International Journal of Sports Science 2012, 2(1): 1-5
DOI: 10.5923/j.sports.20120201.01

Methodology for Studying the Functional Conditions of Athletes' Skin

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Abstract The authors describe an unorthodox methodology for evaluating the functional skin conditions of athletes in various sports and offer their own findings. It is common knowledge that high physical stresses in modern sports affect the entire human organism, including skin conditions. As a specific of aquatic sports, athletes spend much time in water every day and expose their skin to chemical agents that are added to water to cleanse and disinfect it. The goal of this study was to develop a methodology for evaluating the quantity and quality of staphylococci, propionic bacteria, and fungi of genus *Malassezia*. Staphylococcal microflora was inoculated on the visibly healthy skin of 50 athletes engaged in aquatic sports: *St. aureus* was collected from 26 (54.7%) athletes pursuing aquatic sports, *St. saprophyticus* from 2 (3.2%) athletes, *St. intermedius* from 14 (25.2%) athletes, *St. epidermidis* from 6 (10.4%) athletes, and *St. haemolyticus* from 2 (4.8%) athletes. Among athletes engaged in non-aquatic sports, *St. aureus* was detected in 5 athletes (33.3%), *St. saprophyticus* in 1 athlete (6.7%), *St. intermedius* in 1 athlete (6.7%), and *St. epidermidis* in 8 athletes (53.3%). *St. haemolyticus* was not inoculated.

Keywords Skin, Athletes in Aquatic and Non-Aquatic Sports, Skin Micro-Biocenosis

1. Introduction

High physical stresses in modern sports have an effect on the entire human organism, including skin conditions. In turn, skin can be an indicator of an athlete's general reactivity. Human skin is a protective barrier of several layers of specialized cells and antimicrobial substances, and functions as an active immune organ[1].

Excessive training stresses cause strains of microorganisms showing signs of pathogeny to develop on the skin[2]. A great multitude of studies discuss the role of tests for skin auto-microflora as a criterion of resistance and a nonspecific integral indicator of the condition of an organism's immune system among people in manufacturing, students, athletes, and patients under remedial medicine programs[3].

Professional athletes are a unique group of dermatological patients because nearly all of them are confronted with skin problems over their sporting careers[4, 5].Top-performance sports put great physical and psychological strain on athletes, and no subjective sensations caused by skin damage are to be tolerated.

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Published online at http://journal.sapub.org/sports

Swimmers are in a group of risk of skin diseases combined under the common name of "swimmers eczema"[6]. While they are in the swimming pool, their skin is exposed to chemical agents that frequently provoke skin reactions, intensified itching, and other dermatoses[7]. According to literary sources, the sporting performance of professional athletes declines as they develop skin diseases of bacterial and fungal nature, in the first place. Accordingly, there is a need for timely diagnostics of the condition of athletes' skin micro-biocenosis and a variety of preventive measures taken[8].

Studies conducted in the past few years have been focused on establishing the role of bacteria of genera *Propionibacterium* and *Staphylococcus* and fungi of genus *Malassezia* normally living on human skin affected by the most common dermatoses associated with them. Simultaneously, researchers studied groups of clinically healthy people, but similar studies of athletes have never been undertaken[9].

Insemination with staphylococci is of prognostic value in the sense that the higher it is the closer the carrier is to a pathological condition. Staphylococci of genus *Staphylococcus epidermidis* are present most frequently on the skin of healthy people, while genera *Staphylococcus aureus* and *Staphylococcus haemolyticus* have been detected in a majority of carriers of various forms of dermatoses.

The propose of the work was investigation of staphylo-

cocci in athletes and their possible role in dermatoses development.

2. Materials and Techniques

There were 105 subjects in all, and the studies involved two groups of athletes: Group 1 was made up of athletes (51 subjects) involved in aquatic sports, including modern pentathlon, swimming, synchronous swimming, and water polo, and Group 2 (15 subjects) included athletes practicing non-aquatic sports. The control group (Group 3) comprised 39 subjects who were not engaged in professional sports. The subjects had an average age of 21.5 years: athletes pursuing aquatic sports (32 males and 19 females) averaged 20.8 years, and those in non-aquatic sports (14 males and 1 female) were 22.7 years old, on average. The control group (15 males and 24 females) had an average age of 22 years.

All athletes in aquatic sports trained daily in the same swimming pool. The specimens were collected before training sessions.

The improved method comprises collection of skin microflora by scrubbing with a buffer solution containing the Twin 80 deterrent and treating the specimen collected by dithiotreitol to dissipate the agglomerates of propionic bacterial cells. The unorthodox method consists of three stages:

Stage 1: Skin scrub.

Skin scrub was done by the Korting method we have modified ourselves. In particular, the Korting method was modified to be applicable to microorganisms other than *Malassezia* and suitable for dithiotreitol to be used for dissipating agglomerations of propionic bacteria cells and Twin 80 detergent to replace Triton X-100. A glass ring 24 mm in diameter was pressed against a horizontal skin surface and 1 ml of a buffer solution containing 0.1 M of potassium phosphate and 1% of Twin 80 at pH 7.9 was released into it. The skin was rubbed with a cotton tampon for a minute over the full area inside the ring. The resultant suspension was then transferred by a sampler into a test tube and dithiotreitol powder (approximately 20 to 30 mg) was added to the suspension to separate propionic bacteria from one another and from keratinocytes. The mixture was left to stand for 30 minutes at 27 degrees C.

Stage 2: Inoculation of Selective Media

Inoculation of staphylococci: 20 μl of the specimen diluted to one-tenth of its initial concentration was used to inoculate highly inseminated staphylococci (opaque specimen) on a small Petri dish containing yolk-saline agar (YSA) medium. In case of low insemination (almost translucent specimen), 20 μl of the undiluted specimen was used for inoculation. The dish was incubated for 24 hours at 37 degrees C until colonies appeared as seen in microscope. The number of new colonies was multiplied by 50 (if the specimen was not diluted) and divided by 4.5 (area of the ring) to give the number of colony-producing units (CPU) per 1 cm^2.

Inoculation of propionic bacteria: In case of high insemination, 20 μl was used in a small Petri dish to inoculate a medium containing, per 100 ml of water, 1 g of casamino acids, 0.5 g of fungal extract, 2.5 ml of 40% sodium lactate, 2 g of agar, and 20 μl of furazolidone solution in dimexide (10 mg/ml). The same medium was inoculated with 100 μl in a more translucent sample. The dish was placed in anaerostat in an atmosphere of 80% CO_2 in the air and incubated for three to five days at 32 degrees C until white semi- translucent colonies appeared. Typical colonies were examined in microscope, revealing small polymorphic bacilli. The number of colony-producing units was counted similarly. If there were few colonies, they were spread by brushing in the same dish to increase material volume.

Inoculation of fungus *Malassezia*: 100 μl of the specimen was used to inoculate a small Petri dish containing the Dixon medium. The dish was incubated for two to five days at 32 degrees C until dense paste-like cream-colored colonies appeared. The culture was examined in microscope to ascertain the presence of typical cells of fungus of genus *Malassezia*.

Stage 3: Identification

Staphylococci were identified by traditional technique comprising plasma coagulation, mannite fermentation test, and hemolysin presence test.

Propionic bacteria were identified in three tests: formation of indole, hydrolysis of esculine, and fermentation of sucrose, all the three tests following the traditional techniques applied to other microorganisms. It is widely known that *P. acnes* shows a positive reaction to indole, while the other two tests give a negative response. For *P. avidum*, the indole test is negative, while the other two are positive. In the *P. granulosum* test, sucrose fermentation is positive, while the other two tests are negative.

When needed, fungus *Malassezia* was identified by a procedure developed by T. Kaneko. Sensitivity to preparations was measured by the modified disc-diffusion Kirby-Bauer method using two standard discs provided with antibiotics (Pharmacology Science Research Center, St. Petersburg).

Statistics

The results obtained were processed statistically by a program built into Microsoft Excel. The program evaluated the following parameters: arithmetic means, mean deviations, and correlation coefficients.

3. Results

The study evaluated the species making up the staphylococcal microflora and estimates of its quantity in all the groups studied. The results of the study are shown in Table 1. Staphylococcal microflora was inoculated on the visibly healthy skin of 104 subjects: *St. aureus* was collected from 43 (41%) subjects, *St. saprophyticus* from five (4.8%) subjects, *St. intermedius* from 24 (22.8%) subjects, *St. epidermidis* from 22 (20.95%) subjects, and *St. haemolyticus* from 10 (9.5%) subjects. No staphylococcal flora was found on a single subject (0.95%).

Table 1. Occurrence of Staphylococcus Species Detected on the Skin of Subjects in the Groups Studied

Species of staphylococci	Percentage of carriers of the given staphylococcus species		
	Group 1 (n = 51)	Group 2 (n = 15)	Group 3 (n = 39)
St. aureus	54.7	33.3	30.8
St. saprophyticus	3.2	6.7	5.1
St. intermedius	25.2	6.7	23.1
St. epidermidis	10.4	53.3	20.5
St. haemolyticus	4.8	0	20.5

Staphylococcal microflora was inoculated on the visibly healthy skin of 50 athletes engaged in aquatic sports: *St. aureus* was collected from 26 (54.7%) athletes pursuing aquatic sports, *St. saprophyticus* from 2 (3.2%) athletes, *St. intermedius* from 14 (25.2%) athletes, *St. epidermidis* from 6 (10.4%) athletes, and *St. haemolyticus* from 2 (4.8%) athletes. No staphylococcal flora was detected on 1 athlete.

Table 2. Sensitivity of *St. aureus* to antibiotics and antiseptics

	Group 1	Group 2	Group 3
CPU/dm^2	2833 ± 2556	4924 ± 4060,8	3208± 1943
Penicillins			
Oxacillin	2,81 ± 0,34	2,6 ± 048	2,75±0,38
Cephalosporins			
Cefuroxime (Zinacef)	2,85 ± 0,27	3,00 ± 0,00	2,92±0,15
Cefoperazone (Cefobid)	2,85 ± 0,27	3,00 ± 0,00	3,00±0,00
Cefotaxime (Claforan)	2,92 ± 0,15	3,00 ± 0,00	2,92±0,15
Aminoglycosides			
Gentamicin (Garamycin)	2,19 ± 0,87	2,8 ± 0,32	2,67±0,50
Neomycin	2,15 ± 0,78	2,8 ± 0,32	2,83±0,28
Tetracyclines			
Tetracycline	2,19 ± 0,68	1,4 ± 0,64	2,25±0,75
Doxycycline (Vibramycin)	2,38 ± 0,76	1,00 ±0,00	2,50±0,58
Macrolides			
Azithromycin (Sumamed)	1,12 ± 0,21	1, 2 ± 0,32	1,33±0,44
Clarithromycin (Klacid)	1,12 ± 0,20	1,00 ±0,00	1,33±0,44
Erythromycin	1,08 ± 0,14	1, 2 ± 0,32	1,00±0,00
Roxithromycin (Rulid)	1,08 ± 0,15	1,00 ±0,00	1,00±0,00
Lincosamines			
Clindamycin (Dalacin)	2,35 ± 0,65	2,4 ± 0,72	2,42±0,78
Lyncomycin	2,62 ± 0,56	2,6 ± 0,64	2,92±0,15
Fluoroquinolones			
Ciprofloxacin (Cifran)	2,85 ± 0,27	3,00 ± 0,00	2,92±0,15
Ofloxacin (Tarivid)	2,92 ± 0,14	3,00 ± 0,00	2,92±0,15
Chloramphenicol (Levomycetin)	1,69 ± 0,80	1,4 ± 0,48	1,58±0,68
Fusidin	2,77 ± 0,37	2,8 ± 0,32	2,67±0,50

Among athletes engaged in non-aquatic sports, *St. aureus* was detected in 5 athletes (33.3%), *St. saprophyticus* in 1 athlete (6.7%), *St. intermedius* in 1 athlete (6.7%), and *St. epidermidis* in 8 athletes (53.3%). *St. haemolyticus* was not inoculated.

The study of the types of staphylococcal microflora in 39 subjects in the control group showed that *St. aureus* was inoculated to 12 subjects (30.8%), *St. saprophyticus* to 2 subjects (5.1%), *St. intermedius* to 9 subjects (23.1%), *St. epidermidis* to 8 subjects (20.5%), and *St. haemolyticus* to 8 subjects (20.5%).

Since *St. aureus* led in the group of athletes engaged in aquatic sports and in the control group, it was important to evaluate the sensitivity of this staphylococcus species to antibiotics and antiseptics (Table 2).

Studies of the sensitivity to methicillin (oxacillin) have revealed the presence of MRSA strains of *St. aureus* in athletes practicing aquatic sports. It is curious, in our view, that no oxacillin-sensitive strains of *St. aureus* were detected in athletes pursuing non-aquatic sports and in subjects in the control group.

The extent of insemination with *St. aureus* evaluated by imprints for athletes in aquatic sports averaged 2,833 ± 2,556 CPU/dm^2, 4,924 ± 4,060.8 CPU/dm^2 for athletes in non-aquatic sports, and 3,208 ± 1,943 CPU/dm^2 for the control group. Insemination with *St. epidermidis* was 2,578 ± 1,456 CPU/dm^2 for athletes in aquatic sports, 4,975 ± 2,512.5 CPU/dm^2 for athletes in non-aquatic sports, and 1,807.5 ± 1,269.38 CPU/dm^2 for the control group. The staphylococcal microflora inoculation norms for healthy skin range from 0 to 200 CPU/dm^2 for *St. aureus* and from 0 to 1,000 CPU/dm^2 for *St. epidermidis*. This means that insemination with both *St. aureus* and *St. epidermidis* is elevated for all groups of subjects. A significant fact, though, is that even though *St. epidermidis* is the predominant species among athletes in non-aquatic sports, the incidence of, and insemination with, *St. aureus* are higher in this group than they are in the control group.

The sensitivity of *St. aureus* to macrolides and chloramphenycole in the group of athletes in aquatic sports has been found to be low, and the sensitivity to the group of aminoglycosides, tetracyclines, lincosamines, and fuzidine was moderate. The strains detected were also sensitive to preparations in the group of fluoroquinolones.

The group of athletes in non-aquatic sports showed a low sensitivity of *St. aureus* to the group of tetracyclines, macrolides, and chloramphenycole, and moderate sensitivity to the group of lincosamines and fuzidine. The strains found were sensitive to preparations in the group of aminoglycosides, fluoroquinolones, and cephalosporins.

In the control group, *St. aureus* was found to have a low sensitivity to the group of macrolides and chloramphenycole, and moderate sensitivity to the group of aminoglycosides, tetracyclines, lincosamines, and fuzidine. The strains detected were sensitive to preparations in the group of fluoroquinolones and cephalosporins.

4. Discussion

Propagation of methicillin-resistant Staphylococcus aureus as pathogens of skin and soft tissue infections among athletes is studied intensively around the world[10]. American scientists have conducted a major study that revealed maximum growth of MRSA infections among athletes practicing martial arts and game sports[11].

Activity of MRSA as a variety of staphylococcal infection is acquiring epidemiological significance in the population in recent years. Athletes are, more than anyone else, a specific group of people exposed to a potentially high risk of developing MRSA infections[12].

Genus *Malassezia sympodialis* is the most widespread species of fungi. Fungus *Malassezia* occurs in a majority of healthy adults and fulfill a protective function in respect of the host by excreting antagonistic toxins that prevent the growth of other fungi. Their number rises significantly, though, under the effect of unfavorable factors, including overtraining. In several dermatoses, the quantity of fungi of genus *Malassezia* increases above norm, even though the generic composition of these microorganisms is practically unrelated to pathology.

Propionic bacteria, *Propionibacterium acne, granulosum*, and *avidum*, are the most prolific skin inhabitants. They are difficult to count because propionic bacteria can grow in the form of agglomerates, even though insemination count is essential for evaluating the relationship between an existing pathological process and this kind of propionic bacteria.

Imprint, scrub, and biopsy are the principal techniques of microorganism insemination from the skin surface[13] Simple and noninvasive, our method for studying the protective function of skin can be used extensively for prognosticating athletes' performance and preventing development of dermatoses.

This method is suitable for evaluating the quantity and quality of the most widespread groups of the microbial community living on the skin. The method is carried out by backprints, or sterile plastics containers filled with selective media and having a specified area, that have not been used for this purpose before. The agar layer of a backprint applied to the skin surface for 20 seconds is inoculated with staphylococci, whereupon it is placed in a thermostat and the number of new colonies is counted a few hours later. The backprint method is well-suited for inoculating staphylococci and non-lipophilic fungi, but is inadequate for *Malassezia* and propionic bacteria because these microorganisms adhere strongly to the skin surface and can only be collected with the help of an alkaline buffer solution. A similar inoculation method was used previously in respect of fungus of genus *Malassezia*. It suggested, though, that Triton X-100 be used as the detergent that causes partial lysis of propionic bacteria[14].

We have made improvements in the method for registering the quantity and quality of staphylococci, propionic bacteria, and fungus *Malassezia* so that the resultant quantity of microorganisms could be near their real number, a very significant factor indicative of the health of this ecosystem.

The studies we have conducted in the group of athletes engaged in non-aquatic sports have not, therefore, revealed any MRSA strains of *St. aureus*, in a marked difference to the findings of foreign researchers who have detected MRSA strains of *St. aureus* in football players, wrestlers, and fencers[15].

5. Conclusions

St. aureus has been found to predominate in the group of athletes in aquatic sports, and *St. epidermidis*, which belongs in the normal microflora of the skin, predominated in athletes engaging in non-aquatic sports. Another significant fact is that insemination with both *St. aureus* and *St. epidermidis* was the highest among athletes in non-aquatic sports.

MRSA strains were only detected among athletes pursuing aquatic sports, while no oxacillin-insensitive strains of *St. aureus* were detected among subjects in the other groups. No MRSA strains of *St. aureus* were found in the group of athletes in non-aquatic sports, in contrast to the findings of foreign researchers who detected MRSA strains in this group of athletes.

Observation of the skin microflora condition in athletes practicing various sports helps in early diagnostics and prevents the worsening of athletes' professional dermatoses.

Studies of the micro-biocenosis of athletes' skin to evaluate the functional conditions of the skin and the organism as a whole hold much promise for the future and have to be continued.

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