v. 3, n. 95, 2023

International Journal of Health Science

CONCENTRATION AND RESISTANCE TO ANTIBIOTICS OF CULTURAL BACTERIA ISOLATED FROM BIOEROSOLS IN THE CLASSROOM

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Abstract: Occupational exposure to bio-aerosols has been associated with various health effects, as they can contain a wide variety of pathogenic microorganisms, such as bacteria, viruses, fungi and parasites that can pose a threat to humans and animals. In this sense, the purpose of this research is to evaluate the presence and resistance to antibiotics of potentially pathogenic cultivable bacteria in classroom bio-aerosols before, during and after the development of teaching activities. Sample collection was carried out by exposing Petri dishes containing agar media: Blood Agar, Mac Conckey and Baird Parker. Each plate containing sterile culture media will be opened for 30 minutes at a height of 1.5 meters from the floor, arranged in the central part of the room. The plates were incubated at 37oC for 24-48h. There was an increase in bacterial colony-forming units during the course of the class and a reduction after classes. Samples collected before classes showed: *Staphylococcus aureus*, while during and after the development of classes the following were isolated: *Staphylococcus aureus, S. epidemidis, Streptococcus sp, Escherichia coli.* All bacteria showed resistance to several antibiotics.
Keywords: *Staphylococcus aureus, Staphylococcus epidemidis, Streptococcus sp, Escherichia coli, Bioparticles, air*.

INTRODUTION

Air consists of solid and liquid particles, also called aerosols. They can be made up of organic materials (bioaerosols) or inorganic materials (mineral dust, ash) and their origin can be natural, biogenic or anthropogenic. Bioaerosols include microorganisms such as bacteria and fungi, as well as viruses, mites, pollen and their fragments. Its biological activities or components can cause allergies, poisoning or infections in humans and animals (DOUWES et al., 2003, ABELHO, 2013, HAAS et al., 2013). Although the air is

not a favorable habitat for the reproduction of microorganisms, bacterial and fungal bioaerosols, as well as viruses, are a natural component of the air, and pathogenic microorganisms may also exist [HESS-KOSA, 2019].

The quality of indoor air in hospitals, offices, classrooms, libraries, industries, among others, where people spend a large part of their time carrying out their activities, is important for human health and work efficiency (GHOSH et al., 2013; PORTELA, KOZUSNY-ANDREANI (2019); CAVALHEIRO, KOZUSNY-ANDREANI (2020); YE et al., 2021; ZHAO et al., 2022). The presence of microorganisms, for example bacteria, fungi and viruses, has been recognized as an essential issue that can affect indoor air quality.

Exposure to specific airborne bacteria indoors is associated with adverse infectious and non-infectious health outcomes. However, the sources and origins of bacteria suspended in indoor air are not well understood. According to Hospodsky et al. (2012) the high number of bacteria specific to human skin, nostrils and hair found in indoor air and floor dust indicates that floors are an important reservoir of human-associated microorganisms. Human oral and respiratory fluid emitted through coughing, sneezing, speaking and breathing are important sources of microorganisms in suspended particles in closed environments (XIE et al., 2009,

The best-known occupational health effects related to exposure to bio-aerosols are respiratory symptoms, ranging from acute to chronic symptoms, and even life-threatening. Respiratory symptoms can be classified based on inflammatory mechanisms into allergic and non-allergic respiratory diseases. However, these health effects have not been studied extensively and information on their occurrence is limited, especially exposure to bio-aerosols (SMIT et al., 2006; ELFMAN et al., 2008; PATELLA et al. 2018). In this context, the purpose of this research is to evaluate the presence and resistance to antibiotics of potentially pathogenic cultivable bacteria in classroom bio-aerosols before, during and after the development of teaching activities.

MATERIAL AND METHODS

This is a qualitative and quantitative study carried out in the classroom of ``Universidade no Noroeste de São Paulo``, to evaluate the presence and resistance to antibiotics of potentially pathogenic cultivable bacteria in classroom bio-aerosols before, during and after the development of teaching activities. Air samples from the studied environment were collected according to the methodology described by Kalwasińska, Burkowska and Wilk (2012) and Hayleeyesus and Manaye (2014).

Sampling was carried out by exposing Petri dishes containing agar media: Blood Agar, Mac Conckey and Baird Parker. Each plate containing the sterile culture medium was opened for 50 minutes at a height of 1.5 meters from the floor, arranged in the central part of the room. This procedure was carried out before, during (50 and 100 minutes) and after classes (50 and 100 minutes). The plates were capped, identified and individually packaged in plastic bags to avoid possible cross-contamination. Transport to the Microbiology laboratory was carried out in isothermal boxes with an internal temperature of 3oC.

The plates were incubated at 37oC for 24-48h for bacteria and yeast. After the incubation periods, CFU (Colony Forming Units) were counted. After counting, colonies were selected and subjected to Gram staining.

To identify Gram-positive bacteria, catalase, oxidase and coagulase tests were carried out. Gram-negative bacteria were identified by biochemical tests of Motility, Malonate, Indole

Production, Urease Production, Gelatinase Production, Phenylalanine Deaminase Production, Citrate Use, Methyl Red (VM) and Voges-Proskauer Reaction (VP), Lactose fermentation, Esculin Hydrolysis and Nitrate reduction.

All bacterial strains were subjected to susceptibility testing against the following antimicrobials: penicillin (10UI), oxacillin (1mg), ciprofloxacin (5mg), gentamicin (10mg), clindamycin (2mg), erythromycin (15mg), sulfamethoxazole + trimethoprim (25mg) and vancomycin (30mg). To carry out these tests, the isolated strains will be collected in tubes containing 3mL of BHI broth and incubated at 35o C until they reach the standard 0.5 on the MacFarland scale. After incubation, the diluted and seeded cultures with the aid of sterile swabs on plates containing Mueller-Hinton Agar and, after approximately 3 minutes, the time necessary for the surface of the medium to dry, the discs containing the antimicrobials will be placed. The reading was carried out after 18 hours of incubation at 35o C by measuring the inhibition halos, using a millimeter ruler.

The data were tabulated and analyzed using descriptive statistics and tables and figures were created.

RESULTS AND DISCUSSION

Figure 1 presents the results regarding the average values of mesophiles isolated in the classroom before, during and after the presence of students. The average number of students during the three months of the experiment was 48. An increase in microorganisms was observed during the course of the classes, there was an increase in colony forming units (CFU) and a reduction after classes.

In the sampling prior to the start of the class: 605 UFC m^{3-1}, in the first 50 minutes of class were quantified: 2982 UFC m^{3-1}, and after 100 minutes there were 4398UFC m^{3-1}. There was a gradual decrease in microorganisms after classes ended, the average UFC m^{3-1} was 3061 and 1023 after 50 and 100 minutes, respectively (Figure 1).

In work carried out in classrooms at 11 universities, Zhao et al. (2022) found that the average annual concentration of cultivable bacterial aerosols was 494 ± 263 UFC m^{3-1}. The authors point out that human presence becomes the main source of bacterial aerosols cultivable in closed environments.

In research carried out to determine levels and types of microorganisms suspended in the air in areas of the Faculty of Biology at ``Universidad de Murcia`` (Spain), viable bacteria and fungi were evaluated in the presence or absence of personnel to determine contamination introduced by human activity. The results showed that comparing the microbial density inside with that in the outside air indicated a higher bacterial concentration inside. The majority of bacteria identified were Gram-positive cocci of the genera: *Micrococcus*, *Staphylococcus* and *Streptococcus* (SOTO et al., 2009).

Andualem et al. (2019) evaluated the presence of bioaerosols in classrooms in public elementary schools in the city of Gondar and found that the overall total average bacterial load was 2.826,35 UFC m^{3-1} in the morning 4.514,63 UFC m^{3-1} in the afternoon. The bacteria isolated by these authors were *Staphylococcus aureus*, species of *Staphylococcus* coagulase-negative and *Bacillus*.

In the present research, they were isolated in university classrooms before carrying out the activities., *Staphylococcus aureus*, while during and after classes they were isolated: *Staphylococcus aureus, S. epidemidis, Streptococcus sp, Escherichia coli* (Table 1).

Period of sampling	Microrganisms
Before the class	*Staphylococcus aureus*
During the class	*Staphylococcus aureus, S. epidemidis, Streptococcus sp, Escherichia coli*
After the class	*Staphylococcus aureus, S. epidermidis, Streptococcus sp, Escherichia coli*

Table 1: Species of microorganisms isolated in university classrooms, before, during and after activities.

Indoor air quality problems in school environments can be even more serious than in other categories of buildings, due to higher occupancy density, poor sanitation in classrooms and insufficient supply of outside air, aggravated by frequent poor construction and maintenance of school buildings. Poor indoor air quality can also affect student performance and attendance, due to the health risks arising from exposure to environmental hazards (DAISEY et al, 2003, PATELLA et al., 2018). According to Andualem et al. (2019), due attention must be paid to controlling the physical factors that favor the growth and multiplication of bacteria in the internal environment of classrooms to safeguard the health of students and teachers at school.

The study of bacterial resistance to different antibiotics is essential due to the high incidence of bacteria and the high incidence of potentially pathogenic bacteria isolated in the present study. Samples of isolated bacteria were evaluated, *Escherichia coli*, (n=20), *Streptococcus sp* (n=8), *Staphylococcus aureus* (n=45) and *Staphylococcus epidermidis* (n=5)

According to table 2, E. coli was resistant to the antibiotics Chloramphenicol (85%), Sulfazotrim (70%), Ciiprofloxacin (40%), Amikacin, Kanamycin (25%), Streptomycin (10%), 5% of isolates were Resistant to Ampicillin, Enrofloxacin and Clindamycin and Neolmycin, however it was found that 100% were susceptible to Trimethoprim.

In relation to the data analyzed, the bacteria Streptococcus sp were found to be 100% resistant to Ampicillin, 95% to Amikacin, 90% to Clindamycin, 80% to Kanamycin, 70% to Sulfazotrim, 60% to Chloramphenicol, 50% to Cefotaxime, 40% to Trimethoprim and Ciprofloxacin, 35% to Enrofloxacin, 25% to Neomycin and 10% to Streptomycin. It was observed that P. aeruginosa showed broad resistance to antibiotics.

The research showed that *Staphylococcus aureus* was 100% Resistant to Ampicillin and Clindamycin, 70% to Sulfazotrim and Chloramphenicol, 60% Cefotaxime, 40% to CIprofloxacin, 35% to Amicaine, 25% to Streptomycin, 20% to Enrofloxacin and 15% to Neomycin. It is also worth mentioning that 100% of S. aureus antibiotics were susceptible to Trimethoprim and Kanamycin.

The bacteria: *Staphylococcus epidermidis* (Table 2) was 85% resistant to Amikacin, 80% to Ampicillin, 75% to Sulfazotrim, 50% to Kanamycin, 40% to Ciprofloxacin, 25% to Cefotaxime, 20% to Neomycin, Clindamycin and Chloramphenicol, 15% to Trimethoprim, 10% to Streptomycin and, finally, it was 100% susceptible to Enrofloxacin, which is a possible antibiotic of choice.

In recent years there has been a significant increase in antibiotic-resistant bacteria. The occurrence of these microorganisms can be associated with the abusive use of antimicrobials, without indication and in inadequate doses, and poor hygiene of fomites that act as enhancers for the development of resistant strains (ASLAM et al., 2018).

It is noteworthy that actions to control and reduce infections caused by resistant microorganisms are complex and must include not only the restriction of the sale of antibiotics under medical prescription, but other strategies, such as the implementation of educational practices for rational prescription, preparation and implementation of protocols, supervision of prescriptions, hand hygiene campaigns, monitoring,

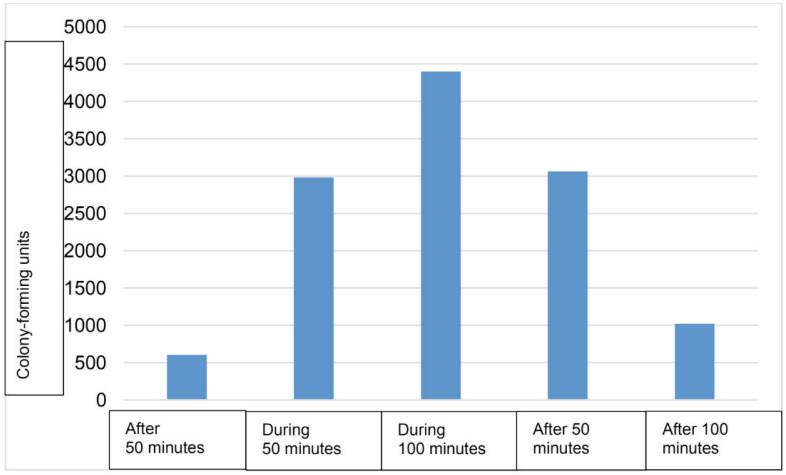

Figure 1: Average values of colony forming units (CFU) of total mesophiles isolated in the classroom before, during and after academic activities.

Antibiotics	*E. coli*	%	*Streptococcus*	%	*S. aureus*	%	*S. epidermidis*	%
Trimetoprim	Resistant	0	Resistant	40	Resistant	0	Resistant	15
Neomycin	Resistant	5	Resistant	25	Resistant	15	Resistant	20
Ampicillin	Resistant	10	Resistant	100	Resistant	100	Resistant	80
Enrofloxacin	Resistant	10	Resistant	35	Resistant	20	Resistant	0
Cefotaxime	Resistant	50	Resistant	50	Resistant	60	Resistant	25
Amikacin	Resistant	25	Resistant	95	Resistant	35	Resistant	85
Clindamycin	Resistant	10	Resistant	90	Resistant	100	Resistant	20
Ciprofloxacin	Resistant	40	Resistant	40	Resistant	40	Resistant	40
Kanamycin	Resistant	25	Resistant	80	Resistant	0	Resistant	50
Sulfazotrim	Resistant	70	Resistant	75	Resistant	70	Resistant	75
Streptomycin	Resistant	25	Resistant	10	Resistant	25	Resistant	10
Cloranfenicol	Resistant	85	Resistant	60	Resistant	70	Resistant	2

Table 2: Antibiotic resistance of *Escherichia coli*, *Streptococcus sp*, *Staphylococcus aureus* and *Staphylococcus epidermidis,* isolated in classrooms.

environmental sterilization measures so that health education can increasingly be achieved (ASLAM et al., 2018; OLIVEIRA et al., 2021). These measures must be carried out not only at the community level, but also at the hospital level. This reinforces the need for continuous assessments of medicine regulatory measures, aiming at the sustainability of reducing the incidence of resistant microorganisms.

Exposure to specific airborne bacteria indoors is associated with adverse infectious and non-infectious health outcomes. Classrooms are typical meeting spaces with high occupant density and mobility, and most classrooms are not well ventilated, which raises further concerns about poor indoor air quality. Unlike school classrooms (students generally aged 6 to 17), university classrooms are used alternately by teachers and students. Therefore, the risks of cross-infection for students and teachers increase greatly due to high mobility inside and outside university classrooms (ZHAO et al, 2022)

According to Andualem et al., (2019), poor indoor air quality is a major problem in the school environment due to the high number of students per classroom, insufficient supply of external air, poor construction and

maintenance of school buildings. Bacteria in indoor air represent a serious health problem. Determining the bacterial load in the indoor environment is necessary to estimate the health hazard and create standards for indoor air quality control. This is especially important in such densely populated installations.

Future interdisciplinary studies focusing on the chemical and biological process of airborne microorganisms, the airborne transmission of emerging pathogens and allergens, and the association between exposure to bioaerosols and the development and variations of the human microbiome and immune response are needed to elucidate the interactions of bioaerosols with the environment and individuals (SHEN, YAO, 2023). Park et al. (2021) suggest that epidemiological and clinical studies are needed to better understand the effect of teaching or classroom microbiomes on the health of staff and students.

FINAL CONSIDERATIONS

The evaluation of bacterial components in classroom bioaerosols and the antibiotic resistance of isolated strains is of great importance to avoid absenteeism in learning processes, as several diseases are triggered by staying in environments with poor air quality. The results of the assessment of bioaerosols in the classrooms of the higher education institution analyzed in this research showed the presence of potentially pathogenic bacteria (*Staphylococcus aureus, S. epidemidis, Streptococcus sp, Escherichia coli*), and the number of these was influenced by the presence of students in the environment. Additional studies are still needed to better elucidate the relationship between the microbiological quality of indoor air and the different factors that can affect it.

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