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# Investigations on Bacterial Load in the Rural and Urban Indoor and Outdoor Environment of Gwalior, Madhya Pradesh, India

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## ABSTRACT

Comparative analysis of airborne bacterial load in the rural and urban indoor and outdoor environment is of utmost importance to evaluate the wellbeing hazards linked with contamination of airborne bacteria in the indoor environment. The present study was conducted during December, 2020 to March, 2021 among 50 randomly selected rural and urban (Adupurajagir and Gwalior, respectively) dwellings to determine the indoor and outdoor bacterial load. The airborne bacterial load 562.35 CFU/$m^3$ and 2593.75 CFU/$m^3$ were recorded in the indoor environment of a modular kitchen in Gwalior city and traditional kitchen in Adupurajagir village, respectively. In addition, bacterial load of respectively 1215.13 CFU/$m^3$ and 783.03 CFU/$m^3$ was calculated in the open space at both study sites. Based on morphological characteristics five bacterial species (spp.) were identified *Staphylococcus aureus* spp, *Bacillus* spp, Coagulase-negative *Staphylococcus* spp, *E-coli* spp, and *Micrococcus* spp. By gram staining method the most common bacteria were gram-positive (+ve) [n=85, 54.48% (37.17% cocci, 17.94% bacilli)] followed by gram-negative (-ve) [n=71, 45.51% (23.07% cocci, 21.79% bacilli)] identified. Pearson's correlation coefficient was employed between bacterial load and physical factors of the indoor environment in the rural traditional kitchen. Bacterial load (CFU/$m^3$) showed a significant correlation with temperature ($p < 0.001$). However, a non-significant correlation was recorded with relative humidity ($p > 0.01$). High bacterial load was found in the rural traditional kitchen's indoor environment compared to urban modular kitchen. Outcomes from this study revealed that bioaerosol sampling could deliver fruitful knowledge about the variation of air quality and prevent possible hospital admissions.

**KEY WORDS:** AIR QUALITY, BIOAEROSOL, GRAM STAINING, PASSIVE AIR SAMPLING, PEARSON'S CORRELATION.

## INTRODUCTION

People spend 90% of their lives indoors, where the contaminated air in dwellings may be making people sick. In these areas, they are exposed to various airborne microorganisms like bacteria. According to previous studies, approx. 10-35% of indoor air contamination is caused by aerial microbial flora (Mirhoseiniet al. 2016; Fujiyoshi et al. 2017; Andualem et al. 2019; Hui et al. 2019). Therefore, poor indoor air quality (IAQ) can lead to disorders known as sick building syndrome (SBS), building-related illness (BRI), chronic inflammatory response sydrome (CIRS) and numerous negative exposures (Mirhoseini et al. 2016; Hui et al. 2019). The most common bacterial species identified in indoor environment were *Bacillus, Staphylococcus, Arthrobacter, Micrococcus, Streptococcus, Diphtheroid, Pseudomonas, Exiguobacterium, Enterobacter, Escherichia coli* (*E-coli*) and *Sphingomonas* (Mirhoseini et al. 2016; Bolookat et al. 2018; Andualem et al. 2019; Sivagnanasundaram et al. 2019).

It has been investigated that pathogenic bacterium like *Staphylococcus aureus* (methicillin resistant) and *Pseudomonas* species were nosocomial infections in nature and developed multi-antibiotic drug resistance which may be accountable ineffective cure (Kunwar et al. 2019). Therefore, indoor air can be more polluted and harmful in terms of health issues including upper respiratory infections

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(URI) and lower respiratory infections (LRI), etc., over outdoor air (Baldacci et al. 2015; Kim et al. 2018; Smith et al. 2000). Though indoor environments are believed to be safer, but they can pollute with micro-pollutants when their load raised from recommended parameters than associated with outdoor exposure. A sampling of bioaerosols is a well-known technique to find out the bacterial load, as it permits a significant evaluation, hence the results were evaluated in the form of colony forming unit per cubic meter (CFU/$m^3$). According to the National Institute of Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH) the recommended parameter of the total count of bioaerosols is 1000 CFU/$m^3$ and the culturable total number of bacteria is 500 CFU/$m^3$ (Cox and Wathes 2020).

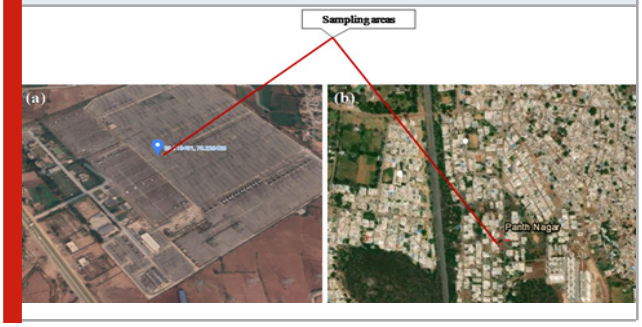
Recent researches have exposed the load of bacteria in different indoor environments (Lazaridis et al. 2015; Mirhoseini et al. 2016; Bolookat et al. 2018; Kunwar et al. 2019; Sivagnanasundaram et al. 2019). However, the indoor airborne bacterial concentration is likely enhanced by the physical parameters of the indoor environment (thermal condition, humidity, and ventilation framework) as well as natural activities of human (sneezing, coughing and talking) by spreading micro droplets (Fujiyoshi et al. 2017; Andualem et al. 2019; Hui et al. 2019; Kunwar et al. 2019; Roslund et al. 2019). Therefore, there is need to investigate bacterial load in the indoors, physical parameters, and micro-climatic variations of the indoor environment. Indoor airborne bacteria can affect human health as various skin and respiratory infections (Fujiyoshi et al. 2017; Roslund et al. 2019; Sharma et al. 2020).

Nonetheless, contamination of bacteria in the kitchen may also be contributable to poor indoor air quality. Hence, kitchens are supposed to be another contributing factor in the burden of airborne bacterial infections. It is important to determine the bacterial load in the indoor environment and its comparison with outdoor environment to find out the risk from indoor environment generated bacterial diseases/disorders. The present work focused on the airborne bacterial load in the indoor environment of rural traditional kitchen and urban modular kitchen and their open space environment. Furthermore, it also analyzes the relationship between high bacterial loads with physical factors of indoor environment of rural traditional kitchen.

## MATERIAL AND METHODS

The present study was accomplished from December, 2020 to March, 2021 among 50 randomly selected dwellings from rural (Adupurajagir village) and urban (Gwalior city) to compare indoor and outdoor airborne bacterial load to analyze the relationship between high bacterial load with the physical environment and cooking pattern of rural and urban people. Therefore, traditional and modular kitchens were selected from rural and urban dwellings respectively as indoor and their open space as outdoor environments. Gwalior is a major city in Central India in the state of Madhya Pradesh. The city is located at 26.22° N latitude and 78.18° E longitudes, 300 km from Delhi. Adupurajagir village is located in the Gwalior districts of Madhya Pradesh. The village is located at 26.113491°N latitude and 78.226425°E longitudes (Fig. 1).

**Figure 1: Areas in (a) Adupurajagir village and (b) Gwalior city where airborne bacterial samples were collected. (Source: https://www.google.co.in/maps)**




Bacterial samples were collected from the kitchen and open space in morning cooking hours at 9.00-11.30 am. The passive air sampling technique was used, the Petri plate was placed at the center of the sampling area for 1 hour, 1 meter away from the wall, and 1 meter above the height of the human breathing zone (1/1/1 standardized method) (Bolookat et al. 2018; Andualem et al. 2019). Physical parameters such as thermal condition, humidity, and ventilation framework were recorded. To minimize the mixing of outdoor bacterial samples, all ventilations were remained closed and movement of inhabitants prohibited during the indoor sampling. At the time of sampling, other microbial safety measures were followed (Napoli et al. 2012; Sharpe et al. 2020). After sampling all samples were transported on the same day without any delay to the CTR (Centre for Translational Research) laboratory, Jiwaji University, Gwalior, and incubated at 37 °C for 24-48 hours. Bacterial colonies growing on culture media were expressed and calculated in the form of colony forming unit per cubic meter (CFU/$m^3$) by applying the equation Andualem et al. (2019).

$$N = \frac{a * 10000}{bt * 0.2}$$

Where N = Indoor airborne bacterial CFU/$m^3$, a = Colony counts per Petri plate,
b= Surface area of Petri plate in
$cm^2$t= Time of air exposure in minutes1

Based on microscopic examination, purification by sub-culturing for another 24-48 hours at 37 °C on the same culture media was used to attain pure culture isolates. The identification of the obtained colonies was based on gram staining and their colony formation characteristics such as shape, size, opacity, and color (Becerra et al. 2016; Mirhoseini et al. 2016). A light microscope was used to assess the morphology of bacteria at 100X magnification under oil immersion. The bacterial generic identity was achieved based on the taxonomic classification (Goodfellow et al. 2012). Descriptive statistics were used to depict the airborne bacterial load. To evaluate comparison, between averages bacterial load in the rural and urban indoor

and outdoor environment one-way analysis of variance (ANOVA) was employed. In addition, to analyze the correlation of airborne bacterial load with physical factors of indoor environment Pearson's correlation coefficients were employed.

## RESULTS AND DISCUSSION

**Bacterial load:** The present study was employed at a preliminary stage to compare the airborne bacterial load in the outdoor and indoor environment of rural traditional and urban modular kitchen among Adupurajagir village and Gwalior city respectively. The minimum and maximum bacterial load were estimated in the urban modular kitchen (511.12 CFU/m$^3$) and a rural traditional kitchen (2621.16 CFU/m$^3$) respectively. The mean bacterial load was 2593.75 CFU/m$^3$ in the rural traditional kitchen, 783.03 CFU/m$^3$ in rural open space, 562.35 CFU/m$^3$ in urban modular kitchen, and 1215.13 CFU/m$^3$ in the urban open space environment. The bacterial load of the indoor environment of rural traditional kitchen among Adupurajagir was found highest with the mean value of 2593.75 CFU/m$^3$. Furthermore, bacterial load of the indoor environment of urban modular kitchen among Gwalior was observed lowest with the mean value of 562.35 CFU/m$^3$ (Table 1).

On the other hand, the sum of outdoor bacterial load of Adupurajagir and Gwalior was 77520.32 CFU/m$^3$ and 120297.87 CFU/m$^3$ with the mean bacterial load of 783.03 CFU/m$^3$ and 1215.13 CFU/m$^3$ respectively (Table 1). Although there is no general threshold estimation concerning airborne bacterial load in the indoor environment, the WHO suggested that a total load of microbes in the indoor environment should not surpass 1000 CFU/m$^3$ (Hänninen 2011). The Sanitary Standards of the European Commission for non-industrial premises reported that > 50 CFU/m$^3$, < 100 CFU/m$^3$, < 500 CFU/m$^3$, and < 2000 CFU/m$^3$ is considered very low, low, high, and very high microbial load (Colbeck and Whitby 2019; Kotgire et al. 2020). Taking these standardized data into consideration, the mean bacterial load of the indoor environment in the rural traditional kitchen much higher than that outdoor. Moreover, the mean bacterial load of the indoor environment in the urban modular kitchen shows lower values.

**Table 1. Descriptive statistics analysis of airborne bacterial load of indoor air environment in kitchen and open space environment among Adupurajagir village and Gwalior city (n = 50).**

| Bacterial CFU/m$^3$ | | | | | | | |
|---|---|---|---|---|---|---|---|
| **RK** | | **RO** | | **UK** | | **UO** | |
| Mean | 2593.75 | Mean | 783.03 | Mean | 562.35 | Mean | 1215.13 |
| Median | 2594.95 | Median | 773.24 | Median | 550.44 | Median | 1192.62 |
| Mode | 2621.16 | Mode | 773.24 | Mode | 537.33 | Mode | 1153.31 |
| Standard Deviation | 23.614 | Standard Deviation | 44.161 | Standard Deviation | 43.992 | Standard Deviation | 68.971 |
| Minimum | 2555.63 | Minimum | 707.71 | Minimum | 511.12 | Minimum | 1048.46 |
| Maximum | 2621.16 | Maximum | 891.19 | Maximum | 655.29 | Maximum | 1310.58 |

RK=Rural Kitchen; RO = Rural Open space; UK = Urban Kitchen; UO = Urban Open space.

**Table 2. One way ANOVA test results on rural and urban airborne bacterial load in indoor environment of kitchen and open space environment at study sites.**

| | Analysis of Variance | | | | | |
|---|---|---|---|---|---|---|
| **Source of Variation** | **SS** | **df** | **MS** | **F** | **P-value** | **F crit** |
| Between Groups | 285805.8 | 99 | 2886.927 | 0.025533 | < 0.001 | 1.684883 |
| Within Groups | 22612981 | 200 | 113064.9 | | | |
| Total | 22898787 | 299 | 115951.8 | | | |

SS = Sum of the Square; df = Degree of Freedom; MS = Mean Square.

One way ANOVA test results was demonstrated to differentiate the mean airborne bacterial load among study sites. Whereas the sum of square between groups was 285805.8 and within groups was 22612981 and total number of mean square was 115951.8 estimated. The ANOVA test reflected that there was a significant mean airborne bacterial load difference among study sites at $p < 0.001$ with total degree of freedom was 299 (Table 2).

**Morphology analysis:** In this study, rural and urban airborne bacterial load in the indoor environment of the

kitchen and open space environment contains a diversity of airborne bacteria. Five bacterial species (spp.) were identified as *Staphylococcus aureus* spp, *Bacillus* spp, Coagulase-negative *Staphylococcus* spp, *E-coli* spp, and *Micrococcus* spp. Gram +ve bacteria were found maximum than gram -ve (Fig. 2).

**Figure 2: Mean of isolated gram +ve and gram -ve bacteria from rural and urban kitchen and open space environments (n = 156).**

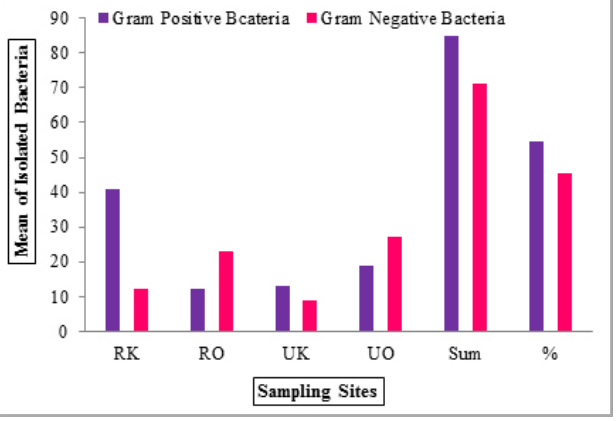
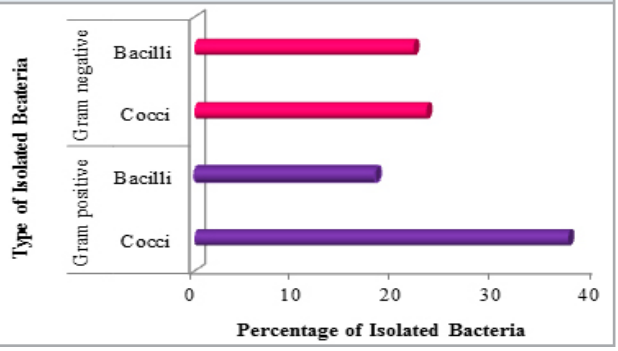



**Table 3. Summary of bacterial isolates from rural and urban kitchen and open space environments (n = 156).**

| Sampling site | No. of isolates | Gram +ve | | Gram -ve | |
|---|---|---|---|---|---|
| | | Cocci | Bacilli | Cocci | Bacilli |
| RK | 53 | 29 | 11 | 4 | 8 |
| RO | 35 | 7 | 5 | 13 | 9 |
| UK | 22 | 9 | 4 | 2 | 7 |
| UO | 46 | 13 | 6 | 17 | 10 |
| Sum | 156 | 58 | 26 | 36 | 34 |

**Figure 3: Percentage of isolated gram +ve and gram -ve bacteria on the basis of their cellular shape (n = 156).**




A total of 156 bacterial colonies were isolated from the rural traditional kitchen (53), rural open space (35), urban modular kitchen (22), and urban open space (46). All bacterial isolates were identified on their colony morphology, shape, size, opacity, and color. Among them, 54.48% and 45.51% belong to gram +ve and gram -ve bacteria respectively. Therefore total 84 gram +ve (cocci = 58, bacilli = 26) and total 70 gram -ve (cocci = 36, bacilli = 34) bacteria were isolated from study sites (Fig. 3). In addition, the highest percentage of gram +ve cocci (23.07%) and lowest percentage of gram +ve bacilli were (17.94%) reported in Adupurajagir village. This was followed by the percentage of gram -ve bacteria in which the highest 23.07% cocci and lowest 21.79% bacilli were reported in Gwalior city. However, the highest percentage of gram +ve cocci were 37.17% and the lowest percentage of gram +ve bacilli was 17.94% estimated in the rural traditional kitchen and outdoor environment among Adupurajagir. Whereas, the mean of 56 gram +ve cocci and 26 bacilli and 36 gram -ve cocci and 34 bacilli were recorded (Table 3). Nonetheless, the high load of gram +ve cocci was observed then gram -ve airborne bacteria. This research also reflects the same outcomes as gram +ve bacteria were isolated more than gram -ve bacteria (Kotgire et al. 2020).

**Characterization of identified bacterial species:** Five bacterial species were identified *Staphylococcus aureus* spp, *Bacillus* spp, Coagulase-negative *Staphylococcus* spp, *E-coli* spp, and *Micrococcus* spp. *Staphylococcus aureus* spp and *Micrococcus* spp be owned by the flora of the human dermis and are usually members of the microbiota of the body, it is aptly that this microbiota may be originated from the dermis flora of the inhabitants of their dwellings. *Micrococcus* spp and *Staphylococcus aureus* were isolated from all the sampling sites (Fig. 2), it is considered to be an emerging nosocomial pathogen.

*Micrococcus* spp can cause pneumonia, septic shock, meningitis, and endocarditis. Conversely, *Staphylococcus aureus* is gram +ve bacteria that may cause disease symptoms through the production of toxins for example food poisoning, human dermis infections, pneumonia, bone infections, urinary tract infections, and diarrhea. *Bacillus* spp can stay alive in the harsh environmental conditions in the air due to its ability to form spores and show resistance against common disinfectants which are used in daily disinfection practice in dwellings. This gram +ve bacterium may be found on dirt particles and paper. Its presence on dirt particles in the air may consequence in settling on food or food contact surfaces, hence ensuring its survival in the kitchen and posing a possible threat to women and their children (< 5 years old) because of determined sex disparities across many proportions, women's household works such as cooking may be more exposed than men's (Kotgire et al. 2020).

In addition, improper cleaning and poor infrastructure (ceiling, mud walls, roof made by wood or leaves, short height of roof, and gap between wall and roof) were observed during sampling in the traditional kitchen among dwellings of Adupurajagir village, which may lead to serious bioaerosol infectivity of food such as queasiness, vomiting, diarrhea, wound, and central nervous system (CNS) infections and people with the weakened immune system are prone to *Bacillus* spp. *E-coli* is the most abundant species in the hospital environment however, in this study it was isolated from the kitchen environment among Adupurajagir village and Gwalior city also. This gram

-ve coliform bacterium of the family Enterobacteriaceae commonly lives in the human intestine. People inside the dwellings among both study sites may be exposed to *E-coli* from contaminated food and water due to poor hygienic practices were observed. Most strains of this bacterium are not harmful but some strains are contagious by produce toxins that cause illnesses such as septicemia, neonatal meningitis, bloody diarrhea, urinary tract infection, and gastroenteritis. Coagulase-negative *Staphylococcus* spp is referred from gram +ve (Kotgire et al. 2020).

This species is commonly found as a food-associated saprophyte and also present on the human dermis and mucous membrane. Overcrowded dwellings with poor ventilation framework found in Adupurajagir village. It was observed that villagers believe in living in joint families rather than nuclear families, which is considered to be the main basis of Coagulase-negative *Staphylococcus* spp occurrence within sampling sites. Coagulase-negative *Staphylococcus* spp is identified as is one of the main pathogens of nosocomial infection also shows methicillin resistance in nature and developed multi-antibiotic drug resistance which may be responsible for the ineffective treatment. Findings say that most of the bacterial species are airborne in the residential environment associated with the human dermis. All identified bacterial species have colonized all the sampling sites within the dwellings (Table 3). It should be considered that all the isolated bacterial species are identified as highly infectious and disease-causing or opportunistic pathogenic. Future research would help to find out the possible source of the subsistence of pathogenic bioaerosols within the kitchen and outdoor environment of the dwellings (Magd et al. 2020; Kotgire et al. 2020).

**Pearson's correlation coefficients between bacterial load and physical factors of indoor environment in rural traditional kitchen:** During measurement of physical factors of the indoor environment in the rural traditional kitchen, it was observed that all measured rural traditional kitchens did not have a ventilation framework. They use unclean fuel (wood, dung cake, and crop residues) over LPG for cooking. The relative humidity (RH %) and indoor temperature (T $^{o}$C) ranged from 61% - 90% and 10 $^{o}$C - 21 $^{o}$C respectively. Bacterial load (CFU/m$^{3}$) showed significant correlation with temperature ($p < 0.001$). However, a non-significant correlation was found with relative humidity ($p > 0.01$). The thermal condition of the indoor environment exhibited a significant correlation with airborne bacterial load in the rural traditional kitchen ($r = 0.9090$) while there was a non-significant correlation with the relative humidity ($r = 0.0006$) (Brągoszewska et al. 2017; Magd et al. 2020).

Hence the airborne bacterial load will increase as the indoor temperature increases and the bacterial load decreased with reduced relative humidity. This is attributed because of decreased metabolism and physiological activities of bioaerosols under dry environmental conditions (Brągoszewska et al. 2017). The difference of bacterial load in the indoor environment of rural and urban kitchen caused by outdoor climate and physical factors of the indoor environment like ventilation framework of kitchens, thermal condition, and relative humidity. The difference amongst the bacterial load of indoor and outdoor environment at the study sites due to the microclimatic variations, construction material, vehicular pollution, outdoor levels, and daily household activities. The bacterial load of the outdoor environment in these settings reflects the variation of biological sources and the geochemical processes affecting indoor and outdoor relationships of airborne bioaerosols (Nasir et al. 2012; Magd et al. 2020).

Taking the account into consideration, the impact of physical factors of the indoor environment of rural kitchens may significantly affect the spread of diseases, as the lungs of exposed persons are more susceptible to infections due to heavy microbial load. In addition, relative humidity and poorly ventilated indoors also affect their health. There is no prominent evidence but the above-mentioned conditions which are analyzed in rural kitchens might influence the spread of dangerous coronavirus due to the poor health conditions and the increased load of aerosols. Recent research also confirmed that the number of positive cases varied between indoor and outdoor environments among rural and urban areas. Therefore, the indoor environment without a ventilation framework with increased temperature may be more vulnerable to the spread of coronavirus infection among residents (Magd et al. 2020).

## CONCLUSION

The study suggests that the microbial air quality analysis of the indoor environment is necessary to provide variation of air quality and prevent possible wellbeing vulnerability allied with it. High bacterial load was found in the indoor environment of the rural traditional kitchen as compared to the urban modular kitchen due to poor ventilation framework and usage of unclean fuel over LPG for cooking. It is important to determine the airborne bacterial load to find out the risk from the indoor environment generated bacterial diseases/disorders. Significance of this study is that bioaerosol sampling could deliver fruitful knowledge about the variation of air quality and in future prevent possible hospital admissions. The study was planned to make a comparison of bacterial load in rural and urban indoor and outdoor environments, to specify the bacterial load in traditional kitchens of rural dwellings.

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**Conflict of Interests:** Authors declare that they have no conflict of interests.

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