# MICROBIAL ASSESSMENT OF TOILET TISSUES SOLD IN OWERRI, IMO STATE, NIGERIA

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## Abstract

Toilet tissue is a widely used hygiene product essential for daily sanitation practices. Despite its intended hygienic purpose, improper handling and poor manufacturing standards may lead to microbial contamination, posing potential health risks to users. This study assessed the microbial safety of different brands of toilet tissues sold in Owerri, Imo State, Nigeria. Six brands labelled A, B, C, D, E and F tissues were analyzed for bacterial and fungal contamination using standard microbiological methods. Samples were serially diluted and cultured on Nutrient Agar, MacConkey Agar, Blood Agar, and Sabouraud Dextrose Agar. Microbial isolates were identified through morphological, biochemical, and microscopic analyses. Results showed varying contamination levels among brands, with the F tissue exhibiting the highest bacterial load ($7.1 \times 10^3$ cfu/ml) and fungal count ($1.0 \times 10^2$ cfu/ml). Enterobacter sp. and *Staphylococcus aureus* were the predominant bacterial isolates, while *Aspergillus* spp. and *Mucor* spp. were the major fungi identified. These findings highlight the need for stricter quality control, improved packaging, and hygienic handling during production and distribution.

**Keywords:** toilet tissue, microbial contamination, Enterobacter, Aspergillus, hygiene, public health

## INTRODUCTION

Toilet tissue is an essential household hygiene product designed primarily for personal sanitation after defecation and urination. Beyond this, it is frequently used for cleaning minor spills, facial

cleaning, and emergency first-aid. Given its widespread use, the hygienic quality of toilet tissue is critical in preventing the transmission of enteric pathogens via the fecal–oral route (Akinmoladun *et al*., 2022; Gendron *et al*., 2012).

Studies have shown that even unused paper towels and tissues can harbor microbial contaminants (Harrison *et al*., 2003). These findings raise concerns regarding the microbiological safety of such consumer products, particularly in developing regions where regulatory standards for paper products are less enforced. In Nigeria, many unbranded or locally produced tissue papers are sold without clear manufacturing details, creating potential risks for microbial contamination.

This study, therefore, aims to assess the hygienic safety and microbial quality of toilet tissues marketed in Owerri, Imo State, Nigeria.

**Aim of the Study**

The aim of this study is to assess the hygienic safety of toilet tissues sold in Owerri, Imo State.

**Objectives of the Study**

The specific objectives of the study are:

1. To isolate and enumerate the microbial load of different samples of toilet tissue sold in Owerri. Imo State
2. To characterize and identify microorganism from different samples of toilet tissue sold in Owerri. Imo State
3. To determine the prevalence of microbial species in different samples.

## MATERIALS AND METHOD

**Sample Collection**

For this study, a total of five different brands of toilet tissue papers were be purchased from Eke-Onunwa Market in Owerri, Imo State, Nigeria. The brands selected for this study include A, B, C, D, E, and F tissue paper. Each brand was purchased in sufficient quantity to ensure adequate sampling for subsequent analysis. The selection of these brands aims to provide a diverse range of toilet tissues for evaluation, potentially revealing variations in microbial contamination across different products.

**Sample Preparation**

The preparation of tissue paper samples for microbial analysis was involving serial dilution. Initially, one gram of each tissue paper sample was carefully weighed using a precision balance. This sample were then be diluted with 10 milliliters of distilled water in a sterile container. The mixture was homogenized thoroughly to ensure an even suspension of microbial contaminants. Following homogenization, the sample was ready for microbial enumeration and further analysis.

**Microbial Enumeration**

To enumerate the microbial load in the tissue paper samples, a portion of the prepared sample was subjected to serial dilution. Specifically, 1 milliliter of the diluted sample was transferred to a sterile tube containing 9 milliliters of distilled water, creating a further diluted solution. This dilution was then be inoculated onto selective and non-selective media using the pour plate method. The media used were include MacConkey agar, Blood agar, and Nutrient agar. These plates were incubated at 37°C for 24 to 48 hours. Additionally, a portion of the diluted sample will be poured onto Sabouraud Dextrose agar to isolate fungi, and these plates were be incubated at 37°C for 72 hours. After the incubation period, colonies on each plate were counted and recorded to determine the microbial load in each tissue paper sample (Ohazuruike *et al.,* 2017; Cheesbrough, 2010).

**Identification of Isolates**

As described by Ohazuruike *et al.,* 2017, the identification of microbial isolates from tissue paper samples was involve a series of detailed procedures, encompassing morphological, microscopic, and biochemical tests.

**Morphological Examination**: Initially, each isolate will be examined morphologically. The colonies were observed for their size, shape, color, margin, and texture. Colony characteristics such as size (in millimeters), shape (circular, irregular), color (opaque, translucent), margin (entire, undulate), and texture (smooth, rough) was noted to provide preliminary classification (Cheesbrough, 2010).

**Microscopic Examination**: Following morphological analysis, isolates were subjected to microscopic examination. For bacterial isolates, a smear was prepared on a glass slide, air-dried, and fixed by passing through a Bunsen burner flame. The slide was then be stained using Gram stain reagents (crystal violet, iodine, decolorizer, safranin). For fungal isolates, a slide will be prepared with lactophenol cotton blue or other appropriate stains. The microscopic analysis was including observations of cellular morphology and arrangement for bacteria, and fungal structures

such as hyphae, conidia, and sporangia for fungi. Parameters such as magnification (100x, 400x) and cellular characteristics were assessed (Cheesbrough, 2010).

**Biochemical Tests**: To further identify the isolates, a series of biochemical tests was performed. Such as **catalase test, coagulase test, indole test, oxidase test, methyl red test, motility test, carbohydrate fermentation tests (**Cheesbrough, 2011)**.**

**Fungal Identification**: For fungal isolates, the morphological and microscopic analyses were complemented by additional identification procedures. The morphology of fungal colonies on Sabouraud Dextrose Agar was observed for characteristics such as color, texture, and growth pattern. For microscopic examination, a slide with lactophenol cotton blue was prepared and examined under the microscope for fungal structures, including hyphae, conidia, and reproductive elements. Parameters such as colony morphology and fungal structures were assessed to accurately identify the fungal isolates.

**Result**

Bacterial and fungal counts varied among brands (Tables 1–2). The unbranded tissue showed the highest bacterial ($7.1 \times 10^3$ cfu/ml) and fungal ($1.0 \times 10^2$ cfu/ml) contamination, while Familia exhibited the lowest bacterial count ($4.5 \times 10^1$ cfu/ml) and no fungal growth.

**Table 1: Total Bacterial Count (TBC) in Tissue Paper Samples (cfu/ml)**

| Sample Brand | Total Bacterial Count (TBC in cfu/ml) |
|---|---|
| A | $4.5 \times 10^1$ |
| B | $3.5 \times 10^1$ |
| C | $5.7 \times 10^1$ |
| D | $4.1 \times 10^1$ |
| E | $5.1 \times 10^2$ |
| F | $7.1 \times 10^3$ |

**Table 2: Total Fungal Count (TFC) in Tissue Paper Samples (cfu/ml)**

| Sample Brand | Total Fungal Count (TFC in cfu/ml) |
|---|---|
| A | - |
| B | - |
| C | $2.0 \times 10^1$ |
| D | $4.0 \times 10^1$ |
| E | - |
| F | 1.0 x 10^2 |

**Table 3: Morphological and biochemical characterization of bacterial isolates**

| Microbial Isolates | Colony Morphology | Gram Stain | Catalase Test | Coagulase Test | Indole Test | Oxidase Test | Methyl Red Test | Carbohydrate Fermentation | Motility | Urease Test |
|---|---|---|---|---|---|---|---|---|---|---|
| *Enterobacter sp.* | Large, smooth, moist colonies, pale or off-white | Gram-negative rods (short, straight or slightly curved) | Negative | Negative | Negative | Negative | Negative | Ferments glucose and other sugars (e.g., lactose) | Motile | Positive |
| *Staphylococcus aureus* | Golden yellow colonies, smooth, opaque | Gram-positive cocci in clusters (grape-like) | Positive | Positive (clot formation in plasma) | Negative | Negative | Negative | Ferments glucose, mannitol (produces acid and gas) | Non-motile | Negative |

**Table 4. Morphological characteristics of fungi isolate**

| Assumed Fungi Species | Microscopic Appearance | Hyphal Structure | Conidia | Colony Appearance (on agar) | Sporulation | Color of Colony (initial) |
|---|---|---|---|---|---|---|
| **Aspergillus spp.** | Conidiophores are branched with a swollen vesicle at the tip, producing conidia. | Septate hyphae (with internal divisions between cells). | Conidia are small, round, and arranged in chains. | Green, yellow, or blackish, velvety or powdery appearance. | Produces conidia from a vesicle in radial pattern. | White, yellowish, green, or brown. |
| **Mucor spp.** | Non-septate, branched hyphae with large sporangium containing sporangia. | Non-septate hyphae (without internal divisions). | Large spherical sporangium containing numerous round sporangia. | White to grayish, cottony or fluffy appearance. | Produces sporangia that release spores when ruptured. | White to cream, turning darker (black or gray). |

**Table 5. Prevalence of Isolates across tissue samples**

| Sample Brand | *Enterobacter sp.* | *Staphylococcus aureus* | *Aspergillus spp.* | *Mucor spp.* |
|---|---|---|---|---|
| A | Present | Absent | Absent | Absent |
| B | Present | Absent | Absent | Absent |
| C | Present | Present | Present | Absent |
| D | Present | Absent | Absent | Present |
| E | Present | Present | Absent | Absent |
| F | Present | Present | Present | Present |

## DISCUSSION

The assessment of the sanitary quality of toilet tissues on sale in Owerri, Imo State, reveals different levels of bacterial and fungal contamination present in various samples. These results correspond with the bacterial contamination literature on hygiene and tissues and shows health challenges that entrenched poor hygiene practices on tissues and tissues products in production processes may pose.

Total Bacterial Count (TBC) has varying contamination levels in the different samples. F tissue presents the greatest level of bacterial contamination with 7.1 x $10^3$ cfu/ml. This finding is consistent with research that shows unbranded or poorly regulated products tend to carry greater microbial loads probably as a result of poor-quality control in production and packaging (Mushiana *et al*., 2018). In contrast, Familia recorded the lowest count of 4.5 x $10^1$ cfu/ml. This difference may imply that branded tissue products may actually exercise more vigorous quality control in order to reduce bacterial contamination. Other studies have documented the incidence of branded products containing less microbial contamination than unbranded products (Akinmoladun *et al*., 2022).

The moderate bacterial counts in brands like B, C, D and E indicate a moderate risk of bacterial contamination, which aligns with the typical finding that some level of microbial growth is often inevitable due to handling and environmental exposure during storage (El-Gohary *et al*., 2021).

In Table 2, the Total Fungal Count (TFC) shows that only C, D, F and E tissue samples exhibited fungal contamination. F tissue, again, shows the highest fungal count at 1.0 x $10^2$ cfu/ml, highlighting a potential concern regarding the storage and handling practices of these products, which may encourage fungal growth. This is consistent with studies that have found tissue products with inadequate packaging or improper storage conditions to be more susceptible to fungal contamination (Adeyemo *et al*., 2020). Fungal growth in tissue papers can lead to respiratory issues and allergic reactions in sensitive individuals, particularly those with weakened immune systems (Mushiana *et al*., 2018). The absence of fungal contamination in other brands like A, B, and E suggests that these brands may have more effective preservation methods in place to prevent fungal growth.

Table 3 provides the identification of bacterial isolates, specifically *Enterobacter* spp. and *Staphylococcus aureus*. The presence of *Enterobacter* spp. in all tissue samples indicates widespread bacterial contamination, which is concerning as *Enterobacter* species are commonly associated with fecal contamination, a common sign of poor hygiene during production (Ogunbanwo *et al*., 2017). The identification of *Staphylococcus aureus* in C, D, E and F samples further raises concerns, as *S. aureus* is a well-known pathogen capable of causing a variety of

infections, especially skin and soft tissue infections. Literature suggests that *S. aureus* can be transferred to consumer products through improper handling or contamination during production (Ogunbanwo *et al*., 2017). These findings emphasize the need for improved hygiene practices in the production and packaging of tissue products.

Table 4 details the fungal species identified, including *Aspergillus spp*. and *Mucor spp. Aspergillus spp*. is known for its ability to grow on organic materials, and its presence in tissue products poses a risk for individuals with respiratory conditions or weakened immune systems (Mushiana *et al*., 2018). *Mucor* spp., which was found in D and F tissue samples, is also a significant concern due to its potential to cause mucormycosis, a life-threatening fungal infection, particularly in immunocompromised individuals (Adeyemo *et al*., 2020). The presence of both *Aspergillus* and *Mucor* species in tissue products suggests inadequate control of environmental conditions during production and storage.

Finally, Table 5 summarizes the prevalence of microbial isolates across tissue samples. The consistent presence of *Enterobacter* sp. across all brands suggests that bacterial contamination is widespread in the tissue products sampled. The detection of *Staphylococcus aureus* in C, E and F, as well as *Aspergillus spp.* and *Mucor spp.* in C and F, further emphasizes the potential health risks posed by these products. Previous study has similarly found that tissue products, particularly those from less regulated brands, can harbor a variety of pathogenic microorganisms, which could lead to health issues if proper hygiene measures are not followed (El-Gohary *et al*., 2021). These results underline the importance of adhering to stringent hygiene standards in the manufacturing, packaging, and storage of tissue products to reduce microbial contamination and safeguard consumer health.

Based on the findings of this study, it is not advisable to declare the tissue papers tested as hygienic enough for public use, particularly given the high levels of microbial contamination observed, especially in unbranded tissue products. In summary, based on the microbial contamination levels observed, these tissue papers do not meet the necessary hygienic standards for safe public use. Further investigation into the production processes, storage conditions, and better-quality control measures are crucial for ensuring the safety of tissue paper products.

## Conclusion

The findings of this study indicate significant microbial contamination in tissue paper samples sold in Owerri, Imo State, with both bacterial and fungal contamination present in various brands. F tissue samples exhibited the highest levels of contamination, both in terms of bacterial ($7.1 \times 10^3$ cfu/ml) and fungal ($1.0 \times 10^2$ cfu/ml) counts, highlighting a potential risk to public health. Bacterial isolates such as ***Enterobacter* sp.** and ***Staphylococcus aureus*,** along with fungal species like

***Aspergillus* spp.** and ***Mucor* spp.,** were identified, further emphasizing the health risks posed by these tissue products. The presence of ***Enterobacter* sp.** in all sample's points to widespread bacterial contamination, while ***Staphylococcus aureus*** and fungal species raise concerns regarding infections and allergic reactions, particularly for immunocompromised individuals.

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