

MICROBIOLOGICAL QUALITY OF AIR AT DIFFERENT SECTIONS WITHIN SPIRITAN UNIVERSITY, NNEOCHI, ABIA STATE NIGERIA

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ABSTRACT

Airborne diseases have been described among debilitating ailments that are ravaging the health of mankind, especially individuals whose immune functions are deficient. This study was carried out to evaluate microbial quality of air at different confined environments at Spiritan University, Nneochi, Abia State. Microbial quality of air at different sections at the University was evaluated using passive method. Three prepared Petri dishes containing different selective media were exposed for 60 min at 10 selected areas. The outcome of the air sampling revealed the presence of *Pseudomonas aeruginosa* (6(25.00%), *Staphylococcus aureus* (4(16.67%), *Escherichia coli* (3(12.50%), *Streptococcus* species (3(12.50%), *Aspergillus fumigatus* (5(20.83%), and *Aspergillus niger* (3(12.50%). The highest microbial load was observed at the University Clinic (83.33 CfU/m³ for bacteria/ 4.17 CfU/m³ for fungi) while the least was observed at the Senate Chamber and Canteen (25.00 CfU/m³ for bacteria/ 2.50 CfU/m³ for fungi). The microbial load in all the sections sampled was within small and medium as stipulated by the European Community Commission (ECC). All the bacteria showed complete haemolysis on blood agar except *Streptococcus* species and *A. niger* that showed partial haemolysis. The susceptibility pattern revealed that *P. aeruginosa* yielded highest resistance against Ciprofloxacin 5(23.80) while *Cassyntha filiformis* extract showed the highest potency (5(23.80) against all the pathogens with zero resistance. This study has shown that pathogenic bacteria and fungi are found at different sections at Spiritan University, Nneochi.

KEYWORDS: Airborne, extract, load, isolate, microbial, susceptibility

1. INTRODUCTION

Microorganisms have been described as ubiquitous due to their presence in every environment such as hospitals, laboratories, lecture halls, market places, motor parks, homes etc. In the field of microbiology and other biological and chemical sciences, laboratory is an apartment that had been carefully constructed for the purpose of scientific researches and diagnoses often found in schools or at separate environments (Alfanda et al. 2023). In laboratories, different samples such as clinical, environmental, industrial, and food are optimized by a certified analyst or a trained medical laboratory scientist and microbiologists (Asif et al. 2018). Laboratory provides an avenue for microbial evaluation at different environments such as microbiological media and equipment.

Several researchers had worked on microbiological quality of air in laboratories found in public universities and hospitals, and other confined environments (market places, motor parks etc.) such as Ugranli et al. (2016), Asif et al. (2018), Baures et al. (2018) and Alfanda et al. (2023) but an indebt study on the microbiological quality of air in private institutions' environments within Abia State and South East geopolitical zone is inadequate based on public health implication.

Moreover, there is an increasing rate of air borne diseases that are emerging on daily basis, and in Nigerian Universities, emergence of these diseases could be disastrous to the entire nations when students and staff cannot be guaranteed safe thereby resulting in a decline in education and low productivity.

Due to delicate services that are performed in universities, laboratories and other confined environments, there is need to ensure clean and safe environment in accordance to the standard stipulated by the Department of Occupational Safety and Health (DOSH) under Industrial Code of Practice (ICOP, 2010) on Indoor Air Quality. According to ICOP, laboratories and related confined environments are to be equipped with Mechanical Ventilation Air Conditioning (MVAC) system in order to eliminate airborne pollutants, and to control temperature and humidity. However, most laboratories, offices, and other confined environments lack ventilation system, and few available ones are dilapidated due to poor maintenance (ICOP, 2010).

Research had shown that microbial contaminants in confined environments emanate from samples, humans, and unsanitary practices such as indiscriminate disposal of water generated, thus promoting conducive environment for proliferation of both bacterial and fungal pathogens (Yan et al. 2016; Awad et al. 2018).

It is worthy to note that evaluation of microbiological quality of air in confined environments is paramount to the health of the public, especially individuals within university environment. (Alfanda et al. 2023). The presence of bacteria and fungi such as *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter*, *Pseudomonas aeruginosa*, *Penicillium*, *Aspergillus*, *Alternaria*, *Microsporum*, *Trichoderma* among others in the air poses threat to humans due to pathogenic potentials of the aforementioned bacterial and fungal pathogens.

Several debilitating illnesses had been attributed to airborne pathogens such as aspergillosis, rhinitis, pneumonia, allergies, asthma, and conjunctivitis among others caused mainly by inhalation of fungal spores and bacterial pathogens (Yan et al. 2016; Awad et al. 2018).

Everywhere you look, fungi thrive quietly around us, sending tiny spores floating on air currents. In Nigeria, the most often found types riding those breezes are *Aspergillus Niger*, *Aspergillus flavus*, *Penicillium spp.*, *Fusarium spp.*, *Cladosporium spp.*, along with *Mucor spp.* [Olise et al., 2023]. Health risks rise when certain kinds step into the spotlight - *Aspergillus* among them - with hidden dangers tucked inside toxic byproducts. Breathing these in might lead to a condition known as aspergillosis, particularly troubling for people already fighting weakened immune defenses.

Research had also revealed that airborne infection had been a major threat to public health globally (Zahid et al. 2016; Asif et al. 2018). Air as an agent of transmission of infectious diseases is dangerous because everyone in the environment inhales oxygen found in the air for survival and this shows that the need to ensuring safe inhalation cannot be undermined (Ugranli et al., 2016). Neglecting the impact of air microbes in the spread of infectious disease could expose many individuals to airborne diseases, which are capable of reducing human population (Asif et al. 2018). This clearly indicates that every section in the environment where humans are found is an important source of airborne pathogens and appropriate measures are to be employed in order to protect the health of individuals in the society, especially those within laboratory setting and University (Asif et al. 2018).

2. MATERIALS AND METHODS

In air sampling, well-accepted method was used as described in the study published by Alfanda et al. (2023). The following media (MacConkey agar, Mannitol salt agar, Nutrient agar, and Potato dextrose agar) were prepared following the instructions of the manufacturer. The prepared and solidified agar plates were exposed at different sections in the laboratory namely; waiting area (A), laboratory entrance (B), working area (C), Consultation room (D), laboratory compound (E), and area far away from the laboratory (Control). All the agar plates were allowed opened for 30 minutes, then covered and incubated at 37°C for 48 h and at 30°C for 5 days. The plates were observed for growth and colonies were counted and documented. The colony forming units (Cfu) obtained were expressed as Cfu/m³ using Omelyansky's formula:

$$N = \frac{5a \times 10^4}{(bt)}$$

Where N= Cfu/m³ of air in the internal environment; b= Diameter of Petri dish (100 mmi) a= Number of colonies; t= time of exposure in minutes.

2.1 CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATES

The isolates were sub-cultured on nutrient agar (Biotech), incubated in inverted position at 37±2°C for 24hrs. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010), biochemical reactions (Cheesbrough, 2010). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates

Gram staining: This was carried out using the modified method of Cheesbrough (2010). In this process, a thin smear of the culture was prepared on a clean grease free slide (75 mm× 25 mm× 1 mm), air dried and heat fixed. The smear was flooded with crystal violet solution for 60 seconds and rinsed with water. It was then covered with Gram's iodine for 60 seconds and rinsed with water. Alcohol (95% w/w ethanol) was used to decolourize the slide content for 10 seconds and rinsed with water. The smear was then counter stained with safranin solution for 60 seconds, rinsed and air dried. The stained smear with a drop of immersion was then observed under the light microscope using oil immersion objective lens.

2.2 BIOCHEMICAL REACTIONS

The following biochemical tests were carried out:

Catalase test: This was carried out as described by Cheesbrough (2010). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then a drop of 30% hydrogen peroxide (H₂O₂) was added on the smear. Prompt effervescence indicated catalase production.

Coagulase test: The test was carried out as described by Cheesbrough (2010). A smear of the isolate was made on a clean grease-free microscopic slide that contained a drop of physiological saline, a drop of human plasma was added, and gently mixed, after 10 seconds, presence of clump indicated positive result.

Oxidase test: The test was carried out as described by Cheesbrough (2010). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

2.3 SCREENING THE BACTERIAL AND FUNGAL ISOLATES FOR PATHOGENIC POTENTIALS

In vitro technique: The *in vitro* pathogenic potentials of the isolates were carried out by testing for the ability of the isolates to produce haemolysis on blood agar as described in the study published by Zahid et al. (2016) and Odebode et al. (2020)

Haemolysis on blood agar: Blood agar was prepared following the manufacturer's instruction using SDA, the medium was allowed to solidify and aseptically streaked using the test isolates. This was incubated in an inverted position at $35\pm 2^{\circ}\text{C}$ for 24 h. The presence of clear zones around the colonies was an indication of β haemolysis, partial zones of inhibition were detected by greenish to gray zones which indicated γ -haemolysis while absence of clear zones as described by Zahid et al. (2016) indicated no haemolysis.

2.4 ANTIBACTERIAL AND ANTIFUNGAL SUSCEPTIBILITY TESTING

The agar well diffusion technique was employed to evaluating the antibacterial and antifungal activities of the selected conventional antifungal agent. Mueller Hinton agar and Sabouraud dextrose agar (SDA) were prepared following the instruction of the manufacturer, and poured on Petri dishes to solidify. Four different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL) of the medicinal plants' extracts, conventional antibacterial and antifungal agents were prepared. The isolates were seeded on the plates using a flamed wire loop followed by boring of wells using flamed cork borer (5 mm diameter). The dilutions were aseptically introduced into the wells using sterile plastic dropper. The plates were incubated at 37°C for 24 – 48 h for bacteria at $30\pm 2^{\circ}\text{C}$ for 5 days, then, observation of the plates was carried out (Jabeen et al., 2013).

2.5 STATISTICAL ANALYSIS

Statistical software: SPSS Version 25.0 was used to analyse the data and p value <0.05 was taken as significant.

3. RESULTS

Table 1 below revealed the sanitary standards for non-industrial premises according to European community commission (ECC) in air quality evaluation. The sampling sections and number of bacteria and fungi isolated are presented in Table 2 below. The result revealed that there are microbial growths in all the sections of the University sampled after 30 minutes exposure. The sampled sections yielded different fungal and bacterial colony forming units as follows; Senate Chamber ($\text{Cfu}/\text{cm}^2/25.00$), FAMS ($29.17 \text{ Cfu}/\text{cm}^2/1.67 \text{ Cfu}/\text{cm}^2$), FL ($26.67 \text{ Cfu}/\text{cm}^2/2.50 \text{ Cfu}/\text{cm}^2$), FNAS ($31.67 \text{ Cfu}/\text{cm}^2/3.33 \text{ Cfu}/\text{cm}^2$), ($\text{Cfu}/\text{cm}^2/\text{Cfu}/\text{cm}^2$), MCB Lab ($33.33 \text{ Cfu}/\text{cm}^2/1.67 \text{ Cfu}/\text{cm}^2$), Male Hostel ($45.83 \text{ Cfu}/\text{cm}^2/2.50 \text{ Cfu}/\text{cm}^2$), and Auditorium ($41.67 \text{ Cfu}/\text{cm}^2/1.67 \text{ Cfu}/\text{cm}^2$). The overall result revealed that the highest number of bacteria and fungi was isolated in plates exposed at the Clinic ($83.33 \text{ Cfu}/\text{cm}^2/4.17$), followed by female hostel ($50.00 \text{ Cfu}/\text{cm}^2/2.50 \text{ Cfu}/\text{cm}^2$) while the least was Canteen ($25.00 \text{ Cfu}/\text{cm}^2/2.50 \text{ Cfu}/\text{cm}^2$).

The cultural and morphological features of the bacterial and fungal isolates are presented in Table 3 below. The result revealed that two Gram positive bacteria and two Gram negative bacteria were isolated while two Molds were the only fungal isolates. The cultural features of the bacterial isolates revealed yellowish colonies on MSA, greenish discoloration on blood agar, colourless on MacConkey agar, and pinkish non mucoidal colony on MacConkey agar and their morphological features were cocci, rod, and cocci, respectively. Similarly, the cultural features of the fungal isolates revealed blue-green colonies and black powdered colony with distinguishing textures and appearances on SDA while their morphology revealed septate hyphae and conidia head with different arrangement of phialides.

The Biochemical features and pathogenic potentials of the bacterial isolates are presented in Table 4 below. All the bacterial isolates were positive to catalase while *Pseudomonas aeruginosa* was only positive to oxidase and *Staphylococcus aureus* was only positive to coagulase. The pathogenic potentials of the isolates revealed that *S. aureus*, *P. aeruginosa*, *Escherichia coli* and *Aspergillus fumigatus* exhibited complete haemolysis while *Streptococcus pneumoniae* and *Aspergillus niger* showed partial haemolysis on blood agar.

The susceptibility pattern of the isolates to conventional antimicrobial agents and medicinal extracts is presented in Table 5. The result revealed that most of the isolates were resistant to Ciprofloxacin and Ampicillin, though Ciprofloxacin was more potent compared to Ampicillin. Meanwhile, medicinal extracts were effective against most of the pathogens, though *Cassytha filiformis* recorded zero resistance 5(23.80) while *Pterocarpus santalinoides* recorded a single resistance by *Pseudomonas aeruginosa*. Furthermore, the conventional antibiotics were not effective against the fungal isolates while the medicinal extracts exert a substantial antifungal effect on the fungal isolates 3(14.29).

Table 1: Sanitary standards for non-industrial premises according to European community commission (ECC) in air quality evaluation

Group of microbe	Range of Value (CFU/m ³)	Degree of pollution
Bacteria	Less than 50	Very small
	50 -100	Small
	100-500	Medium
	500 -2000	High
	Greater than 2000	Very high
Fungi	Less than 25	Very small
	25 -100	Small
	100-500	Medium
	500 -2000	High
	Greater than 2000	Very high

Table 2: Sampling sections and distribution of microorganisms

Sample	Sampling section	Number of plate exposed	Number of bacterial colony isolated	Number of fungal colony isolated	Cfu/cm ³ For Bacteria	Cfu/cm ³ for Fungi
A	Senate chamber	3	30	3	25.00	2.50
B	Canteen	3	30	4	25.00	3.33
C	FAMS	3	35	2	29.17	1.67
D	FL	3	32	3	26.67	2.50
E	FNAS	3	38	4	31.67	3.33
F	MCB Lab	3	40	2	33.33	1.67
G	Clinic	3	100	5	83.33	4.17
H	Male Hostel	3	55	3	45.83	2.50
I	Female Hostel	3	60	3	50.00	2.50
J	Auditorium	3	50	2	41.67	1.67

FAMS= Faculty of Arts, Management and Social Sciences; FL= Faculty of Law; FNAS= Faculty of Natural and Applied Sciences

Table 3: Cultural and morphological features of the isolates

Bacterium	Cultural feature	Morphological feature	Medium
<i>Staphylococcus aureus</i>	Yellowish colonies	Gram positive cocci in clustered shape	MSA
<i>Pseudomonas aeruginosa</i>	Colourless, smooth, slightly raised colonies	Gram negative rod, which appears singly mostly	MacConkey agar
<i>Escherichi coli</i>	Pinkish, non-mucoid,	Gram negative rod, which appears singly	MacConkey agar
	Smooth colonies		
<i>Streptococcus</i> species	Greenish discoloration of the agar plates due to alpha haemolysis (partial lysis of blood cells), gray-white colonies, smooth, moist, and translucent/opaque	Gram positive cocci arranged in chains	Blood agar
Fungus			
<i>Aspergillus fumigatus</i>	Blue-green colonies, colorless reverse pigmentation, powdery, and velvety texture	Septate and clear hyphae with columnar conidia heads with uniseriate (single row of phialides)	SDA
<i>Aspergillus niger</i>	Black seriated, powdered, colourless reverse pigmentation	Branched, septate hyphae and brush0like clusters of conidiophores(phialides)	SDA

SDA= Sabouraud dextrose agar

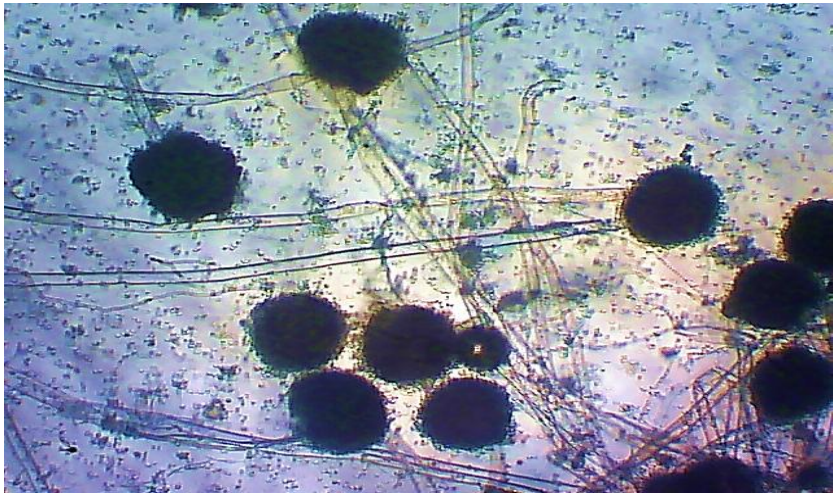


Plate 1: *Aspergillus niger*



Plate 2: *Aspergillus fumigatus*

Table 4: Biochemical features and pathogenic potentials of the bacterial isolates

Isolate	Catalase	Coagulase	Oxidase	Blood agar
Staphylococcus aureus	Positive	Positive	Negative	Beta haemolysis
Pseudomonas aeruginosa	Positive	Negative	Positive	Beta haemolysis
Escherichia coli	Positive	Negative	Negative	Beta haemolysis
Streptococcus pneumoniae	Positive	Negative	Negative	Alpha haemolysis
Aspergillus fumigatus	Not applicable	Not applicable	Not applicable	Beta haemolysis
Aspergillus niger	Not applicable	Not applicable	Not applicable	Alpha haemolysis

Note: Beta haemolysis indicates complete haemolysis of red blood cells (potential pathogens)
Alpha haemolysis indicates partial haemolysis of red blood cells (may or may not be pathogenic)

Table 5: Susceptibility pattern of the isolates to conventional antimicrobial agents and medicinal extracts

Isolate	Number of Isolate	CPX R %	S	AMP R %	S	PS R %	S	CF R %	S
<i>Pseudomonas aeruginosa</i>	6	5(23.80)	1(4.76)	3(14.29)	3(14.29)	1(4.76)	5(23.80)	0(0.00)	6(28.57)
<i>Staphylococcus aureus</i>	4	3(14.29)	1(4.76)	4(19.05)	0(0)	0(0.00)	4(19.05)	00.00	4(19.05)
<i>Escherichia coli</i>	3	1(4.76)	2(9.52)	3(14.29)	0(0)	0(0.00)	3(14.29)	0(0)	3(14.29)
<i>Streptococcus pneumonia</i>	3	1(4.76)	2(9.52)	3(14.29)	0(0)	0(0.00)	3(14.29)	0(0)	3(14.29)
<i>Aspergillus fumigatus</i>	5	5(0.00)	0(0.00)	5(0.00)	0(0.00)	0(0.00)	5(23.80)	0(0.00)	5(23.80)
<i>Aspergillus niger</i>	3	3(0.00)	0(0.00)	3(0.00)	0(0.00)	0(0.00)	3(14.29)	0(0.00)	3(14.29)

R= Resistance; S= Susceptible; CPX= Ciprofloxacin; AMP= Ampicillin; PS= *Pterocarpus santalinoides*; CF= *Cassytha filiformis*

4. DISCUSSION

Airborne diseases have been described among debilitating ailments that are ravaging the health of mankind, especially individuals whose immune functions are deficient. Screening for the quality of air at different areas that humans frequently dwell has been described as appropriate to achieving global health goal. This study was carried out to assess the air quality at different sections within Spiritan University, Nneochi, Abia State. The highest number of bacteria and fungi observed at the Clinic could be attributed to frequent visit of patients, and research had revealed that those common human pathogens such as *Staphylococcus aureus* and *Escherichia coli* are liberated mostly from humans. Similar observation was reported by Ana et al 2025) who screened air quality at tertiary University hospital in Ibadan and Adebowale et al. (2015) who evaluated bioaerosols in Osun State motor parks and markets and reported the presence of *S. aureus*, *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Aspergillus* spp., and *Fusarium* spp. Generally, the bacteria and fungi discovered in this study supported the documentation of several researchers (Dong et al. 2016; Okunola et al. 2020; Oluwafemi et al. 2021). The microbial load (expressed as colony forming unit per cubic meter (CFU/m³) observed at all the sections in the University was within small and medium as stipulated by the European community commission (ECC) for sanitary standards for non-industrial premises, which indicates that the environment has not been heavily contaminated by human pathogens unlike the microbial load obtained by Okunola et al. (2020) at tertiary University hospital in Ibadan that yielded 2962.96 CFU/m³. It is worthy to note that another study at a private institution in Benin, Nigeria obtained low microbial load (7–440 CFU/m³ and fungal aerosols of 3–45 CFU/m³) that corresponds to the findings in this study. This further supports the fact that most private Universities in Nigeria are not overcrowded like the State and Federal Universities.

The cultural, morphological, and biochemical features of microorganisms observed in this study conform to the features documented by several researchers (Adebowale et al. 2015; Amengialue et al. 2017; Oke et al. 2026) who evaluated air quality at different environments such as Universities, hospitals, market places, motor parks etc. However, the pathogenic potentials of the isolates in this study call for serious attention because diseases that are caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Echerichia coli*, and *Aspergillus fumigatus* could be highly devastating. Similar findings were reported by several researchers (Adebowale et al.2015; Okunola et al. 2020; Amengialue et al. 2017).

The low efficacy observed in the conventional antibiotics (Ciprofloxacin and Ampicillin) was due to high antimicrobial resistance. These common human pathogens have been a threat to the public due to emergence of resistance strains, and this study has also observed the proliferation of the pathogens in the presence of the antimicrobial agent. This resistance could be attributed to the presence of resistance gene in their plasmids, which enable them to counter the activities of antibiotics using different mechanisms such as drug deactivation, alteration of structure etc. This supports the deduction made by several researchers (Adebowale et al. 2015; Okunola et al. 2020). However, the extracts from medicinal plants (*Pterocarpus santalinoides* and *Cassytha filiformis*) were effective against the pathogens, especially *Cassytha filiformis* that yielded zero resistance. This high efficacy could be attributed to the presence of bioactive components such as flavonoids, tannins, alkaloids, saponins, glycosides etc. This observation corresponds to the documentation by several researchers (Adebowale et al. 2015; Oluwafemi et al. 2021; Okunola et al. 2020) who investigated susceptibility pattern of common human pathogens from environmental samples.

5. CONCLUSION

This study has shown that different sections or confined environments at Spiritan University, Nneochi contain bacteria and fungi such as *Pseudomonas areuginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* species, *Aspergillus fumigatus*, and *Aspergillus niger*. The microbial load of these microorganisms was small and medium, which indicates that the environments are safe for human inhabitation as stipulated by European community commission (ECC) for sanitary

standards for non-industrial premises. The microorganisms observed were potential pathogens, except *Aspergillus niger*. Also, the pathogens showed resistance to Ciprofloxacin and Ampicillin while the extracts from *Pterocarpus santalinoides* and *Cassipoupa filiformis* inhibited the growth of all the pathogens. Therefore, it is worthy to note that all the confined environments investigated at Spiritan University, Nneochi are safe for inhabitation though appropriate care ought to be taken due to the presence of potential pathogens in minute microbial load.

6. RECOMMENDATION

At the end of this research, the following were recommended.

- (i) Sanitary measures such as nursing barrier (use of nose masks, washing of hands etc.) should always be employed
- (ii) Frequent testing of students
- (iii) Proper disposal of refuses and clearing of bushes
- (iv) Frequent air evaluation within the University

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