OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com/sjpm

Original Research Article

Bacteriological Probing of Outdoor Air Quality Using Sterile Food Samples as Air Sampling Substrates

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DOI: 10.36348/sjpm.2020.v05i02.017 | **Received:** 17.02.2020 | **Accepted:** 24.02.2020 | **Published:** 29.02.2020

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Abstract

The presence of airborne microbes and their relationship to disease has become an important area of study, as many bioaerosols in indoor and outdoor environments have been found to cause adverse health effects. This study was carried out to assess the application of sterile food samples as a novel technique in the bacteriological probing of air quality. In this study, different food items (pawpaw, meat and yam) were sterilized by autoclaving, and exposed to an outdoor air condition, in order to isolate bacteria capable of causing contamination of sterile materials, including processed food samples exposed to the air environment. Five (5) grams each, of the various food samples were exposed at varying elevation to a maximum height of 40 feet above ground level and studied at daily intervals for five consecutive days. The bacterial population dynamics as well as diversity was determined and was observed to vary with respect to the food type used as sampling substrate and exposure duration. In the overall analysis, meat had more bacterial load than pawpaw, while yam was the least. The results obtained from the investigation showed that *E. coli* **(**25.9%), *Klebsiella pneumonia* (16.9%), *Pseudomonas auroginosa* (15.6%), *Staphylococcus aureus* (12.9), *Shigella spp.* (11.7%), *Bacillus spp* (9.1%), and *Salmonella spp* (7.9%) were the most frequent bacterial isolates in the air environment studied. The study reveals a novel method of air quality determination using sterile food samples. The study further recommends proper waste management at the tropospheric level to prevent upward movement of particles.

Keywords: Sterile food; bacteriological probing, air quality, outdoor environment, tropospheric level.

INTRODUCTION

Human existence is negatively affected by the quality of air inhaled. Outdoor air quality is of great public health concern as this in part influences the indoor air quality through regular outside air inflow into interiors, eventually resulting in biological contamination of the indoor environment [1]. The air inhaled by people is abundantly microorganisms which may be through aerosolized droplets [2]. Bio-aerosol is a colloidal suspension, formed by liquid droplets and particles of solid matter in the air, whose components contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues. In particular, all particles and substances of biological origin or with biological activities diffused in the air are defined as bioaerosol [3], including bacteria, fungi, viruses, pollen and spores as well as their by-products (i.e., toxins and allergens) [4].

Air quality determination is recently receiving much attention (Hurst, 1991) due to the ever increasing cases of respiratory associated ailment attributable to indoor and outdoor air environment. Microbial damage in indoor and outdoor areas is caused most frequently by molds and bacteria. The most significant environmental factors influencing the viability of microorganisms are temperature, relative humidity and wind velocity [3].

Air sampling can be done by many methods ranging from simple to complex. Sedimentation (settle plate), impactors (slit samplers, sieve samplers, centrifugal samplers and impingement samplers) and gelatin membrane filtration have been in use for decades [5]. Passive sampler is becoming a more prominent and effective alternative for conventional active sampler in exposure and health effects studies, given its simplicity and low cost. Also, many passive samplers are capable of providing comparable performance to active samplers in terms of sensitivity and reproducibility [6].

Traditional sampling involving the use of Petri dishes provides for the estimation of the microbial population per overall viable cell counts, but the contributions from previously deposited cells, quiescent cells and the new deposition of still viable cells may not be discriminated using the settle plate method of air sampling [7]. By implication, conventional or in other words, well-known and widely used methods of air quality determination have one or several limitations ranging from the inability to carry out successional studies as well as studies aimed at understanding the microbial kinetics in the indoor and outdoor environment with respect to time. Exposure time, among others is one limitation with the use of the settle plate (Petri dishes) method, as the method is carried out within 5 to 30 minutes, depending on the population density of cells in that environment and other prevailing circumstances.

The determinants of the indoor and outdoor air quality are not static. Accurate measurement of air pollutants in outdoor, indoor is critical for assessing exposure to air pollution and potential health effects. Many samplers are however, subject to effects of temperature, sampling duration, wind speed, and air concentrations [6]. Also, enumerating microbial population is quite tasking. And most of the available methods require one form of conversion or the other, to estimate the microbial cell density in the air. And comparisons among different indoor environments are difficult due to the variability of the methods used in studies [8]. There is therefore, the need to explore a more sensitive, reliable, inexpensive, and friendly air sampling technique for microbiological air quality determination.

Microbial activities in foods can be viewed from the perspective of the food as a ‘‘selective environment’’ despite the diversity of microorganism that contaminate the surface of the raw materials. The selectivity is imposed by the physical and chemical characteristics of the food, the additives it contains, the processing techniques, the packaging materials and storage conditions.

Carica papaya commonly known as pawpaw, a poor man’s banana is a member of the small family Caricaceae allied to the Passifloraceae. This family comprises of Jamilla, Jacarta, Cycliomorpha and Carica genera [9].

Nutritionally, pawpaw is a good source of calcium, and an excellent source of vitamins A. Biochemically, its leaves and fruits are complex containing several proteins and alkaloids with important pharmaceutical and industrial applications. Due to the high nutritional value, particularly sugar and low pH, fruits serve as a breeding substrate for microorganisms whose activities constitute the most important causes of spoilage.

Meat is a nutritious, protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used. It is the first-choice source of animal protein for many people all over the world. A significant portion of this is lost due to microbial spoilage.

Yam (Dioscorea spp.) is an important farm product in many countries. They are the third most consumed crops in the Sub-Saharan region, especially in West Africa and are also largely consumed in South America, India, and South-East [10].

This study therefore explored the potential of these food sources, serving as a medium for bacterial growth in the outdoor air environment, with the ultimate aim of applying them as a novel technique in catching and probing bacterial community lurking the outdoor air environment. This was done with the view of using these sterile food samples to determine the outdoor air quality at heights significantly above ground level. While this instant paper looks at the potential of the food samples, subsequent papers will evaluate the impact of altitude and seasonal variation on the bacteriological quality of outdoor air environment as well as the molecular characterisation and antibiogram of the bacterial isolates.

MATERIALS AND METHOD

Study area and Sampling Techniques

The air sampling was conducted at the River State University, Harcourt, Nigeria. All microbiological analysis was carried out at the Microbiology Laboratory of the River State University, Harcourt, Nigeria.

Samples (pawpaw, meat and yam) were purchased randomly from vendors in Port Harcourt, Nigeria and transported immediately to the laboratory for analysis.

Five (5) grams each of the food samples (yam, pawpaw, and meat) were wrapped in aluminum foil, sterilized using the autoclave and then transported for sampling in the experimental site using sterile nylon bags. At the experimental site, the sterile food samples were then exposed to the air environment and sampled at regular intervals.

The samples were exposed elevations, at 10ft intervals from ground level to a maximum of 40ft using a metal water tank stand adjacent to a Lecturers’ office building, about 100 meters away from the Microbiology Laboratory.

Study period, sampling frequency and duration.

The study was carried out between the months of May 2018 to May 2019. The samples were studied at daily intervals for five consecutive days in two seasons (wet and dry). The data in the result section of this

paper are presented as mean values of the outcomes recorded.

Determination of bacterial population dynamics during the study period

The changes in the bacterial population over time was determined by accessing the population density. One gram (1g) of each sample was smashed and transferred into 9 ml of physiological saline to achieve a 10-fold dilution. Further dilutions of the sample was done by transferring 1 ml from the last dilution into another tube containing 9 ml of normal saline. An aliquot of the serially diluted samples were inoculated on freshly prepared agar plates and incubated for 24 to 48 hours at 37^0 C for growth. The population of cells was enumerated by expressing the plate counts as a quotient of X in cfu/ml. Where X is the product of the dilution and volume plated. That is, bacterial population = Counts / (dilution x volume plated).

Isolation of Pure Culture

To get a pure culture, an inoculum of the colonies was taken and subcultured on fresh agar plates using the streak plate method and incubated for 24-48 hours.

Identification of Bacterial Isolates

The bacterial isolates were identified based on standard microbiological procedures as described by [11, 12]. Cultural morphology of the isolates was studied based on their physical appearances such as colour, shape, size, elevation and margin. Isolated colonies from pure cultures as described above, were identified using standard biochemical test methods [13].

Precautions

A destructive sampling technique was employed in this method of air sampling, where each of the experimental samples were sacrificed after each stage of the analysis. This implies the samples were not returned to the experimental site after it was taken to the laboratory for analysis.

RESULTS AND DISCUSSION

Profiling of Bacterial Community Structure in an Outdoor Air Environment using Sterile Food Samples

The bacterial profile of an outdoor air quality was assessed using sterile food samples, and it was observed that *E. coli* (24.6%) was the most occurring bacterial isolate, followed by *Bacillus* spp *(*16%), *Pseudomonas* spp. (14.8%), *Staphylococcus* spp. (12.3%), *Salmonella* spp (12.3%), *Shigella* spp. (11.1%) and *Klebsiella* spp (8.6%) as shown in Table 1 below.

Airborne bacterial emanates from different sources including soil, water, plants surfaces and even humans. Scientists in recent years have developed several means of isolating airborne microorganisms such as the use of impingers, cyclones, impactors, filters, spore trapper, settle plates and others [5] but this study focused on the use of sterile food substrate as a sampling tool to determine the distribution pattern of bacteria in an outdoor air environment. Sterilization of the food samples renders them free of viable forms of microorganism and ensures that any organism isolated is from the air environment sampled.

This study which relied on the principle of microbial colonization of cooked or processed food, noticed that the bacterial genera isolated in this study are known to be associated with different transmission routes and cause a variety of infection in man, such as food poisoning, contact dermatitis, respiratory ailment, amongst others. This shows that the air biome is a reservoir of diverse organisms and transported by aerosols of different nature. This is supported by the fact that most of the bacterial population may not proliferate in the atmosphere without an appropriate substrate for growth.

The presence of these food spoilage agents at levels significantly higher above ground level shows that these organisms may be of an anthropogenic origin and transported upwards through meteorological vehicles. This therefore provides an insight in the mode of disease spread in the air environment to different parts of the globe. This is supported however, by the fact that airborne diseases are transmitted as both small, dry particles, and as larger liquid droplets [14]. A recent meta-analysis concluded that "outdoor air and unidentified sources dominated the sources for indoor air environments," accounting for an average of 52 and 43 %, respectively, of observed bacteria [15].

This study has reported the dominant bacterial population to include *E. coli* > *Bacillus* spp > *Pseudomonas* spp. > *Staphylococcus* spp > *Salmonella* spp > *Shigella* spp. > *Klebsiella* spp (Table 1). This is however different from bacterial community structure in the air environment identify by other researchers. Bouillard *et al*. [8] found that *Micrococcus* spp., *Staphylococcus* spp., and *Streptococcaceae* spp. were the most common species found in the air of a healthy office building. These bacteria are representative of the normal human flora, providing further evidence that human occupancy shapes the bacterial communities in indoor air to some degree. In another study by [16] *Staphylococcus* spp., *Micrococcus* spp., *Acinetobacter* spp., *Bacillus* spp., and *Streptomyces* spp. were found to be part of the normal human skin flora. A research by [17] also found high relative abundances of *Staphylococcaceae* spp., *Propionibacteriaceae* spp., *Corynebacteriaceae* spp., *Streptococcaceae* spp., *Veillonellaceae* spp., *Prevotellaceae* spp., *Fusobacteriaceae* spp., and *Neisseriaceae* spp. in healthy human nasopharynx and oropharynx tracts, and

many of these have been identified in indoor air, and therefore implicates humans as sources of air contamination. The bacterial genera identified in this study are associated with different habitat. These differences are therefore, attributable to the sampling techniques, nature of the environment sampled as well as the prevailing environmental conditions.

Table-1: Bacterial diversity in the outdoor air environment sampled

Isolate	Frequency (%)
E. coli	20 (24.7)
Pseudomonas aeruginosa	12 (14.9)
Staphylococcus aureus	10 (12.3)
Salmonella spp	10 (12.3)
Klebsiella pneumonia	7 (8.7)
Bacillus spp	13 (16)
Shigella spp	9 (11.1)
Total	100%

Influence of substrate type on the survival of pathogens in the air environment.

From the result obtained, the microbial population in air was much higher in meat having a total of 34 bacterial isolates, followed by pawpaw (23 isolates), with yam having the least (20 isolates) number of isolates as shown in Figure 1. It was also observed that some of the food substrates used were seemingly selective for the growth of certain bacterial species while favouring the proliferation of other bacterial groups. It followed that yam used as air sampling substrate did not support the growth of *Salmonella* and *Shigella* species while *Escherichia coli*, *Staphylococcus, Klebsiella, Bacillus, Klebsiella* and *Pseudomonas* species grew on the yam substrate. Pawpaw on other hand supported the growth of all the viable and culturable bacterial population identified in this study except for *Salmonella* spp. In similar fashion, meat used as a probing substrate for air quality, supported the growth of all the bacterial population identified in this study, except for *Bacillus* spp. *as* shown in Figure 2.

The occurrence of the different bacterial isolates in the food samples was also noted as it was observed that in the yam sample, *E. coli* (30%), *Bacillus* spp. (25%). *Pseudomonas aeruginosa* (15%), *Staphylococcus aureus* (15%) and *Klebsiella spp* (15%) were the prominent bacterial contaminants. The bacterial concentration however, appeared differently in pawpaw as *E. coli* and *Staphylococcus* were more dominant with 21.7% each, followed by *Pseudomonas* spp. and *Klebsiella spp* with 17.4% each, while *Bacillus* spp (8.7%). and *Shigella spp*. (13.1) were the least. Same pattern was observed in the meat samples as *E. coli* was the dominant species of bacteria with a frequency of 27.7% followed by *Salmonella* spp., *Klebsiella* spp. and *Shigella* spp. having the same frequency of 18.2%. *Pseudomonas* had a frequency of 12.1% while *Staphylococcus* recorded the least with 6.0%. In this present study *Bacillus spp*. was not isolated from meat. This is however attributable to species variation, as the prevalence of bacteria in the genera, Bacillus is influenced by the habitat. For instance, the prevalence of *Bacillus cereus* is different from *B. subtilis* per habitat.

The According to [18], the common genera found in meat includes; *Staphylococcus, Bacillus, Campylobacter, Salmonella*. There are three mechanisms for the spoilage of meat; microbial spoilage of meat, lipid oxidation and enzyme autolysis. Meat provides an enabling environment for microorganisms to strive due to its nutritional values and favorable PH (5.5-7.0) leading to slime formation, dehydration, off odors and change in appearance. The changes could be as a result of an enzymatic action by specific enzymes such as lipase, esterase and phospholipase [19].

Pawpaw has a high nutritional content such as sugar, low PH and even water which serves as breeding grounds for organisms to grow. According to research conducted by Nwachukwu and H.U. Osucha, 2014 *Staphylococcus aureus* (60%), *Shigella spp* (50%) and *Pseudomonas* spp. (30%) were isolated from sliced pawpaw fruit. Their findings are in agreement with results obtained in this study. The outcome of yam recording the least number of isolates could be as result of low PH values, inhibiting microbial growth.

From the results, it was observed that the air quality assessment is influenced by the type of substrate used in the determination of the microbiological air quality parameters. This observation is based on the observation that some of the organisms were isolated from all three food substrates used, while others either from one or two of the food samples. This implies that air quality determination is influenced by the properties of the sampling media. This observed variance could be as a result of differences in the nutritional compositions, pH, and other intrinsic factors associated with the food types [18]. Hence the use of more than one type of sampling media is important in air quality determination.

The study also reveals a novel method of air quality determination using sterile food samples as an

easy method of estimating bacteria population compared to conventional air sampling methods with rigorous formulas.

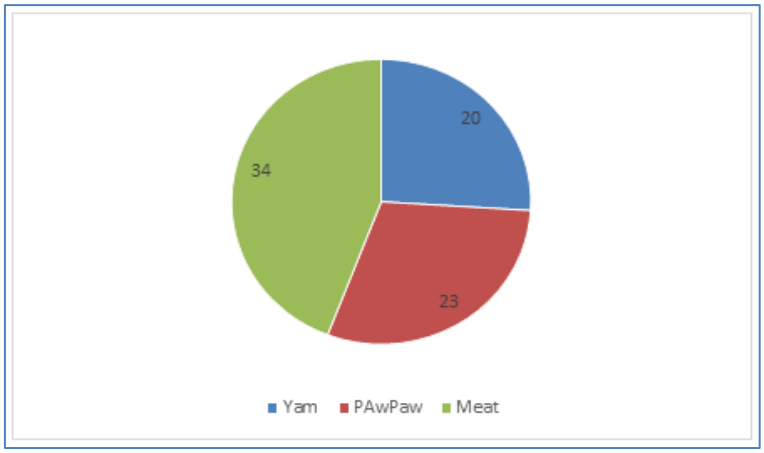

Fig-1: Number of bacterial Isolates from the various food samples

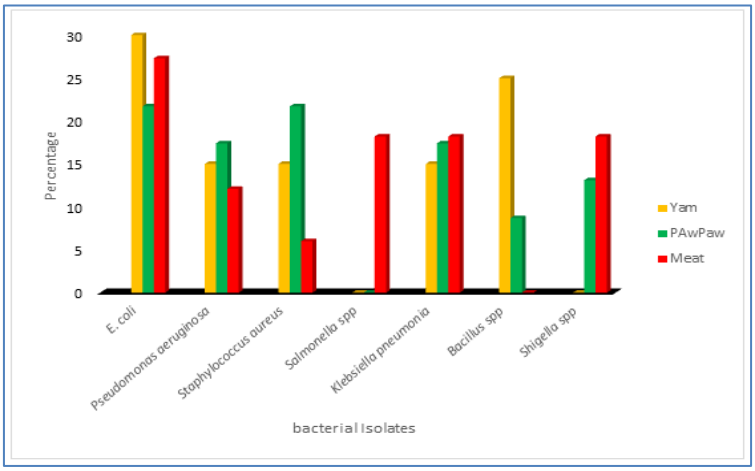

Fig-2: Bacterial diversity in the different food types used as substrate for air quality determination

Changes in the population density of bacterial determinants of outdoor air quality

The bacterial growth kinetics in an outdoor air environment was evaluated using the different food substrates, to decipher the role of environmental substrate availability in the spread of pathogens in air. The results reported in this section are based on data obtained for elevations 10 feet above ground level. A feature publication shall evaluate the impact of altitude on the growth dynamics of pathogens at varying elevations, up to 40 ft. Most bacteria found in the air environment, including pathogens, are believed to be transmitted in the form of bio-aerosols. Bio-aerosols on the other hand have different characteristics that influence the type of organism transported in the air environment.

In this study, the importance of substrate type on the growth dynamics of bacteria in the air environment, with respect to time, was determined. The result obtained showed that the population density of the bacterial isolates increase with increase in time. It follows that the initial bacterial load in the food substrates changed from a lower value to a higher value as the number of days increased during the dry and wet season samples, albeit a seemingly different trend was observed to be associated with the pawpaw sampled during the dry season. It was observed that while the other food substrate maintained similar pattern for both dry and wet season, pawpaw used as substrate showed a different pattern during the dry season, as the population density increased progressively from the first day to the fourth day and later declined at the 5th day (Figure 3 and 4). This reduction after a period of time during the dry season may be due to reduction in water activity during the dry season.

It was also, observed that the growth rate of the bacterial species varied with respect to the different food substrates used in this study. From Figure 3 and 4, higher rates of change in bacterial population density was seen to be associated with the pawpaw substrate, followed by yam and meat. This difference in the growth rate of bacteria in air is attributable to the inherent properties of the food type that favour the proliferation of bacteria in a particular food type.

In this work, measuring the contamination of sterile materials (food samples) was used to determine

the air quality of the region studied. This method is in line with the work of other researchers. Previous research has shown that measuring surface contamination could be a more convenient measure of air quality than measuring air contamination, as sampling systems (i.e., plates, swabs) are easily available and can be monitored without additional equipment [20].

They observed that methods that provide a direct indication of the microbial contamination are the most reliable method to measure surface contamination [21]. Several attempts to establish a relationship between culture counts in air and on surfaces have been carried out, and in some cases a linear regression was found [22]. However, only a few studies have investigated microbial fallout on sterile surfaces in air [23, 24], and have been specifically directed towards understanding and quantifying the mechanisms controlling particle dynamics when an aerosol particle adheres to a surface upon contact [25]. The result from this result has provided useful information regarding the pattern of bacterial colonization of sterile substances in air. The study showed that the air environment is not free of bacterial population, but the growth is a function of substrate availability in the air environment.

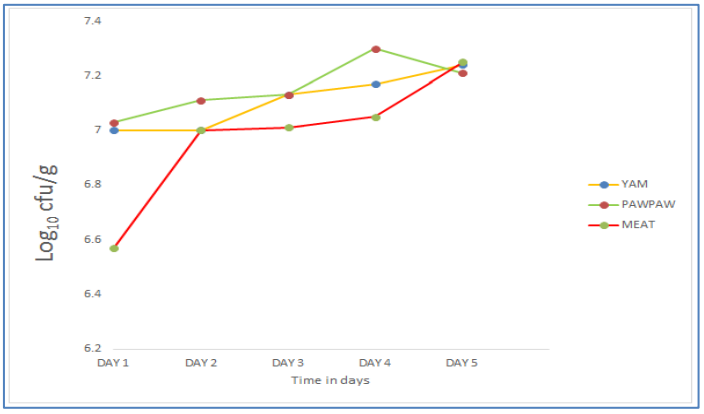

Fig-3: Dry Season Bacterial Growth Kinetics in the outdoor air environment, with respect to time and substrate type

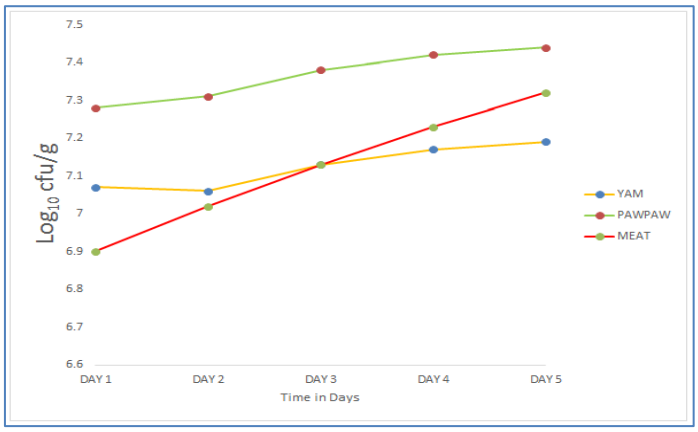

Fig-4: Wet Season Bacterial Growth Kinetics in the outdoor air environment, with respect to time and substrate type

CONCLUSION

This novel method of air quality determination using sterile food samples has shown that the bacteriological quality of air is determined by the type of substrate used to capture the bacterial population lurking the air environment. From the results obtained, this research concludes that meat remains the best food sample with intrinsic factors that support the probing of the bacterial community structure of a particular air environment, albeit no food type served as a general purpose substrate. Therefore the determination of the bacterial concentration in the air depends on the type of substrate used.

The study has also revealed that the majority of bacterial populations in the air environment are of enteric origin. This shows their presence in the air environment must have been through aerosols (droplets particles) and this further implicates aerosols in the transmission of airborne diseases.

This study further showed that sterile materials can be contaminated at elevated positions and the growth dynamics of these agents is influenced by the properties of the substrates. This method is therefore, an easy method of estimating bacterial population compared to conventional air sampling methods with

rigorous formulas. It is also a suitable method for successional studies involving bacterial growth kinetics with respect to time.

The study recommends proper waste management at the tropospheric level to prevent upward movement of particles.

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