.87	2.13	3.00	7.50
105	0.0	0.0	2				
	0.0	0.0	2				
	0.0	0.0	7	0.00	0.00	3.67	0.00
106	0.0	0.0	0				
	0.0	0.0	1				
	0.0	0.2	3	0.00	0.07	1.33	0.03
107	0.0	0.0	15				
	0.0	0.1	16				
	0.0	0.0	8	0.00	0.03	13.00	0.02
109	0.2	0.0	2				
	0.1	0.0	45				
	0.1	1.1	15	0.13	0.37	20.67	0.25
110	0.0	0.0	1				
	0.0	0.1	2				
	0.0	0.0	48	0.00	0.10	17.00	0.05
111	0.2	1.3	1				
	0.3	0.5	24				
	0.9	4.2	2	0.47	2.00	9.00	1.23
114	0.7	0.2	17				
	0.0	0.2	24				
	0.8	0.3	2	0.50	0.23	14.33	0.37

[a] The three sampling sites of the pools.
[b] The mean of the three observations.

Table 8. Total Coliforms Isolated From Each Sampling Site of the Swimming Pools with the Respective Means During the Three Observation Periods.

Swim Pool Code	Total Coliforms per 100 ml X_1[a]	Total Coliforms per 100 ml X_2[a]	Total Coliforms per filter X_3[a]	Mean Total Coliforms per 100 ml $\bar{X}_1$[b]	Mean Total Coliforms per 100 ml $\bar{X}_2$[b]	Mean Total Coliforms per filter $\bar{X}_3$[b]	$\frac{\bar{X}_1+\bar{X}_2}{2}$
101	0.0	0.0	0				
	0.1	1.8	2				
	0.0	0.3	0	0.03	0.70	0.67	0.37
104	0.1	3.6	0				
	0.3	0.2	0				
	0.0	0.0	0	0.13	1.27	0.00	0.13
105	0.0	0.0	0				
	0.2	0.6	2				
	0.0	0.0	0	0.07	0.20	0.00	0.13
106	0.0	0.0	0				
	0.0	0.1	0				
	0.0	0.0	0	0.00	0.03	0.00	0.02
107	0.1	0.1	0				
	0.0	0.2	1				
	0.0	0.0	0	0.03	0.10	0.30	0.07
109	0.0	0.0	0				
	0.1	0.2	8				
	0.0	0.0	0	0.03	0.07	2.67	0.05
110	0.0	0.0	0				
	0.0	0.0	0				
	0.0	0.0	3	0.00	0.00	1.00	0.00
111	0.1	0.1	0				
	0.2	1.0	3				
	0.1	0.3	1	0.13	0.47	1.33	0.03
114	0.2	0.4	4				
	0.0	0.0	0				
	0.0	0.0	0	0.07	0.13	1.33	0.10

[a] The three sampling sites of the pools.
[b] The mean of the three observations.

Table 9. Total Staphylococci Isolated From Each Sampling Site of the Spas with the Respective Means During the Three Observation Periods.

Spa Code	Total Staphylococci per 100 ml X_1[a]	Total Staphylococci per 100 ml X_2[a]	Total Staphylococci per filter X_3[a]	Mean Total Staphylococci per 100 ml $\bar{X}_1$[b]	Mean Total Staphylococci per 100 ml $\bar{X}_2$[b]	Mean Total Staphylococci per filter $\bar{X}_3$[b]	$\frac{\bar{X}_1+\bar{X}_2}{2}$
102	0.0	0.0	20				
	0.0	0.0	19				
	0.0	0.0	1	0.00	0.00	13.33	0.00
103	0.0	0.0	5				
	6.7	7.1	10				
	7.6	2.0	0	4.77	3.03	5.00	3.90
108	0.0	0.0	6				
	0.0	0.0	0				
	0.0	0.0	5	0.00	0.00	3.67	0.00
112	0.3	0.3	10				
	12.2	4.5	9				
	12.2	4.5	9	8.23	3.10	9.33	5.67
113	0.0	4.3	3	0.00	4.30	3.00	2.15

[a] The three sampling sites of the pools.
[b] The mean of the three observations.

Table 10. Total Coliforms Isolated From Each Sampling Site of the Spas with the Respective Means During the Three Observation Periods.

Spa Code	Total Coliforms per 100 ml X_1[a]	Total Coliforms per 100 ml X_2[a]	Total Coliforms per filter X_3[a]	Mean Total Coliforms per 100 ml $\bar{X}_1$[b]	Mean Total Coliforms per 100 ml $\bar{X}_2$[b]	Mean Total Coliforms per filter $\bar{X}_3$[b]	$\frac{\bar{X}_1+\bar{X}_2}{2}$
102	2.5	3.2	3				
	0.0	0.0	0				
	0.0	0.0	0	0.83	1.07	1.00	0.95
103	0.0	0.4	1				
	0.1	0.6	0				
	0.0	0.0	0	0.03	0.33	0.33	0.18
108	1.2	0.3	0				
	0.0	0.0	0				
	0.0	0.0	0	0.40	0.10	0.00	0.25
112	0.1	0.2	2				
	0.2	0.0	0				
	0.2	0.0	0	0.17	0.07	0.67	0.12
113	0.0	0.2	1	0.00	0.20	0.10	0.10

[a] The three sampling sites of the pools.
[b] The mean of the three observations.

A correlation value, r = -.1365 (p = .1673), was obtained for staphylococci and free chlorine, while r = .32091 (p = .28374) was derived for staphylococci and bathing load. The same statistics were used to measure the correlation exhibited by the coliform organisms. It yielded r = -.0949 (p = .37349) and r = .1348 (p = .32297) when associated with free chlorine and bathing load, respectively.

Although the correlations did not show any significance, these findings implied that the number of staphylococci and coliform organisms was in-

versely proportional to the level of free chlorine in the water, while both organisms showed direct proportion to the bathing load.

Table 11 presents the correlation coefficient values of total staphylococci and total coliforms obtained from each sampling site during the three observation periods. To get a closer estimate of the relationship, results during these periods were combined and a correlation representing each sampling site was obtained (Table 12).

Table 11. Correlation Coefficient (r) of Total Staphylococci & Total Coliforms During the Three Observation Periods.

Observation Periods	Sampling Sites 1	2	3
1	-.1911 (.512)	-.0836 (.776)	.7739 (.001)
2	.6949 (.008)	.1266 (.680)	.8143 (.001)
3	.4569 (.115)	.6099 (.026)	.4470 (.124)

Note: Numbers in parentheses are the corresponding p values.

Table 12. Correlation Coefficient (r) of Total Staphylococci & Total Coliforms Averaged Over the Three Observation Periods.

Sampling Sites 1	2	3
-.04146 (.44405)	-.06767 (.40910)	.58361 (.01423)

Note: Numbers in parentheses are the corresponding p values.

The use of the Millipore membrane filter to sample the surface microlayer of the water provided a higher percentage of recovery of both or-

ganisms. Correlation of results of total staphylococci and total coliforms was found to be significant using this sampling procedure ($r = .5836$, $p = .01423$). However, this relationship was not consistent with the other two sampling sites.

Due to a limited number of observations and the variations of the number of organisms in each observation, there were situations when the strength of relationship was not significantly detected.

Discussion

Since free chlorine and bathing load were not measured in the three sampling sites of every pool accordingly when samples were collected, this might have introduced an error when these variables were correlated with total staphylococci and total coliforms. But even if the correlation values were not significant, results showed that organism density decreases with increasing chlorine concentrations and more organisms were isolated as the bathing load increases.

Results of this study revealed a significant correlation between total staphylococci and total coliforms using the membrane filter to sample the surface microlayer of the water. This finding was consistent with the fact that most bacteria shed by bathers are found in body oils, saliva, and other secretions that layer the water surface. Because the organisms found in the surface microlayer were not exposed to free chlorine as much as those that were found in the inlet and outlet sites, more organisms were recovered from the surface using the membrane filter. A reverse pattern of association was observed in the inlet and outlet sites.

Effort was made to collect water samples at peak times when the swimming pools and spas were most loaded with bathers. However, due to

time constraints, there were a few occasions when this was not possible. This could account for situations when very few or no microorganisms were isolated from the samples collected.

This investigation has been constrained with time and money, so the number of observations made was limited, which in some instances was not sufficient to detect any significance in the correlations.

V. SUMMARY AND CONCLUSIONS

Summary

Water samples were collected on three occasions from three sampling sites of 14 public, indoor, and chlorinated swimming pools and spas in Linn and Benton Counties using staphylococci as a microbiological indicator. Evaluation of water quality was based on guidelines of the Oregon State Health Division that no pathogen (in this study *S. aureus*) should be present from any samples collected and tested in accordance with Standard Methods (48).

Because coliforms are still the indicator of choice of water quality in swimming pools and other recreational waters, water samples were also tested for the presence of total coliforms.

The pathogen *S. aureus* was identified from the total staphylococcal isolates on the basis of colony morphology, gram stain reaction, catalase reaction, cellular morphology and arrangements, and coagulase activity using API-Staphase III test kit.

Staphylococcus aureus was isolated and identified in 33 percent of the swimming pools at three different times. Pearson's r and scattergram revealed that the number of staphylococci and coliform organisms from the inlet and outlet sites of the pools was inversely proportional to the level of free chlorine, but a direct proportion was noted to the bathing load.

The surface microlayer of the water was sampled using a Millipore membrane filter and statistics showed a significant correlation of both organisms in this sampling site. Furthermore, it was found that more staphylococci and coliforms were recovered using this sampling procedure.

Conclusion

The conclusions derived from the findings of this investigation are:

1. Thirty-three percent of the swimming pools in Linn and Benton Counties were found to be unsanitary on three occasions due to the presence of the pathogen S. *aureus*.
2. Although the correlation values were not significant, the results implied that the staphylococcal density increases as the free chlorine level decreases and a high number of staphylococci was associated with an increasing bathing load. The same pattern of association was observed for the coliforms.
3. Higher number of organisms were recovered from the surface microlayer of the water using the Millipore membrane filter and a significant correlation (r= .5836, p = .01423) was found between the number of total staphylococci and total coliforms from this sampling site. This outcome is consistent with the fact that most bacteria shed by bathers are found in body oils, saliva, and mucus discharges that layer near the water surface (15, 48).

Recommendations

Because this investigation was limited to public, indoor, and chlorinated swimming pools and spas in Linn and Benton Counties, conclusions

from the results should be applied with caution and only to facilities with similar conditions. Therefore, it is highly recommended that:

1. Further research should be done to include outdoor facilities that are only operational during summer.
2. Bathing load and free chlorine measurements should be carried out whenever and wherever water samples are collected.
3. Health authorities should look into the potentials staphylococci have over the coliforms as better indicators of recreational water quality.

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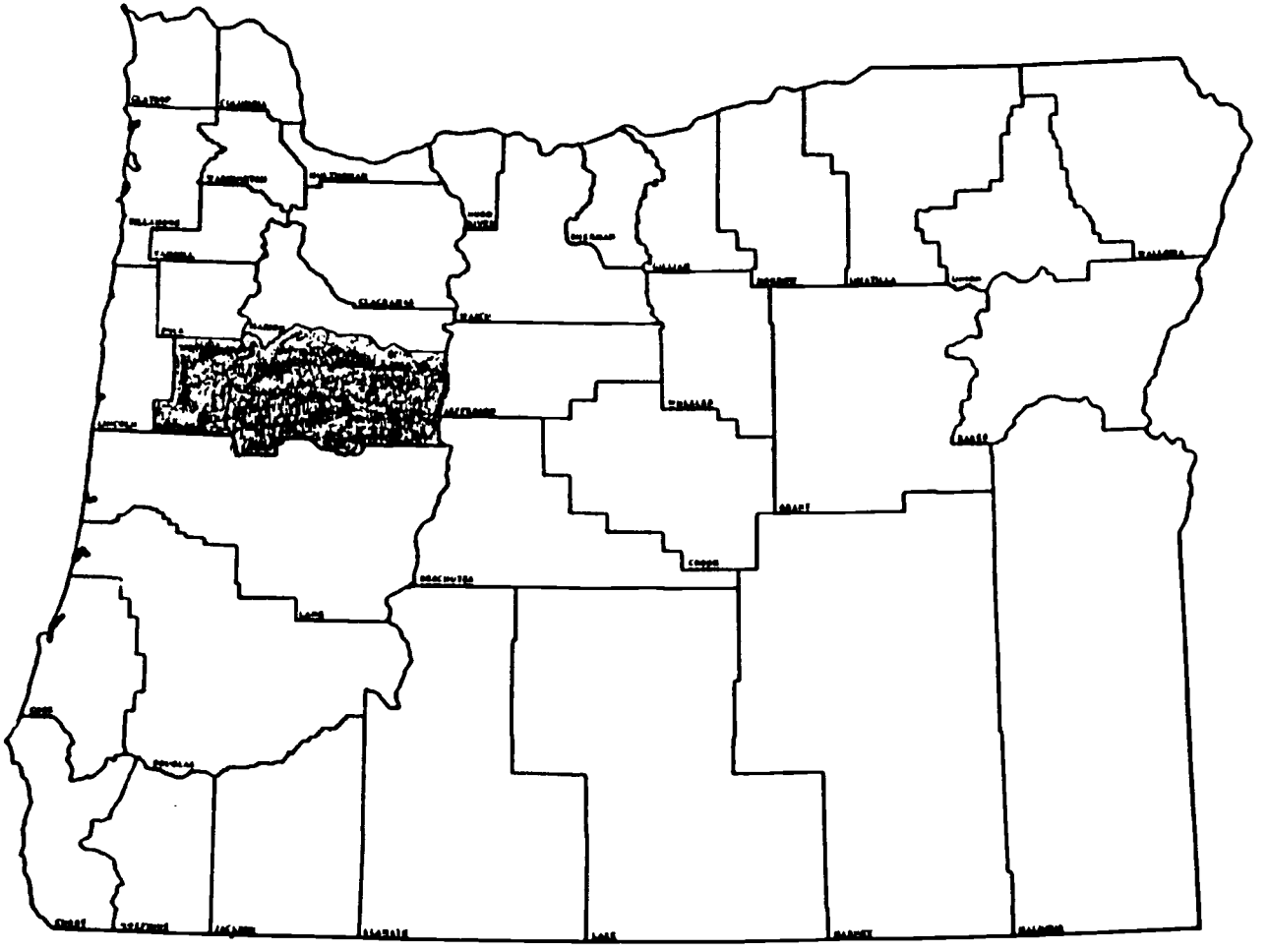
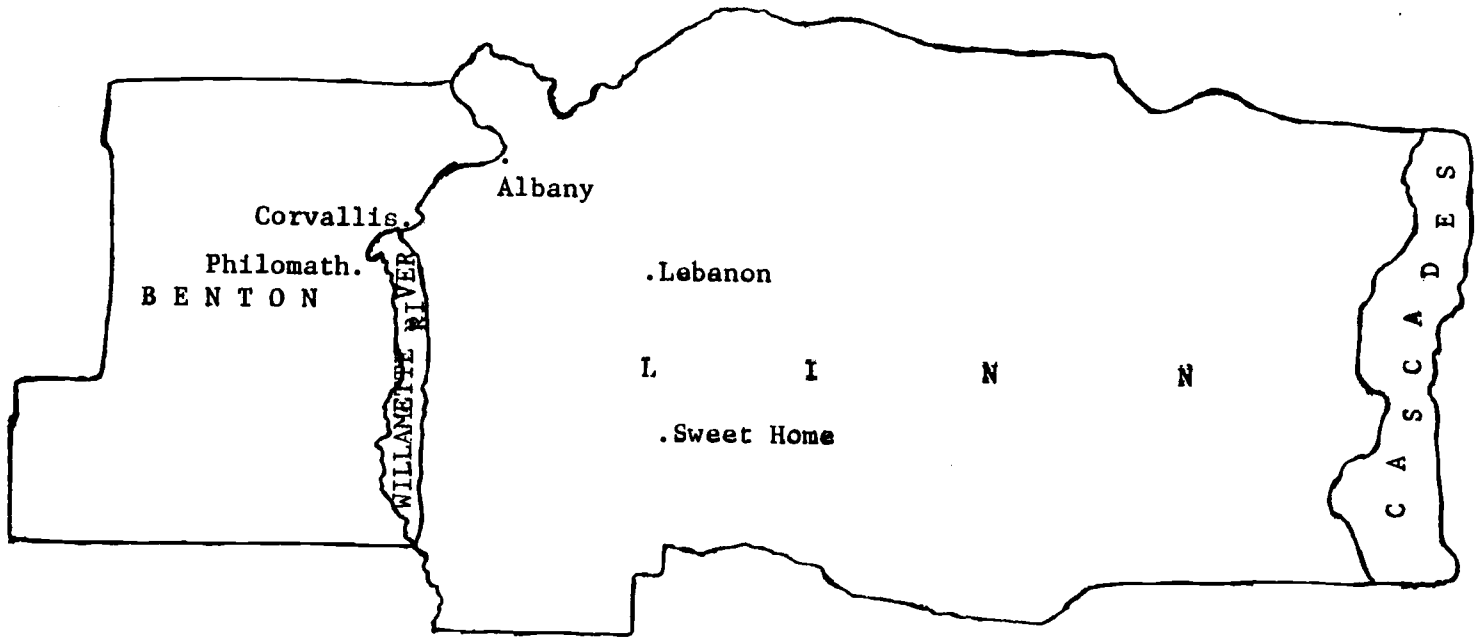
APPENDICES

APPENDIX A

MAP OF THE STUDY AREA



COUNTY MAP OF THE STATE OF OREGON. THE SHADED AREAS ARE LINN AND BENTON COUNTIES.



MAP OF LINN AND BENTON COUNTIES SHOWING THE FIVE PLACES WHERE THE SWIMMING POOLS AND SPAS WERE LOCATED.

APPENDIX B

LETTER OF INTRODUCTION

Department of Health Oregon State University Corvallis, Oregon 97331-6406 (503) 754-2686

One of our graduate assistants, Bechaida Rivera, is conducting a research study on environmental health management for her Master's thesis. She is currently in the process of gathering data and information on swimming pool/spa waters in this locality.

She would like to collect water samples from your facility as part of the research project. This project is supported by this department and I have asked her to contact you as soon as possible.

I look forward to your support and cooperation which I believe are key factors toward the success of this project.

If you have any questions, please feel free to contact me.

Sincerely,

David C. Lawson, Ed.D.
Chair and Associate Professor

APPENDIX C

WORKSHEET

DEPARTMENT OF HEALTH
OREGON STATE UNIVERSITY

Col. No.

I.D. NUMBER _____ (1-3)

SWIMMING POOL AND SPA SURVEY WORKSHEET

1. NAME OF FACILITY:_____

2. OWNER'S NAME:_____

3. LOCATION ADDRESS:_____

4. PHONE NUMBER:_____

5. POOL DIRECTOR/INCHARGE:_____

OPERATIONAL DATA

1.	TYPE OF FACILITY:	1) GENERAL USE	(5)
		2) LIMITED USE	
		3) UNKNOWN	
2.	SIZE OF POOL:	WIDTH (FEET)	(7-11)
		LENGTH (FEET)	(13-17)
		88888) UNKNOWN	
3.	DEPTH (DEEPEST):	(FEET)	(19-22)
		888) UNKNOWN	
4.	WATER CAPACITY:	(GALLONS)	(24-29)
		888888) UNKNOWN	
5.	TYPE OF DISINFECTANT:		
		1) CHLORINE	(31)
		2) BROMINE	

3) OTHERS_____

8) UNKNOWN

6. IF CHLORINE, TYPE OF CHLORINATION: (33)

1) GAS

2) SODIUM HYPOCHLORITE

3) SODIUM DICHLOROISOCYANURATE

4) OTHERS

8) UNKNOWN

7. TYPE OF FILTER 1) SAND (35)

2) DIATOMACEOUS EARTH

3) OTHERS_____

8) UNKNOWN

8. AVERAGE BATHING LOAD/DAY:

PERSONS (37-39)

888) UNKNOWN

9. TYPE OF WATER OPERATION SYSTEM: (41)

1) RECIRCULATING

2) OVER-FLOW REFILL

3) UNKNOWN

10. SWIMMING HOURS: OPENING TIME: (43-45)

CLOSING TIME: (47-50)

8888) UNKNOWN

11. ADMISSION FEE: $_____/PERSON/MONTH (52-56)

MEMBERSHIP FEE: $_____/PERSON/MONTH

(CLUB)

88888) UNKNOWN

PHYSICAL AND CHEMICAL DATA

		DATE:	(1-6)
		TIME:	(8-11
1.	WATER CLARITY:	1) ACCEPTABLE*	(13)
		2) UNACCEPTABLE**	
		8) UNKNOWN	
2.	TEMPERATURE:	(DEGREES CENTIGRADE)	(15-16)
		88) UNKNOWN	
3.	pH		(18-20)
		888) UNKNOWN	
4.	FREE CHLORINE:	(ppm)	(22-24)
		888) UNKNOWN	
5.	TOTAL ALKALINITY:	(ppm)	(26-28)
		888) UNKNOWN	
6.	NO. OF BATHERS DURING SAMPLE COLLECTION:		(30-32)
		PERSONS	
		888) UNKNOWN	

BACTERIOLOGICAL RESULTS

SAMPLING SITE 1 (Inlet)

1.	TOTAL NUMBER OF COLIFORM COLONIES	(35-36)
2.	TOTAL NUMBER OF STAPHYLOCOCCI COLONIES	(38-40)

*Water clarity is considered acceptable if a standard 2" diameter clarity disc which is divided into alternate black and red quadrants is clearly visible and the separate colors discernible through 15 feet (4.57 m) of water.

**Water clarity is considered unacceptable if a standard 2" diameter clarity disc which is divided into alternate black and red quadrants is not clearly visible and the separate colors discernible through 15 feet (4.57 m) of water.

SAMPLING SITE 2 (Outlet)

1. TOTAL NUMBER OF COLIFORM COLONIES (42-43)

2. TOTAL NUMBER OF STAPHYLOCOCCI COLONIES (45-46)

SAMPLING SITE 3 (Surface Microlayer)

1. TOTAL NUMBER OF COLIFORM COLONIES (48)

2. TOTAL NUMBER OF STAPHYLOCOCCI COLONIES (50-51)

IDENTIFICATION OF STAPH. AUREUS

SAMPLING SITE ____

MACROSCOPIC EXAMINATION (COLONIES ON VOGEL-JOHNSON AGAR):

1. MORPHOLOGY
   1) ROUND (53)
   2) IRREGULAR
   3) OTHERS_____
   8) UNKNOWN

2. COLOR
   1) BLACK (55)
   2) GREY
   3) OTHERS_____
   8) UNKNOWN

3. SIZE
   1) SMALL (57)
   2) PINHEAD
   3) OTHERS_____
   8) UNKNOWN

4. APPEARANCE:
   1) SMOOTH (59-61)
   2) WELL-DEFINED
   3) RAISED
   4) OPAQUE

5) MUCOID

6) OTHERS_____

888) UNKNOWN

5. MANNITOL FERMENTATION: (63)

1) POSITIVE

2) NEGATIVE

8) UNKNOWN

6. CATALASE REACTION: 1) POSITIVE (65)

2) NEGATIVE

8) UNKNOWN

MICROSCOPIC EXAMINATION

1. GRAM STAIN REACTION: (67)

1) POSITIVE

2) NEGATIVE

8) UNKNOWN

2. CELL MORPHOLOGY:

1) COCCI (69)

2) OTHERS_____

3) UNKNOWN

3. CELL ARRANGEMENT: (71-75)

1) CLUSTERS

2) TETRADS

3) SHORT CHAINS

4) PAIRS

5) SINGLE CELLS

6) OTHERS_____

88888) UNKNOWN

COAGULASE REACTION 1) POSITIVE (77)

2) NEGATIVE

8) UNKNOWN

IDENTIFICATION OF ISOLATE:

1) *STAPHYLOCOCCUS AUREUS* (79)

2) STAPHYLOCOCCUS SPECIE

DATE ANALYSIS COMPLETED: _____ (81-86)

/JBTR

APPENDIX D

GROUPING OF FACILITIES

In the interest of distance, time, and convenience in sampling and transit, the facilities were grouped into four based on their geographical locations so that the time difference between sample collection and analysis will not be more than six hours:

GROUP A

1. Timberhill Athletic Club (spa & swimming pool)
2. Philomath High School (swimming pool)
3. OSU Women's Bldg. (swimming pool)

GROUP B

1. Albany Athletic Club (women's hot tub)
2. Osborn Aquatic Center (swimming pool)
3. Osborn Aquatic Center (swimming pool)

GROUP C

1. Albany Community Swimming Pool
2. Mennonite Home (spa 1 and 2)
3. YMCA (swimming pool & spa)

GROUP D

1. Sweet Home High School (swimming pool)
2. Lebanon High School (swimming pool)

APPENDIX E

WATER QUALITY PARAMETERS FOR SWIMMING POOLS

Water Quality Parameters

FOR SWIMMING POOLS

OREGON ADMINISTRATIVE RULES
(333-60-200)

	Parameters	Min.	Ideal	Max.
(a)	free chlorine	0.8 ppm	1.0-1.5 ppm	3.0 ppm
(b)	combined chlorine	0	0	0.5 ppm
(c)	pH	7.2	7.2-7.6	7.6
(d)	total alkalinity as CaCO3	80 ppm	80 ppm	200 ppm
(e)	cyanuric acid	0 ppm	30 ppm	150 ppm
(f)	calcium hardness (recommended)	175 ppm		
(g)	turbidity (water clarity):	O/F.T.U. 0.0-5/F.T.U. 1.0/F.T.U. or such that a standard 2" (5cm) diameter clarity disc which is divided into alternate black and red quadrants is clearly visible and the separate colors discernible through 15 feet (4.57m) of water. NOte: F.T.U. = Formazin Turbidity Unit.		
(h)	bacteria:	Coliform organisms shall not be present in more than 15 percent of any series of samples and *pseudomonas aeruginosa* or other human pathogen shall not be present in any samples tested using Standard Methods for Testing Water and Waste Water 16th Edition. (Note: it is not required that this parameter be checked routinely but shall be monitored at the discretion of the Health Division.)		

APPENDIX F

WATER QUALITY PARAMETERS FOR SPAS

Water Quality Parameters

FOR SPA
(333-62-165)

OF THE
OREGON ADMINISTRATIVE RULES

	Parameters	Min.	Ideal	Max.
(a)	free chlorine	1.5 ppm	3.0 ppm	5.0 ppm
(b)	combined chlorine	0	0	0.2 ppm
(c)	pH	7.2	7.2-7.6	7.6
(d)	total alkalinity as CaCO3	80	Plaster & tile spas 80-125	175
(e)	cyanuric acid	30		150
(f)	calcium hardness (recommended)	100		200
(g)	water temperature			104°C
(h)	total dissolved solids			1500 ppm
(i)	turbidity (water clarity):	O/F.T.U. 0.0-5/F.T.U. 1.0/F.T.U. or such that a standard 2" (5cm) diameter clarity disc which is divided into alternate black and red quadrants is clearly visible and the separate colors discernible through 15 feet (4.57m) of water. NOte: F.T.U. = Formazin Turbidity Unit.		
(j)	bacteria:	Coliform organisms shall not be present in more than 15 percent of any series of samples tested using Standard Methods for Testing Water and Waste Water 14th Edition. (Note: it is not required that this parameter be checked routinely but shall be monitored at the discretion of the Health Division.)		

APPENDIX G

INGREDIENTS OF CULTURE MEDIA

INGREDIENTS OF CULTURE MEDIA

VOGEL-JOHNSON AGAR

(Ingredients per liter)

Bacto-Tryptome	10.000 g
Bacto-Yeast Extract	5.000 g
Bacto-Mannitol	10.000 g
Dipotassium Phosphate	5.000 g
Lithium Chloride	5.000 g
Glycine	10.000 g
Bacto-Agar	15.000 g
Phenol Red	0.025 g

Final pH: 7.2 ± 0.1 at 25°C

M-ENDO BROTH MF

(Ingredients per liter)

Bacto-Yeast Extract	1.500 g
Bacto-Casitone	5.000 g
Bacto-Thiopeptone	5.000 g
Bacto-Tryptose	10.000 g
Bacto-Lactose	12.500 g
Sodium Desoxycholate	0.100 g
Dipotassium Phosphate	4.375 g
Monopotassium Phosphate	1.375 g
Sodium Chloride	5.000 g
Sodium Lauryl Sulfate	0.050 g
Sodium Sulfite	2.100 g
Bacto-Basic Fuschsin	1.050 g

Final pH: 7.2 ± 0.2