

# Prevalence of *Staphylococcus aureus* carriage by young Malaysian footballers during indoor training

J L William, S Radu, S A Aziz, R A Rahim, Y K Cheah, A Liwan, S Lihan

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**Background:** Research has shown that athletes are carriers of *Staphylococcus aureus* during physical activity.

**Objective:** To estimate the mean total plate count of *S aureus* carried by footballers before and after training at an indoor venue.

**Methods:** Forty Malay and 20 Indian students volunteered to participate. There was also a control group consisting of 40 Malay and 20 Indian students who were not active. The experimental group were active footballers who had played at school or club level. The subjects were healthy and free of skin infection. The experiment was divided into three sessions, with 20 subjects present at each. At each session, the subjects trained for one hour. Swabs were taken from the skin, nose, and ear before and after training. For the control group, swabs were taken only once from the skin, nose, and ear. The swabs were subjected to biochemical tests and then streaked and cultured aerobically in Baird Parker agar plates for 24 hours at 37°C. Black colonies with a clear zone were presumed to be *S aureus*, and the mean total plate count of the colonies was estimated. Gram staining, catalase, coagulase slide, coagulase tube, acetoin production, o-nitrophenyl β-D-galactopyranoside (ONPG), and mannitol fermentation tests were used to confirm the colonies as *S aureus*. A haemolysin test was conducted with human blood to confirm haemolytic activity.

**Results:** All subjects in the experimental group were carrying *S aureus* both before and after training. The estimated mean total counts of colonies from the skin, ear, and nose for the Malays before training were 33, 71, and 312 respectively. Counts after training were 21, 44, and 452 respectively. The results for the Indians were 72, 80, and 309 respectively before training and 55, 200, and 466 respectively after training. The positive results for Gram staining, catalase, coagulase slide, coagulase tube, acetoin production, ONPG, and mannitol fermentation tests were 100%, 96%, 95%, 95%, 93%, 93%, and 90% respectively. All subjects in the control group were also carrying *S aureus*.

**Conclusions:** All of the players were carriers of *S aureus* during training. The decrease in total count from the skin for both races may be due to lysozyme activity lysing the bacterial cells. Contamination of the environment with these bacteria may have increased the estimated total plate count in the nose. The experimental group face a higher risk of infection because of lower immunity during training and higher rate of injuries compared with the control group.

See end of article for authors' affiliations

Correspondence to:  
J L William, University  
Putra, Malaysia;  
laurence\_john@  
hotmail.com

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Over the past decade physical activity has become important in our daily lives, and the number of participants in various forms has increased considerably in recent years.<sup>1,2</sup> This increase in physical activity participation is not surprising because of the potential benefits.<sup>3–5</sup> However, a major problem is the high incidence of injury.<sup>3</sup> Most injuries occur in physical contact sports. Participants in such sports need to be optimally fit to withstand the constant physical contact that may cause injury.<sup>6</sup>

The athlete may also be exposed to bacterial infection. A common bacterium that may cause harm to an injured athlete is *Staphylococcus aureus*, which is found in moist warm places such as the skin and mucous membranous area of the nose, mouth, and throat.<sup>7,8</sup> The name comes from “staphyle” meaning a bunch of grapes and “coccus” meaning round or spherical.<sup>9</sup> This bacterium can be grouped with the opportunistic pathogens and can produce serious infection.<sup>9,10</sup> *S aureus* is an important cause of soft tissue infection.<sup>11,12</sup> The pathogen usually colonises host tissues that are in contact with the external environment, and therefore open wound injuries are at risk. In later stages, it is able to invade the bloodstream.<sup>13</sup> Invasion of host defence mechanisms can be life threatening in an injured athlete. In general, once the bacterium has colonised the host's defences, it releases toxins

into the bloodstream. This may cause diseases such as sepsis, endocarditis, pneumonia, enteritis, osteomyelitis, hepatic abscess, impetigo, and cellulitis.<sup>13,14</sup> A number of studies have been carried out on *S aureus* infection in athletes. Stacey *et al*<sup>15</sup> found that five rugby players were infected with *S aureus*. One was found to be carrying the bacterium in his nose. He was treated with either erythromycin or clarithromycin and recovered well. A similar study was carried out by Hodde *et al*<sup>16</sup> on a 21 year old collegiate wrestler with acute pain at the left lower quadrant abdominal. A blood sample showed *S aureus* infection.

The objective of our research was to determine the number of athletes carrying *S aureus* before and after a session of indoor training.

## MATERIALS AND METHODS

### Subjects

An experimental group comprised 60 male students, 40 Malays and 20 Indians. A control group also comprised 60 male students with the same ratio of Malays and Indians. The students were all medically fit, free from injuries and skin infection. All were from a secondary school in Malaysia and ranged in age from 13 to 15 years. They were active footballers and had played for either the school or clubs. Before the study, the students provided written informed

consent. Individual biodata, name, date of birth, race, and health status were obtained before the study. During the period of the study, none of the subjects were taking any prescribed drugs.

### Orientation test

All subjects in the experimental group had two orientation sessions before the experiment to familiarise themselves with the test procedures and techniques. They were not allowed to consume alcohol and drinks with caffeine during the experiments. Both the orientation tests were held in the morning at an indoor venue. All the subjects followed the rules and conditions of the test during the orientation sessions.

### Test protocol

The experiment was divided into three training sessions, with 20 subjects at each. Subjects in the control group rested in a room during the period of the experiment. Each training session was conducted for an hour during the morning. The subjects wore their own sports attire during training sessions and used their own towels but soap was provided. They reported to the training venue an hour before the experiment. They were then instructed to bathe with the soap provided. Sterile cotton buds were used to take swabs from the skin (thigh), nose, and ear of each subject after they had bathed. The swabs were stored in individual sterile universal bottles at 0°C for 45 minutes and taken immediately to the laboratory for isolation and further biochemical tests. Three colonies were isolated from each plate and stored for further biochemical tests to identify *S aureus* species.

### Biochemical tests

All cotton swabs were streaked on to specific prepared Baird Parker agar plates which were incubated under aerobic condition for 24 hours at 37°C. Colonies with black and clear zones were presumed to be *S aureus* species, and the mean total count of colonies was estimated from each plate. Three colonies from each plate were selected and stored in glycerol at -20°C. Twenty colonies from Malays and 10 from Indians were selected randomly for further biochemical tests to confirm the strain of *S aureus*. The biochemical tests were conducted at the Microbiology Laboratory, University Putra, Malaysia. The colonies were selected for identification on the basis of morphological characteristics. The standard identification tests were Gram staining and catalase and coagulase production by the slide agglutination test and confirmed by the tube coagulase test, acetoin production, *o*-nitrophenyl  $\beta$ -D-galactopyranoside test (ONPG) and mannitol fermentation. We also carried out the haemolysin test with human blood to confirm the haemolytic activity of the bacteria.

## RESULTS

### Basic data on the subjects

The mean (SD) age of the subjects was 14.5 (0.5) years (range 13–15). The age gap between the subjects was small. In contrast, there was a large difference in weight between the subjects: minimum 35 kg and maximum 58 kg. Both the Malays and Indians had a mean (SD) weight of 49.3 (5.5) kg. Mean (SD) height was 155.7 (3.5) cm (range 152.5–175.2).

### *S aureus* carriers

Table 1 shows the number of subjects carrying *S aureus* before and after training. Before training, all of the Malays and Indians were carrying the organism on their skin, 90% of the Malays and 100% of the Indians were carrying it in their nose, and 80% of the Indians and 100% of the Malays carried it in their ear. After training, the numbers of carriers in the nose for both the Malays and Indians was the same as before

**Table 1** Number of *Staphylococcus aureus* carriers during indoor training (n = 60)

Site	Before training		After training	
	Malay	Indian	Malay	Indian
Skin	40 (40)	20 (20)	24	20
Nose	36 (38)	20 (19)	36	20
Ear	40 (40)	16 (19)	40	20

Numbers for the control group are given in parentheses (n = 60).

training. However, only 60% of the Malays carried the organism on their skin after training compared with 100% before training, whereas for the Indians the numbers remained the same after training. However, more Indians carried it in the ear after training; the number had increased to 100%, whereas for the Malays the number remained at 100%. Table 1 also gives the results for the control group.

### Total plate count of *S aureus*

Table 2 shows the estimated total count of *S aureus* colonies from the three sites. The estimated mean total count from the Malays was reduced, after training, at the skin and ear (from 33 to 21 and from 71 to 44, respectively) but had increased in the nose (from 312 to 452). The results for the Indians differed. The count for the skin was 72 before training and 55 after training. At the other two sites, the count was increased after training, from 80 to 200 for the ear and from 309 to 466 for the nose.

### Biochemical and haemolysis tests

In the biochemical tests, we found that 96% were positive for catalase, whereas for the coagulase slide, coagulase tube, acetoin production, *o*-nitrophenyl  $\beta$ -D-galactopyranoside (ONPG), and mannitol fermentation tests, the result were 95%, 95%, 93%, 93%, and 90% respectively. In addition, we found that 100% of the strains were Gram positive, and 87% of the strains produced either  $\alpha$  or  $\beta$  haemolysis.

## DISCUSSION

One interesting finding is that most of the students in both the experimental and control group were carriers of *S aureus*. However, although subjects in the control group were carriers, the risk of the bacteria causing them harm was lower than for the active students. Athletes carrying out strenuous intense training are at higher risk of infection. Intense training affects certain immune variables that are important to the host in fighting bacterial infection.<sup>17 18</sup> During intense training, pathogens may invade the bloodstream and cause health problems. Open wound injuries in athletes provide another channel for infection as the tissues are exposed to the environment. *S aureus* is a normal flora and can be found in many conditions. Furthermore, the species can grow over a wide temperature range (6.5–50°C), and the mean body temperature during training was 40°C. This may explain why most of the subjects were carriers of *S aureus*.

A large number of *S aureus* strains produce either  $\alpha$  or  $\beta$  haemolysis, indicating their potential to cause medical problems. Both toxins are able to lyse red blood cells, but the most important is the  $\alpha$  toxin, which can cause necrosis

### Take home message

Maintaining good health and hygiene is a key factor in athletic performance.

**Table 2** *Staphylococcus aureus* plate count before and after training at an indoor venue (n = 60)

Site	Total count (Malay)				Total count (Indian)			
	Before		After		Before		After	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Skin	33	1-95	21	0-85	72	5-94	55	15-86
Ear	71	5-110	44	0-106	80	44-120	200	14-294
Nose	312	0-350	452	2-520	309	0-410	466	3-495

of the skin.<sup>19</sup> Hence these toxins could play an important role in affecting performance if infection were present.

From our results, it can be seen that a large proportion of the colonies were positive in the catalase and coagulase slide and tube tests. Nai *et al*<sup>13</sup> obtained a similar result where a large number of the strains were positive for the coagulase test. This also indicates the potential danger from the bacteria if an athlete has an infection.

Our findings also show that the estimated total plate count for both Malays and Indians on the skin had decreased after training. During aerobic training, the core body temperature may increase to above 40°C. Performance may be impaired when the core temperature is very high. The core temperature is controlled by the hypothalamus. If core temperature is above the set point of the hypothalamus, there is a discharge of efferent impulses from its anterior portion to induce mechanisms for heat removal.<sup>20</sup> Sweating is usually needed to dissipate the extra heat caused by the increase in metabolic rate. Sweat is produced from the skin and contains lysozyme. Lysozyme is a natural body defence substance which protects the body from infection and has the ability to lyse and inhibit bacteria of many species.<sup>21</sup> It may be one explanation for the reduced bacteria on the skin of both Malays and Indians after training.

In contrast, our findings show that *S aureus* had increased in the nose after training in both Malays and Indians. The demand for oxygen is high during aerobic training. Oxygen is the main element used in aerobic glycolysis, where glycogen or glucose is broken down to ATP. ATP is the energy substrate required to perform aerobic activity, and therefore the subjects needed to inhale more oxygen through the nose and mouth during training for energy production. During inhalation, large amounts of bacteria from the air probably enter the nose and are trapped in the nasal passages. Our findings show that the estimated total plate count for swabs from the nose increased for both the Malays and Indians. This may be the main point of entry for bacteria into the body of the host causing higher risk of infection.

The information from this study should help athletes to avoid health problems. Meanwhile other than training, it is important for athletes to pay attention to daily hygiene to avoid health problems and bacterial infection. Wearing clean kit, having a good shower, and proper treatment of wounds are some additional suggestions for reducing bacterial infection in athletes.

#### Authors' affiliations

J L William, S Radu, S A Aziz, R A Rahim, Y K Cheah, A Liwan, S Lihan, University Putra, Malaysia

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