Research Article

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Health Science Journal
ISSN 1791-809X

2015
Vol. 9 No. 6:6

Evaluation of Physical Characteristics and Public Health Significance of Easily-Culturable and Clinically-Relevant, Inhalable Microbial Flora of Unused Toilet Rolls

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Abstract

Background: Research into prevalence and public health importance of easily-culturable and inhalable indicator microbial flora of unused toilet-rolls had not been previously reported in Nigeria. This study therefore, aims at determining physical characteristics and microbial profiles of unused toilet rolls, and their public health importance.

Methods: Using microbial cultural methods and standard phenotypic taxonomic tools, 200 easily-culturable bacterial strains, randomly isolated from 371 unused Nigerian toilet-rolls obtained from 10 states, in a three-year period: year 1 [n = 111]; year 2 [n = 59] and year 3 [n = 30] were identified. Antibiotic susceptibility profiles of the isolated bacterial strains were determined by agar disc-diffusion method.

Results: Most of the unused toilet rolls had pH of 7–8; significantly high microbial loads and high concentrations of paper dusts. Isolated indicator microbial flora included *Bacillus*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Micrococcus*, *Morganella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Micrococcus*, *Staphylococcus*, *Aspergillus*, *Botyriodiplodia*, *Candida*, *Cephalospora*, *Curvularia*, *Fusarium*, *Malbranchea*, *Neurospora*, *Penicillium* and *Scopulariopsis* species, some of which were toxigenic. Most-prevalent microbial species were *Bacillus cereus*, *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staph. aureus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Botyriodiplodia*, *Candida* and *Penicillium chrysogenum* species. Gram-positive and Gram-negative bacterial flora, which were maximally sensitive to undiluted disinfectants, exhibited varying antibiotic resistance profiles, with significant resistance to amoxicillin, augmentin (90.0-100%), cotrimoxazole (40.0-90.0%) and tetracycline (33.3-90.0%).

Conclusions: This first reported study, highlighted prevalence of inhalable paper dusts, some toxin-producing fungi, and multiple antibiotic-resistant indicator bacteria of clinical importance in unused contaminated toilet rolls, which without adequate process control have harmful domestic and occupational health implications.

Keywords: Environmental health; Paper products; Inhalable respiratory diseases; Microbial toxins; Toilet rolls

Received: March 25, 2015, **Accepted:** September 10, 2015, **Published:** September 30, 2015

Introduction

Toilet rolls (tissue paper) have been available since the end of 19th century, and as today, several billion rolls of toilet tissues are used each year all over the world. 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 / pant liners, and also for emergencies like first aids, to stop / clean light bleedings due to cuts, wounds and bruises [1-3]. 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). With millions of toilet rolls used daily, toilet rolls manufacturing will always be necessary, while demand for toilet rolls will never stop because it is one of the commodities that cannot be done without [4-6].

In modern usage, toilet rolls would seem to play an important role as barrier to transmission of enteric infection by faecal-manual-oral route. However, although scientific reviews revealed a dearth of information on this issue, remarkable compliance with hygienic practice of toilet paper use is in contrast to the more limited compliance with hand-washing policies that are touted universally, as a sound infection-control measure [4]. After proper hand-washing, most people usually dry their hands, especially with toilet papers but there can be transmission of bacteria from contaminated toilet rolls [7,8]. According to some authors [8-12], 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. It was even reported that average toilet paper dispenser was found to have more than 150 times the amount of bacteria than the average toilet seat [13].

In 2004, the federal government of Nigeria banned importation of finished toilet rolls, serviettes and face tissues, in order to encourage local production and create a much needed employment [14]. Whereas, post-consumer wastes, which are papers that had already been used for the final and intended purpose [1] are commonly recycled paper that serve as raw material for the production of most Nigerian toilet rolls. 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 utilisation rates, it is still important to note that products with recycled contents, like toilet rolls, may contain certain toxic bioaerosols and chemicals during production.

Airborne pathogenic bacteria and fungi from toilet roll dusts can be suspended in air as respirable bioaerosols that can be readily inhaled [15]. Although non-infectious bioaerosols may not cause frequent mortality, infectious bioaerosols, 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 [16,17]. 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. The aims of this study therefore, are to determine the microbial profiles of Nigerian toilet rolls; the physico-chemical parameters of the toilet rolls that can enhance microbial growth, and likely public health issues of associated bacterial and fungal

Figure 1 Plate counts on PCA.

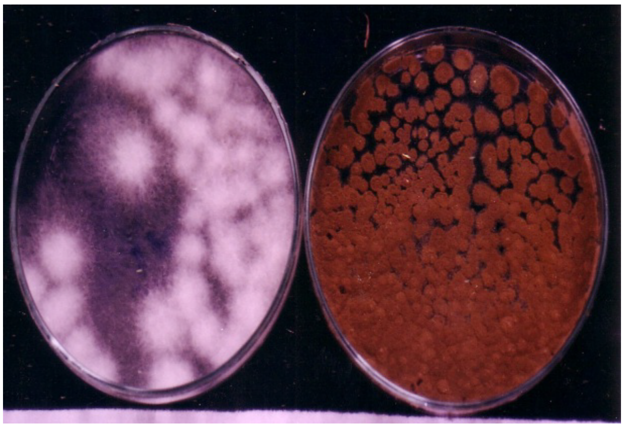

Figure 2 Plate counts on PDA,

Figure 3 Plate counts on YEA.

Figure 4 Plate counts on MEA.

Figure 5 Plate counts on SDA.

floral of significant clinical importance.

Methods

Collection of samples

Three hundred and seventy one toilet roll samples were purchased from various markets of 10 states of Nigeria, namely Lagos (129), Ogun (13), Oyo (129), Osun (14), Ondo (17), Kwara (17), Edo (13), Kogi (15), Enugu (13), Rivers (11) **(Figures 1-5)**.

Determination of physical properties of toilet roll samples

Quality / physical properties of the toilet roll samples determined were absorbance / soaking abilities, colour, texture, foil demarcations, fluffiness, foil designs, foil leaflets (number of plies), foil leaflets (length), size, packaging and cost.

pH determination of toilet roll samples

Five plies of each toilet roll samples were soaked in 30 ml of sterile distilled water with intermittent shaking for 3 hours. Ten ml of each homogenate was aseptically dispensed into sterile McCartney bottle for pH determination, while the remaining 20 ml of each homogenate was aseptically dispensed into another set of sterile McCartney bottles for microbiological studies. The pH of each toilet roll homogenate was then determined using a Pye unican pH meter equipped with a glass electrode inserted into 10 ml of each toilet roll homogenate. Determinations were done in duplicates.

Determination of lactose fermenters / total and faecal coliforms of toilet roll samples

Presence of lactose and non-lactose fermenters was determined as total plate counts on sterile MacConkey agar (Lab M, Basingstoke, England) plates, using pour plate method. Presence of total and faecal coliforms was determined by dispensing each toilet roll sample homogenate into sterile test tubes containing sterile MacConkey broth (Lab M, Basingstoke, England) and inverted Durham tubes, followed by incubation at 35°C and 45°C respectively for 24 and 48 h. Confirmation of total and faecal coliforms was based on colour change of MacConkey broth from pink to yellow and production of gas in the inverted Durham tubes after incubation.

Determination of total plate counts of toilet roll samples

Isolation of easily recoverable, Gram-positive and Gram-negative bacteria from each homogenate of the toilet roll samples was by pour-plating culture method on blood agar, cystein-lactose-electrolyte deficient agar, eosin methylene blue agar, MacConkey agar, plate count agar, nutrient agar and *Salmonella-Shigella* agar (Lab M, Basingstoke, England). Pure isolated bacterial strains were characterised using phenotypic protocols, according to standard bacterial taxonomical methods [18-22].

Determination of antibiotic susceptibility / resistance of bacteria isolated from toilet rolls

Antibiotic susceptibility determination of isolated bacteria from unused toilet rolls to various antibiotic (discs)- ampicillin (AMP; 25 µg), amoxycillin (AMX; 25 µg), chloramphenicol (CHL; 25 µg), cotrimoxazole (COT; 25 µg), cloxacillin (CXC; 5 µg), erythromycin (ERY; 5 µg), nitrofurantoin (NIT; 300 µg), gentamicin (GEN; 10 µg), nalidixic acid (NAL; 30 µg), ofloxacin (OFL; 30 µg), augmentin (AUG; 30 µg) penicillin (PEN), streptomycin (STR; 10 µg) and tetracycline (TET; 30 µg), was carried out using agar disc-diffusion method [23] Entire agar surface of each sterile Mueller-Hinton agar (Lab M, Basingstoke, England) plate was seeded with each test bacterial isolate and the antibiotic discs were later placed on the agar surfaces, followed by incubation of the plates at 35°C for 24-48 hours. Zones of inhibition after incubation were measured and recorded in millimetre diameter, [24] while absence of zones or zones less than 10.0 mm in diameter was recorded as resistant.

Determination of fungal flora of toilet roll samples

Isolation of easily recoverable fungal flora from each homogenate of unused toilet roll samples was by pour-plating culture procedure on potato dextrose agar, malt extract agar, yeast extract agar and Sabouraud dextrose agar (Lab M, Basingstoke, England). Isolated fungal strains were subcultured, and pure cultures of the fungal isolates were characterised using standard phenotypic protocols [25-27].

Determination of *in vitro* bacteriostatic potentials of two disinfectants on bacterial species isolated from toilet roll samples

Inhibitory activities of two disinfectants- Dettol and Roberts on bacterial flora isolated from unused toilet rolls were determined in this study, using a modification of agar well-diffusion method

[28]. Wells (6.0 mm in diameter) were bored into sterile Mueller-Hinton agar plates, followed by surface sterilisation of the agar plates by flaming the agar surfaces with Bunsen burner. Bacterial strains (500 µl of 10^5 cfu/ml) previously inoculated into sterile peptone water and incubated at 37°C for 18-24 hours were seeded on cooled Mueller-Hinton agar plates by streaking the entire surface of each sterile plate with each test bacterial strain. Modification procedure involved dispensing and homogenising 500µl of each undiluted and diluted concentrations of disinfectants separately into sterile semi-solid Mueller-Hinton agar in different sterile McCartney bottles, before being dispensed into agar wells to avoid spreading of the disinfectants from the agar wells unto agar surfaces. Plates were then incubated at 35°C for 24-48 hours and zones of inhibition were measured and recorded in millimetre diameter. Zones less than 10.0 mm in diameter or absence of inhibition zones were recorded as resistant (negative).

Results

Table 1 showed the sampling sources and presence of lactose fermenters in 371 unused toilet roll samples. Generally, more unused toilet roll samples were obtained in the first two years of sampling. Lactose-fermenters were present in 40.0-100% of the toilet rolls from each of the 10 states, with least recovery occurrence of 40.0-46.7% lactose-fermenters in samples from Osun, Ondo and Edo states. All the unused toilet roll samples from Rivers state were positive for presence of lactose fermenters, while lactose fermenters were present in more than 50.0% of samples from other six (Lagos, Ogun, Oyo, Kwara, Kogi and Enugu) states.

According to the observed physical characteristics recorded for the sampled unused toilet rolls in this study, they were either light brown, white, cream or greyish white in colour but most of them were white. Their textures were either soft, very soft, thick or very thick, while foil demarcations were either present or absent. Some of the toilet rolls were fluffy, while most were non-fluffy or papery. Absorbency / soaking ability of the toilet rolls indicated that about half of them 181 (48.8%) were highly absorbent, while the remaining samples were slowly absorbent. Very few 92 (24.8%) of the toilet rolls had dotted / line imprints as foil designs; whereas, most were plain without foil designs. Most of the unused toilet rolls that were brown and papery had no foil plies but others had between 30 and 50 foil plies, depending on sizes (small / medium / large) of the toilet rolls. Foil plies (length vs. breadth) were mostly between 12 cm vs. 10 cm (length vs. breadth), and while most were wrapped with branded paper or nylon, few, which were mostly the small, brown and papery samples were unwrapped. Cost of toilet rolls was between #25 (12.6 cents) and #160 (65 cents), depending on quality of the unused toilet rolls; however, the small, brown and papery toilet rolls were the cheapest. The pH values of the 371 unused toilet rolls randomly obtained from 10 states of Nigeria were between pH 5 and 8 but most of the samples (72.8%) had pH around neutral (pH 7-8) range. There was also presence of total and faecal coliforms in (20.0-26.7%) and (13.3-20.0%) of the toilet roll samples respectively **(Table 2)**.

Table 1 Sampling sources and presence of lactose fermenters in unused toilet roll samples.

Sampling sources	Samples / sampling period			Total no of samples	Lactose fermenters
	Year 1	Year 2	Year 3		
Lagos	58	53	18	129	78.90%
Ogun	10	3	-	13	66.70%
Oyo	48	43	38	129	77.30%
Osun	4	5	5	14	40.00%
Ondo	7	3	7	17	44.40%
Kwara	5	9	3	17	63.60%
Edo	3	5	5	13	46.70%
Kogi	5	5	5	15	61.50%
Enugu	3	9	1	13	66.70%
Rivers	6	2	3	11	100%
Total no. of samples	149	137	85	371	

Microbial loads of the unused toilet roll samples, as colony forming units (cfu), were too numerous to count (TNTC) from dilutions 10^{-2} - 10^{-4} on plate count agar, nutrient agar and cysteine lactose electrolyte deficient agar plates. Apart from some plates containing TNTC colonies, varying viable colonial counts of 1.4 x 10^2 - 4.5 x 10^3 cfu g^{-1} (Lagos and Ogun), 1.0 x 10^2–2.8 x 10^3 cfu g^{-1} (Oyo), 1.0 x 10^2–5.6 10^3 cfu g^{-1} (Osun and Kwara), 1.0 x 10^2–1.5 x 10^3 cfu g^{-1} (Kogi and Edo), 8.0 x 10^2 –2.0 x 10^4 cfu g^{-1} (Ondo), 1.3 x 10^3–4.0 x 10^4 cfu g^{-1} (Rivers and Enugu) were recorded on MacConkey agar plates. However, most plates on which *Proteus mirabilis* strains grew were swarming, which led to inhibition of growths of other bacterial colonies.

Table 2 Physical, pH and coliform characteristics of unused toilet roll samples.

Physical characteristics	Observed physical characteristics of toilet rolls		
Colour	light brown / white / cream / greyish white		
Texture / softness	soft / very soft/ thick / very thick		
Foil demarcations	present / absent		
Fluffiness	fluffy / non-fluffy (papery)		
Absorbency / soaking ability	highly absorbent / absorbent /slowly absorbent		
Foil designs	plain (no design) / dotted imprints/ line imprints		
Foil plies (number)	none / 30 / 50		
Foil plies (length v. breadth)	12 cm v. 10 cm (length vs. breadth)		
Size	large / medium / small		
Packaging	wrapped (paper / nylon) / unwrapped		
Cost	N 25 – N160 [12.5 - 65 cents]		
pH	**% of samples**		
5	17.60%		
6	9.80%		
7	37.30%		
8	35.50%		
Total coliforms (37°C)		**Faecal coliforms** (45°C)	
Time	% of samples	Time	% of samples
24h	20.00%	24h	13.30%
48h	26.70%	48h	20.00%

One hundred and one (n = 48 Gram-positive; n = 63 Gram-negative) bacterial strains randomly isolated from toilet roll samples in first year were identified as *Bacillus cereus* 13 (11.7%), *Bacillus subtilis* 13 (11.7%), *Clostridium perfringens* 3 (2.70%), *Citrobacter aerogenes* 5 (4.5%), *E. coli* 25 (22.5%), *Enterobacter aerogenes* 1 (0.9%), *Klebsiella pneumoniae* 7 (6.31%), *Klebsiella aerogenes* 2 (1.8%), *Morganella morganii* 1 (0.9%), *Proteus mirabilis* 8 (7.23%), *Salmonella typhi* 4 (3.6%), *Shigella dysenteriae* 4 (3.6%), *Shigella sonnei* 1 (0.9%), *Staphylococcus aureus* 19 (17.1%) and *Pseudomonas aeruginosa* 5 (4.5%) **(Table 3)**.

In the second year, 59 (n = 8 Gram-positive; n = 51 Gram-negative) bacterial strains were identified as *Bacillus cereus* 2 (3.4%), *Citrobacter aerogenes* 4 (6.8%), *E. coli* 20 (33.9%), *Enterobacter aerogenes* 4 (6.8%), *Klebsiella pneumoniae* 5 (8.5%), *Klebsiella aerogenes* 2 (3.4%), *Micrococcus luteus* 2 (3.4%), *Proteus mirabilis* 11 (18.6%),*Salmonella paratyphi* 5 (8.5%) and *Staphylococcus aureus* 4 (6.8%). The 30 (n = 11 Gram-positive and n = 19 Gram-negative) bacterial flora strains isolated in the third year were identified as *Bacillus cereus* 2 (6.7%), *Corynebacterium* sp. 2 (6.7%), *E. coli* 7 (23.3%), *Enterobacter aerogenes* 3 (10.0%), *Klebsiella pneumoniae* 3 (10.0%), *Micrococcus luteus* 3 (10.0%), *Proteus mirabilis* 3 (10.0%), *Salmonella typhi* 1 (3.3%) and *Staphylococcus aureus* 6 (20.0%). Overall, most-prevalent bacterial flora were *Bacillus cereus* (8.5%), *Bacillus subtilis* (6.5%), *E. coli* (26.0%), *Klebsiella pneumoniae* (7.5%), *Proteus mirabilis* (11.0%) and *Staphylococcus aureus* (14.5%) species **(Table 3)**.

Fungal flora isolated from the unused toilet roll samples were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Botyriodiplodia*, *Candida*, *Cephalospora*, *Curvularia*, *Fusarium*, *Malbranchea*, *Neurospora crassa*, *Penicillium chrysogenum*, *Penicillium gratulanum* and *Scopulariopsis* species. The most prevalent fungal flora were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Botyriodiplodia*, *Candida* and *Penicillium chrysogenum* species **(Table 4)**.

As shown in **Table 5**, except among *Staphylococcus aureus* (3.4-79.3%), Gram-positive bacterial species exhibited highest resistance against amoxycillin and augmentin (90.0-100%), cotrimoxazole (40.0-90.0%) and tetracycline (33.3-90.0%) antibiotic discs. Lowest resistance rates were recorded for ofloxacin (0.0-10.0%) and gentamicin (0.0-20.0%) but percentage multiple antibiotic resistance (%MAR) were mostly between 25.0 and 100%. Each Gram-negative bacterial strain exhibited varying antibiotic resistance patterns but most of the strains displayed ≥50.0% resistance against the antibiotics, with the exception of tetracycline (0.0-50.0%). Overall resistance rates of toilet roll-borne Gram-negative bacteria were 18.2-72.7%, while %MAR was between 25.0 and 100% **(Table 5)**.

Table 6 further showed the characteristic multiple antibiotic resistance profiles of bacterial flora isolated from Nigerian-produced unused toilet rolls. In this study, four different characteristic multiple antibiotic resistance profiles (AMX-AUG-COT-TET/AMX-AUG-COT-NIT-NAL-TET/AMX-AUG-COT-NIT-GEN-NAL-TET/AMX-AUG-COT-NIT-GEN-NAL-OFL-TET) were recorded for Gram-positive; while, five different characteristic multiple antibiotic resistance profiles (CHL-CXC-ERY-STR/AMP-CHL-CXC-PEN-TET/AMP-CHL-ERY-GEN-PEN-STR-TET/AMP-CHL-CXC-ERY-GEN-PEN-STR/AMP-CHL-CXC-ERY-GEN-PEN-STR-TET) were recorded for Gram-negative bacteria isolated from unused toilet rolls.

Table 3 Recovery rates and percentage occurrence of viable and culturable bacterial flora from unused toilet roll samples.

Bacterial species	Recovery rates (strains)			Total no / (%) occurrence
	Year 1	Year 2	Year 3	
Bacillus cereus	13 (6.5%)	2 (1.0%)	2 (1.0%)	17 (8.5%)*
Bacillus subtilis	13 (6.5%)	-	-	13 (6.5%)*
Citrobacter aerogenes	5 (2.5%)	4 (2.0%)	-	9 (4.5%)
Clostridium perfringens	3 (1.5%)	-	-	3 (1.5%)
Corynebacterium sp.	-	-	2 (1.0%)	2 (1.0%)
E. coli	25 (12.5%)	20 (10.0%)	7 (3.5%)	52 (26.0%)**
Enterobacter aerogenes	1 (0.5%)	4 (2.0%)	3 (1.5%)	8 (4.0%)
Klebsiella aerogenes	2 (1.0%)	2 (1.0%)	-	4 (2.0%)
Klebsiella pneumoniae	7 (3.5%)	5 (2.5%)	3 (1.5%)	15 (7.5%)*
Micrococcus luteus	-	2 (1.0%)	3 (1.5%)	5 (2.5%)
Morganella morganii	1 (0.5%)	-	-	1 (0.5%)
Proteus mirabilis	8 (4.0%)	11 (5.5%)	3 (1.5%)	22 (11.0%)**
Pseudomonas aeruginosa	3 (1.5%)	-	-	3 (1.5%)
Pseudomonas alkaligenes	-	-	2 (1.0%)	2 (1.0%)
Salmonella paratyphi	-	5 (2.5%)	-	5 (2.5%)
Salmonella typhi	4 (2.0%)	-	1 (0.5%)	5 (2.5%)
Shigella dysenteriae	4 (2.0%)	-	-	4 (2.0%)
Shigella sonnei	1 (0.5%)	-	-	1 (0.5%)
Staphylococcus aureus	19 (9.5%)	4 (2.0%)	6 (3.0%)	29 (14.5%)**
Total / (%) bacterial recovery rates	111 (55.5%)	59 (29.5%)	30 (15.0%)	200 (100%)

Keys: ** = most prevalent bacterial species; * = moderately prevalent bacterial species

Table 4 Recovery rates and percentage occurrence of viable and culturable fungal flora from unused toilet roll samples.

Fungal species	Recovery rates (strains)			Total no / (%) occurrence
	Year 1	Year 2	Year 3	
Aspergillus flavus	111 (6.7%)	123 (7.5%)	49 (3.0%)	283 (17.2%) **
Aspergillus fumigatus	96 (5.8%)	103 (6.3%)	36 (2.2%)	235 (14.3%) **
Aspergillus niger	49 (3.0%)	124 (7.5%)	51 (3.1%)	224 (13.6%) **
Aspergillus terreus	16 (1.0%)	38 (2.3%)	12 (0.7%)	66 (4.0%)
Botyriodiplodia sp.	51 (3.1%)	49 (3.0%)	72 (4.4%)	172 (10.4%) **
Candida	104 (6.3%)	95 (5.8%)	63 (3.8%)	262 (15.9%) **
Cephalospora sp.	23 (1.4%)	-	-	23 (1.4%)
Curvularia sp.	41 (2.5%)	18 (1.1%)	19 (1.2%)	78 (4.7%)
Fusarium sp.	38 (2.3%)	-	-	38 (2.3%)
Malbranchea sp.	20 (1.2%)	41 (2.5%)	5 (0.3%)	66 (4.0%)
Neurospora crassa	-	62 (3.8%)	-	62 (3.8%)
Penicillium chrysogenum	25 (1.5%)	71 (4.3%)	-	96 (5.8%) *
Penicillium gratulanum	-	15 (0.9%)	-	15 (0.9%)
Scopulariopsis sp.	15 (0.9%)	3 (0.2%)	9 (0.5%)	27 (1.6%)
Total no. (%) of fungal flora	589 (35.8%)	742 (45.1%)	316 (19.1%)	1647 (100%)

Keys: ** = most prevalent fungal species; * = moderately prevalent fungal species

Table 5 Antibiotic resistance rates of bacterial species from unused toilet roll samples (antibiotic discs).

Gram-positive bacterial species	Antibiotics (µg/l)								
	AMX	AUG	COT	NIT	GEN	NAL	OFL	TET	%MAR
Bacillus cereus [10]	90	90	90	30	10	60	10	50	25.0-100
Bacillus subtilis [10]	100	100	80	70	20	60	0	90	25.0-100
Cl. perfringens [3]	100	100	66.6	0	0	0	0	33.3	25.0-100
Micrococcus spp. [5]	100	100	40	20	0	20	0	80	50.0-100
Staph. aureus [29]	41.4	31	79.3	27.6	3.4	58.6	3.4	62.1	37.5-50.0
Total [58] % Overall resistance	**68.4**	**63.1**	**77.2**	**33.3**	**7**	**52.6**	**3.5**	**64.9**	**25.0-100**

Gram-negative bacterial species	Antibiotics (µg/l)								
	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	% MAR
Citrob. aerogenes [1]	R	R	R	0	0	R	0	R	
Ent. aerogenes [4]	33.3	33.3	0	66.7	100	100	33.3	0	37.0-50.0
Escherichia coli [30]	75	100	75	100	75	50	75	0	50.0-75.0
Kleb. pneumoniae [9]	33.3	33.3	83.3	16.7	88.9	83.3	88.9	50	50.0-87.5
Proteus mirabilis [6]	100	66.7	83.3	16.7	50	50	50	0	25.0-75.0
Ps. aeruginosa [2]	80	60	40	60	80	100	60	20	37.5-87.5
Sal. paratyphii [4]	62.5	50	62.5	37.5	62.5	50	62.5	25	25.0-75.0
Sal. typhii [4]	62.5	50	62.5	37.5	62.5	50	62.5	25	25.0-75.0
Sh. dysentariae [3]	0	100	100	100	0	0	100	0	50.0-100
Total [63] % Overall resistance	**72.7**	**66.7**	**63.6**	**45.5**	**66.7**	**66.7**	**60.6**	**18.2**	**25.0-100**

Keys: AMX = amoxycillin; AUG = augmentin; COT = cotrimoxazole; NIT = nitrofurantoin; GEN = gentamicin; NAL = nalidixic acid; OFL = ofloxacin; TET = tetracycline

Undiluted Dettol and Roberts were the most-inhibitory disinfectants, with percentage susceptibility rates being 100% and 96.8% respectively, while percentage susceptibility rates and zones of inhibition decreased with increasing dilution values. Most of the bacterial flora from unused toilet rolls was inhibited *in vitro* with narrow zones of inhibition (10.0-20.0 mm in diameter) but overall, Dettol was slightly more inhibitory than Roberts **(Table 7)**.

Discussion

Non-infectious bioaerosols may cause obstructive airways diseases, hypersensitivity reactions and cardiovascular diseases [29]. Whereas, chronic obstructive pulmonary disease is projected to become the fourth leading cause of death by 2025, especially among those living in low and middle income countries [30]. Meanwhile, the brownish or greyish-white and papery toilet rolls sampled in this study, which would probably not be produced in many other countries, were of extreme low quality, and mostly with much paper dusts that can serve as bio-aerosols. Also, the bio-aerosols may contain culturable endotoxin-producing microbial contaminants, which may even significantly exceed suggested safe levels. It may not be obvious that certain illnesses can be implicated with production and usage of such contaminated unused toilet paper but there can be latter

Table 6 Antibiotic resistance profiles of bacterial species from unused toilet roll samples (antibiotic discs).

Gram-positive bacteria	Antibiotic profiles								%MAR overall profiles
1. *Cl. perfringens* [3]	AMX	AUG	COT					TET	50
2. *Micrococcus* spp. [5]	AMX	AUG	COT	NIT		NAL		TET	75.5
3. *Bacillus subtilis* [10]	AMX	AUG	COT	NIT	GEN	NAL		TET	87.5
4. *Bacillus cereus* [10]	AMX	AUG	COT	NIT	GEN	NAL	OFL	TET	100
5. *Staph. aureus* [29]	AMX	AUG	COT	NIT	GEN	NAL	OFL	TET	100
Gram-negative bacteria	Antibiotic profiles								%MAR overall profiles
1. *Shigella dysenteriae* [3]		CHL	CXC	ERY			STR		50
2. *Citrobacter aerogenes* [1]	AMP	CHL	CXC			PEN		TET	62.5
3. *Ent. aerogenes* [4]	AMP	CHL		ERY	GEN	PEN	STR		75
4. *Escherichia coli* [30]	AMP	CHL	CXC	ERY	GEN	PEN	STR		87.5
5. *Proteus mirabilis* [6]	AMP	CHL	CXC	ERY	GEN	PEN	STR		87.5
6. *Kleb. pneumoniae* [9]	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	100
7. *Ps. aeruginosa* [2]	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	100
8. *Salmonella paratyphi* [4]	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	100
9. *Salmonella typhi* [4]	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	100

Keys: CXC = cloxacillin; CAZ = fortum; CRX = ciprofloxacin; GEN = gentamicin; CTX = claforan; AUG = augmentin; NIT = nitrofurantoin; OFL = ofloxacin

Table 7 *In vitro* bacteriostatic potentials of two disinfectants on bacterial species from unused toilet roll samples.

Dettol				Concentrations (ml v-1)	Roberts			
U	**10^{-1}**	**10^{-2}**	**10^{-3}**		**U**	**10^{-1}**	**10^{-2}**	**10^{-3}**
100%	88.90%	66.70%	18.60%		96.80%	76.20%	46.00%	6.30%
(10.0-35.0)	(10.0-30.0)	(10.0-27.0)	(10.0-20.0)		(10.0-30.0)	(10.0-28.0)	(10.0-20.0)	(11.0-12.0)
15	32	35	18 {100}	[10.0-20.0]	27	39	29	4 {95}
31	23	7	- {61}	[21.0-30.0]	32	9	-	- {41}
16	1	-	- {17}	[≥31.0]	1	-	-	- {1}
62	**56**	**42**	**18**	**{178}**	**60**	**48**	**29**	**4 {137}**

implications of nasopharyngeal and respiratory infections due to associated bio-aerosols. Furthermore, exposure to high levels of paper dusts (> 5 mg/m^3) has been found to cause respiratory diseases among workers in the pulp and paper industries [31-35]. 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 [32-35] but not pathogenic microorganisms. In spite of the varying viable colonial counts on different culture plates, microbial loads of the sampled unused toilet rolls in this study were significantly high, irrespective of location of purchase. This may be due to contamination problem arising from pulp and paper industry, where some species of airborne Enterobacteriaceae distinctly prevailed in examined paper mill factories [32-35]. Contaminated machinery and increase of nutrients in water circuits can contaminate paper products; thereby, favouring microbial 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 [36,37]. Moreover, it is quite alarming that N. gonorrhoeae was even known to survive for brief periods on different surfaces, including toilet paper (up to 3 hours) [38]. 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 [39] 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. Most times, pH level has been found to be contributory to high colony counts in paper products and effluents; [39] whereas, with most (72.8%) of the unused toilet rolls having between pH 7 and pH 8, and others between pH 5 and 8, these obtained results in the current study indicated that pH range of 5-8 must have favoured the isolated culturable microbial flora.

Earlier studies on pulp and paper mill processing plants, which evaluated for bacterial concentrations, traced the presence of faecal coliform, *Klebsiella pneumoniae* and significant microbial counts from early pulping stages to water processing reuse systems; suggesting degraded water quality [33,34,39,40]. Detection of quite significant indicator (coliform) bacterial pathogens in unused toilet rolls in this study is also indicative of environmental contamination by environmental and faecal bacteria, which is of public health importance. Since toilet rolls are also most times used as kitchen rolls / napkins, and thus, come in direct contact with some food during food packaging, they can also serve as source of toxins and food-borne microbial contaminants, which can lead to food-borne disease outbreaks or/ and food spoilage microorganisms. Such contaminated toilet rolls can thus, be quite harmful, especially to immunocompromised individuals, children and elderly, while complications may also occur, resulting in more severe health cases.

The most-prevalent microbial flora recovered from unused toilet rolls in this study included the potentially pathogenic, spore-forming and toxin-producing species, *B*. *cereus*, as well as *E. coli*, *Staphylococcus aureus*, *Aspergillus*, *Penicillium* species etc. Considering that potential harmfulness of aerobic mesophilic bacilli and thermophilic bacteria and fungi from different paper mill samples have been earlier reported; [34,41-44] 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 [35]. Recovery of pathogenic microbial flora in toilet rolls may induce toxic shock syndrome during usage of toilet rolls as sanitary towels and when used for cleaning bruises or wounds. There is also the possibility of contaminated toilet rolls inducing urinary tract infections when used as sanitary towels or pant liners, for which they are sometimes used for.

Although the public health importance of significant recovery of multi-drug (antibiotic) resistant indicator bacterial species from toilet rolls is yet to be investigated by other workers, current study reported that bacterial species associated with unused toilet rolls exhibited significant resistance and multiple antibiotic resistance against some of the commonest test antibiotic discs used for routine antibiotic susceptibility testing in the country. Antibiotic resistant bacteria often carry resistance genes that can be spread to other bacterial pathogens, and this can lead to hidden reservoir of antibiotic resistance. Moreover, bacteriological failure and bacteriological relapse in clinical conditions are some of the effects of antibiotic resistance that make countries all over the world spend billions of their national currencies to combat. Even many infectious diseases that were previously controlled by antibiotics are re-emerging due to antibiotic resistance. Similarly, pathogenic fungi have many complex mechanisms of resistance to antifungal drugs, and information about the clinical, cellular and molecular factors contributing to antifungal-drug resistance continues to accumulate. There is the strong possibility of isolated fungal species in this study also exhibiting significant resistance to test antifungals.

If some species of Enterobacteriaceae distinctly prevail in paper effluents and products, [43-46] the incidence of microbes in unused toilet rolls can therefore, be traced to woods or the recycled papers, which were the raw materials [47]. Environmental impact of wastewater emanating from paper mill industries had been of particular concern, so, there is the strong possibility of processing water in the toilet roll manufacturing companies, serving as source of microbial contaminations in unused toilet rolls. Assay for inhibitory potentials of two most-commonly used disinfectants, Dettol and Roberts on isolated bacterial species from unused toilet rolls indicated that undiluted disinfectants, and at dilution of 10^{-1} were the most-inhibitory, while inhibitory potentials decreased with increasing dilution ratios. Except for the fact that toilet rolls are sometimes used for food packaging, it would have been appropriate that antimicrobial-inhibiting disinfectants be incorporated in toilet tolls, since that could assist in minimising microbial growth in highly contaminated locally-produced toilet rolls in the country. Such toilet rolls can thus, be exclusively used for toilet and hygienic purposes.

Current study for the first time; highlighted the prevalence of diverse species of easily-culturable Gram-positive and Gram-negative bacteria and fungi in Nigerian-produced, unused toilet rolls. The only similar study which reported that unused toilet rolls had been found to be overgrown with bacteria was that of Gendron et al. [12]. 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, [16,17] should be of serious concern.

Conclusion

Much might have been achieved in terms of local production of toilet rolls in Nigeria but in their present forms, Nigerian-produced unused toilet rolls are significantly microbially-contaminated, especially with easily-culturable fungal and multi-drug resistant, indicator bacterial species. This points at contaminated unused toilet rolls as a major source of multiple antibiotic resistant bacteria, which had not been previously reported. Prevalence of indicator microbial flora is indicative of microbial contamination in toilet roll industries, and this can provide a rational basis for development of an effective industrial control measures. Studies to trace and identify contamination routes of indicator 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 in the country.

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