Analysis of Bacterial Flora in Dohyo Soil

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Abstract

Objectives: Sumo wrestling is one of the most popular sports in Japan. Injuries are not uncommon as this is a vigorous contact sport. Sumo wrestlers have little in the way of protective clothing; their main garb is the mawashi, making them prone to exposure to any microorganisms in the dohyo. The bacterial flora of the dohyo has received little attention. If the constituent flora is identified, then appropriate treatment or prevention of any bacterial lesions or infections incurred by the wrestlers is possible.

Methods: The Vitek AMS system used in this study was developed by McDonnell Douglas Corporation. In this system, the physiological and biochemical properties of Gram-positive and -negative bacilli, Gram-positive and -negative cocci, and fungi isolated from clinical materials and environments are examined using test cards specifically for each microorganism group, and the results are automatically read by a computer and encoded. Obtained codes are compared with a built-in database, and bacterial species of test strains are identified.

Results: In this study, using the automatic identification kit VITEK or ATB, we describe the aerobic bacterial flora found in the dohyo over the four seasons of the year. We also investigated the effect of salt on the bacterial flora as sumo wrestlers toss salt on the dohyo before each match. We show the relationship between salinity changes and variations in the flora observed upon the addition of salt. Without salt, at the beginning of a match, Gram-negative bacteria predominate. When salt is added, there is a transient decrease in the incidence of flora followed by an increase in the incidence Gram-positive cocci.

Conclusions: Sixteen bacterial genera were identified using the bacterial identification systems in dohyo soil samples during the year. The number of identified bacterial species was 32. Even in the presence of salt, there is a measurable amount of bacterial flora in dohyo soil; salt does not act as an antibacterial agent.

Key words: aerobic bacterial flora, identification, sumo wrestler, salt tossed on dohyo

Introduction

Despite the importance of promoting safety in sports, there have been only a few studies on bacterial flora in sport facilities, equipment, and protectors (1). To clarify the present status of bacterial flora living in the natural environment, we have performed detailed surveys of bacteria found in educational/ workplace facilities, sport equipment, sport protectors, and uniforms using the rapid automatic bacterial identification systems VITEK and Automatic Tests in Bacteriology (ATB) as high-technology systems in our department. Our previous studies clarified the presence of bacterial flora in kendo equipment, water in pools, wrestling mats, and dance floors, and evaluated the bactericidal effects of various disinfectants in terms of hygiene, public hygiene, and epidemiology (2–5).

Sumo wrestling is a vigorous contact sport using the entire body on the dohyo, and sumo wrestlers wear only “mawashi”.

Therefore, injuries and disorders tend to occur in sumo wrestlers. Minami et al. (6) performed a survey of pathogenic bacteria in the soil of dohyos for sumo wrestling in universities to identify bacteria causing purulent prepatellar bursitis in student sumo wrestlers. For safety in sport activities, we consider it important to clarify bacterial flora derived from dohyo and take appropriate preventive measures when necessary. The purpose of this study was to clarify aerobic bacterial flora in dohyo soil for sumo wrestling using automatic bacterial identification systems throughout a year. In addition, changes in bacterial flora with changes in the concentration of salt tossed on the dohyo were investigated.

Materials and Methods

1. Collection of samples

Soil samples obtained from the dohyo of Nippon Sport Science University (the university hereafter) and those from the centre of the dohyo in the sumo stadium Kokugikan of Nihon Sumo Kyokai were used for experiments.

Collection of soil: For the isolation of aerobic bacteria, about 5 g of soil was collected from the surface of the dohyo of the university. In the Ryogoku Kokugikan, soil samples were obtained from the center of the dohyo between the first day of the New Year’s Sumo Tournament to the last day of the grand sumo tournament.

2. Culture methods for bacteria

Collected soil samples were diluted with sterile physiological saline, and the bacterial solution was inoculated in normal agar (Eiken Chemical Co., Ltd.), aerobically cultured in a thermostat at 37°C for 24 hours, and further incubated at 20–25°C for 3 days. Colonies formed on the medium were counted.

The frequency of bacterium was calculated, as follows:

\[
\text{frequency} = \frac{\text{number of colonies (CFUs/g) of each bacterial species}}{\text{total number of bacterial species}} \times 100
\]

3. Determination of salt concentration

Standard sodium chloride solution was prepared, and the normality of silver nitrate was determined. Using this silver nitrate, chlorine concentration in extracts from soil was determined.

4. Salt concentration and bacteria count

Bacterial suspension prepared from collected soil samples was inoculated into media with different salt concentrations (0.5–15%). The subsequent procedure was similar to that for the culture of aerobic bacteria, and colonies formed on media were counted.

5. Identification using automatic systems

For aerobic bacteria, colonies obtained from the agar medium were isolated and purified. After Gram staining, a sample was observed under a microscope. Subsequently, appropriate identification cards (VITEK or ATB) were selected on the basis of Gram staining. Each card was filled with the suspension of the test bacteria, and bacteria were identified using the rapid automatic bacteria identification systems (VITEK and ATB) (7, 8).

Results

I. Analysis of bacterial flora in dohyo soil

Figures 1a–d show bacterial species identified using the rapid automatic bacterial identification systems according to the 4 seasons. The 3-month periods from March to May, June to August, September to November, and December to February were corresponded to spring, summer, autumn, and winter, respectively. The frequency of each bacterial species was expressed as the percentage of all bacterial species identified at 3-month intervals. The following bacterial genera were identified using the bacteria identification systems in dohyo soil samples during the year: Acinetobacter, Bacillus, Bordetella, Escherichia, Kocuria, Micrococcus, Moraxella, Pasteurella, Plesiomonas, Proteus, Providencia, Pseudomonas, Sphingobacterium, Sphingomonas, and Staphylococcus. The number of identified bacterial species was 32 (Figs. 1a–d). The total bacterial numbers in the four seasons were calculated by the mean of standard plate counts. The total number (4.3×10^4 CFUs/g) was highest in spring, followed by summer (3.4×10^4 CFUs/g), autumn (2.9×10^4 CFUs/g) and winter (2.3×10^4 CFUs/g) in this order.

In spring, 20 bacterial species of nine genera were isolated and identified. In the case of Gram-positive bacilli, nine species were identified: Bacillus sphaericus, B. pumilus, B. cereus, B. subtilis, B. megaterium, B. lentus, B. thuringiensis, B. firmus, and B. licheniformis. The incidences of B. megaterium and B. pumilus were 10% or higher. The incidences of B. thuringiensis, B. cereus, and B. subtilis, Pseudomonas stutzeri and Sphingomonas paucimobilis were 5% or more. In the case of Gram-positive cocci, Staphylococcus hominis, S. colnii, and S. xylosus were identified, and their incidences were low. Pasteurella multocida and P. haemolytica were isolated and identified (Fig. 1a).

As Fig. 1b shows, 18 bacterial species of 10 genera were identified in summer. In the case of Gram-positive bacilli, nine species were identified in spring, and the incidences of Bacillus sphaericus, B. cereus, and B. megaterium were about 15%. B. subtilis, B. pumilus, B. thuringiensis, B. firmus, B. licheniformis, and B. coagulans were also identified. The other species frequently isolated were Staphylococcus xylosus, Bacillus subtilis, and B. thuringiensis. In addition, Escherichia coli as a representative intestinal bacterial species was identified (Fig. 1b).

In autumn, 13 bacterial species of five genera were isolated and identified. In the case of Bacillus, there were seven bacterial species: B. megaterium, B. sphaericus, B. cereus, B. subtilis, B. pumilus, and B. licheniformis. As shown in Fig. 1c, the incidence of B. megaterium was about 20%. Pathogenic bacteria were Staphylococcus aureus, Bacillus cereus, and Pasteurella multocida, and their incidences were about 5%. In addition, Micrococcus lylae, M. roseus, and Sphingomonas paucimobilis were also detected.

In winter, 15 bacterial species of eight genera were iso-