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Microbial Contamination in Shaker Bottles among Members of Fitness Centers

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ABSTRACT

Silveira MB, Scudese E, Senna GW, Ferreira AP, Dantas EHM, Ribeiro LCP, Alvares AFH, Guedes PG. Microbial Contamination in Shaker Bottles among Members of Fitness Centers. **JEPonline** 2018;21(4):134-142. The purpose of this was to analyze the presence of different bacterial strains and to determine resistance to antimicrobials in used bottles (UB) from the members of fitness centers and new non-used bottles (NUB). A total of 60 shakers (30 UB and 30 NUB) were selected and submitted to microbiological analysis. The samples were collected through Swabs containing Stuart's medium and delivered to the laboratory. Gram staining and biochemical tests were performed for the identification of microorganisms and antimicrobial susceptibility. The Cochran Q test presented significant difference (P=0.001) in contamination status (UB vs. NUB). All the NUB tests showed 100% absence of contamination while 90% of the UB showed bacterial contamination. For the 60 samples investigated, we were able to isolate six species of different microorganisms. It is important to note that 16.6% had no bacterial growth. The antibacterial susceptibility test revealed a varied range of resistance profile. In conclusion, six pathogenic microorganisms were isolated from poorly sanitized bottles highlighting that the post-use hygiene must be made appropriately to avoid the proliferation of pathogenic bacteria.

Key Words: Bacteria, Exercise, Health, Microbiology

INTRODUCTION

In recent years, the general concern for health and well-being has increased significantly with more people pursuing a more conscious lifestyle. This phenomenon is in line with the growth in the total number of fitness centers around the world. A gym environment is a place where individuals engage in regular exercise to improve their quality of life and health (3). Among this population, a consensus on the importance of overall nutrition and hydration for achieving their health and fitness goals are well known. For instance, adequate water intake is vital to prevent dehydration and its adverse effects, such as headaches and urinary lithiasis during exercise (1).

Nutrition is an important tool within sports practice and when well elaborated promotes the maintenance of the health of the physical exercise practitioner (2). Previous studies have demonstrated both acute and chronic ergogenic effects of different types of supplementation in subjects undergoing strength training routines (10). Additionally, different substances may require distinct intake approaches and strategies. For instance, the window of opportunity for hypertrophy may be associated with the use of whey protein hours after training (9) as opposed to pre-workout/stimulants (20). The fact of the matter is that independent of the timing, many individuals carry shakers bottles along their gym routine.

The propagation of this knowledge is allied to the growth of the supplement industry that has increased the use of shaker bottles among gym members (2). However, what is normally not well-known is that improper use of shaker bottles may favor the growth of microorganisms on the walls of the container. While the practice of regular exercise increases the demand of water intake and, often times, the use of some commonly prescribed supplements (such as branched-chain amino acids (BCAA) and Whey Protein), this practice might lead people to use the same shaker for both hydration and supplementation.

Water quality control is a universal need, requiring attention from health authorities and consumers in general. Specifically, regarding water intended for human consumption, there is a concern due to its potential to become a vehicle capable of transmitting various pathogens that threaten the well-being and health of gym members (23). Hence, if the bottle is not adequately cleaned, instead of drinking pure water, individuals might be drinking contaminated water that may cause symptoms in some individuals. One should also consider the lack of hygiene of the hands as a source of contamination of the container since the hands are essential sources of cross infection.

Thus, the purpose of the present study was to analyze the shaker bottles conditions for the possible presence of different bacterial strains from two distinct fitness centers and to determine its resistance to antimicrobials.

METHODS

Sample

A total of 60 bottles were selected for this study. Thirty of these bottles were purchased in different stores and sampled as the non-used bottles (NUB). These samples were obtained from three distinct brands, chosen for their high prevalence in the Brazilian fitness market. In addition, different batches were chosen for each brand to minimize possible contamination

due to poor transportation or storage of one batch in particular. For the used shaker bottles (UB), we randomly selected from 30 gym members of two distinct fitness centers (15 at each) in the city of Petrópolis, RJ, Brazil, from November 2016 to January 2017. The most commonly used dietary supplements by the UB sample were: (a) whey protein; (b) compounds derived from meat protein; (c) carbohydrate supplements; (d) branched chain amino acids (BCAA); and (e) creatine.

Procedures

The samples were collected in a dry and empty bottle state at the pre-use moment. To avoid any attempt of exacerbating cleaning of the shakers, the subjects were asked to participate at the time of the data collection, without previous knowledge about the research. Immediately after collection, the subjects were interviewed regarding basic hygiene knowledge and bottle usage purposes. Samples were collected by smooth friction through sterile Swabs in the inner walls of the shakers. The Swabs were immediately introduced into the Stuart medium and directly sent to the Microbiology Laboratory via temperature-controlled boxes.

The Swabs were inoculated into brain-heart infusion (BHI) broth and incubated at 35°C ± 1°C for 24 hrs. Subsequently, the samples were seeded in blood agar, MacConkey agar, and mannitol salt agar from Probac™ (Brazil), and then incubated at 35°C ± 1°C, 24 hrs in the aerobic incubator model ECB 1.2 Digital Odontobras™. After bacterial growth, the isolated colonies were submitted to gram staining and biochemical identification tests such as oxidase test, Bactray 1 and 2 - Laborclin tests, for the identification of Gram-negative glucose-fermenting bacilli and Bactray 3 for non-fermenting bacteria. In addition to the catalase assays, the coagulase was performed for *Staphylococcus aureus* identification. After identifying the bacterial genera and species, the strains isolated were submitted to the Antimicrobial Susceptibility Test according to EUCAST (2017).

For the following bacteria: *Escherichia coli; Proteus vulgaris,* and *Serratia* sp, the following antibiotics were tested: Ampicillin + Sulbactam, Amoxicillin + Clavulanic Acid, Piperacillin + Tazobactam, Cefoxitin, Cefotaxime, Ceftriaxone, Imipenem, Meropenem, Ertapenem, Aztreonam, Levofloxacin, Ciprofloxacin, Amikacin, Gentamycin, Sulfamethoxazole + Trimethoprim, always observing the intrinsic resistance of each microorganism. The antibiotics tested for *Pseudomonas* sp were: Piperacillin + Tazobactam, Cefepime, Imipenem, Meropenem, Levofloxacin, Ciprofloxacin, Amikacin, Gentamicin. For *Staphylococcus aureus* the following antibiotics were tested*:* Amoxicillin + Clavulanic Acid, Oxacillin + Tazobactam, Cefadroxil, Cephalexin, Cefoxitin, Cefepime, Ceftriaxone, Cefuroxime, Imipenem, Meropenem, Levofloxacin, Ciprofloxacin, Moxifloxacin, Amikacin, Gentamycin, Azithromycin, Clarithromycin, Clindamycin, Erythromycin, Doxycycline, Linezolid, Rifampicin, Sulfamethoxazole + Trimethoprim. The antibiotics tested for *Acinetobacter* sp were: Imipenem, Meropenem, Ciprofloxacin, Levofloxacin, Amikacin, Gentamycin, Sulfamethoxazole + Trimethoprim.

Statistical Analyses

All results were presented by percent delta and/or the absolute number. The Cochran Q test was applied to verify significant differences between two sample groups for its binary characteristics (0 or 1). The significance level was set at $P \leq 0.05$. The software used for all statistical analyses was the SPSS, version 21.0 (IBM, Inc).

RESULTS

From the data obtained by Cochran Q analysis, there was a significant difference (P=0.001) in contamination situation to compared distinct shaker bottle conditions (UB vs. NUB). Specifically, the analysis of the NUB showed 100% absence of contamination. Conversely, contamination (i.e., presence of microorganism) was found in 90% of the UB.

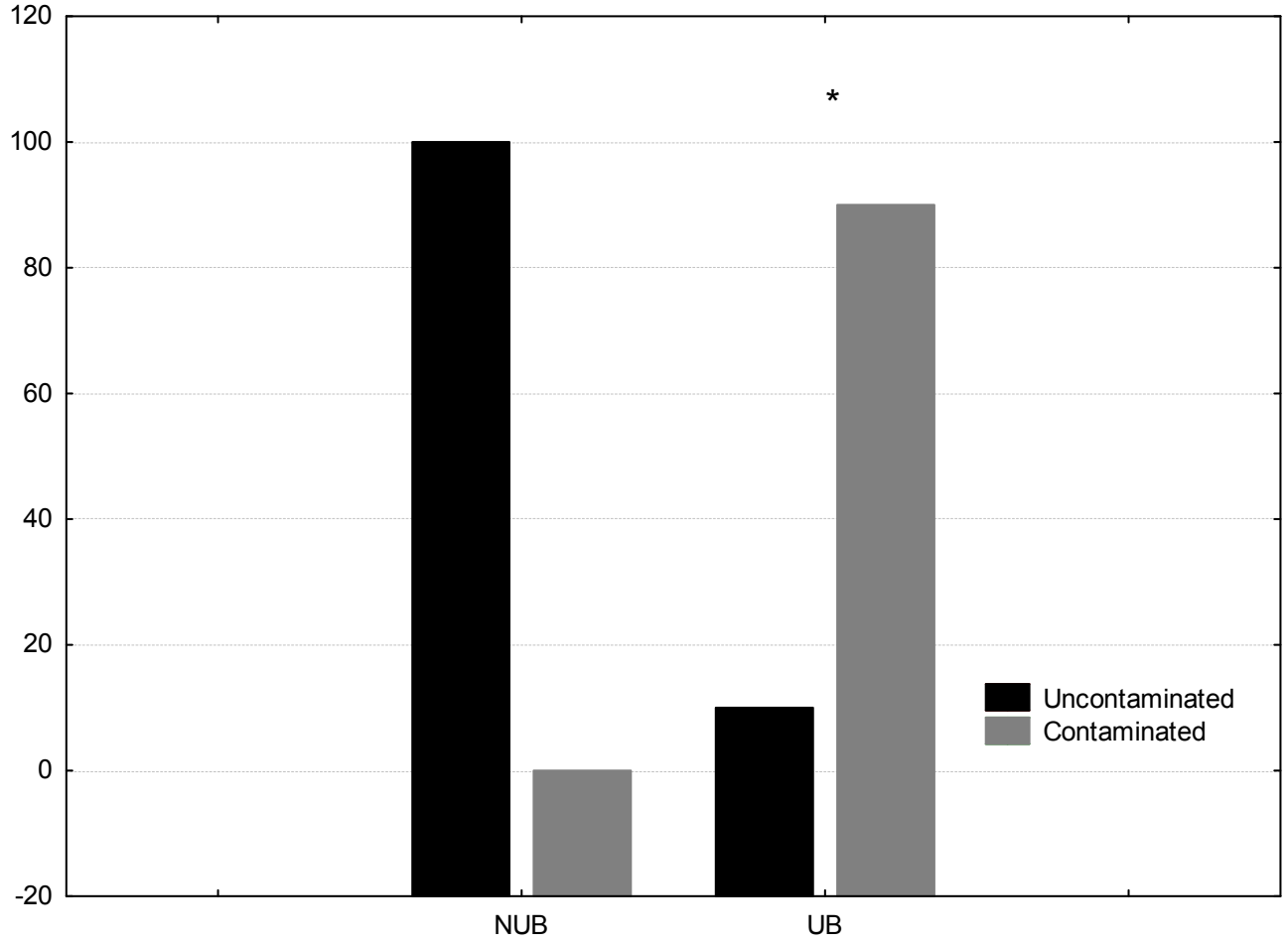

Figure 1. Percentage Contamination from Used Bottles (UB) and Non-Used Shaker Bottles (NUB). *Significant difference to UB sample.

For the 30 samples analyzed in UB, we observed bacteria growth in 25 (Δ% = 83%) of the verified shaker bottles. Specifically, we verified that the *Staphylococcus aureus* (Δ% = 26.66%) and *E. coli* (Δ% = 16.66%) were the most frequent species. The percentage prevalence of isolated bacteria species is shown in Figure 2.

Table 2 shows the results of the antimicrobial susceptibility test for the isolated strains, showing the resistance profile. Briefly, most cases of contamination have demonstrated antimicrobial resistance to different types of substances. Specifically, the *Proteus vulgaris* had a 100% resistance score to most antimicrobials tested (APS, AMC, AZT, SUT, CFM); *Escherichia coli* presented 100% resistance to SUT; and *Serratia* had 100% resistance for APS, AMP, AMC, CFO and CFM.

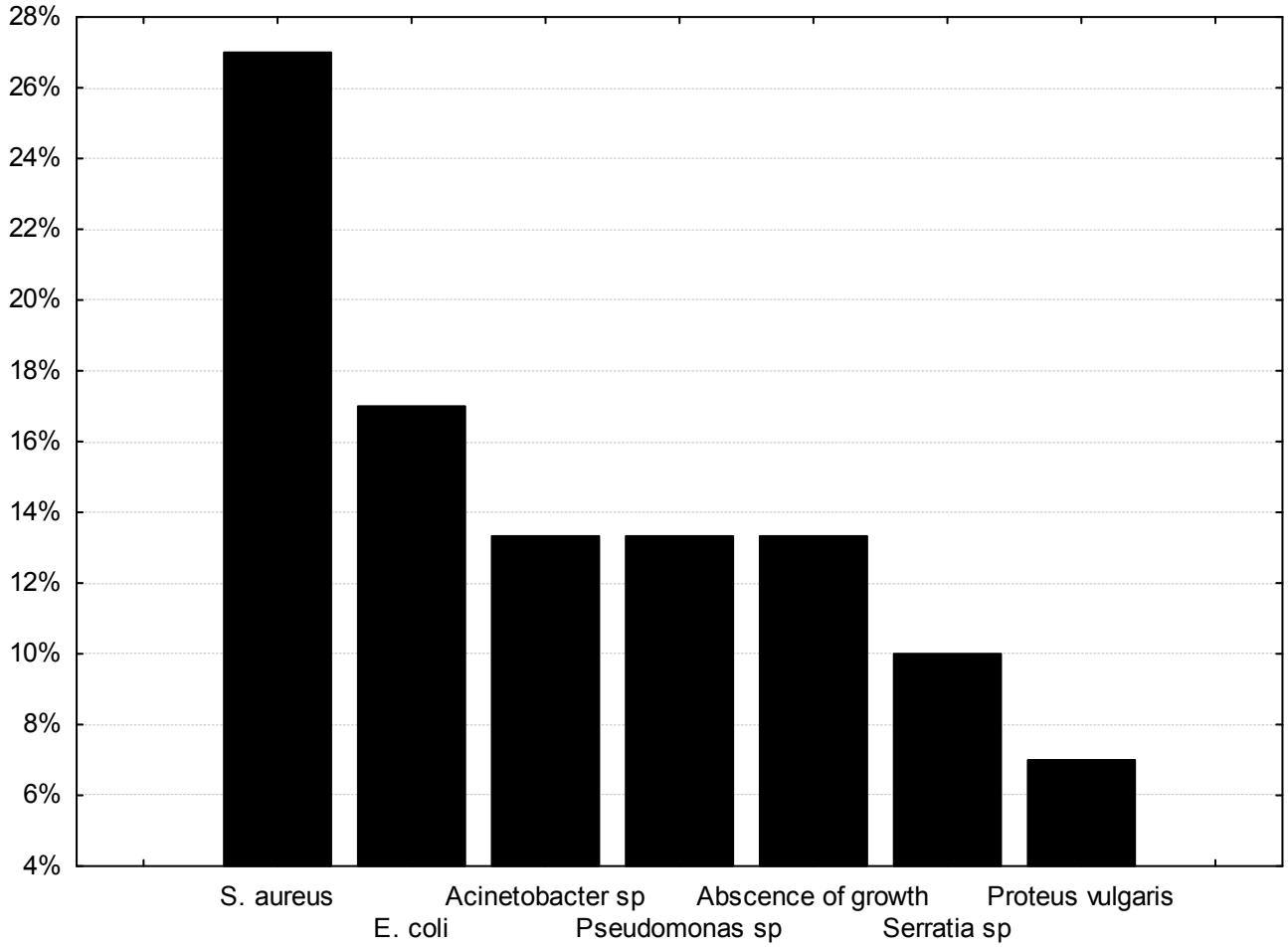

Figure 2. Percentage Prevalence of Isolated Microorganism from Shaker Bottles.

Table 1. Antimicrobial Resistance Profile of Isolated Strains.

Bacteria	Resistance Observed	Perceptual (%)
Proteus vulgaris	CFO, CRO.	50%
	APS, AMC, AZT, SUT, CFM.	100%
E. coli	LEV, CIP.	25%
	SUT.	100%
Staphylococcus aureus	CFO, LEV, CIP, OXA.	12.5%
	SUT.	25%
Acinetobacter sp.	LEV.	25%
Pseudomonas	IMI, MER.	25%
	AMI, GEN.	25%
Serratia	APS, CFO, CFM.	100%

APS= Ampicillin + Sulbactam; **LEV** = Levofloxacim; **CIP** = Ciprofloxacim; **CFO** = Cefoxitin; **CRO** = Ceftriaxone; **IMI** = Imipenem; **MER** = Meropenem; **OXA** = Oxacillin; **SUT** = Sulfamethoxazole + Trimethoprim; **AMC** = Amoxicillin + Clavulanic Acid; **AMI** = Amikacin; **GEN** = Gentamicin; **AZT** = Aztreonam; **CFM** = Cefuroxime.

DISCUSSION

Among the major findings of the present study, we observed that microbial growth was present in 90% in the used bottles (UB). The inadequate hygiene of the container leads to the growth of environmental microorganisms and even pathogens, which may pose a risk to human health. Our tests conducted on the shaker bottles of gym members demonstrate the growth of strains of antibiotic-resistant bacteria. Antimicrobial resistance has become a threat to public health due to its association between the development of bacterial resistance (i.e., *S. aureus*, and *enterococci*), increases in mortality, and costs of health care. Patients with infections due to antimicrobial resistant organisms have higher costs (i.e., $ 6,000 - $ 30,000) than patients with antimicrobial infections. The cost difference is even greater when patients infected with antimicrobial resistant organisms are compared with patients without infection (5).

Specifically, the prevalence of *Staphylococcus aureus* strains was found in 26.6% the used shakers and was sensible to most of the antibiotics tested. *Staphylococcus aureus* is a prominent etiological agent in infections acquired both in the community and in the hospital environment, and is considered an important pathogen due to its high capacity to cause disease (22). In fact, the *S. aureus* is the most commonly isolated human bacterial pathogen. It is an important cause of skin and soft tissue infections, endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis (7). This specie may colonize the anterior nares and other body sites (16). Gorwitz et al. (12) identified a significant association between obesity and colonization with *S. aureus* in adults. Similar association has been reported for adult patients who had undergone general, cardiothoracic, or neurologic surgery (14). The reasons for this association are unclear, but may include physical, biochemical, or hormonal factors that predispose these individuals to colonization with *S. aureus* (12). The prevalence of *S. aureus* colonies has also been demonstrated previously in males (14), which was our population tested. Our data portray the need for an ideal hygiene of shakers bottles mainly for males.

Proteus species are widespread in the environment and are part of the normal flora of the human gastrointestinal tract as well as mammals. They cause a variety of diseases acquired in the community, including urinary tract infections, wounds, and the bloodstream (18). Drug resistance has been increasingly reported for this genus, and the predominant mechanism for resistance to β-lactam antibiotics is for the synthesis of β-lactamases. Among β-lactamases, the production of extended-spectrum β-lactamases (ESBLs) and β-lactamases AmpC is more common (21). Belonging to the same family, *Serratia* sp., *Escherichia coli* and *Klebsiella* sp., are responsible for urinary infections, being E. coli more prevalent. The *Escherichia coli* is also known to be a very well adapted entero-invasive pathogenic able to enter epithelial cells of colon, multiplicate within them, and move between adjacent cells. This pathogen is one of the most recorded infection agents worldwide, as documented by recent outbreaks in the industrialized countries (19).

Additionally, we found the presence of *Pseudomonas* species in four shakers. This pathogen is one of the major microorganism causing hospital-acquired infections and can be more aggressive towards immune-suppressed individuals. This agent presents a large genome, and it can develop antibiotic resistance involving almost all classes of antibiotics. This trait can be correlated to chromosomal mutations or by horizontal acquisition of resistant

determinants. The habitat of this microorganism is water and soil and can cause infections in several body systems and parts such as urinary, skin, bone, and blood, being more severe in the hospital environment (4).

The *Acinetobacter* specie was isolated in four shakers. It is also found in soil and water, most commonly in dry environments. The *Acinetobacter* was originally identified in 1938. It is ubiquitous in the environment as fresh water, vegetables, and animals (5). Several *Acinetobacter* strains have been identified as causing infections in humans such as pneumonia, sepsis, skin infections and infected wounds, according to the site affected (11). This agent is a nosocomial pathogen that causes ventilator-associated as well as bloodstream infections in critically ill patients, and the spread of multidrug-resistant *Acinetobacter* strains is cause for concern. This microorganism is also considered opportunistic, rarely causing community infections, except in cases of comorbidities such as alcoholism, smoking, diabetes, and chronic obstructive pulmonary disease where much of the success can be directly attributed to its plastic genome, which rapidly mutates when faced with adversity and stress (13). Sporadic cases have occurred occasionally in healthy patients exposed to environmental sources (8).

An estimated four billion cases of diarrhea annually represented 5.7% of the global disease burden in the year 2000 (24). The scientific literature has accumulated over the decades a good understanding of the transmission of several pathogens that cause diarrhea and other diseases through drinking water (15). It has previously been observed that the microbiological quality of water in domestic vessels is lower than at source, suggesting that contamination is widespread during water collection, transport, storage, and extraction (17). We found that most of the microorganism species that colonized the shakers were enterobacteria. Some are present in the intestinal microbiota, which leads us to believe that not being hand sanitized or incorrectly used may be contaminating the shakers. We must also consider the non-hygiene practice of gym members who do not clean their shakers, thus facilitating bacterial growth.

CONCLUSIONS

The isolation of pathogenic microorganisms from shakers used by physical activity practitioners at fitness centers such as Staphylococcus aureus, Acinetobacter sp, Pseudomonas sp, Methicillin-resistant Staphylococcus aureus, and members of the family Enterobacteriaceae presented a varied profile of antimicrobial resistance. It is worth mentioning that most of the bacteria isolated in this study belong to the group of enterobacteria, present in the intestinal microbiota and are pathogenic, emphasizing that manipulation with contaminated hands may contribute to the colonization of the shakers. We conclude that the best way to avoid bacterial proliferation in the shakers is make sure they are correctly and frequently cleaned, such as daily washing with neutral soup in association with proper hand hygiene to prevent contamination.

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