



The importance of environments and surfaces hygienization in pandemic times (Covid-19): a microbiological analysis in places for physical activity practice

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ABSTRACT. The objective of this study was to analyse microbiological organisms in different locations and regions for physical activity in the city of João Pessoa, Brazil. Samples were collected on various objects used, such as: mattresses, drinking fountains, gloves, cell phones and others. The samples were collected in João Pessoa-PB, following the Standard Operating Procedure-SOP/ Microbiology of a specialized laboratory. The collection took place in the five macro-regions: North, South, East, West and Center. For each region samples were collected in one public place (square), a private one (gym) and one school (public or private), totaling fifteen collected sites and 450 samples. The following microorganisms were studied in all analyzed surfaces: *Bacillus sp*, *Escherichia Coli*, *Klebsiella spp* or *Enterobacter spp* and *Coag. Neg. Staphylococcus*. All regions had a high contamination level by some microorganism. The highest rates were found in the western, central and northern regions - 96, 94 and 93% respectively. The *Coag. Neg. Staphylococcus* presented the highest and lowest incidence rates in the South and East regions, with 43.33 and 6.67%, respectively, as well as *Klebsiella spp* or *Enterobacter spp*, which presented high levels. It is concluded that there is a microorganisms' contamination in the most varied places and regions where physical activity practices are developed, with a predominance of *Coag. Neg. Staphylococcus* and *Klebsiella spp* or *Enterobacter spp*. These results lead to a warning about the hygiene importance in places for physical activity practice, especially in pandemic times (COVID-19), since almost all the evaluated surfaces were contaminated.

Keywords: contaminants; microbiology; physical exercise; hygiene; health.

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Introduction

The regular physical activity practice is of great importance in promoting quality of life, providing a health improvement in its biopsychosocial aspects (Pitanga, 2008), thus promoting positive changes to the body physiological levels. For this effect to be even more significant, it is important that some precautions regarding health safety are observed. There are several microorganisms' species, some of them harmful to our health, which find on solid surfaces, in other living beings or even in the air, a proper environment to their maintenance and propagation (Pinheiro & Stopiglia, 2016; Alberts et al., 2017;).

The most commonly found microorganisms are protozoa, algae, fungi, bacteria and viruses (Carvalho, 2016), and specifically since December 2019 (Yang, Liu, Li, & Zhao, 2020), when the first case of SARS-CoV-2 (COVID-19) infection was registered, the world has been facing a viral pandemic disease that causes severe acute respiratory syndrome. According to the data from the World Health Organization, until October 2, 2020, there were already more than 34 million cases worldwide (World Health Organization [WHO], 2020), which shows its high contagion and dissemination capability (Awadasseid, Wu, Tanaka, & Zhang, 2020). So, the attention focused on individual care and environmental correct hygiene have stood out in the eyes of society as a mean of prevention.

In order to minimize the action of these infectious agents, there is the use of medicines and vaccines, but a great alibi in the fight against pathogenic microorganisms, at a relatively low cost, are the prophylactic measures that meet the correct environmental hygiene and with individual care (Ferreira & Silveira, 2004).

Considered as places of enormous potential in changing the population habits, the gyms stand out, for offering various services for guidance, monitoring and supervision of physical exercise practices (Liz & Andrade, 2016), as well as parks and public squares, constantly used by physical activity practitioners, equipped with public urban facilities, generally coordinated by the municipal government through public policies (Presotto et al., 2016). It is also important to mention the schools, which are places where children and adolescents spend a good part of their day and offer this type of activity and where they are usually initiated to such practice (Silva, Gonçalves, Silva, & Silva, 2019).

However, the use of these environments requires good usability conditions, with an adequate environment sanitation, equipment in good conditions, garbage dumps distribution and properly sanitized drinking fountains. All these factors corroborating physical activity practice in a safe and healthy way (Presotto et al., 2016).

A study by Guedes, (Guedes, Maia, Santos, Santos, & Menezes, 2018) aimed to evaluate the antimicrobial activity of a certain substrate in microorganisms frequently found in gyms' equipment. Through this study it was observed and classified the percentage of four main microorganisms in 11 evaluated devices as it follows: *Pseudomonas sp.* 100%, *Staphylococcus aureus* 45.5%, *Streptococcus spp.* 36.4%, *Escherichia coli* 27.3% and fungi that in total had a percentage below 10%.

It is known of the improvement that physical activity performed in a continuous and orderly manner will bring to the immune system (Abd El-Kader & Al-Shreef, 2018), which is fundamental for fighting aggressive external agents. However, combined with prophylactic care, these, in addition to minimizing the negative impacts that a pathogen proliferation conducive environment cause, can bring a significant increase in the number of people who will become physically active through physical exercise constancy (Legnani, Guedes, Legnani, Barbosa Filho, & Campos, 2011).

When reviewing the relevant literature, only one study on the topic was observed. Guedes et al. (Guedes et al., 2018) evaluated the antimicrobial activity of the oil obtained from *Corymbia citriodora* biomass residue *in vitro* on microorganisms found in gym equipment. In this way, it is possible to observe gaps involving regions and diversification due to the collection sites, data that involve the public and private sector, as well as in-depth studies that work with a specific audience, seeking to confirm or not, the presence of contaminating agents in the environments and objects used during the practice of physical activity in gyms, public places and schools.

Therefore, this work aimed to carry out a microbiological analysis in different places and regions for physical activity practice in the city of João Pessoa, Paraíba, Brazil. Thus, being able to identify which contaminating microorganisms are present in the equipment and places, which is relevant nowadays due to the COVID-19 pandemic the world has been facing.

Methodology

Sample

The samples were collected in the city of João Pessoa, Paraíba, Brazil, in public and private places where physical activity practices are carried out, such as gyms, schools and squares in different regions of the capital of Paraíba. At the gyms, samples were collected on mattresses, cupboards, dumbbells, bench press, abductor machine support cushion, leg machine backrest, squat bar pad, seat cushion support, free stool, extensor bench, armrest scot, pulley rope, mat or handrail support, bench and exercise bike handlebar, cloth used to clean the exercise mat, trigger sprinkler, gym reception bench, bathroom sink, weight bench, tatami mat or artificial grass. In schools, collections took place on the court floor, water fountain, spout outlet, hula hoop, (Medicine) net, beam, grandstand, ropes, volleyball iron or columns, teacher whistle, door or gate bolts, mat, basketball, soccer ball, in addition to other types of balls, sports room (if any), internal part of the cabinets where sports materials and class diaries were kept. In the squares, collections took place on the floor, benches, fixed bar, in addition to the sample from which people pick, touch, sit or step on each of the devices present in this environment. The collection sites were selected geographically and divided into five macro-regions: North, South, East, West and Center, so that a reliable profile of the city reality could be drawn, where for each macro-region one public square gym, one private gym and a school (public or private) were chosen. The schools and gyms were intentionally selected by the researcher, as for the squares, it occurred according to the proximity of the respective schools and gyms. As an inclusion criterion, the voluntary authorization of owners or persons responsible for these sites by signing the consent letter was used. The study was approved by the ethics committee of the Centro Universitário de João Pessoa - UNIPÊ (nº 2,890,929 CAAE nº 91325618.5.0000.5176).

Data-collecting instruments

Data collection was performed by the laboratory technicians in compliance with the Standard Operating Procedure (SOP). After collection, the laboratory forwarded the report with results by location and object analyzed during the research. Stuart's Medium (FIRSTLAB) was used as transport medium and for enrichment, the Thioglycolate Broth (MICROMED). As a culture medium we had: a) Blood Agar Base (KASVI) + 5% sheep blood; b) Macconkey Agar (KASVI); c) Ss Agar (KASVI); d) Thayer Martin Agar (LABORCLIN). The classic method based on morphological and biochemical characteristics was used for identification: a) Mannitol Agar (KASVI); b) Simmons Citrate Agar (KASVI); c) Tsi Agar (KASVI); d) Epm Mili (PROBAC). And for the automated technique method, Maldi-Tof mass spectrometry was used.

Data Analysis

The collected data were analyzed using the R project software. The chi-square test for independence and relative and absolute frequency was used. In calculating the odds ratio for each microorganism to be identified in each region, the odds calculation was used, which corresponds to:

$$Odds_{occurrence} = \frac{probability_{occurrence}}{1 - probability_{occurrence}}$$

The odds ratio calculation, or odds ratio (RCG1 / G2) is given by the equation below:

$$RC_{G1/G2} = \frac{Odds_{G1}}{Odds_{G2}}$$

Where, G1 and G2 correspond to the events: Odds for identification of microorganisms in region 1, Odds for identification of microorganisms in region 2, respectively. The level of significance was set at $p < 0.05$.

Results

All regions had a high contamination percentage by some type of microorganism, those with the highest rates are the west, center and north regions with 96, 94 and 93%, respectively. While the lowest indexes are in the southern, with 88%, and east, with 87%.

When comparing the microorganisms separately by region, a statistically significant difference is observed for *Klebsiella spp* or *Enterobacter spp*, with a p-value equal to 0.015, and *coag. neg. Staphylococcus*, with p-value of 0.013. The highest percentage of *Klebsiella spp* or *Enterobacter spp* was found in the North, corresponding to 40% of the total of 40 identified items. Regarding *coag. Neg. Staphylococcus*, the highest percentage was identified in the South, representing 43.33% of the total of 30 identified items, as it can be seen in Table 1.

Table 1. Microorganisms present in the evaluated sites, distinguished by region and Chi-square independence test of p-value.

Microorganisms	Regions										Total	p-value ¹	
	Center		East		North		West		South				
	N	%	n	%	n	%	n	%	n	%	n	%	
<i>Bacillus sp</i>	45	23.68	38	20.00	33	17.37	42	22.11	32	16.84	190	100.0	0.506
<i>Escherichia coli</i>	7	18.42	12	31.58	6	15.79	4	10.53	9	23.68	38	100.0	0.298
<i>Klebsiella spp</i> or <i>Enterobacter spp</i>	6	15.00	9	22.50	16	40.00	6	15.00	3	7.5	40	100.0	0.015*
<i>Coag. Neg. Staphylococcus</i>	3	10.00	2	6.67	7	23.33	5	16.67	13	43.33	30	100.0	0.013*
Total	61	20.47	61	20.47	62	20.80	57	19.13	57	19.13	298	100.0	0.983

¹Chi-square independence test. *Significant difference ($p < 0.05$).

Table 2 shows the absolute and relative frequencies of the microorganisms present in the three sites evaluated in the different regions of João Pessoa. It is observed that *Bacillus sp* are more frequently present in the items of the three locations (gym, school and square), corresponding to 63.76% of the total identified microorganisms in the items. It is also possible to verify that, in second place, at the gym, the most commonly found microorganism was *coag. neg. Staphylococcus* (6.38%), while at the school and square, the second most frequent microorganism was *Escherichia coli*, with values of 6.04% and 4.36%, respectively. The Chi-square test showed a p-value of 0.0005, which results in a strongly significant difference (at the level of 1% of significance) between the types of microorganisms and the locations in which they were found.

Table 2. Microorganisms present in the evaluated sites and the Chi-square independence test p-value.

Microorganisms	Sites						Total	p-value ¹
	Gym		School		Square			
	n	%	n	%	n	%	n	
<i>Bacillus sp</i>	59	19.80	68	22.82	63	21.14	190	63.76
<i>Escherichia coli</i>	7	2.35	18	6.04	13	4.36	38	12.75
<i>Klebsiella spp</i>	6	2.01	1	0.33	2	0.68	9	3.02
<i>Enterobacter spp</i>	6	2.01	17	5.71	8	2.68	31	10.40
<i>Coag. Neg. Staphylococcus</i>	19	6.38	4	1.34	7	2.35	30	10.07
Total	97	32.55	108	36.24	93	31.21	298	100.00

¹Chi-square independence test.

Table 3 presents the Odds by microorganisms for each region. Such values were used in the Odds ratio calculation, or odds ratio, which is the probability of the environment being contaminated by a certain microorganism. Thus, it is observed that the same microorganism can suffer changes in the contamination levels by region (major - minor), between 11% (*Bacillus sp*) and 69% (*coag. Neg. Staphylococcus*). Regarding the contamination level of different microorganisms for the same region, this variation was between 16% (West region) and 68% (South region).

Table 3. Odds for identification of microorganisms by region: Odds of the microorganism identified in the objects of each region.

Microorganisms	Regions				
	Center	East	North	West	South
<i>Bacillus sp</i>	0.31	0.25	0.21	0.28	0.20
<i>Escherichia coli</i>	0.23	0.46	0.19	0.12	0.31
<i>Klebsiella spp</i> or <i>Enterobacter spp</i>	0.18	0.29	0.67	0.18	0.08
<i>Coag. Neg. Staphylococcus</i>	0.11	0.07	0.30	0.20	0.76

As mentioned, the microorganisms that showed a significant difference between the regions were *Klebsiella spp* or *Enterobacter spp* and *coag. Neg. Staphylococcus*, and these were selected to calculate the odds ratio in order to identify which region is most likely to have such microorganisms. Tables 4 and 5 show such results. Thus, it is observed that for *Klebsiella spp* or *Enterobacter spp* the North region is the place that has the greatest chance of identifying such microorganisms, with odds ratios varying between 2.30 (compared with the Eastern region) and 8.22 (compared with South region). Regarding *coag. Neg. Staphylococcus*, it is observed that the greatest odds ratios are found in the South region, with a variation between 2.51 (compared to the North region) and 10.71 (compared to the East region), that is, in this region there is a greater chance of identifying such a microorganism.

Table 4. Chance ratio for *Klebsiella spp* or *Enterobacter spp* to be identified in the objects of the region in question.

<i>Klebsiella spp</i> ou <i>Enterobacter spp</i>		Region 2			
Region 1	Center	East	North	West	South
Center	-	0.61	0.26	1.00	2.18
East	1.64	-	0.43	1.64	3.58
North	3.78	2.30	-	3.78	8.22
West	1.00	0.61	0.26	-	2.18
South	0.46	0.28	0.12	0.46	-

Table 5. Chance Ratio of *coag. neg. Staphylococcus* to be identified in the objects of the region in question.

<i>Coag. Neg. Staphylococcus</i>		G2 Occurrence			
G1 Occurrence	Center	East	North	West	South
Center	-	1.56	0.36	0.56	0.14
East	0.64	-	0.23	0.36	0.09
North	2.74	4.26	-	1.52	0.40
West	1.80	2.80	0.66	-	0.26
South	6.88	10.71	2.51	3.82	-

From the microbiological analysis of equipment and places for physical activity practices in the city of João Pessoa-PB, comprised in five macro-regions (north, south, east, west and center), bacterial contamination of several genera was observed in a large amount of the analyzed items, as shown in Figure 1.

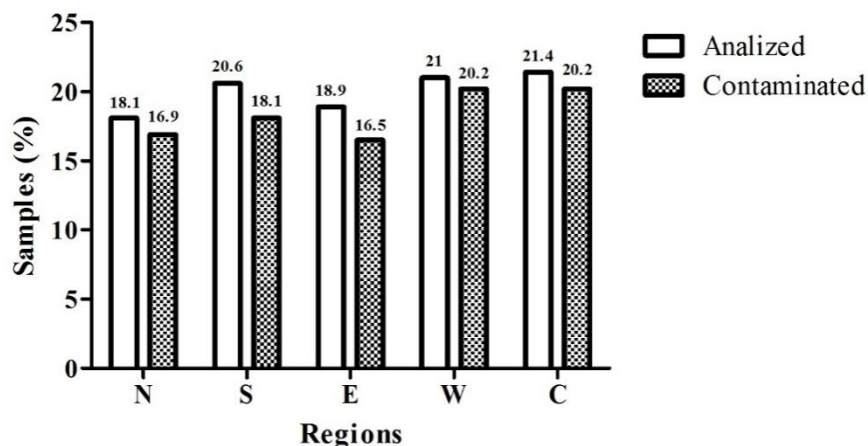


Figure 1. Percentage of surfaces analyzed for contaminated surfaces. N: North region; S: South region; E: East region; W: West region; C: Central region

Discussion

This work carried out a microbiological analysis in different places for physical activity (gyms, schools and public squares) and in different regions of the city of João Pessoa (north, south, east, west or center). This study is shown as the only one of this profile in sample quantification, as well as in places and regions where physical activity is practiced and can be a reference for other and more diverse possible studies on the subject, seeking the best clarification on it and presenting important information for professionals and users who practice physical activity in the examined places, mainly in pandemic times.

As a first finding, a higher prevalence of *Klebsiella spp* was observed, frequently involved in human body infections (Levinson, 2016), with this microorganism being found in about 40% (North region) of the total analyzed surfaces, and *coag. neg. Staphylococcus* identified in 43.3% (South region) of the total sample, consequently with a higher prevalence of the contamination Chance Ratio for these regions compared to the others. It is observed that both are opportunistic pathogens (Bernardi, Pizzolitto, & Pizzolitto, 2007; Chirindze et al., 2018) and as immunological factors significantly influence the infection occurrence (Bernardi et al., 2007), it is important to take the necessary precautions, since even if not affected by the pathogen, the individual can serve as a contaminating vector.

The treatment of these infections has become an increasingly challenge for public health, since *coag. neg. Staphylococcus* has shown resistance to multiple antimicrobial drugs (Bernardi et al., 2007). Surface disinfectants, such as quaternary ammonium compounds, widely used in hospitals, often also become ineffective against these hospital pathogens, which can be evidenced by the emergence of resistant strains or presenting reduced susceptibility to these compounds (Bernardi et al., 2007).

The second finding of this study is the microorganisms' frequency at the sites, whether in gyms, schools or squares, realizing that *Bacillus sp* was the one that had the highest frequency found, both relative, reaching an index of 22.82% in schools, and absolute, with a representation of 63.76%. Despite the high values found in the analysis, *Bacillus sp* is considered a non-pathogenic agent (Ayala et al., 2017).

Another microorganism found was *Escherichia coli*, the second largest in total frequency, with 12.75%. It is a bacterium that reaches the urethra through the feces (therefore the correct hygiene is one of the most simple and effective methods to avoid this type infection). This bacterium can colonize the gastrointestinal or urinary tract and meninges, reaching the bloodstream and thus causing sepsis, which leads to believe the possibility that users are susceptible to this infection (Kaper, Nataro, & Mobley, 2004).

As for *Enterobacteriaceae*, it is noteworthy that they are found mainly in the humans and animals colon. The endotoxins in their cell walls come from the fact that they are gram-negative and, despite being taxonomically classified as a set, the *Enterobacteriaceae* family can cause several diseases through different pathogenic mechanisms (Levinson, 2016) such as gastroenteritis (Paterson, 2006), which is an inflammation in the digestive tract, bringing symptoms such as diarrhea, abdominal pain, nausea and vomiting.

Finally, when checking the total location number, the regions observed through the contaminated surfaces represent 91.7% of the total analyzed sample, that is, of the 243 surfaces from the three locations and the five regions, 223 surfaces were found contaminated by one or more microorganisms.

Thus, the findings of this study are relevant to the academic community, as in addition to properly identifying the microorganisms that the population using physical activity locations are susceptible to. This paper brings a diagnosis that can alert everyone involved in the process of practicing physical activity, from physical education professionals, private places owners, public authorities and especially users to have better prevention and asepsis when practicing physical activity, thus bringing greater security for everyone.

Thus, preventive care to minimize the diseases development caused by microorganisms is necessary and should be done by simple methods such as educational campaigns that clarify the contamination risks, hands, equipment and environment hygiene, thus avoiding your own contamination when touching the skin, mucous membranes and when carrying out any different ingestion in these environments, avoiding any possible microorganisms associated pathologies. In addition to this, it is also important to carry out a health mapping in order to understand for which reasons in certain places there are more pathogenic agents than others, being these multi-causal factors, that range from environmental to psychosocial factors (Massa & Chiavegatto Filho, 2020), that is, from pollution of the environment itself as a determining factor of direct contamination, to indirect forms such as the cultural or educational level of the population as part of a preventive factor.

Therefore, the commitment of all those involved, from the act of each citizen to the Public Policies developed for this purpose, are basic conditions for the successful implementation of good practices and the use of these environments, seeking health promotion in a way that matches the different regions of a city, given that factors related to social and economic determinants, with poverty as one of the strong indicators of sanitation in a given region (Mara, Lane, Scott, & Trouba, 2010), in addition to cultural factors and also environmental factors, such as basic sanitation (Aguiar, Cohen, Maciel, & Kligerman, 2020), end up influencing the occurrence of these practices due to a higher contamination level. For such measures, periodic training is recommended for employees who will work in these environments, as well as reports to all users, whether direct or indirect.

This study has some limitations in its development, such as the non-quantification of 100% of the analyzed surfaces, because of the amount of exams and swab made available by the laboratory, it was necessary to divide the surfaces to be analyzed by location, giving preference to those that had direct contact with the user's body or body part, mainly unprotected parts with direct contact between surface and human skin. The sample selection, which was made by convenience to facilitate the permission for collecting, mainly in gyms and schools. Another point worth mentioning was the collecting date, as it occurred before the COVID-19 pandemic, so there was no direct collection of the new coronavirus that is responsible for COVID-19, but as the potential for vertical transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has called the attention of the world, presenting data that can alert public and private agents is relevant to enhance inspections in different environments and regions in which physical activity is practiced.

Conclusion

This work demonstrated the existence of microorganisms' contamination in majority analyzed places where physical activity practices are developed. The microorganisms with the highest contamination degree was *Coag. Neg. Staphylococcus* in the South and *Klebsiella* spp in the North. Therefore, these data serves to alert about the importance of hygiene in places where physical activity is practiced, especially in COVID-19 pandemic times. Finally, it is suggested that new research in other capitals/cities are developed, with other microorganisms (eg, the SARS-CoV-2 virus), as well as others surfaces which people touch and possible preventive measures that can be adopted.

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