

Study of Antibiotic Sensitivity Pattern and Enterotoxigenicity of Staphylococci Isolated from Swimming Pools in Ibadan, Nigeria

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Abstract: Staphylococci were isolated from some swimming pools in Ibadan, Nigeria. Twelve strains of the *Staphylococcus aureus* and three strains of the coagulase-negative staphylococci isolates were resistant to methicillin. Eighteen strains of the *Staphylococcus aureus* isolates were found to be enterotoxigenic. The enterotoxin types were Staphylococcal enterotoxin A (SEA) (16 strains) and Staphylococcal enterotoxin C (SEC) (2 strains). The findings pose health hazard to swimmers, particularly children, who may ingest the water while swimming and hence exposed to the risk of gastrointestinal disorders. The risk of skin infections is also highlighted.

Key words: Staphylococci • Recreational water • Antibiotic resistance • Enterotoxin

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen which has emerged as a significant cause of nosocomial infections. In the recent times the incidence of community-acquired infections associated with the organism has been on increase [1]. Charenca and Fujioka [2,3] have assessed of *Staphylococcus* bacteria in Hawaii recreational waters and their association with skin infections and reported a high counts of *Staph. aureus* being shed from people while swimming or engaging in other recreational activities. Their findings thus suggest that recreational waters could be a source of transmission of community-acquired infection involving the organism. Thus, recreational waters, for example swimming pools could be considered a potential risk factor for many diseases involving the organism especially infections of the skin. Little information is available on the antibiotic resistance survey and enterotoxin production potential of *Staph. aureus* isolated from recreational waters in Nigeria. It is very essential therefore to know the distribution of antibiotic resistant and enterotoxigenic strains of *Staph aureus* in any environment because of its public health importance. The antibiotic resistance patterns of isolates from clinical samples have been studied extensively in Nigeria [4-11]. This study has been undertaken to evaluate the

prevalence of antibiotic resistant and enterotoxin producing staphylococci from some swimming pools in Ibadan, Nigeria.

MATERIALS AND METHODS

A total of 90 samples of water from three swimming pools located in Ibadan, Nigeria were collected (30 samples per swimming pool) over a period of three months using sterile universal bottles. The samples were collected in the morning and evening. The samples were brought to the laboratory and were plated on Blood Agar (BA) and Mannitol Salt Agar (MSA) and incubated at 37°C. The total bacterial count was done using Tryptone Soy Agar (TSA, Oxoid, U.K). The isolates were subjected to morphological and biochemical studies using tests such as Gram staining, catalase, coagulase, carbohydrate fermentation, DNase and phosphatase tests as outlined in Barrow and Feltham [12] and Bergey's Manual [13] and the identity of the isolates were confirmed to staphylococci. Other isolates recovered were also characterised following standard procedures outlined in the above manuals.

Antibiotic Sensitivity Testing: Antimicrobial sensitivity testing of staphylococci isolates was performed according to the Kirby-Bauer's disc diffusion method [14].

The antibiotics tested included: penicillin, gentamicin, oxacillin, ciprofloxacin, cotrimoxazole, clindamycin, erythromycin, vancomycin, amoxycillin, rifampicin, chloramphenicol and streptomycin. However, oxacillin sensitivity was performed on Mueller-Hinton agar supplemented with 4% NaCl, following standard method [15]. *Staph. aureus* strains are reported to be susceptible to oxacillin when its minimum inhibitory concentration (MIC) is = 2mg/l and resistant when its MIC is = 4mg/ml. In addition, a 5µg methicillin disc (Oxoid, U.K) was used to confirm the resistance or otherwise to methicillin. Strains of *Staph. aureus* that were resistant to oxacillin were tested for beta- lactamase production and for their ability to produce enterotoxins [16]

RESULTS AND DISCUSSION

The total bacteria mean counts as recorded on TSA in the swimming pool waters were of the order 10^2 - 10^7 cfu/ml (Table 1). The mean counts of *Staph aureus* were between 35.46- 103.25 cfu/ml with the highest value recorded for swimming pool B followed by swimming pools C and A in that order (Table 2). The results revealed that staphylococci were isolated from all the samples of water from the swimming pools from the three locations. They were relatively high for the samples collected during evening time for all the swimming pools. This is expected since most swimming activities take place during the evening when people are less busy to engage in recreational activities.

Table 1: Total bacteria count from the swimming pools

Sampling location designate	No of samples	Mean count (cfu/ml)
A	30	1.6×10^3
B	30	1.2×10^7
C	30	2.1×10^2

Although there seemed to be no generally accepted threshold limit values concerning the level of staphylococci in recreational water, the counts for all the location were particularly high for recreational waters in view of the 100cfu of *Staph. aureus* per 100ml of water proposed by Mackereth *et al.* [17]. Since the pools from the location did not meet the standard recommended by Mackereth *et al.* [17], there is a serious public health concern from the microbiological points of view especially if such water is ingested by swimmers which include children. Moreover, the risk of exposure to staphylococci via the swimming pool was stressed by the facts that some of the isolated strains were coagulase positive, enterotoxin producers and hence potential disease-causing strains. The major enterotoxin encounter in this study is staphylococcal enterotoxin A (SEA), which is associated with staphylococcal food poisoning. The other organism encountered in this study from the blood agar cultures include *Pseudomonas aeruginosa*, isolated from two locations, A and C and *E.coli* which occurred in all locations. The presence of *Pseudomonas aeruginosa* has potential health hazards, especially since the organism has been variously reported to be resistant to antibiotic and disinfectants and has been associated with wound infections.

Table 2: Average count (cfu/ml.) of staphylococci in the three swimming pools

Sampling location designate	Time of collection (No of samples)	Mean count of staphylococci
A	Morning (15)	35.47
	Evening (15)	49.27
B	Morning (15)	54.73
	Evening (15)	103.53
C	Morning (15)	37.93
	Evening (15)	61.13

Table 3: Rates of resistance of staphylococci isolated from the swimming pools

Antibiotic	<i>Staph. aureus</i> (n = 64) No. (%)	CONS (n = 26) No (%)
Gentamicin(10µg)	16 (25.0)	19 (73.1)
Oxacillin(5µg)	46 (71.9)	15 (57.7)
Penicillin(10i.u)	63 (98.4)	20 (76.9)
Ciprofloxacin(5µg)	14 (21.9)	6 (23.1)
Cotrimoxazole(25µg)	11 (17.2)	16 (61.5)
Clindamycin(5µg)	43 (67.2)	18 (69.2)
Erythromycin(5µg)	18 (28.1)	15 (57.7)
Vancomycin(30µg)	4 (6.3)	2 (7.7)
Amoxycillin(10µg)	10 (15.6)	18 (69.2)
Rifampicin(10µg)	2 (3.1)	2 (7.7)
Chloramphenicol(30µg)	12 (18.8)	11 (42.3)
Streptomycin(10µg)	21 (32.8)	14 (53.8)

The resistant rates of the staphylococci from the swimming pool waters to different antibiotics is shown in Table 3. The isolates of *Staph aureus* were susceptible to a large extent to rifampicin, vancomycin, amoxycillin, cotrimoxazole, ciprofloxacin, chloramphenicol and gentamicin. Majority were resistant to penicillin, oxacillin and clindamycin and 96.9% produce beta-lactamase enzyme. The percentage resistance of the coagulase negative staphylococci (CONS) to all the antibiotics were higher than those recorded for *Staph aureus* although the number of CONS isolates investigated was lower than that of *Staph aureus*.

The emergence of community-acquired *Staph aureus* strains which are virulent and which possess resistance to antibiotics, particularly methicillin has been reported [18]. That *Staph aureus* with these characteristics are emerging in community facilities like swimming pools is worrisome and calls for adequate treatment of swimming pool waters to prevent such disease as skin ulcers, conjunctivitis, ear infections, eczema and skin rashes [19,20]. Gabutti *et al.* [21] had reported that high counts of *Staph aureus* in recreational waters is often considered a risk factor for contracting many disease affecting such areas of the body as skin, eyes and ears. There is need for continued microbiological evaluation of swimming pools to assess their sanitary quality to avoid outbreak of these disease

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