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Microbial Quality Assessment of Tissue Paper Sold within Sokoto metropolis, Nigeria

***[1]S.M. Jodi and [2]Z.M. Abdullahi**

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, UsmanuDanfodiyo University, Sokoto, Nigeria
[2]Department of Microbiology, Faculty of Science, UsmanuDanfodiyo University, Sokoto, Nigeria
Correspondence Email: saadatumjodi@gmail.com

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Abstract

Ten (10) brands of tissue paper commercially available in Sokoto metropolis were assessed for microbial contaminants using Total bacterial plate count, Test for the presence of coliform and test for fungal load. The bacterial colony count ranges from 1.8×10^4 CFU/g -5.2×10^4 UCF/g. The bacterial species identified include *Bacillus cereus* (45%), *Staphylococcus aureus*(15%), *Escherichia coli* (15%), *Bacillus lichenformis*(1%), *Bacillus subtilis*(1%), and *Pseudomonas aeruginosa*(1%). The fungi isolated include: *Aspergillusniger*(36.8%), *Aspergillusfumigatus*(10.5%), *Rhizopusstolonifer* (10.5%), *Candida albicans*(15.7%), and *Aspergillusfumigatus*(10.5%). In the coliform test, only two brands were found to be coliform positive. Rose belle has the highest with three positive tubes corresponding to 17 coliform per 100 ml on the MPN table, while Jet Aime has two corresponding to 7 coliform per 100 ml on MPN Table. All the other brands show negative results. This indicates that 80% of tested tissue paper can be a source of contamination and therefore should be produced and handled appropriately to reduce level of cross contamination.

Keywords: Tissue paper, Bacteria, Fungi, Coliform

Introduction

Tissue paper is soft light- weight paper product made from virgin pulp, recycled pulp or the mixture of both. The fiber material used for making tissue paper contain cellulose, lignin and other materials suitable for the growth of micro-organisms present in the paper making environment [9]. The possibility of transmitting various infections by cross contamination from the tissue paper when produced or handled inappropriately has been reported [8]. Studies previously demonstrated that microbial contaminants in tissue paper product include strains from genera *Enterobacter, Pseudomonas, Staphylococcus*, *Streptococcus* and *Bacillus*haemolytic *Streptococcus*, and *Enterobacter*([40]. The aim of this study is to assess the microbial qualities of ten brands of tissue paper sold in Sokoto metropolis.

Material and Methods

Sample collection

Ten (10) tissue paper brands (designate 1: to 10), commercially available in Sokoto metropolis were bought from four (4) different location within Sokoto metropolis; the sample tissue paper in their original wrappings are taken to the microbiology laboratory of the department of microbiology, Sokoto State university Sokoto in an aseptic condition.

Preparations of the media

The media used include; Nutrient agar (ANTEC), Potatoes dextrose agar (TITAN) and othei media for biochemical tests. All the media are prepared according to the manufacturer instructions and autoclaved for 121°c for 15 minutes.

Preparation of the sample

About ten (10) gram of the tissue paper samples is weighed on a balance, and then dissolved into 100 ml of sterile normal saline. The mixture is allowed to stand for ten minutes and then shaken gently; this solution series as a stock solution, and serial dilution from 10^{-1} to 10^{-4} of dilutionfrom is performed [1]

Enumeration of mean viable mesophilic bacterial count

An aliquot of 0.1 ml from the dilution of 10^{-2} is transferred with the aid of a sterile and pipette onto sterile solidified Nutrient agar plate. The inoculum is spread with a sterile e bent glass- rod and then incubated at 37°C for 48 hours in an inverted position. After the incubation, the colonies are counted and recorded (Caroline *et al*., 2012).

Detection of Coliform

Nine (9) test tubes are arranged each with 10 ml of MacConkey broth, the first three tubes were inoculated with l0inl from the stock solution, the second set of three tubes were inoculated with 0.1ml from the stock solution and the third set of three tubes were inoculated with 0.1 ml from the stock solution. Each test tube contained a Durham tube placed in an inverted position; the test

tubes are capped and incubated for 48 hours at 37°C. After 48 hours, the tubes are removed out of the incubator, and the presence or absence of' the gas in the Durham's tube together with change in colour of the broth due to the formation of acid is observed and later refers to the MPN table[14].

Identification of bacterial Isolates

After 48 hours of incubation, Colony-Forming Units (CFU) is counted on each plate and the culturable microbial load on each test paper brand is determined. Two colonies from distinct colonial morphology is picked and re-streaked on nutrient agar plates and incubated for 24 hours and the pure isolates are obtained and identified using microscopy, spore staining and biochemical tests as described by previously [13].

Detection of fungal contaminants

An aliquot of 0.1 ml from the 10^{-2} dilution was transferred with aid of sterile syringe and pipette onto sterile molten potatoes dextrose agar plate. The droplet was spread with bent glass rod; the plates were sealed, labeled with masking tape, and then incubated at room temperature for six days. A small portion of the fungal culture was cut out with sterile inoculating needle and placed in a drop of distilled water on a glass slide and covered with cover slide. It was then pressed slightly and viewed under microscope with lower power of (X10) objective lens. The fungal isolates were identified based on their morphological characteristics and microscopic observation and finally confirmed with the help of mycological atlas.

Results and Discussion

The results obtained from this study shows that all the ten tissue paper samples analyzed prove positive for bacterial and fungal load. Table 1 shows the bacterial colonies count from the tissue paper; Jet Aime contained $1.08x10^5$ CFU/g, St. Michael tissue contained 2.0x10 CFU/g. Rose Belle contained $2.4x10^5$ CFU/g, Robust contained $2.7x10^4$ CFU/g, Finex contained $3.2x10^4$ CFU/g, Seven Starts had $4.2x10^4$ CFU/g, Laurel tissue contained $4.2x10^4$ CFU/g super serviette had $4.4x10^4$ CFU/g and King size contained $5.2x10^4$ CFU/g.

Table 2: shows the results of Coliform test on the tissue paper. The result which is indicated by formation of acid and gas in the tubes shows that only two brands of the tested tissue paper are Coliform positive. Jet Aime contain two positive tubes corresponding to seven (7) Coliform on MPN table. Rose Belle had three positive tubes corresponding to seventeen (17) Coliform on MPN table. All the other tested brands were Coliform negative.Table 3: shows the biochemical identification of bacterial species isolated from tissue paper tested. The bacterial species isolated were *Bacillus cereus* (45%), *Escherichia coli* (15%), *Staphylococcus aureus*(15%), *Bacillus licheniforms*(15%), *Bacillus subtilis*(5%) *and Pseudomonas aeruginosa*(5%).Table 4: Shows the growth and identification of fungi isolated from tissue paper samples. The fungi identified includes; *Aspergillusniger*(36.8%), *Candida albicans*(15.7%),*Rhizopusstolonifer*(10.5%),*Aspergillusflavus*(26.%) *and Aspergillusfumigatus*(10.5%).Table 5: shows the occurrence of the bacterial isolated from tissue paper in which *Bacillus cereus* occurred at (9%), *Staphylococcusaureus*(15%), *Bacillus licheniformis*(15%), *Bacillus subtilis*(5%) *and Pseudomonas aeruginosa*at (5%). Table 6: shows the occurrence of the fungal species grown on tissue paper samples as *Aspergillusniger* (36.8%), *Aspergiliusflavus* (26.3%), *Rhizophusstolonifer* (10.5%), *Candida albican* (15.7%) and *Aspergillusfumigatus* (10.5%).

Table 1: Bacterial Colony counts from tissue paper brands

S/N	Manufacturer	CFU/g
1.	Paloma	2.8×10^4
2.	Jet Aime	1.08×10^5
3.	Super Serviette	4.4×10^4
4.	St. Michael tissue	2.0×10^4
5.	Finex	3.2×10^4
6.	Rose Belle	2.4×10^5
7.	King size joy	5.2×10^4
8.	Seven Starts	4.1×10^4
9.	Robust	2.7×10^4
10.	Laurel tissue	4.2×10^4

Table 2: Biochemical identification of bacterial isolate from tissue paper

Isolate	Morphology	Gram reaction	Glucose	Sucrose	Lactose	Gas	H_2S	Motility	MR	VP	Urease	Indole	Citrate	Catalase	Coagulase	Spore	Starch	Organism
1	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
2	Rod	-	+	+	+	+	-	+	+	-	-	+	-	-	-	-	-	*E. coli*
3	Rod	-	+	+	+	+	-	+	+	-	-	+	-	-	-	-	-	*E. coli*
4	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
5	Cocci	+	+	+	+	-	-	-	-	+	+	-	-	-	+	-	-	*S. aureus*
6	Cocci	+	+	+	+	-	-	-	-	+	+	-	-	-	+	-	-	*S. aureus*
7	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
8	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
9	Rod	+	+	+	+	-	-	+	-	+	+	-	+	-	-	+	+	*Bacillus licheniformis*
10	Cocci	+	+	+	+	-	-	-	-	+	+	-	-	-	+	-	-	*S. aureus*
11	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
12	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
13	Rod	+	+	+	+	-	-	-	-	+	+	-	+	-	-	+	+	*Bacillus licheniformis*
14	Rod	-	+	+	+	+	-	+	+	-	-	+	-	-	-	-	-	*E. coli*
15	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
16	Rod	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+	*Bacillus subtilis*
17	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*
18	Rod	-	+	+	+	+	-	+	-	+	-	-	+	+	-	-	-	*P. aeruginosa*
19	Rod	+	+	+	+	-	-	+	-	+	+	-	+	-	-	+	+	*Bacillus licheniformis*
20	Rod	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	*Bacillus cereus*

Table 3: Growth and Identification of fungi isolates from tissue paper

S/N	Manufacturer	Observation Days							Fungi identified
		1	2	3	4	5	6	7	
1	Paloma	-	-	-	-	+	++	+++	*A. niger* *A. flavus* *A. fumigatus*
2	Jet Aime	-	-	-	+	+	++	+++	*A. niger* *C. albicans*
3	Super Serviette	-	-	-	+	+	++	+++	*A. flavus*
4	St. Michael tissue	-	-	-	+	+	++	+++	*A. niger* *A. flavus*
5	Finex	-	-	-	+	+	++	+++	*A. niger*
6	Rose Belle	-	-	-	+	+	++	+++	*A. niger*
7	King size Joy	-	-	-	+	++	+++	+++	*A. niger* *A. fumigatus*
8	Seven Stars	-	-	-	+	+	++	+++	*A. flavus* *R. stolonifer*
9	Robust	-	-	-	-	++	++	+++	*C. albicans*
10	Laurel Tissue	-	-	-	+	+	++	+++	*A. niger* *A. flavus* *C. albicans*

Keys: - = No growth; + = Growth, ++ = Moderate growth, +++ = Full growth

Table 4: Frequency of occurrence of the bacterial isolates in tissue paper samples

Organism	Number	Frequency
Bacillus cereus	9	45
S. aureus	3	15
E. coli	3	15
Bacillus licheniformis	3	15
Bacillus subtilis	1	5
P. aeruginosa	1	5
Total	**20**	**100**

Table 5: Frequency of occurrence of fungal isolates from tissue paper

Organism	Number	Frequency
Aspergillusniger	7	36.8
Aspergillusflavus	5	26.3
Rhizopusstolonifer	2	10.5
Candida albican	3	15.7
Aspergillusfumigatus	2	10.5
Total	**19**	**100**

Discussion

This study investigated the microbial load of 10 different brands of tissue paper identified commercially available in the Sokoto metropolis. From the analysis carried out all the tissue paper analysed was positive for bacterial load ranging from $1.08x10^4$ CFU/g to $5.2x10^4$ CFU/g. The standard acceptable mead viable mesophilic bacterial count limit is $10x10^3$ CFU/g. This indicated that all the brand of tissue paper analyzed have passed the quality limit of safety set up by the International Standard Organisation. The presence of bacterial contaminant in the tissue paper may occur due to poor conditions and handling of tissue paper, use of untreated water, unsterilized processing equipment during processing and packaging. Bacterial isolates were identified by biochemical test. The identified species are; Bacillus cereus, Staphylococcus *cerues, Staphylococcus aureus, E. coli, bacillus licheniformis, Bacillus subtilis*and *Pseudomonas aeruginosa*as indicated in Table 4.The presence of *Bacillus* species in tissue paper is not surprising, as they are known to be widely distributed in nature. Most lives as saprophytes in soil, dust and water and on vegetation as they are able to form resistance spores that can withstand heat and the temperature of processing machinery germinate and grow in the tissue paper end products if the conditions are favourable. *Bacillus* is Gram positive, aerobic and facultative, spore forming rod found in diverse environment. Several bacillus species are amylolytic and some are cellulolytic, thus it's not surprising that these bacteria are isolated from tissue paper, the product which is in rich in starch and cellulose as reported by[44]. *Staphylococcus aureus*is a normal flora of the nose, and it's found in the hands, skin of animals and humans. The presence of this bacterium in the tissue paper samples is probably due to contamination of the tissue paper by those workers that usually touch their nose and skin with their hands and switch on to packaging of tissue paper without disinfecting their hands. This gives room to the bacterium to spread over the paper end products. *S. aureus* has been known to cause food poisoning by virtue of the enterotoxin it produced in food.For *E. coli*, it's one of the many bacteria that are harmless in a normal circumstance, but become pathogenic when the immune system becomes weak. The presence of *E. coil* in the tissue paper products may be due to the use of contaminated water with faecal material in it and use untreated or not adequately treated and use in the processing of tissue paper by the manufacturing companies. *Pseudomonas aeruginosa* is commonly inhibits soil, water and vegetation. It is found on the skin of some healthy persons and has been isolated from the throat of non-hospitalized patients. *Pseudomonas aeruginosa* may found its way into the paper products through the use of untreated water or inadequately sterilized manufacturing equipment.The fungal species isolated from tissue paper analyzed after 7 days incubation period at room temperature were *Aspergillusniger* (36.8%), *Aspergillusflavus* (26.3%). *Rhizopusstolonifer* (10.5%), *Candida albicans* (15.7%) and *.Aspergillusfumigatus*(10.5%). *Aspergillus*spp was reported in many literatures as among the fungal species capable of deteriorating paper products [38][1][38]. The implication of this finding is that *Aspergillus*spp is responsible for the production of mycotoxin: *Aspergillus*spp is found on grains and they decolourised the infected grains and reduce germination of the seeds, these organisms can cause deterioration of wide range of products including paper products. This study reestablished that tissue paper contain high number of culturable bacteria and fungi. The microorganisms isolated on this study are probably not the only culturable species encountered on tissue paper. Different culture conditions could perhaps offer a larger insight on the vast microbial community of tissue paper.From all the bacterial species found oh tissue paper in this study, *Bacillus cereus* is possibly the most toxigenic specie that may cause harmful effect or the quality and safety of consumable products. Some strains of *Bacillus cereus* have been shown to cause food poisoning at low concentration (10^{10} bacterium/g) as described by[2][36]. *Bacillus cereus* is increasingly associated with infections like eye infection, pneumonia, sepsis and central nervous system infections.

Conclusion

The results obtained in this study demonstrate that diverse communities of microorganisms contaminate issue paper and that some of these organisms may be toxin producers. But despite the presence of the diverse microbial community in the tissue paper, 80% of the tissue paper brands analyzed in this study were safe for used as the passed the quality control limit Furthermore, the possible transfer of these microbes from tissue paper to individuals during hand drying can be another problem. This study does not that tissue papers are unsafe, but it point to the possibility of unwanted contamination on tissue paper products.

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