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Bacteriological Assessment of Tissue Papers Sold in Eke Awka Market in Anambra State, Nigeria

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Abstract

The tissue paper originates from the French word "Tissue", meaning cloth. This work was carried out to determine the Bacteriological assessment of tissue paper in an unused tissue papers sold in Eke awka market and to deduce their sources of contamination. A total of 15 tissue papers were collected from Eke-Awka market, Anambra State, Nigeria. Serial dilution was carried out on 15 samples of tissue papers. Most of the unused tissue paper posseses high microbial loads and high concentrations of dusts. Different microscopic and biochemical test were employed to identify the bacterial isolates. After the analysis the organisms were identified, some of which were toxigenic. The highest plate count was recorded in sample 9 whose plate count was 298 while the least was recorded by sample 2. Most-Prevalent organisms were *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Staphylococcus and Salmonella species.* Prevalence of bacteria of clinical importance in unused contaminated toilet rolls, have harmful domestic and occupational health implications. Discovery of microbial flora in toilet roll is indicative of microbial contamination in toilet roll industries, and this can provide a rationale for development of an effective industrial control measures. Studies to trace and identify contamination routes of bacterial and fungal flora of public health importance, occurring in toilet roll industries are on-going in our laboratories. This is in order to develop an effective microbial control programme in wood processed and recycled paper processing for the production of unused toilet rolls. Good hygiene practices should be maintained starting from choice of raw materials, the manufacturing processes to packaging and the equipment used must be properly cleaned to reduce contamination.

Keywords

Tissue Paper, *Bacillus cereus*, Bacteriological Assessment, Toilet Rolls and *Eke market*

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1. Introduction

They are general-purpose, soft paper products for toilet needs of the entire family, most especially for maintaining personal hygiene after human defecation or urination. They can also be used for other purposes, such as food packaging, kitchen cleaning of plates and dishes, cups, cutleries; as sanitary towels or pant liners, and also for emergencies like first aids, to stop or clean light bleedings due to cuts, wounds and bruises [1]. Other uses of toilet rolls include removal of facial makeup, nose-blowing or absorbing/cleaning common spills around the house (although paper towels are more used for this particular purpose). In modern usage, toilet rolls would seem to play an important role as barrier to transmission of enteric infection by fecal manual- oral route. According to some authors [2, 3] bacteria, which can be transmitted to humans during usage, have been known to be present on paper products, such as unused paper towels and toilet rolls.

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Although toilet paper with highly recycled content may be inexpensive or easily obtained, such that it was reported that toilet tissue manufacturers have one of the highest recycled paper utilization rates, it is still important to note that products with recycled contents, like toilet rolls, may contain certain toxic bio- aerosols and chemicals during production. Airborne pathogenic bacteria and fungi from toilet roll dusts can be suspended air as respirable bio aerosols that can be readily inhaled [4]. Although non-infectious bio aerosols may not cause frequent mortality, infectious bio aerosols, such as paper dusts have long been known to cause mortal respiratory infections, while infectious microorganisms have also reportedly caused more serious respiratory infections like dust-induced pulmonary conditions, including acute airway inflammation, mucous membrane irritation, chronic bronchitis, and hypersensitivity [5, 6]. Likely unhygienic and microbial-laden unused toilet rolls call for scientific attention in every country, especially since associated pathogenic microbial species can be geographic dependent.

2. Materials and Methods

Collection of Samples: A total of 15 tissue papers were collected from Eke-Awka market, Anambra State, Nigeria.

Determination of physical properties of Tissue paper samples

Quality or physical properties of the tissue paper samples determined were absorbance or soaking abilities, color, texture, foil demarcations, fluffiness, foil designs, foil leaflets (number of plies), foil leaflets (length), size, packaging and cost.

Quantitative Analysis:

Serial Dilution: Serial dilution was carried out on 15 samples of tissue papers. One gram of the sample was weighed and diluted with 10 ml distilled water and homogenized. 1 ml from the test sample was carefully transferred to the tube with 9 ml of distilled water and aspetically inoculated using pourplate method and incubated at 37°C for 24-48 hours. Then Isolation and identification of the organisms were carried out.

Biochemical Test: The following biochemical test was carried out; Catalase test, Coagulase test, Indole test, Oxidase test, Methylred test, motility and carbonhydrate fermentation tests.

3. Results

The toilet rolls were purchased from Eke Awka market randomly from different shops especially those manufactured by different companies. Table 1, shows the number of samples, the plate counts and the dilution factor in the unused tissue analyzed. The highest plate count was recorded in sample 9 whose plate count was 298 while the least was recorded by sample 2. Table 2, shows the number of organisms isolated from their mixed colony after examination of the plates. It was isolated based on their colony morphology which includes shapes, margin, sizes, texture, optical properties and pigmentation. Table 3 shows the biochemical tests carried out on the pure isolates to identify the organisms, the isolates are *Staphylococcous specie, Escherichia coli, Bacillus specie Salmonella specie and Klebsiella pneumonia*

Table 1. Number of samples, the plate counts and the dilution factor in the unused tissue analysed.

Sample	Plate counts	Dilution Factor
1	104	10^{-2}
2	100	10^{-2}
3	160	10^{-2}
4	176	10^{-2}
5	116	10^{-2}
6	119	10^{-2}
7	198	10^{-2}
8	142	10^{-2}
9	298	10^{-2}
10	262	10^{-2}
11	276	10^{-2}
12	221	10^{-2}
13	123	10^{-2}
14	186	10^{-2}
15	109	10^{-2}

Table 2. Colony Morphology of the Isolates.

Isolates	Shape	Margin	Elevation	Size	Texture	Appearance	Optical properties	Pigmentation
1	Circular	Entire	Flat	Moderate	Moisture	Shiny	Opaque	Cream
2	Circular	Entire	Flat	Punctiform	Moisture	Shiny	Opaque	Grey
3	Circular	Entire	Flat	Large	Mucoid	Shiny	Opaque	Cream
4	Circular	Entire	Convex	Small	Moisture	Dull	Transluscent	White
5	Circular	Entire	Convex	Large	Mucoid	Dull	Transluscent	Cream
6	Circular	Entire	Flat	Moderate	Moisture.	Shiny.	Transluscent	Cream

Table 3. Gram Staining and Biochemical Properties of the Isolates.

Isolates	Gram staining	Oxidase	Catalase	Coagulase	Methyl Red Test	Citrate Test	Indole Test	Motility
1	Gram positive cocci	-	+	-	-	ND	ND	-
2	Gram positive cocci	-	+	-	-	ND	ND	-

Isolates	Gram staining	Oxidase	Catalase	Coagulase	Methyl Red Test	Citrate Test	Indole Test	Motility
3	Gram negative rod	-	-	ND	+	-	+	+
4	Gram positive rod	-	+	-	-	+	-	+
5	Gram negative rod	-	-	ND	+	-	-	+
6	Gram negative rod	-	-	ND	+	+	-	-

Table 3. Continued.

Isolates	Gram staining	Glucose Acid and Gas	Lactose Acid and Gas	Sucrose Acid and Gas	Maltose Acid and Gas	Probable Organism
1	Gram positive cocci	+	+	+	+	*Staphylococcus spp*
2	Gram positive cocci	+	+	+	+	*Staphylococcus spp*
3	Gram negative rod	+	+	-	-	*Escherichia coli*
4	Gram positive rod	+	-	-	+	*Bacillus spp*
5	Gram negative rod	+	-	-	+	*Salmonella spp*
6	Gram negative rod	+	+	+	+	*Klebsiella pneumoniae*

KEY:
ND- Not determined,-= Negative reaction, += Positive reaction

4. Discussion

Exposure to high levels of paper dusts (> 5 mg/m) has been found to cause respiratory diseases among workers in the pulp and paper industries [7, 8]. Thus, prevalence of paper dusts observed among most of the analysed toilet rolls in this study suggests a harmful occupational and domestic health hazards to producers and users of such toilet rolls.

Previous studies on paper machine operators have to a large extent focused on endotoxins as possible health hazard [8] but not pathogenic microorganisms. In spite of the varying viable colonial counts on different culture plates, some of the bacteria loads of the sampled unused toilet rolls in this study were significantly high, irrespective of location of purchase. Contaminated machinery and increase of nutrients in water circuits can contaminate paper products; thereby, favouring bacteria growth and fouling. Bacteria can also thrive on recycled paper because they contain some binding ingredients like starches and fillers, which can serve as nutrients to microorganisms [9, 10].

Moreover, it is quite alarming that *Neissariae gonorrhoeae* was isolated and it is in contrast to my findings. *Neissariae gonorrhoeae* even known to survive for brief periods on different surfaces, including toilet paper (up to 3 hours) [11]. All these could infer that most of the unused toilet roll samples were contaminated, probably due to processing defects. In spite of high processing temperature [12] that the evaluated unused toilet roll samples in this study could have been subjected to during production, significant recovery rates of diverse easily culturable microbial flora were still recorded. This could be due to the microbial species being post-production contaminants or thermo-tolerant microbial flora, most especially since spore formers can likely survive various procedures encountered during paper-making processes. The most-prevalent bacteria recovered from unused toilet rolls in this study includes; s *Escherichia coli, Staphylococcus epidermidis, Staphylococcus saprophyticus, Klebsiella pneumonia, Salmonella species and Bacillus species*. Considering that potential harmfulness of aerobic mesophilic bacilli and thermophilic bacteria from different paper mill samples have been earlier reported; [13, 14, 15] if these potentially pathogenic microbial species are respirable fraction flora in toilet rolls, when such contaminated toilet rolls are used for nose blowing / cleaning, they may induce allergenic, immunotoxic and/or infectious conditions, just as also detected among paper mill workers [8].

Gendron reported that they isolated *Morganella, Pseudomonas, Shigella, Citrobacter, Corynebacterium, Enterobacter, Proteus mirabilis, Staphylococcus aureus, Paenibacillus, Exiguobacterium and Clostridium, Bacillus spp, Klebsiella, Salmonella*. [3] The most prevalent ones isolated from Gendron works includes *Bacillus cereus, Bacillus subtilis, Klebsiella pneumonia, Proteus mirabilis, Escherichia coli, Staphylococcus aureus,* while from our own study *Escherichia coli, Staphylococcus saprophyticus, Staphyloccus epidermidis, Salmonella were* the most prevalent.

Since chronic obstructive pulmonary disease is a major leading global public health problem, the significance of contaminated unused toilet rolls as likely pollutants with high particulate pollution, as well as likelihood of transmission of pathogenic or and toxigenic foodborne microorganism that cause significant morbidity and mortality rates, [5, 6], should be of serious concern.

5. Conclusion and Recommendation

Discovery of microbial flora in toilet roll is indicative of microbial contamination in toilet roll industries, and this can

provide a rationale for development of an effective industrial control measures. Studies to trace and identify contamination routes of bacterial and fungal flora of public health importance, occurring in toilet roll industries are on-going in our laboratories. This is in order to develop an effective microbial control programme in wood processed and recycled paper processing for the production of unused toilet rolls. Good hygiene practices should be maintained starting from choice of raw materials, the manufacturing processes to packaging and the equipment used must be properly cleaned to reduce contamination.

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