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Comparison of Vogel-Johnson and Baird-Parker Media for Membrane Filtration Recovery of Staphylococci in Swimming Pool Water

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Previous studies have indicated that the coagulase-positive *Staphylococcus* (*Staphylococcus aureus*) has potential as a useful indicator of the infection hazard associated with the use of swimming pools and other recreational waters. However, before this indicator system can be used effectively, a recovery system that is sufficiently selective, accurate, and reliable for the enumeration of *S. aureus* must be developed. In this study, Vogel-Johnson (VJ) and Baird-Parker (BP) agars were compared for efficacy in the primary isolation and recovery of *S. aureus* from swimming pool water. For equal sample volumes of pool water containing adequate free chlorine residual, VJ agar was found to be more selective for staphylococcal species and less inhibitory to general cell growth than was BP agar. However, neither medium was found to be sufficiently differential to permit the accurate identification of *S. aureus*. In contrast, water samples obtained from a swimming pool containing very low levels of chlorine (none of which was in the free form) showed abundant growth of staphylococci on both test media, with both VJ and BP agars showing increased sensitivity for the detection of *S. aureus*. Thus, VJ and BP agars show increased sensitivity for the detection of coagulase-positive staphylococci from unchlorinated versus chlorinated waters.

There has been much debate during the past 40 years as to the wisdom of using a coliform standard to determine the infection hazard of certain recreational waters (16, 19). Staphylococci have been suggested as an alternative indicator to the coliforms for swimming pools because they are ubiquitous in this environment and generally are much more resistant to halogen disinfectants than are coliforms (6, 9, 13). The coagulase-positive staphylococci, herein used synonymously with the species *Staphylococcus aureus*, are normal inhabitants of the nose, skin, and intestinal tract of humans, are readily shed by swimmers, and thus are a major component of the bacterial flora found in swimming pools of high bather density (20). Crone and Tee (6) determined that two-thirds of the staphylococci in pools studied were *S. aureus*. Furthermore, several investigators have found *S. aureus* to be particularly resistant to the levels of halogens used in swimming pool disinfection (9, 13). In addition, *S. aureus* is a proven pathogen and notably responsible for a variety of skin abscesses and pustules (2). The major significance of *S. aureus* in swimming pool water lies in situations in which it might infect the eyes, ears, or cuts and scratches on the skin of bathers.

The major obstacle to the use of *S. aureus* as an indicator of potential infection hazard is the lack of a recovery system that is sufficiently selective, differential, and reliable for their enumeration from swimming pool water. The evaluation of media and methods was not the prime focus of the few studies on staphylococci in water which are cited in the literature. Nevertheless, membrane filtration with either *Staphylococcus* broth or *Staphylococcus* medium 110 has been found to be neither sufficiently selective nor differential for the quantitative recovery of staphylococci, especially *S. aureus* (1). Although Vogel-Johnson (VJ) agar, in conjunction with membrane filtration, has been used for the selective enumeration of *S. aureus* in swimming pools, this method has never been rigorously evaluated (8, 9, 16). There are no reports in the literature of the use of Baird-Parker (BP) agar for the isolation of *S. aureus* from swimming pool water, although that agar is the medium of choice for enumeration of contaminant *S. aureus* in foods (17). However, there is a recent report of the successful use of Baird-Parker agar to evaluate *S. aureus* in hydrotherapy pools (7). In an effort to bridge the gap in the literature, the following study was conducted to evaluate the comparative efficacy of VJ and BP agars for the

primary isolation and recovery of *S. aureus* from swimming pool water.

MATERIALS AND METHODS

Sampling. Samples (500 ml) from three chlorinated, recirculation-type swimming pools were collected in compliance with the specifications outlined in Standard Methods for the Examination of Water and Wastewater (3). Sodium thiosulfate sufficient for complete dechlorination of specimens was present in sample bottles. All samples were processed within 3 h after collection.

Physical parameters. Pool water was analyzed for chlorine content, pH, and temperature at the time of sampling for bacteriological parameters. Both free and combined chlorine were determined by titration, using Palin's ferrous ammonium sulfate method with *N,N*-diethyl-*p*-phenylene-diamine as the indicator (3). pH was assessed with a digital pH/mv meter (Orion Research).

Bacteriological analysis. All samples were collected during times of peak bather load from chlorinated pools. Four 100-ml aliquots of each sample were membrane filtered according to *Standard Methods* (3). Millipore filter holders, Millipore HA membranes (pore size, 0.45 μm), and disposable plastic petri dishes (diameter, 60 mm) with loose-fitting lids were used. Of the four membrane filters resulting from each sample, two each were incubated on VJ agar (BBL Microbiology Systems) and BP agar (Difco Laboratories). Incubation was carried out in a circulating air incubator at 35 to 37°C for 24 to 48 h.

Characterization of isolates. Each colony isolated from membrane filters on VJ or BP agar was immediately transferred to tryptic soy broth (Difco). The overnight tryptic soy broth culture was then streaked for isolation on Standard Methods agar (Difco). After six passages on Standard Methods agar, each isolate was maintained on a nutrient agar slant. Catalase activity was determined by the rapid ebullition of gas upon contact of a nutrient agar slant culture with 3% hydrogen peroxide solution (10). Controls were *S. aureus* ATCC 25923 (positive control) and *Streptococcus pyogenes* ATCC 19615 (negative control). Coagulase activity was determined by the direct tube method with rabbit plasma (Difco) according to the recommendations of the Subcommittee on Taxonomy of Staphylococci and Micrococci (24). The degree of clotting was scored according to the four-point scheme proposed by Turner and Schwartz (26). Production of heat-stable DNase (thermonuclease) was determined by the metachromatic agar-diffusion technique of Lachica et al. (14). Test organism tryptic soy broth cultures were heated for 15 min at 100°C before thermonuclease testing. Controls for coagulase and thermonuclease tests were *S. aureus* ATCC 25923 (positive control) and *Staphylococcus epidermidis* ATCC 12228 (negative control). Lysostaphin sensitivity was determined by an agar overlay method previously described by Schleifer and Kloos (21), using lysostaphin (Sigma Chemical Co.) at a concentration of 200 μg/ml. Controls were *S. aureus* ATCC 25923 (strong positive control), *S. epidermidis* ATCC 12228 (weak positive control), and *Micrococcus luteus* (negative control). Each isolate was streaked for isolation on VJ and BP agars to facilitate the assessment of colony morphology, and was Gram stained.

RESULTS

A total of 45 samples were collected from chlorinated swimming pools containing a minimum free chlorine residual of 0.6 ppm. Since each sample was divided into four 100-ml aliquots for membrane filtration with two membrane filters incubated on each of the two test media, the results presented herein represent the evaluation of 90 membrane filters incubated on VJ agar and 90 on BP agar. The total number of isolates from membrane filters incubated on VJ agar was 356. Of these, 355 proved to be lysostaphin-sensitive, catalase-positive, gram-positive cocci. Thus, VJ agar appears to be highly selective (99.7%) for staphylococcal species. In contrast, of the 142 isolates yielded from membrane filters on BP agar, only 122 (85.9%) were confirmed as lysostaphin-sensitive, catalase-positive, gram-positive cocci. In addition to being somewhat less selective for staphylococcal species, BP agar isolated 60.1% fewer colonies than VJ agar from an identical group of 45 water samples. This may be due to a combination of the inherent inhibitory effects of BP medium components and the competition afforded by non-staphylococcal species growing on plates.

For the 45 samples assayed, an attempt was made to correlate "typical colony morphology" on each medium with identification as a coagulase-positive *Staphylococcus* sp. On VJ agar, coagulase-positive staphylococci form smooth, black colonies (due to the reduction of tellurite) which are often surrounded by a yellow zone due to the formation of acid from mannitol. Of the 355 *Staphylococcus* sp. isolates on VJ agar, 49 produced black colonies surrounded by yellow zones, with 2 of these exhibiting a +4 coagulase reaction and 47 exhibiting a negative coagulase reaction. A random sample consisting of 20% of the remaining 306 mannitol-negative VJ isolates was selected and tested for coagulase production. All were found to be negative. The production of thermonuclease has been shown to correlate quite well with +3 and +4 coagulase reactions (15, 18, 22, 25). Thus, the thermonuclease test appears to be a suitable ancillary test and subsequently was performed on all mannitol-negative VJ isolates. Each of the 306 isolates was negative for thermonuclease production. All of the 122 staphylococcal isolates from BP agar were tested and found to be negative for coagulase production. It should be noted that only seven of these 122 isolates exhibited colony morphology on BP agar typical of coagulase-positive staphylococci (black colonies surrounded by a clear zone). Thus, it can be concluded

that the accepted description of colony morphology on either VJ or BP medium correlates poorly with the identification of the isolates as coagulase-positive staphylococci.

Of special interest were five water samples obtained from a swimming pool containing a free chlorine residual of 0 ppm and a combined chlorine residual of 0.17 ppm. Although the volume of water filtered was reduced to 25 ml, all plates exhibited abundant growth. In fact, filters incubated on BP agar exhibited nearly twice the colony growth observed on filters incubated on VJ agar, for equal volumes of the same water sample. VJ agar was somewhat more selective than BP agar for staphylococcal species (Table 1), a trend shown to hold true for chlorinated and unchlorinated swimming pool water samples alike. However, it is evident from a comparison of the data here that both VJ and BP agars show increased sensitivity for the detection of coagulase-positive staphylococci from unchlorinated versus chlorinated waters.

DISCUSSION

Since staphylococci are a major bacterial contaminant of swimming pools that have a high bather density, and since a significant proportion of these staphylococci are *S. aureus*, enumeration of these organisms appears to provide a useful index of not only the level of contamination but also the concurrent effectiveness of the filtration and chlorination procedures used. However, there is no reliable analytical procedure which establishes a clear, quantitative correlation among factors such as number of staphylococci present, bather load, and chlorination-filtration procedures, and how these factors affect the incidence of microbial infection of bathers. A primary reason for the lack of suitable data to determine this correlation is the absence of a recovery system that is sufficiently selective, accurate, and reliable. Each of the past three editions of Standard Methods has advocated the enumeration of total staphylococci or *S. aureus* organisms in swimming pool water. However, these methodologies have remained in a tentative status due to the lack of data documenting their accuracy and precision when applied to the examination of swimming pool waters.

The present study was designed to evaluate two media for efficacy in the selection and differentiation of *S. aureus*. Although VJ agar appears to be slightly more selective than BP agar for the isolation of staphylococcal species, neither medium was sufficiently differential to permit the accurate identification of *S. aureus* from swimming pool water containing adequate free chlorine residual. However, when samples collected from a swimming pool containing no free chlorine and a combined chlorine residual of 0.17 ppm were analyzed, the differentiation of *S. aureus* on both VJ and BP medium markedly improved. In fact, the results on VJ agar for these samples more nearly approximated the data published by Favero et al. (9; Table 2). Favero's group determined that VJ agar was superior to Chapman-Stone and phenol red mannitol salt agars for the selection of *S. aureus*. This group also showed a high degree of correlation between mannitol fermentation and coagulase activity for staphylococci isolated on VJ agar. However, no data concerning the concentration of free or combined chlorine residual of the samples used in the Chapman-Stone–phenol red mannitol salt–VJ comparison were presented. In contrast, three independent studies conducted in our laboratory of chlorinated and brominated swimming pools containing adequate free halogen residuals indicated a minimal degree of correlation between mannitol fermentation and coagulase activity. Apparently, the identification of coagulase-positive staphylococci cannot confidently be based on colony mor-

TABLE 1. Efficacy of VJ and BP agars for the primary isolation and detection of coagulase-positive staphylococci from swimming pool water

Agar and water	No. of isolates picked	No. of *Staphylococcus* spp. isolates	No. of isolates exhibiting colony morphology typical of coagulase-positive staphylococci[c]	No. of typical colonies that were coagulase positive (+4 level)	No. of typical colonies that were coagulase positive (+1–3 level)
VJ agar					
Chlorinated water[a]	356	355	49	2	0
Unchlorinated water[b]	77	77	40	37	0
BP agar					
Chlorinated water	142	122	7	0	0
Unchlorinated water	73	58	35	20	15[d]

[a] Chlorinated water contained a minimum of 0.6 ppm free chlorine residual.

[b] Unchlorinated water contained no detectable free chlorine residual.

[c] On VJ agar, these were black colonies with surrounding yellow zones; on BP agar, they were black colonies with surrounding clear zones.

[d] All of these colonies exhibited a +3 coagulase reaction and a positive thermonuclease reaction.

TABLE 2. Correlation between mannitol fermentation and coagulase activity of staphylococci isolated on VJ agar from swimming pools

Source of isolates (reference)	Total no. of isolates	No. of isolates with the following characteristics[a]:		
		Mannitol and coagulase positive	Mannitol positive and coagulase negative	Mannitol and coagulase negative
Chlorinated pools				
Klapes[b]	77	37	3	37
Favero et al. (9)[c]	106	104	0	2
Klapes[d]	355	2	47	306[e]
Klapes[d]	53	0	50	3
Brominated pool				
Klapes[d]	111	25	54	32

[a] No isolates were mannitol negative and coagulase positive.
[b] These samples had no detectable free chlorine residual.
[c] No chlorine concentration data were available.
[d] These samples contained a minimum of 0.6 ppm free halogen residual.
[e] The implied coagulase-negative character was based on negative results from a 20% random sample of the total and thermonuclease negative results for each of the 306 isolates.

phology on VJ agar when the isolates are obtained from adequately halogenated swimming pool water samples. The observation supports the theory that environmental stress can produce sublethal physiological injury as well as lethal injury. Additionally, evidence of the reduced ability of environmentally stressed cells to perform as expected on selective media is well documented (4, 11, 23).

The abundance of staphylococci, a high percentage of which are isolates of *S. aureus*, in swimming pool water is well documented (6, 9, 20). In addition, Keirn and Putnam (13) have determined that the chlorine resistance of *S. aureus* and *S. epidermidis* is essentially equivalent. These data taken collectively suggest that *S. aureus* should survive long enough in swimming pool water to be enumerated, yet die within a reasonable time period. However, we see only a small percentage of coagulase-positive staphylococci among the staphylococcal species recovered on VJ agar from adequately chlorinated swimming pool water. It remains to be seen whether the remainder of the staphylococci recovered from this environment are truly coagulase-negative staphylococci or *S. aureus* which have been physiologically or genetically altered or both as a result of exposure to hypochlorous acid.

Although disinfection by chlorine has been extensively used for the last six decades, the mode of action of chlorine and its compounds on microbial life is not fully understood. Some of the more recently considered modes of chlorine action include disruption of the cell membrane and protein synthesis, reactions with nucleic acids, purines, and pyrimidines, creation of chromosomal aberrations, and induction of DNA lesions with accompanying loss of DNA transforming ability (5, 12, 27). Chlorine injury has been shown to be reversible under suitable conditions, and staphylococci surviving chlorination may be capable of repairing the resultant injury in some instances. However, if chlorine produces a nonlethal mutation, the staphylococci isolated from chlorinated environments may be altered genetically and thus may not be accurately identified by currently accepted species identification schemes. In addition, the range of responses observed in cells exposed to chlorine may very well be a dose-dependent relationship. In our laboratory, results from samples of chlorinated swimming pool water show a significant increase in the composite yield of staphylococci isolated on 1% pyruvate-supplemented VJ agar compared with standard formulation VJ agar. If we desire optimal enumeration of these indicator bacteria from chlorinated water, we must gain an understanding of chlorine injury physiology in staphylococci and begin to identify the compounds and growth conditions which favor the recovery of these injured cells on selective media. This is the necessary first step in the development of a recovery system suitable for the selective isolation, enumeration, and accurate identification of coagulase-positive staphylococci in swimming pool water.

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LITERATURE CITED

1. **Alico, R. K., and C. A. Palenchar.** 1975. *Staphylococcus aureus* recoveries on various brands of membrane filters. Health Lab. Sci. 12:341–346.

2. **Aly, R., and H. I. Maibach.** 1978. Clinical skin microbiology—pathogenic bacteria and pathogenic viruses. Charles C Thomas, Springfield, Ill.
3. **American Public Health Association.** 1981. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Inc., New York.
4. **Bissonnette, G. K., J. J. Jezeski, G. A. McFeters, and D. G. Stuart.** 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. **29**:186–194.
5. **Camper, A. K., and G. A. McFeters.** 1979. Chlorine injury and the enumeration of waterborne coliform bacteria. Appl. Environ. Microbiol. **37**:633–641.
6. **Crone, P. B., and G. H. Tee.** 1974. Staphylococci in swimming pool water. J. Hyg. **73**:213–220.
7. **Evans, J. B.** 1977. Coagulase positive staphylococci as indicators of potential health hazards from water, p. 126–130. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazards associated with water, ASTM STP 635. American Society for Testing and Materials, Philadelphia.
8. **Favero, M. S., and C. H. Drake.** 1966. Factors influencing the occurrence of high numbers of iodine-resistant bacteria in iodinated swimming pools. Appl. Microbiol. **14**:627–635.
9. **Favero, M. S., C. H. Drake, and G. B. Randall.** 1964. Use of staphylococci as indicators of swimming pool pollution. Public Health Rep. **79**:61–70.
10. **Finegold, S. M., W. J. Martin, and E. G. Scott.** 1978. Reagents and tests, p. 481–495. *In* Bailey and Scott's diagnostic microbiology. C. V. Mosby Company, St. Louis, Mo.
11. **Flowers, R. S., S. E. Martin, D. G. Brewer, and Z. J. Ordal.** 1977. Catalase and enumeration of stressed *Staphylococcus aureus* cells. Appl. Environ. Microbiol. **33**:1112–1117.
12. **Haas, C. N., and R. S. Engelbrecht.** 1980. Physiological alterations of vegetative microorganisms resulting from chlorination. J. Water Pollut. Control Fed. **52**:1976–1989.
13. **Keirn, M. A., and H. D. Putnam.** 1968. Resistance of staphylococci to halogens as related to a swimming pool environment. Health Lab. Sci. **5**:180–193.
14. **Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich.** 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. **21**:585–587.
15. **Lachica, R. V. F., K. F. Weiss, and R. H. Deibel.** 1969. Relationships among coagulase, enterotoxin, and heat-stable deoxyribonuclease production by *Staphylococcus aureus*. Appl. Microbiol. **18**:126–127.
16. **Palmquist, A. F., and D. Jankow.** 1973. Evaluation of *Pseudomonas* and *Staphylococcus aureus* as indicators of bacterial quality of swimming pools. J. Environ. Health **36**:230–232.
17. **Rayman, M. K., J. J. Devoyod, U. Purvis, D. Kusch, J. Lanier, R. J. Gilbert, D. G. Till, and G. A. Jarvis.** 1978. ICMSF methods studies. X. An international comparative study of four media for the enumeration of *Staphylococcus aureus* in foods. Can. J. Microbiol. **24**:274–281.
18. **Rayman, M. K., C. E. Park, J. Philpott, and E. C. D. Todd.** 1975. Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus aureus*. Appl. Microbiol. **29**:451–454.
19. **Ritter, C., and E. L. Treece.** 1948. Sanitary significance of cocci in swimming pools. Am. J. Public Health **38**:1532–1538.
20. **Robinton, E. D., and E. W. Mood.** 1966. A quantitative and qualitative appraisal of microbial pollution of water by swimmers: a preliminary report. J. Hyg. **64**:489–499.
21. **Schleifer, K. H., and W. E. Kloos.** 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. Int. J. Sys. Bacteriol. **25**:50–61.
22. **Sperber, W. H., and S. R. Tatini.** 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. Appl. Microbiol. **29**:502–505.
23. **Suarez, G., and I. Araquistain.** 1981. Comparative study of selective media to enumerate normal and stressed *Staphylococcus aureus* cells. Zentralble Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 **10**(Suppl.):113–126.
24. **Subcommittee on Taxonomy of Staphylococci and Micrococci.** 1965. Recommendations. Int. Bull. Bacteriol. Nomencl. Taxon. **15**:109–110.
25. **Sukroongreung, S., C. Nilakul, and S. Sirinanunta.** 1981. Free coagulase and thermostable nuclease reactions of staphylococci: a comparison. Am. J. Med. Technol. **47**:237–239.
26. **Turner, F. J., and B. S. Schwartz.** 1958. The use of a lyophilized human plasma standardized for blood coagulation factors in the coagulase and fibrinolytic tests. J. Lab. Clin. Med. **52**:888–894.
27. **Venkobachar, C., L. Iyengar, and A. V. S. Prabhakara Rao.** 1977. Mechanism of disinfection: effect of chlorine on cell membrane functions. Water Res. **11**:727–729.