Microbiological Quality of Sanitary Toilet Tissue Paper Manufactured in Nigeria And the Implication of their Indiscriminate Exposure to Public Health

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(Received May 29, 2019; Revised August 23, 2019; Accepted August 25, 2019)

Abstract

In Nigeria, there is a dearth of available data relating to the microbiological quality of sanitary toilet tissue paper manufactured in Nigeria. Thus, this study aimed at determining the microbiological quality of sanitary toilet tissue paper manufactured in Nigeria and the public health implications of their indiscriminate exposure after usage. Total aerobic plate count (APC), total coliform count (TCC) and total coagulase-positive *Staphylococci* count (SC) were measured in all the samples by the spread plate technique. Isolated bacteria were identified with phenotypic techniques. All the samples were also examined for the presence of protozoan parasites. Ninety per cent of the intact commercially packed toilet tissue paper brands had APC that were below or within international recommended limits (2.78 to 3.00 $\log_{10}$ CFU/g). All the intact commercially packed toilet tissue paper brands also complied with the zero tolerance for faecal coliform bacteria, coagulase-positive *Staphylococci* and haemolytic *Streptococci* stipulated by international regulatory authorities. On the contrary, 70% of the brands of toilet tissue paper that were exposed to the environment after usage was found to be significantly contaminated as indicated by APC values that exceeded internationally acceptable standards. While only *Bacillus* species were isolated from the intact batch, *Bacillus*, *Micrococcus*, *Enterobacter* and non-haemolytic *Streptococcus* species, as well as *Staphylococcus aureus*, were all isolated from the exposed batch of toilet tissue paper. Thus, there is a need for the relevant agencies to educate the public on the proper handling of toilet tissue paper during usage to mitigate potential health hazards.

***Keywords**: Coagulase-positive Staphylococci, Bacillus species, toilet tissue paper*

1. Introduction

Toilet tissue paper is a sanitary paper that comes into direct contact with the body. Hence, they must be manufactured in such a way that they do not constitute a danger to human health. In Nigeria, there is no specific documentation of guidelines for assessing the quality of sanitary papers, but general guidelines employed by Standard Organization of Nigeria (SON) for assessing the quality of products are also applied to evaluate the quality of sanitary papers manufactured and sold in Nigeria. In Germany, the BfR (Bundesinsitut für Risikobewertung)-German Federal Institute for Risk Assessment has published guidelines for evaluating sanitary papers [1]. Also in the Carribean, particularly in Jamaica, guidelines for evaluating sanitary papers have been published by the Jamaican Bureau of Standards [2]. From sanitary point of view, certain microbiological indicators, such as, total quantity of bacteria (indicated by the aerobic plate count [APC]) present in the toilet tissue paper, as well as the presence/absence of some specific pathogens such as faecal coliforms, *Staphylococcus aureus*, haemolytic *Streptococci* and parasitic organisms are often evaluated in order to establish the quality of toilet tissue papers [3]. Most international guidelines [1, 2, 4] stipulate that acceptable limits of total quantity of bacteria should range from 600 to 1000 bacterial colony-forming units (CFU)/g (2.78 to 3.00 $\log_{10}$ CFU/g), while faecal coliforms (particularly, *Escherichia coli*),

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Staphylococcus aureus, haemolytic streptococci and parasitic organisms must be absent in all sanitary papers for them to be considered safe for human health.

The raw materials used, manufacturing process and the quality of the finished product are often the basic areas that must be controlled to ensure the safety of tissue paper for the end-users [4]. For raw materials, there is typically a positive list of functional additives and process chemicals. There are also restrictions for substances not to be used, like the carcinogenic or mutagenic ingredients. The manufacturing should be made according to GMP (good manufacturing practice). Good manufacturing practice is based on the principles of relevant quality management systems, such as the ISO 9000 series. It is also based on the relevant principles of a risk management system, such as HACCP (Hazard Analysis Critical Control Points).

Microbes have been regularly isolated from pulp and paper factories situated all over the world. Toilet tissue paper product produced in France, India, United States, Spain, Germany and New Zealand were contaminated with microbes [5, 6, 7, 8]. Microbes that have been isolated from toilet tissue paper include *Pseudomonas* species, *Bacillus* species, *Enterobacter* species, *Aeromonas* species, and *Staphylococcus* species [9, 10]. However, in Nigeria, there is a dearth of available data relating to the microbiological quality of sanitary toilet tissue paper manufactured in Nigeria. Thus, this study was carried out to determine the microbiological quality of sanitary toilet tissue paper manufactured in Nigeria and the public health implications of their indiscriminate exposure after usage.

2. Materials and Methods

2.1 Experimental design

Eighty toilet tissue paper samples consisting of 10 different commercial brands were purchased from different retail outlets in Nigeria. Each of the commercial brands consists of eight tissue paper samples that were randomly selected from the retail outlets. The 80 tissue paper samples were divided into two equal batches with all the 10 commercial brands equally represented. One of the batches represented the portion of tissue paper samples that were directly exposed to the environment after usage, as practised by some end-users; while the other batch of tissue paper samples were kept intact, as produced by the manufacturers. Both batches were subsequently analyzed. The sample size in each batch was divided into 10 different independent groups based on the different commercial brands. The measurements that were performed include total aerobic plate count, total coliform count, and total coagulase-positive *Staphylococci* count as well as the parasitological examination of the samples. Statistical significance in the variability of the value of the mean datasets obtained from the different brands of the tissue paper samples was estimated with the one-way analysis of variance (ANOVA); while statistical significance in the variability of the value of the mean datasets obtained from the exposed and intact batches of samples was estimated with the Student's t-test. Levene test of homogeneity and the Shapiro-Wilk normality test were also performed, and their outcomes were respectively used to determine the preferred ANOVA (parametric or non-parametric) and Student's t-test (paired or unpaired) that were employed for the statistical analysis.

2.2 Isolation and enumeration of bacteria

The tissue paper samples were prepared for microbial analysis according to a previously described protocol [2, 11]. Ten grams of each sample from the batch of intact tissue paper was weighed using the tissue paper from the middle of the toilet roll, while those tissue paper samples in the exposed batch were weighed using tissue paper from the toilet rolls that had been directly exposed to the environment for 5 days. The weighed tissue paper was transferred with a sterile forceps into a sterile glass jar blender containing 500 ml of distilled water with 0.025 % Tween 20. The mixture was then shredded for 30 seconds and serial dilutions ($10^{-1.7}$ to 10^{-4}) of the suspension were subsequently prepared. Ten microliters of each of the dilutions were separately spread on tryptic soy agar plates and Baird-Parker agar plates. The agar plates were incubated at 37^{o}C for 48 hours. After incubation, colony forming units were counted

on the tryptic soy agar plates to determine the total aerobic plate counts (APC). Black colony-forming units counted on the Baird-Parker agar plates were used to deduce the coagulase-positive *Staphylococci* counts (SC).

2.3 Enumeration of coliform bacteria

Total coliform count (TCC) was estimated with the most probable number (MPN) technique [2]. This test was sequentially performed in three stages (presumptive, confirmed and completed tests). For the presumptive test, all the 6 tubes were arranged in two series. In the first series, 50 ml of the toilet tissue paper suspension from the 1 in 50 dilutions was aseptically dispensed into a tube containing 50 ml double strength lactose broth and an inverted Durham tube. In the second series, 10 ml of the toilet tissue paper suspension from the 1 in 50 dilutions was dispensed into each of the 5 tubes containing 10 ml double strength lactose broth and inverted Durham tubes. All the tubes were then incubated at 37 ºC for 48 hours. The presence of acid in the broth and production of gas in the Durham tubes was recorded as a positive presumptive test for coliforms. For the confirmed test, a loopful of lactose broth from the presumptive positive tubes was respectively inoculated into 3 ml brilliant green lactose broth and a nutrient agar slant. This was followed by incubation at 37°C for 24 to 48 hours. After incubation, Gram-stained preparations were made from the agar slants and examined under the microscope; while the brilliant green lactose tubes were observed for the production of gas. The formation of gas in the broth and the confirmation of Gram-negative bacilli in the Gram-stained preparation confirmed the presence of a member of the coliform group. The MPN value of the total coliforms, which was expressed as MPN/100 gram sample, was inferred by matching the probability values of the confirmed positive tubes to those in the MPN statistical table (McCrady's table). For the completed test, positive brilliant green lactose broth tubes were inoculated by streaking a loopful of the broth onto sterile Eosin Methylene Blue (EMB) agar plates and tryptophan broth. The agar plates and tryptophan broth were then incubated at 37ºC for 24 to 48 hours and then evaluated for the appearance of typical colonies with dark centres and metallic sheen as well as the formation of a red coloured complex in the tryptophan broth. Colony-forming units with dark centres and metallic sheen on the EMB agar plates as well as the formation of a red coloured complex in the tryptophan broth confirmed the presence of *Escherichia coli*.

2.4 Identification of bacterial isolates

The bacterial isolates obtained from the tryptic soy agar plates were identified with previously described phenotypic tests [12]. The phenotypic traits of the bacterial isolates which were examined include: bacterial cell wall morphology (Gram staining), production of haemolysin (haemolysis test), biodegradation of tryptophan to produce indole (indole test), production of stable acids from glucose fermentation (methyl red test), utilization of citrate as a sole carbon source (citrate test), urea utilization (urease test), cytochrome oxidase enzyme production (oxidase test) and acetoin production (Voges Proskauer test).

2.5 Parasitological Examination

All the toilet tissue paper samples were examined for the presence of protozoan parasites using the zinc sulphate centrifugal floatation method [13]. One millilitre of each of the samples obtained from the 1 in 50 dilutions was suspended in 20 ml of zinc sulphate solution (specific gravity = 1.18). An Aliquot of the suspension was poured into the centrifuge tubes and centrifuged for 2 minutes at 2000 revolutions per minutes. The tubes were then removed from the centrifuge and filled to just over the top of the tube with coverslips placed over the top of the tube for 10 minutes. A drop of iodine was placed on slides and the coverslips transferred onto the drop of iodine. The slides were then examined for the presence of the ova of protozoa with the ×10 magnification objective lens.

2.6 Statistical Analysis

Descriptive statistics of the bacterial concentration datasets was performed with NCSS ver. 12 data analysis software. Shapiro–Wilk normality test,

Levene test of homogeneity, Fisher (F) one-way ANOVA test for normally distributed datasets with equal variances, Kruskal–Wallis nonparametric one-way ANOVA test and unpaired Students t-test were also performed with NCSS ver. 12 data analysis software.

3. Results

3.1 Extent of contamination of toilet tissue paper with microbes

The concentration of bacteria in the different brands of both the intact commercially packed toilet tissue paper and the exposed toilet paper are presented in Table 1. Total aerobic plate counts (APC) in the batch of intact commercially packed toilet tissue paper ranged from 1.00 ± 1.00 $\log_{10}$ CFU/g to 3.06 ± 0.02 $\log_{10}$ CFU/g; while in the batch of exposed toilet tissue paper, APC ranged between 2.87 ± 0.09 $\log_{10}$ CFU/g and 3.90 ± 0.02 $\log_{10}$ CFU/g. While the APC datasets of the intact batch of toilet tissue paper were non-normally distributed ($P < 0.05$), the APC datasets of the exposed batch of toilet tissue paper was normally distributed ($P > 0.05$) as indicated by the Shapiro-Wilk normality test.

The median APC datasets of the different brands of the intact commercially packed toilet tissue paper were also found to be significantly different ($P < 0.05$) from each other as indicated by the Kruskal-Wallis non-parametric ANOVA test. However, the results of the Fisher ANOVA test indicated that the mean APC datasets of the different brands of the exposed toilet tissue paper were not significantly different ($P > 0.05$) from each other. Levene test of homogeneity showed an unequal variance ($P < 0.05$) between the APC datasets of the intact and exposed batches of the toilet tissue paper; while the results of unpaired Students t-test indicated that there was a statistically significant difference ($P < 0.05$) between the mean APC datasets of the intact batch of toilet tissue paper and the exposed batch of toilet tissue paper. An infinitesimal concentration of total coliform bacteria (< 1.00 ± 0.00 MPN/100 g) were probably present in all the samples in the intact batch of the commercially packed toilet tissue paper; but in the batch of exposed toilet tissue paper, the concentration of total coliform bacteria ranged between 5.50 ± 0.65 MPN/100 g and 8.25 ± 0.75 MPN/100 g. There was also an unequal variance ($P < 0.05$) between the TCC datasets of both the intact and exposed batches of the toilet tissue paper. The mean TCC datasets of the intact batch of toilet tissue paper were not significantly different ($P > 0.05$)

Table 1: Concentration of Bacteria in the Intact and Exposed Batches of Toilet Tissue Paper

Commercial brands	Bacteriological analysis					
	Total Aerobic Plate Count (APC) analysis		Total Coliform Count (TCC) analysis		Total coagulase-positive *Staphylococci* (SC)	
	Intact toilet paper Mean APC (Log_{10} CFU/g) (N = 4)	Exposed toilet paper Mean APC (Log_{10} CFU/g) (N = 4)	Intact toilet paper Mean TCC (Coliform/100 g) (N = 4)	Exposed toilet paper Mean TCC (Coliform/100 g) (N = 4)	Intact toilet paper Mean SC (CFU/g) (N = 4)	Exposed toilet paper Mean SC (Log_{10} CFU/g) (N = 4)
1	1.00 ± 1.00	3.51 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.24 ± 0.24
2	2.59 ± 0.11	3.49 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.15 ± 1.15
3	2.60 ± 0.00	3.90 ± 0.02	0.00 ± 0.00	8.25 ± 0.75	0.00 ± 0.00	0.00 ± 0.00
4	3.06 ± 0.02	3.68 ± 0.05	0.00 ± 0.00	6.50 ± 0.87	0.00 ± 0.00	2.39 ± 0.09
5	2.54 ± 0.06	3.51 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.48 ± 0.00
6	2.54 ± 0.06	3.09 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.65 ± 0.05
7	2.83 ± 0.13	2.97 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
8	2.00 ± 0.00	2.93 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
9	1.15 ± 1.15	2.87 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.15 ± 0.05
10	1.15 ± 1.15	3.38 ± 0.06	0.00 ± 0.00	5.50 ± 0.65	0.00 ± 0.00	0.00 ± 0.00

Bacterial counts represented as mean ± standard error. N is the total number of toilet tissue paper samples per brand for the different batches examined.

Table 2: Characterization of Bacterial Isolates Obtained from the Intact and Exposed Batches of Toilet Tissue Paper

Isolates	Cultural/morphological characteristics			Biochemical characteristics							Probable bacteria on tryptic soy agar plates	Relative abundance of isolated bacteria			
	Tryptic soy agar plates	Gram staining	Haemolysis test	Co	Ca	Ur	Ci	In	Mr	Vp		Intact toilet paper (n_1 = 148)	(%)	Exposed toilet paper (n_2 = 190)	(%)
1	Mucoid colony	Positive rods	γ	-	+	-	+	-	-	-	*Bacillus* species	26/148	17.57	12/190	6.32
2	Dry colony	Positive rods	γ	-	+	-	+	-	-	-	*Bacillus* species	122/148	82.43	151/190	79.47
3	Mucoid colony	Positive cocci in clusters	β	+	+	+	+	-	+	-	*Staphylococcus aureus*	0/148	0.00	2/190	1.05
4	Mucoid colony	Positive cocci	γ	-	+	+	+	-	+	-	*Micrococcus* species	0/148	0.00	18/190	9.47
5	Mucoid colony	Negative rods	γ	-	+	-	+	-	-	+	*Enterobacter* species	0/148	0.00	6/190	3.16
6	Mucoid colony	Positive cocci in chains	γ	-	-	-	+	+	+	-	*Streptococcus* species	0/148	0.00	1/190	0.53

Co: coagulase test; Ca: catalase test; Ur: urease test; Ci: citrate test; In: indole test; Mr: methyl red test; Vp: Voges-Proskauer test. n_1 and n_2 were the total number of bacterial isolates that was respectively examined in the intact batch and exposed batch of toilet tissue paper samples. % represented the relative abundance of bacterial isolates expressed in percentage.

from the mean TCC datasets of the exposed batch of toilet tissue paper. *Escherichia coli* was not isolated from all the positive brilliant-green lactose broth tubes of the coliform-contaminated exposed toilet tissue paper samples that were inoculated onto eosin methylene blue (EMB) agar plates. Coagulase-positive *Staphylococci* was also not isolated from all the samples in the intact batch of the commercially packed toilet tissue paper. However, the concentration of coagulase-positive *Staphylococci* in the batch of exposed toilet tissue paper ranged from 0.00 ± 0.00 $\log_{10}$ CFU/g to 2.65 ± 0.05 $\log_{10}$ CFU/g. Levene test of homogeneity also indicated an unequal variance ($P < 0.05$) between the coagulase-positive *Staphylococci* datasets of the intact and exposed batches of the toilet tissue paper. The mean coagulase-positive *Staphylococci* datasets of the intact batch of toilet tissue paper were significantly different ($P < 0.05$) from the mean coagulase-positive *Staphylococci* datasets of the exposed batch of toilet tissue paper. Ova of protozoan parasites were not seen in all samples of intact and exposed batches of the toilet tissue paper.

3.2 Characterization of bacterial isolates

The phenotypic characterizations of bacterial isolates obtained from samples in both the intact and exposed batches of toilet tissue paper are presented in Table 2. *Bacillus* species were the only bacterial isolates that were found in all samples in the intact batch of the commercially packed toilet tissue paper. In the batch of exposed toilet tissue paper samples, *Bacillus* species, *Staphylococcus aureus*, *Micrococcus* species, *Enterobacter* species and *Streptococcus* species were isolated. *Streptococcus* species were the least abundant in the pool of bacterial isolates obtained from exposed batches of toilet tissue paper, while *Bacillus* species were the most abundant.

3.3 Prevalence of contaminated toilet tissue paper

The prevalence of toilet tissue paper contaminated with isolated microbes is presented in Table 3. All samples in both batches of the intact commercially packed toilet tissue paper and the exposed toilet paper were contaminated with *Bacillus* species. *Streptococci* species were the least bacterial contaminant, with only 2.5 % of the exposed tissue paper samples contaminated with these bacterial species. Both intact and exposed toilet tissue paper samples were

Table 3: Prevalence of Contaminated Toilet Tissue Paper In the Intact and Exposed Batches

Isolated microbes	Prevalence of contaminated toilet paper			
	Intact toilet paper (H_1 = 40)	(%)	Exposed toilet paper (H_2 = 40)	(%)
Bacillus species	40/40	100.00	40/40	100.00
Micrococcus species	0/40	0.00	11/40	27.50
Staphylococcus aureus	0/40	0.00	2/40	5.00
Enterobacter species	0/40	0.00	4/40	10.00
Streptococci species	0/40	0.00	1/40	2.50
Protozoan parasites	0/40	0.00	0/40	0.00

H_1 and H_2 represented the total number of toilet tissue paper samples that were respectively examined in the intact and exposed batches.

found not to be contaminated with protozoan parasites.

4. Discussion

Of all the tissue papers examined, only one particular brand had APC values (Table 1) which exceeded internationally acceptable limits of 2.78 to 3.00 $\log_{10}$ CFU/g for APC [2, 4] in intact commercially packed toilet tissue paper. This anomaly may largely be attributed to the inefficiency of the sanitary strategies that were employed during the production operations in this company. However, since a largely significant proportion of the manufacturing companies producing toilet tissue paper in Nigeria had APC, TCC, and SC values (Table 1) that were well within internationally acceptable standards, it may be safely asserted that the overall sanitary practices during the production operations of toilet tissue paper in Nigeria complied with international best practices. No parasitic protozoa were also recovered from all the intact toilet tissue paper examined (Table 2). However, *Bacillus* species were the main bacterial species that were isolated from the intact commercially packed toilet tissue paper samples. This present finding agreed with the results of the few studies on tissue paper [9, 14]. Besides the spore-forming properties of the *Bacillus* species, the fact that *Bacillus* species are also cellulolytic and amylolytic may be the cause of its high abundance in pulp and paper factories where the tissue products are rich in starch and cellulose [9, 14].

Upon indiscriminate exposure of the toilet tissue paper after usage, seven brands of the toilet tissue paper were found to be significantly contaminated as indicated by APC values (Table 1) that exceeded internationally acceptable standards [2, 4]. Because a significant number of the exposed tissue paper had unacceptable APC and SC values, it generally indicates that upon indiscriminate exposure of the intact commercially packed tissue papers, they could become grossly contaminated by transfer of microorganisms of human or air origin to the exposed tissue paper [15, 16]. The TCC values of the exposed toilet tissue papers (Table 1) were not significantly different ($P > 0.05$) from those obtained in intact toilet tissue papers (Table 1), indicating that upon indiscriminate exposure, exposed tissue papers were not significantly contaminated with coliforms. The bacterial species identi- fied in the exposed tissue paper samples include *Bacillus*, *Micrococcus*, *Enterobacter* and non-haemolytic *Streptococcus* species as well as *Staphylococcus aure- us* (Table 2). The presence of *Staphylococcus aureus* in an insignificant fraction (Table 3) of the exposed batch of toilet tissue paper samples was due to cross-contamination from the environment or human handling during usage. The presence of *S. aureus* in a toilet tissue paper is of significant health concern because this bacterium is a dominant human pathogen that is an etiologic agent of an array of clinical infections [17]. It is the main cause of bacteremia and infective endocarditis. It is also the cause of skin and soft tissue infections as well as device-related infections [18, 19, 20].

5. Conclusion

Results of the present study revealed that sanitary practices employed in the production of toilet tissue paper in Nigeria do not significantly deviate from international best practices. However, the indiscriminate exposure of toilet tissue paper during usage by humans may grossly distort its safety to humans. Thus, cross-contamination of the toilet tissue paper with pathogenic microbes may result in pathologic infections of grave consequence, especially in immune-compromised humans that use them. Therefore, the relevant agencies may need to educate the public on the proper handling of toilet tissue paper during usage to mitigate potential health hazards.

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