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Full Length Research

Microbial air quality assessment of some selected food industries in Oluyole Ibadan, Nigeria

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ABSTRACT: Air assessment is an essential component of environmental monitoring systems, helping to evaluate air quality, the health of personnel, and product safety in food industries. This study aimed to assess the microbial air quality of selected food industries in Ibadan and to investigate the presence of antibiotic-resistant bacteria. Microbial load enumeration was carried out using the settle plate method, followed by biochemical identification, pathogenicity, and antibiotic sensitivity tests. Selected isolates were also characterized molecularly. The results revealed that two out of the three sites exceeded the European Union (EU) and World Health Organization (WHO) Good Manufacturing Practices limit of 50–100 CFU for air microbial counts. The isolates identified in this study included Serratia, Bacillus, Staphylococcus, Proteus, Micrococcus, and Enterobacter. Three isolates exhibited positive reactions to hemolysis and DNase tests. While 100% of the isolates were susceptible to ciprofloxacin and 89.5% were susceptible to meropenem, resistance was observed against ceftriaxone, tetracycline, penicillin, vancomycin, gentamicin, and trimethoprim/sulfamethoxazole. Alarmingly, 57.8% of the isolates displayed multiple antibiotic resistance patterns, posing significant threats to public health. Therefore, the finding indicates the need for enhanced air quality control and hygiene practices in food industries, as well as periodic monitoring of the air environment.

Keywords: Air assessment, environmental monitoring, EU, food industries, microbial quality, WHO GMP.

INTRODUCTION

The food industry represents an occupational environment that contains raw materials, flour, flavourings, and other ingredients. Workers in such environments may face exposure to bioaerosols, which include bacteria, viruses, fungi, algae, and dust (Theisinger and de Smidt, 2017). The suspensions of microscopic solid or liquid particles in the air are defined as aerosols (Ferguson et al., 2019). The air is one of the microbial vectors that is thought to contribute to cross-contamination (Masotti et al., 2019). Therefore, microbial analysis of air is one of the most vital investigations for determining microbial air pollution (Moelling and Broecker, 2020). Bioaerosols contain microorganisms and their components such as fungi. bacteria, endotoxins, mycotoxins, and allergens. Such organisms are well-known as normal components of both indoor/outdoor air (Niazi et al., 2015).

Global expansion of drug resistance makes illnesses more difficult to cure and fatal, antibiotics are becoming

less and less effective. Despite its emerging significance to public health concerns, the presence of antibiotic resistance genes (ARGs) in urban air has not received significant attention (Li et al., 2018). According to this research, airborne transmission may actively contribute to environmental proliferation and exposure antimicrobial resistance, in addition to other modes of transmission (Lis et al., 2009). Most of the time, complex mixes of allergens, toxins, and other diverse substances result in the development of bioaerosols (Humbal et al., 2018). Exposure to bioaerosols is frequently linked to several negative health impacts (e.g., Cancer, allergies, acute toxic effects, and infectious diseases). The most extensively researched topic has been respiratory symptoms and lung function impairment, which are among the most significant health issues brought on by bioaerosols (Chretien et al., 2015).

Bio-aerosol is a source of biological hazards in food

production environments and, antibiotic-resistant bacteria in the air is an emerging health concern. Therefore, microbial contamination of air in food production facilities is a major problem. There is a dearth of information about bioaerosols in the food industry and with the COVID-19 pandemic, it became essential to observe the aerosols in our environment, which have an adverse effect on the health of the public. This study aimed to assess the microbial air quality and product quality of different food industries in Oluyole Ibadan, as well as determine the presence of antibiotic-resistant bacteria.

MATERIALS AND METHODS

Sample site

The study sites are located at the Oluyole industrial axis, in Oluyole Local Government Area, Ibadan, Southwestern Nigeria. Ibadan is the capital of Oyo state and the third largest metropolitan area by population also one of the fastest growing industrial states in Nigeria, the largest metropolitan geographical area, Ibadan is located between latitude 7° 02' 49" and 7° 43' 21"N longitude 3° 31' 58" and 4° 08' 20"E. The mean annual temperature is 28°C, ranging between 37°C and the mean annual rainfall is 1,205mm, with a relative high humidity of about 74.5% all year round (UNDATA, 2013).

This study was conducted in three food industries where they produce vegetable cooking oil, bread and drinking water. These three food industries were coded as Sites A, B and C respectively. To ensure that all locations were covered, samples were collected at various sections of the food industry. Points where the samples were collected include the laboratory, stores, crushing section, refinery plant, solvent extraction plant, reception, and packaging unit.

Collection of samples

Air samples were collected using the settle plate method, as described by Olaitan and Bashir (2018). Standard Petri dishes containing media were exposed to the air for 15–30 minutes to collect biological particles that settled on the media. All samples were immediately transported to the Department of Microbiology laboratory, University of Ibadan, for incubation and further analysis. Sampling was conducted between March and July 2022. For each industry, samples were collected in two batches: one during the dry season and the other during the rainy season. This approach was based on previous studies, such as Pepper and Gerba (2015), which demonstrated differences in microbial load between the two seasons.

Enumeration of bacteria and fungi

The aerobic mesophilic bacteria and fungi counts from the

air samples were determined using the settle plate method, involving standard Petri dishes containing appropriate media. The Petri dishes were exposed to the air for 15–30 minutes to collect biological particles that settled on the media. Nutrient agar and potato dextrose agar were prepared and used as the culture media. After the collection of air samples, they were transported to the University of Ibadan for incubation and further analysis, following the protocol described by Olaitan and Bashir (2018).

Identification of bacteria

The biochemical tests conducted include Catalase, Oxidase, Gram's staining, Indole, Triple Sugar Iron (TSI), Methyl Red (MR), Urease, Carbohydrate fermentation test, Starch hydrolysis test, Citrate utilization test. This test was done to identify the bacteria. Molecular Characterization of bacteria isolates was done using 16S rNA sequencing. Hemolysis, Gelatinase, Lecithinase and DNase test was conducted to determine the pathogenicity of the bacteria.

Antibiotics susceptibility profile

The antibiotic susceptibility patterns of the isolates were determined using the disc diffusion method on Mueller-Hinton agar. Inhibition zone sizes were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2021). Eight antibiotics discs were used, and these include Tetracycline (TE; 30ug), penicillin (PG; 10ug), ciprofloxacin (CIP; 30ug), ceftriaxone (CFM; 30ug), trimethoprim/sulphanethoxazole (SXT; 1.25ug/23.75ug), gentamicin (CN; 10ug), vancomycin (VA; 30ug), meropenem (MEM; 10ug) (Kabir et al., 2016). A fresh culture of the isolate was obtained to carry out the antibiotic susceptibility test, a sterile cotton swab was dipped into the sterile test tube containing the suspension, and the cotton swab was rotated at the edge of the test tube to remove the excess liquid. The swab was spread across the petri dish containing Mueller Hinton agar, then the antibiotic disc was impregnated on the agar. The plates were inverted and incubated at 37° C after 18 -24 hours the plates were observed, and the inhibition zones were measured using a meter rule to the nearest whole millimetre (mm) and interpreted using (CLSI, 2021).

RESULTS

The results of the total aerobic mesophilic bacteria and fungi count present in the air of the three food industries are shown in Table 1. The highest microbial air count is 164 CFU for site A and the lowest is 32 CFU for site C. According to the result, the rainy season which is 2nd batch has more microbial load due to the activities in the air that season due to low temperature.

Table 1. Aerobic mesophilic bacteria and fungi count for the two batches of samples.

| Parameters | Samples | Sites | Mean of 1 st batch | Mean of 2nd batch | Standard Deviation | Limit for viable Particles |
|------------|-----------|-------------|----------------------------------|-------------------|-----------------------|-------------------------------|
| AMBC | Air (CFU) | A B C | 144 95 32 | 164 138 44 | 56.5 97.3 36.6 | 50-100 CFU (EU&WHO GMP) |
| AMFC | Air (CFU) | A B C | 8 7 6 | 11 10 6 | 3.8 4.4 4.3 | 50-100 CFU (EU&WHO GMP) |

Key: AMBC=Aerobic Mesophilic Bacteria Count, AMFC= Aerobic Mesophilic Fungi Count.

Table 2. Pathogenicity tests of bacteria isolates obtained from air samples at different points in Sites A, B, and C.

| Organisms | Haemolysis | DNase | Lecithinase | Gelatinase | |
|-------------------------|------------|-------|-------------|------------|--|
| Micrococcus luteus | - | - | - | - | |
| Staphylococcus sp. | + | + | - | - | |
| Proteus sp. | - | - | - | - | |
| Enterobacter hormaechi | - | - | - | - | |
| Serratia marcescens | + | + | - | - | |
| Myroides marinus | - | - | - | - | |
| Providencia sp. | - | - | - | - | |
| Pluralibacter gergoviae | - | - | - | - | |
| Bacillus sp. | + | + | - | - | |

Table 3. The frequency of occurrence of isolates obtained from the air sample of Sites A, B and C. (n=38).

| Probable organisms | Site A | Site B | Site C | Con | Total | Percentage (%) |
|-------------------------|--------|--------|--------|-----|-------|----------------|
| Proteus mirabilis | 3 | 1 | - | 1 | 5 | 13.2 |
| Proteus vulgaris | 3 | 2 | 1 | - | 6 | 15.8 |
| Micrococcus luteus | 4 | 5 | - | 1 | 10 | 26.3 |
| Bacillus aerius | 2 | 1 | - | 1 | 4 | 10.5 |
| Bacillus paramycoides | 2 | - | - | - | 2 | 5.3 |
| Staphylococcus spp. | 2 | 2 | - | - | 4 | 10.5 |
| Enterobacter hormaechi | 2 | - | - | - | 2 | 5.3 |
| Myroides Marinus | - | 1 | - | - | 1 | 2.6 |
| Serratia marscens | 1 | - | - | - | 1 | 2.6 |
| Providencia spp. | 2 | - | - | - | 2 | 5.3 |
| Pluralibacter gergoviae | - | - | 2 | - | 2 | 5.3 |

Key: Con: - Controlled environment.

A total of 38 microorganisms were isolated from all plant air samples and they were characterized and identified through biochemical tests. Some isolates were identified as *Proteus*, *Micrococcus luteus*, *Staphylococcus*, *Providencia*, *Enterobacter*, *and Bacillus*. The pathogenic activity of the isolates was examined in Table 2, three isolates showed pathogenic activity and were identified as *Serratia marcescens* and *Bacillus paramycoides*.

The percentage frequency of occurrence of the isolates obtained from the air assessment of site A, site B, site C and Controlled Environment is shown in (Table 3) which

shows that *Micrococcus luteus* had the highest frequency of occurrence (26.3%), followed by *Proteus vulgaris* (15.8%) with *Myroides Marinus* and *Serratia marscens* being the least (2.6%).

All isolates were susceptible to ciprofloxacin as the highest (100%) followed by meropenem (89.5%) and penicillin as the least (2.6%). The Antibiotics Susceptibility Profiling (%) of the bacteria isolates from Site A, Site B, Site C and Controlled Environment was shown in (Table 4). From the antibiotics susceptibility profiling test, eight antibiotics were used for the analysis the antibiotics

Table 4. Antibiotics susceptibility profiling (%) of bacteria isolates from air samples of sites A, B and C.

| Antibiotics | N | Resistant | Intermediate | Susceptible |
|-------------------------------|----|-----------|--------------|-------------|
| Ciprofloxacin | 38 | 0 | 0 | 38(100%) |
| Tetracycline | 38 | 24(63%) | 2(5.3%) | 12(31.6%) |
| Trimethoprim/Sulfamethoxazole | 38 | 8(21.1%) | 2(5.3%) | 28(73.7%) |
| Meropenem | 38 | 1(2.6%) | 3(7.9%) | 34(89.5%) |
| Ceftriaxone | 38 | 24(63.2%) | 0 | 14(36.8%) |
| Penicillin | 38 | 12(31.6%) | 25(65.8%) | 1(2.6%) |
| Vancomycin | 24 | 20(83.3%) | 3(12.5%) | 1(4.7%) |
| Gentamicin | 14 | 4(28.6%) | 7(50.0%) | 3(21.4%) |

Key: - N: - Number of isolates.

Table 5. Multiple Antibiotic resistance profile of isolates from different sites.

| Isolates | Resistance pattern | Multiple R-type | Frequency |
|---------------------|---------------------|-----------------|-----------|
| Drotous ann | TE, CRO, VA | 3 | 5 |
| Proteus spp. | TE, SXT, CRO, VA | 4 | 2 |
| | TE, CRO, VA | 3 | 2 |
| Missossossossossos | SXT, PG, VA | 3 | 1 |
| Micrococcus luteus | TE, SXT, VA | 3 | 1 |
| | TE, CRO, VA, CN | 4 | 1 |
| 01 | TE, CRO, VA | 3 | 2 |
| Staphylococcus spp. | SXT, CRO, VA | 3 | 1 |
| D ''' | SXT, CRO, PG | 3 | 1 |
| Bacillus spp. | TE, CRO,PG, VA | 4 | 1 |
| DI 1" ' | TE, CRO, VA | 3 | 1 |
| Pluralibacter spp. | TE, PG, VA | 3 | 1 |
| - | TE, CRO, PG, VA | 4 | 1 |
| Enterobacter spp. | TE, CRO, VA | 3 | 1 |
| Myroides marinus | TE, CRO, PG, VA, CN | 5 | 1 |
| Providencia spp. | TE,CRO, VA | 3 | 1 |
| Serratia marcescens | TE, PG, VA | 3 | 1 |

Key: CIP:- ciprofloxacin; TE:- tetracycline; SXT:- trimethoprim/sulphanethoxazole; MEM:- meropenem; CRO:- ceftriaxone; PG:- penicillin; VA:- vancomycin; CN:- gentamicin.

include: Ciprofloxacin, Tetracycline, Trimethoprim/ Sulphanethoxazole, Meropenem, Ceftriaxone, Penicillin, Vancomycin. Gentamicin. Vancomycin was used for grampositive isolates and gentamicin was used for gramnegative isolates. The results show that most of the isolate was not resistant in nature with the highest resistant antibiotic as ceftriaxone (63.2%) followed by Tetracycline (63%), with the least as Ciprofloxacin which showed no resistance at all.

Sixteen (42.1%) of the isolates obtained were resistant to two or more of the eight antibiotics, and 22(57.8%) showed multiple antibiotic resistance (MAR) patterns of varying degrees. Seventeen (44.7%) were resistant to three of the antibiotics, 4(10.5%) were resistant to four antibiotics and 1(2.6%) of the isolate obtained was resistant to 5 antibiotics which is shown in (Table 5)

The MAR index of the isolates is shown in Table 6. The

result showed that 34.2% were found to have an index less than 0.2 while none had an index of 0.7-1.0 (not being resistant to all the antibiotics tested against the isolates). Sixty-five-point seven percent (65.7%) were greater than 0.2.

The crude material and product from each site were examined to ascertain the quality of the product. The results shown in Table 7 show that the product from Site A and Site C is safe for consumption and are within the European Union and World Health Organization, good manufacturing practice limits, the highest microbial load was Site B product (15.5CFU) with the least as the product from site A with (3 CFU) this product passes through high temperature before it is packaged.

Molecular characterization was done for some of the isolates that showed unique features. Figure 1 shows the phylogenetic tree of the isolates. The isolates were

Table 6. Multiple Antibiotics Resistant (MAR) index of isolates obtained from air samples of Sites A, B and C.

| MAR Index | No. of isolates (%) |
|-----------|---------------------|
| 0.0 | 1 (2.6%) |
| 0.1 | 7(18.4%) |
| 0.2 | 5(13.2%) |
| 0.3 | 3(7.89%) |
| 0.4 | 17(44.7%) |
| 0.5 | 4(10.5%) |
| 0.6 | 1(2.61%) |
| 0.7 | 0(0) |
| 0.8 | 0(0) |
| 0.9 | 0(0) |
| 1.0 | 0(0) |

Table 7. Microbial load of raw materials and product of sites A, B and C.

| Parameters | Sample | Site | 1st Batch | 2nd Batch | Average | Limit for viable particles |
|------------------------|------------------------|------|-----------|-----------|---------|--------------------------------|
| Crude (CFU/Plate) | Palm oil | Α | 46 | 79 | 62.5 | |
| | Untreated tap water | В | 23 | 60 | 41.5 | 25-50 (CFU/25cm ²) |
| | Flour | С | 20 | 21 | 20.5 | |
| Product (CFU/plate) | Vegetable oil | Α | 5 | 1 | 3 | |
| | Treated drinking water | В | 10 | 21 | 15.5 | 25-50 (CFU/25cm ²) |
| (Ci O/piate) | Bread | С | 2 | 5 | 3.5 | |

identified as *Proteus mirabilis* (Con4), *Myroides marinus* (B7), *Enterobacter hormaechi* (A9), *Proteus mirabilis* (B8), *Bacillus aerius* (A21), *Pluralibacter gergoviae* (C1), *Proteus mirabilis* (A16) and *Bacillus paramycoides* (A6).

DISCUSSION

In this study, air samples that were collected from selected food industries in Ibadan showed that the rainy season had more microbial load than the dry season. Table 1 result is consistent with the work of Oyet et al. (2020) who obtained a higher microbial load during the rainy season in their assessment of different foods such as yam, fish and plantain from food vendors in Port Harcourt, Nigeria. Oyet et al. (2020) stated that the dry season had lower bacteria counts compared to the rainy season and attributed it to the distribution of microorganisms by environmental factors such as temperature, humidity of the season, pH, and intensity of sunlight.

One of the most important investigations to ascertain the level of microbial air pollution in the environment is the microbiological quality assessment of industrial air. The settle plate method was used to assess the bacterial and fungal aerosol concentrations in the atmosphere of the

food industries in Ibadan, and the results obtained ranged from 30.5–163.6 CFU. This is within the range obtained by Olaitan and Bashir (2018). The overall bioaerosol of the food industries (Site A and B) with a mean microbial load of 154 CFU and 116.5 CFU respectively exceeded the acceptable limits of World Health Organization Good Manufacturing Practices (WHO, 2000; European Union, 2008) with 50-100 CFU as the standard microbial load of food industries. Table 1 result also indicates that the hygiene of food industries is not taken seriously.

Staphylococcus sp., Serratia marcescens and Bacillus sp. were positive for the pathogenicity test, the Table 2 result correlates to the findings of Kabir et al. (2016) who the presence of pathogenic showed bacteria (Staphylococcus sp.) from the air samples of a university environment, in Bangladesh. Bioaerosols usually harbour microorganisms as Gram-positive cocci and Gramnegative rods are present alongside their components such as β glucans, mycotoxins, endotoxins, and allergens (Kim et al., 2018). These bioaerosols are capable of causing serious diseases in humans such as respiratory diseases, cancer and infection when they are been inhaled or ingested (Kim et al., 2018).

It was observed that *Micrococcus luteus* was the predominant isolate followed by *Proteus vulgaris*, *Bacillus*

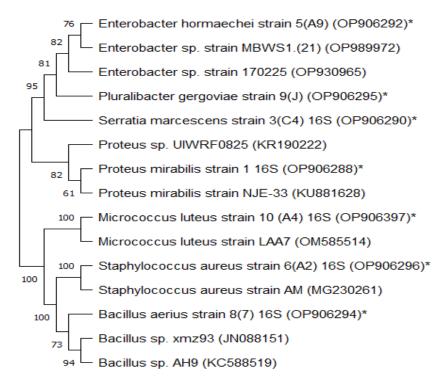


Figure 1. Phylogenic tree of isolates obtained from air samples of Sites A, B and C in relation to isolates in the gene bank.

sp. and Staphylococcus sp. This finding in Table 3 agrees with the works of Olaitan and Bashir (2018) and Oyet et al. (2020), who discovered that Micrococcus luteus was predominant in the air of pharmaceutical industries in Kano, Nigeria. Micrococci luteus can grow well in an environment with little water or high salt concentration. They occur in a wide range of environments, including dust (air), water and soil. *Micrococcus* is generally thought of as a harmless bacterium, though there have been rare cases of Micrococcus infection in people with compromised immune systems, as occur in HIV patients. Similar work was reported by Sabharwal and Sharma (2015) who observed Micrococcus luteus and Staphylococcus sp. as the predominant isolates obtained from the air of a school environment. Other isolates obtained include Providencia sp., Enterobacter sp., Serratia marcescens, Citobacter freudii and Pluralibacter sp.

Antibiotic-resistant genes have been examined and discovered in the air (Li et al., 2018). Hence, after the collection and isolation of bacteria from the air samples, the isolates were subjected to an antibiotic susceptibility test with eight antibiotics namely, Ciprofloxacin, Meropenem, Gentamicin, Ceftriaxone, Vancomycin, Tetracycline, Penicillin and Trimethoprim/Sulfamethoxazole. All the isolates were majorly susceptible to Ciprofloxacin (100%) while 89.5% were susceptible to Meropenem. Sixty-three per cent of the isolates were resistant to Tetracycline and 63.2% showed resistance to Ceftriaxone this finding is consistent with the study of Kabir et al. (2016) that reported 100% susceptibility by Meropenem and

Ciprofloxacin with resistance to Ceftriaxone (50%) and Tetracycline (31.3%). A similar finding has also been reported by Hayleeyesus and Manaye (2014).

Sixteen (42.1%) of the isolates obtained were resistant to one or two of the eight antibiotics; 22(57.8%) showed multiple antibiotic resistance (MAR) patterns on varying degrees. Of the 22, 17(44.7%) were resistant to three of the antibiotics, 5(13.2%) were resistant to four antibiotics and 1(2.6%) of the isolate obtained was resistant to 5 antibiotics. Eleven (28.9%) of the isolate showed the same phenotypic antibiotic-resistant pattern as Tetracycline, Cetriaxone, and Vancomycin, as shown in Table 5 agrees with the work of Kabir *et al.* (2016). Isolates with multiple antibiotic-resistant patterns can serve as reservoirs for antibiotic-resistant genes through horizontal gene transfer which poses a great threat to humans.

The findings in this study revealed the highest multiple antibiotic resistance rates, in that 34.2% were found to have a MAR index less than 0.2 while none had an index of 1.0 (not being resistant to all the antibiotics tested against the isolates). This finding shown in Table 6 correlates with Ayandele *et al.* (2020), who showed that isolates from a tertiary institution in Oyo had no MAR index of 1.0.

The products from Site A and Site C are safe for consumption as their microbial load was within the European Union and World Health Organization, good manufacturing practice (WHO, 2000; European Union, 2008) limits. The highest microbial load was observed in Site B product (15.5CFU) and Table 7 shows the need for

proper hygiene in the food industries.

It is alarming to detect *Serratia marcescens* produced from the air of the laboratory air conditioner system in Site A. Which was both resistant to multiple antibiotics and pathogenic. This finding agrees with Kim *et al.* (2018) who obtained antibiotic-resistant bacteria from the dust air particles of indoor air conditioner filters indicating that the air conditioner system aids the spread of microorganisms in the environment. The personnel in the food industries are to ensure personal protective equipment is worn during operations.

Conclusion

This study highlights the critical need for improved hygiene in food industries, as two out of the three sites assessed did not comply with World Health Organization (WHO) Good Manufacturing Practices. The detection of pathogenic microorganisms and multiple antibioticresistant bacteria in the air poses a significant threat to human health. Additionally, the microorganisms present in the air could act as reservoirs for antibiotic-resistance genes. Therefore, further studies are necessary to identify the specific resistance genes in the bacteria isolated from the air. It is recommended that periodic air quality assessments be conducted in food industries to monitor bioaerosol compositions. The use of personal protective equipment (PPE) should be prioritized, particularly for workers in food industries, as exposure to airborne microorganisms can adversely affect human health. Furthermore, adherence to Good Manufacturing Practices (GMP), including stringent hygiene protocols and effective air filtration systems, should be enforced to ensure a safer industrial environment.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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