

Aeromicrobial profiles of some selected eateries in Sagamu axis of Ogun State, Nigeria

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ABSTRACT

Background: This study investigated some selected eateries frequently patronized in Sagamu axis of Ogun state, for air-borne microorganisms of clinical importance that can cause food borne illness and respiratory infection.

Methods: Bacteriological media appropriate for selected microbes were exposed for 10 minutes in each designated site of sample collection and incubated at 37°C for 24 - 48 hours for bacteria, and at 25±3°C on Sabouraud's dextrose agar medium for 5-7 days for fungi. The colonies obtained were enumerated, antibiogram of the isolates were determined and meteorological values considered.

Results: Main Gate Eatery (MGE) morning counts range from 185±2cfu/mL and 283±5cfu/mL from the cooking site and outdoor counts while the evening counts ranges from 242±2 cfu/mL and too numerous to count from the cooking site, outdoor and serving table. Ikene Sharp corner eatery (ISCE) exhibited a higher counts range from 235±3 cfu/mL (outdoor), 298±8 cfu/mL (indoor) and too numerous to count (serving table) in morning while the evening counts ranges from 196±2 cfu/mL, 235±3 cfu/mL, 280±6 cfu/mL and too numerous to count from the cooking site, indoor, outdoor and serving table respectively. Isale Oko garage eatery (IOGE) elicited 225±2 cfu/mL outdoor morning counts, 270±2cfu/mL from serving table. Antibacterial and antifungal resistance were detected. The meteorological parameter observed varied remarkably.

Conclusions: The preponderance of antibacterial and antifungal resistance from the isolates capable of transferring resistance factors could be a threat to therapeutic management of these infections. There is a need for surveillance of the eating places by Public Health Inspectors to enforce standard.

Keywords: Aeromicrobial profile, Eateries, Sagamu, Nigeria

1. INTRODUCTION

Aeromicrobiology is a study of airborne particles of biological origin, which includes, bacteria, fungi, viruses, and their metabolic components such as mycotoxins, glucans and endotoxins. These air-borne particles are generated from indoor human activities, namely coughing, sneezing, talking, eating, walking, washing and cleaning surfaces and as well as outdoor activities like composting and waste recycling[1]. These aeromicrobes vary in sizes ranging from 20 nm to 100 µm and are easily transported in the environment. The inhalable fraction can penetrate the deeper parts of the respiratory system. The settling of air-borne microbes depends on sizes, density and meteorological factors such as temperature and humidity. Various studies revealed that in many cases exposure to airborne microorganisms can lead to several health problems such as contagious and

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respiratory diseases [2]. About 90% of the food available for local consumption are produced by small and medium scale road side eateries popularly called ‘Bukataria’ in southwest Nigeria. The organogram of most of the eatery’s buildings are not all that primarily designed for such duty types and are vulnerable to a lot of air-borne microbes of pathogenic potential due to poor ventilation and other associated anomalies. There are potential undetermined hazards due to lack of information on the microbial characteristics of spoilage organism deposition in these food from the atmosphere. Furthermore, it has been established that the incidence of diseases caused through food borne bacteria is at least ten times more prevalent than actually reported [3]. Most people have a habit of eating out in the canteen to save time for other tasks not minding the influence of handling and preparation on the microbial counts and sequel to this, small and medium scale canteens are springing up and flourishing. Air-borne microbes are the primary sources of contamination in food items, these microbes causing food deterioration due to the nutrient components of the foods that support microbial growth. However, their growth could be impaired by temperature, water availability and or pH [4]. Fungi are found in air frequently as spores. Spores are generally able to survive harsh environmental conditions due to protective small molecules (amino acids, sugars, and sugar alcohols) the possession of a thicker cell wall, and expression of heat shock proteins. These features enable fungal spores to remain viable in air for longer periods than their vegetative forms [5]. *Staphylococcus spp*, *Bacillus sp* and fungi such as *Aspergillus*, *Rhizopus*, *Penicillium*, *Alternarium* and related families have been implicated as vehicle of food-borne illness, allergies and poisoning for many decades. Other diseases like pulmonary anthrax, tuberculosis and Legionellosis have been traced to *Bacillus anthracis*, *Mycobacterium tuberculosis* and *Legionella pneumophila* respectively. The past phenomenon of Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), H1N1 infection, the potential danger of bird flu pandemic and the bioterrorism incident in America in 2001 related to airborne *Bacillus anthracis* spores (have attracted worldwide attention on bioaerosols [6]. This study therefore investigated the occurrence of air-borne bacteria and fungi of pathogenic potential from selected frequently patronized eateries namely; Main gate eatery, Ikene Sharp Corner eatery, and Isale-Oko garage eatery within Sagamu axis of Ogun state of Nigeria.

1. MATERIALS AND METHODS

2.1 Materials

2.1.1. Equipment

The equipment used for this study include; autoclave, Sterilin Petri-plates, colony counters, hand held environmeter, laminar flow hood, graduated Pyrex glasswares, water bath, disc dispensers.

2.1.2 Biological materials

The biological consumables used for the study include; bacteriological media - Nutrient agar media, cetrimide nutrient agar, mannitol salt agar media, Sabouraud’s dextrose agar, ketocanazole antibiotic.

2.1.3 Reagents

Chemical consumables and reagents used for this study include; (500 gm)fermentable sugars Kemlight Laboratories PVT., India, (1ltr)hydrogen peroxide (Ranjo Medix Laboratories LTD., India) (25g) crystal violet(Kemlight Laboratories PVT., India), (25g) Lugol iodine Kemlight Laboratories PVT., India), (25g)safraein Kemlight Laboratories PVT., India), methylated spirit(Ranjo Medix Laboratories LTD., India, peptone broth(HI media, India), methyl red, Kemlight Laboratories PVT., India), (25g) lactophenol Kemlight Laboratories PVT., India),.

2.2 Methods

2.2.1 Study Location

The three frequently patronized eateries were located in Isale–Oko the most popular interstate garage, Ikene Sharp Corner and the eatery outside and opposite the Main gate campus of the Olabisi Onabanjo University Teaching Hospital, within Sagamu metropolis in Ogun state were explored. The exact site of sample collections were indoor, outdoor area of the canteen, cooking hub/site and serving tables.

2.2.2. Bacteriological Procedure

Three different bacteriological culture media namely, Nutrient agar media, cetrimide nutrient agar, mannitol salt agar media and Sabouraud’s dextrose agar media were aseptically prepared according to the manufacture’s specifications. Petri dishes prefilled with suitable agar media as appropriate were exposed for 10 minutes followed by incubation at 37°C for 24-48 hours for bacteria and 25±3°C for 5 -7 days for fungal growth. The study was carried out in the morning at (9-10AM) and evening at (5-6 PM). Samples were collected in triplicates for bacteria and fungi and the mean values were used for statistical analysis.

2.2.3. Biochemical Identification

Distinct colonies were selected on the basis of morphology, texture, sizes and color for Gram staining and conventional biochemical tests which include; catalase test, methyl red test, coagulase test, starch hydrolysis test, sugar fermentation test for bacteria and lactophenol-in- cotton blue test for fungi.

2.2.4. Enumeration of bacterial and fungal isolates

A volume of 1000mL of nutrient agar fortified with 16mg/mL of Nystatin, to suppress the growth of fungi and Sabouraud's dextrose agar fortified with 40mg/mL of gentamicin to suppress the growth of bacteria were prepared separately. Exactly 0.1mL from serially diluted overnight culture of each organism (bacteria and fungi) separately were cultured on the above-mentioned media for the enumeration of aerobic viable count. The plates were incubated at 37°C for 24-48 hours and 25±3°C for 3-5 days for bacteria and fungi respectively. The colony were counted and expressed in colony forming units per millimeter (cfu/mL) all the counts were done in triplicate.

2.2.5. Antibiogram

Antimicrobial susceptibility patterns of the isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus sp.* and *Micrococcus sp.* on selected antibiotics were determined using the Kirby-Bauer modified agar diffusion technique. Control strains were used as standard for each represented microbe. A volume of 0.1mL of the 24 hours broth culture of each isolate was pipetted in to 9.9mL of sterile distilled water in the vials. The inocula were standardized by diluting the broth culture to match the 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into each of the standardized suspension, drained and used for inoculating 20mL of Mueller Hinton agar (Oxoid, UK) on 100-mm disposable plate (Sterlin, UK). The inoculated plates were air dried for 30 min, and antibiotic discs (Oxoid, UK) were placed on the agar using flamed forceps and were gently pressed down to ensure maximum contact. Discs containing the conventional antibiotics as appropriate for each organism as elicited in the results below were used for the susceptibility testing. The plates were incubated aerobically at 37°C for 24 hours before measuring the diameter of zones of growth inhibition. Susceptible, intermediate and resistant strains were marked S, I and R respectively and were analyzed as specified by Clinical Laboratory Standard Institute (CLSI, 2016 guide).

2.2.6. Antifungal susceptibility testing

A tablet of ketoconazoles which contains 200mg/mL was suspended in 1000mL of sterile distilled water to give a stock concentration of 200µg/ml. 1mL of the aliquots of 200µg/ml was pipetted into 49mL of sterile distilled water to give 4 µg/mL. Thereafter, 30 µL were impregnated on each of the disc of 6mm Whatman filter paper were aseptically kept in a sterile desiccator for 24 hours. A sterile cotton swab was dipped in to the adjusted cell suspension of the overnight broth dilution of 10⁶cells/ml, firmly pressed against the inside wall of the test-tube to drain excess liquid and swab the surface of the agar, the plates were left to dry for 10 minutes and the disk impregnated with ketoconazoles were applied and pressed gently to ensure complete contact, the plates were incubated at 35±2°C for 18 to 24hours and the zone of growth inhibition was measured to the nearest millimeter at the point of prominent reduction in growth .

2.2.7. Meteorological data

Wind speed, Temperature and Relative humidity were recorded at each canteen using handheld envirometer automatic calibrated device (Fisher Scientific, TX, USA).

2.2 Statistical Analysis

Data collected were analyzed using SPSS 15 and graph was plotted using Graph Pad prism 8.1 Version 5 for Windows.

3. RESULTS

A total of 10 organisms comprises 4 bacteria and 6 fungi were isolated from various specific sites, namely; serving table, cooking site, indoor and outdoor locations of the selected food canteens as shown in Table 1.

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Table 1: Aeromicrobes isolated from selected sites

S/ No.	Microbes	Site of Isolation
1	<i>Pseudomonas sp.</i>	Serving table
2	<i>Staphylococcus sp.</i>	
3	<i>Micrococcus sp</i>	Cooking site
4	<i>Bacillus sp</i>	
5	<i>Rhizopus sp.</i>	
6	<i>Penicillium sp.</i>	Indoor
7	<i>Aspergillus sp</i>	
8	<i>Helminthosporium Sp.</i>	
9	<i>Trichophyton sp.</i>	Outdoor
10	<i>Epidermophyton sp.</i>	

Colonial and cultural method employed as shown in Table 2 aid in identifying the isolates of bacteria exhibited conventional appearances that indicates their profiles on general purpose media and different selected media used, *Pseudomonas sp*, *Staphylococci sp*, *Micrococcus sp* and *Bacillus sp* were identified for further biochemical study.

Table 2: Colonial and morphological appearance of aerobacteria isolated on culture media

S/n	Characteristics	Bacterial isolates			
1	Gram's staining	<i>Pseudomonas sp</i>	<i>Staphylococcus sp.</i>	<i>Micrococcus sp</i>	<i>Bacillus sp.</i>
2	Colony	Greenish colony growth,	Yellowish colony, smooth, regular margin	Yellowish convex	Opaque growth, smooth, margin, irregular
3	Cell shape	Rod	cocci	Spherical	Rods
4	Cell arrangement	Polar	In cluster	Tetrads	In chains
5	Catalase test	Positive	Positive	Positive	Positive
6	Lactose	Positive	Positive	Positive	Negative
7	Dextrose	Positive	Positive	Positive	Positive
8	Sucrose	Positive	Positive	Positive	Positive
9	Starch hydrolysis	Positive	Negative	Negative	Positive
10	MR	Negative	Positive	Positive	Negative
11	VP	Negative	Negative	Negative	Negative
12	Nitrate reduction	Positive	Positive	Positive	Negative

Varied growth appearances were trapped on the culture media exposed of which were further sub-cultured for further purification. The growth appearances (Figure 1) suggest staphylococci sp., *Bacillus sp.* with contaminant overgrowth, *Micrococcus sp.*, *Pseudomonas sp.* and fungal isolates on the selected representative plates. Figure 1(A, B, C, D, E).



A



B



C



D

E

Figure 1: The growth appearance (Figure A, B, C, D, E); staphylococci sp, *Bacillus* sp. with contaminants overgrowth, *Micrococcus* sp and fungi on selected sample plates.

The average mean of triplicate colony count of bacteria culture obtained from this study varied from one site of isolation to the others. Morning counts were notably less than the evening counts. From the Main Gate Eatery (MGE) - the morning count ranges from 185 ± 2 CFU as indicated on the cooking site and 283 ± 5 CFU as the highest as elicited on the outdoor counts while the evening counts ranges from 242 ± 2 CFU as shown in cooking site and too numerous to count as indicated on outdoor and serving table of the MGE counts. Ikenne Sharp corner eatery (ISCE) exhibited a higher count that ranges from 235 ± 3 CFU (outdoor), 298 ± 8 CFU (indoor) and too numerous to count TNTC (serving table) in morning while the evening counts ranges from 196 ± 2 CFU, 235 ± 3 CFU, 280 ± 6 CFU and TNC from the cooking site, indoor, outdoor and serving table respectively as shown in Table 3. Isale Oko garage eatery (IOGE) elicited a notable different from other canteens explored for aeromicrobes, 225 ± 2 CFU outdoor morning count was recorded and 270 ± 2 CFU from serving table while indoor and cooking site were too numerous to count. The evening count also ranged from 200 ± 3 CFU (indoor counts and too numerous to count as indicated on serving tables as shown in Table 3.

Table 3: Enumeration of bacterial colonies isolated (Average of triplicates)

		Morning(9AM-10AM) (Mean of CFU \pm S.E)	Evening(5PM-6PM) (Mean of CFU \pm S.E)
A	Main Gate Eatery.		
1	Indoor	203 ± 4.0	248 ± 7.0
2	Outdoor	283 ± 5.0	TNTC
3	Cooking site	185 ± 2.0	242 ± 2.0
4	Serving table	215 ± 5.0	TNTC
B	Ikene Sharp Corner Eatery		
1	Indoor	298 ± 8.0	235 ± 3.0
2	Outdoor	285 ± 2.0	280 ± 6.0
3	Cooking site	235 ± 6.0	196 ± 2.0
4	Serving table	TNTC	TNTC
C	Isale - Oko Garage Eatery		
1	Indoor	TNTC	200 ± 3.0
2	Outdoor	225 ± 2.0	285 ± 3.0
3	Cooking site	TNTC	245 ± 2.0
4	Serving table	270 ± 2.0	TNTC

The colony counts in Fungi examined in this study exhibited a lesser value in comparison with bacteria colony counts. The morning and evening counts from the Main Gate Eatery (MGE) were relatively closer from indoor to serving table values as indicated in the table below. The same trend was observed from Ikene Sharp corner eatery (ISCE) and Isale Oko garage eatery (IOGE) and the factors attributable to these values were explained at the discussion.

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Table 4: Enumeration of fungal colonies isolated (Average of triplicates)

			Morning (9AM-10AM) (Mean of CFU ± S.E)	Evening (5PM-6PM) (Mean of CFU ± S.E)
A	Main Gate Eatery			
1	Indoor		10±2.0	12±2.0
2	Outdoor		9±2.0	12±3.0
3	Cooking site		12±1.0	14±2.0
4	Serving table		12±3.0	15± 3.0
B	Ikene Sharp Corner Eatery			
1	Indoor		15±2.0	12±4.0
2	Outdoor		12±2.0	10±4.0
3	Cooking site		25±1.0	15±2.0
4	Serving table		17±2.0	17±3.0
C	Isale - Oko Garage Eatery			
1	Indoor		13±10	10±10
2	Outdoor		16±3.0	12±2.0
3	Cooking site		18±2.0	14±1.0
4	Serving table		13±3.0	12±3.0

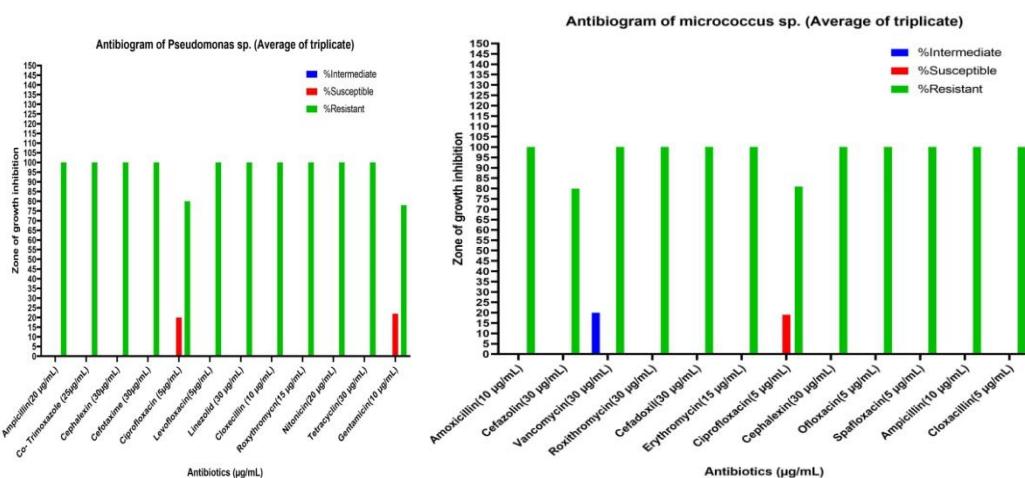


Figure 2: Antibiogram of *Pseudomonas* sp. Figure 3: Antibiogram of *Micrococcus* sp.

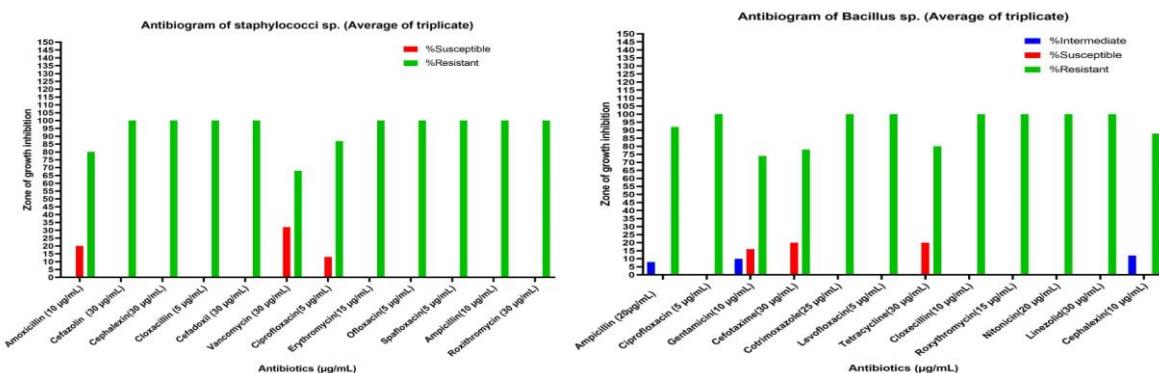


Figure 4: Antibiogram of *staphylococci* sp

Figure 5: Antibiogram of *Bacillus* sp.

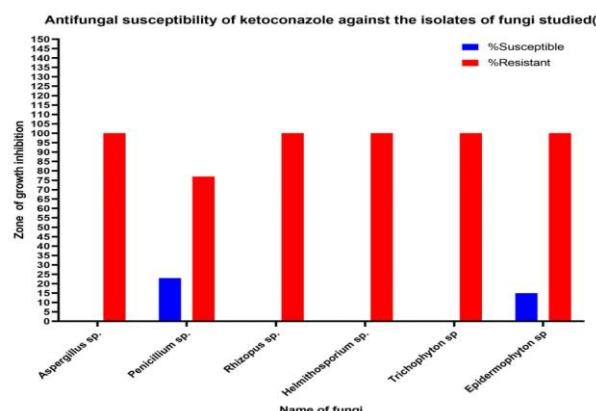


Figure 6: Antifungal resistance to ketoconazoles

The antibiogram study of the isolates of bacteria and fungi were mostly resistant to the antibiotics and antifungal tested. *Pseudomonas* sp were resistant to 10 of the 12 antibiotics used with the exception of degree of susceptibility to ciprofloxacin and gentamicin that elicited some degree of pronounced zones of growth inhibition as shown in Figure 2. Ten (10) of the 12 antibiotics were resistant to *Micrococcus* sp with exception of ciprofloxacin antibiotic that elicited a notable zone of growth inhibition, an indication of susceptibility as shown in Figure 3. *Staphylococci* were totally resistant to 9 of the 12 antibiotics of varied concentration exposed but susceptible to amoxicillin, vancomycin and ciprofloxacin with zones of growth inhibition of 20mm, 30mm and 10mm respectively as elicited in Figure 4. *Bacillus* sp were also resistant to all the antibiotics with the exception of gentamicin, cefotaxime and tetracycline that elicited some degree of zones of growth inhibition 15mm, 20mm and 20mm respectively as exhibited in Figure 5. All the fungi isolates were resistant to ketoconazoles with the exception of *Penicillium* sp and *Epidermophyton* sp that showed some degree of susceptibility as indicated by the 15mm and 10mm zones of growth inhibition as indicated in Figure 6.

The meteorological data, a function of prevailing climate on the site and at the time of collection were recorded. The morning data were significantly varied from the evening data. The temperature values in the morning were lower than the evening values from the selected canteens. The relative humidity and the wind speed also varies from morning to evening values from all the selected site of isolation monitored by envirometer as shown in Table 5.

Table 5: Meteorological Values Recorded at the A.M. and P.M. Period of the Samples Collection

			Temperature (°C)	Relative Humidity (%)	Wind speed(m/s)
A	MGE	AM	21.3	45	6.80
		PM	32.0	27	8.75
B	ISCE	AM	20	42	10
		PM	33	25	9.3
C	IOGE	AM	25	38	10
		PM	40	22	10.7

Key: MGE: Main Gate Eatery

ISCE: Ikene Sharp Corner Eatery IOGE: Isale – Oko Garage Eatery

4. DISCUSSION

Air, an entity on its own, can be considered one of the unsuitable hospitable environments for microbes because of its minimal nutrient composition and its tendency to support fewer organisms for growth. Thus, the study of living microorganisms suspended in air traditionally gets less attention than other branches of microbiology. However, mounting evidence of airborne infectious agent's transmission as occurred in Covid-19 era has generated novel interest in the field of Aeromicrobiology [7]. Air as a medium for microbial transmission is supported in this study with isolation of both bacterial and fungal species- four different bacterial species and six fungal species. Various environmental factors could have accounted for their isolation; a site of sampling, period

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of the day, meteorological factors were found to influence the microbial counts obtained [8]. The variation obtained in the morning count and evening count could be attributed to frequency and number of the people visiting as at that time of isolation, the temperature and the prevailing climate that has the capacity to influence and determine the survival of the isolates associated with a specific environment. As the day progresses the number of the people visiting eateries increases and hence the higher number enumerated in the evening than the morning [9]. Higher frequency of patronage, noise and smoke pollution of the environment, and other related factors could also have influenced the comparative differences in the enumerated microbial colony. It is noteworthy that the fungi isolated showed less value compared with the bacterial colony count. This corroborates the study of Tang (2009) on the effect of environmental parameters on the survival of airborne infection [10]. The bacterial isolates showed varied levels of resistance to twelve antibiotics used in the antibiogram, with the exception of ciprofloxacin and gentamicin against *Pseudomonas spp* and *Micrococcus spp.*, amoxicillin, vancomycin and ciprofloxacin against staphylococci spp and gentamicin, cefotaxime and tetracycline against *Bacillus spp*. Similarly, all the fungal isolates exhibited resistance to ketoconazoles, with the exception of Penicillium and *Epidermophyton* which agreed with study of Pathak and Verma (2009) on aerobacteriological study of vegetable market at Jabalpur [11].

5. CONCLUSION

The preponderance of antibacterial and antifungal resistance obtained in this study, could be attributed to the individual's status of immunity that determined the carriage loads- the inherent underlying factors of the individual visiting the selected canteen, continuous exposure of the bacterial and fungal to selective pressure in the environment, and total embodiments of the prevailing environmental milieu around the selected eateries. Hence, the need for a study such as this designed to evaluate the potential of public eateries to serve as routes of transmitting pathogens of infectious diseases.

Conflict of interest: None

Contributions of Authors

O. L. Okunye - Conception, data collection, design, analysis and manuscript preparation

C. O. Babalola - Data collection, analysis and manuscript preparation

O. E. Adeleke - Data collection, analysis and manuscript preparation

O. O. Elijah - Data collection, analysis and manuscript preparation

O. O. Joseph - Data collection, analysis and manuscript preparation

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