

MICROBIOLOGICAL HAZARDS ASSOCIATED WITH THE USE OF POLYTHENE BAGS AS FOOD CONTAINERS

*Mendie, E.U. and *Egwari, L.O.

*Department of Pharmaceutics & Pharmaceutical Technology and

*Department of Microbiology, University of Lagos, Lagos.

ABSTRACT

Contamination and health risks associated with the use of polythene bags normally used in package ready to eat foods were investigated. One thousand, five hundred and fifty (1,500) bags were sampled for microbial contamination, with fifty percent of the bags opened by squeezing. The remaining 50% were opened by mouth blowing.

The results showed that a high proportion of the bags was contaminated (71.90%). However, the risk of contamination was significantly higher ($p < 0.05$) in mouth-blown bags (89.0%), than in bags opened by squeezing. There was a significantly strong association between mouth blowing of the bags and the rate of contamination of the bags ($p < 0.05$). Blowing did not only result in increased contamination, but introduced microorganisms of oral origin into the bags.

Clinically important bacterial isolates included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Escherichia coli*, *Klebsiella aerogenes* and *Pseudomonas aeruginosa*; while *Candida* spp, *Torulopsis* spp, *Trycophyton* spp, and *Microsporium* spp constituted the fungal isolates. Generally, significantly higher ($p < 0.05$) proportions of these isolates were recovered from mouth blown bags.

Such high rates of contamination of the bags, and the diversity of isolates so obtained is of high public health interest, and portends a major health risk to consumers of products packaged in these bags. It calls for appropriate hygienic controls on their use. In the event of their being used as food containers, they should be squeezed open rather than mouth blown to prevent the introduction of oral organisms into the bags.

INTRODUCTION

Polythene bags commonly called nylon bags in Nigeria are frequently used by traders and product manufacturers to pack edible products most of which require no further treatment before consumption. These products include bread, fried groundnut, powdered melon, water, powdered milk, cocoa beverage, etc.

Polythene bags are relatively cheap and affordable even by the petty traders. Moreover, their transparent nature gives the buyers an apparent security and assurance of the content against adulteration. However, the problem of microbial contamination which may arise from its use as a primary food container with its attendant microbiological hazards warranted our interest in this project. More

so, when one considers the level of cleanliness and personal hygiene normally exhibited by the calibre of individuals using nylon bags in Nigeria, then our objective of evaluating the contamination potentials of these bags in line with commonly in-use habits becomes highly desirable.

Negretti,⁽¹⁾ examined 4200 samples of pharmaceutical packaging materials and accessories (glass, plastic bottles, flexible metal tubes, droppers, blisters, and capliners), and the rates of contamination were found to be capliners 96.40%, plastic bottles 94.80%, droppers 93.86%, flexible metal tubes 89.70%, glass bottles 54.60%, and blisters 33.50%. In a similar manner, Hugbo and Akpan,⁽²⁾ reported an in-use contamination rate for intravenous fluids of 32.10% for glass bottles, 54.50% for plastic bottles, and 46.90% for plastic bags with overall rate of 44%; which was attributed to airborne contaminants. With nylon bags, their very nature makes them easily prone to extrinsic air-borne and touch contamination.

It is a common practice amongst the users of these bags to blow open with their mouth during insertion of materials into the bags. This sort of unhygienic practice may certainly introduce some oral

microbes into the bags. The consequence of such introduction, the type, nature and quantity of such contaminants will depend on the level of cleanliness and oral hygiene of the operatives. Apart from the health-risk that may arise from such in-use habits, the presence of physical defects such as fragility and leakability, or thermolability may predispose nylon bags to a high level of

extrinsic contamination. These may serve as potential sources of transmission of pathogenic spoilage organisms from product to consumers. The incidence of diarrhoeal and other gastrointestinal diseases^(3,4) or even respiratory tract infections⁽⁵⁾ may not be unconnected with the use of this type of packaging material for food products^(6,7).

With the possibility that polythene bags may be inherently or

incidentally contaminated, the use of mouth to blow them open may actually complicate this problem. The findings from this study will therefore highlight the contamination potentials of nylon bags and the microbiological hazards associated with their use; viz- viz the in-use habits of blowing the bags open during product insertion.

Table 1.0: Classification of Isolates Recovered from Contaminated Nylon Bags

ISOLATES	SQUEEZED	SAMPLES	MOUTH BLOWN	SAMPLES
	Number of bags Contaminated	% Contamination	Number of bags Contaminated	% Contamination
Bacterial growth only	274	35.35	377	48.65
Fungal growth only	85	10.97	85	10.97
Bacterial and Fungal growth	198	25.55	228	29.42
Total	557	71.87	690	89.03

MATERIALS AND METHODS

Media:

The following media which are products of Biomerieux France, were used in this study: Brain heart infusion agar, Mac-Conkey agar, Desoxycholate citrate agar, Sabouraud dextrose agar, Corn meal agar, and Nutrient broth. Blood agar plates were prepared by adding human blood, 10% to sterilized Brain heart infusion agar at 45°C. Physiological saline was used as diluent.

POLYTHENE BAGS:

A total of 1,550 bags were

purchased from open markets in Lagos, Nigeria. All polythene bags were sealed at both ends at the time of sampling.

SAMPLING METHODS:

One thousand, five hundred and fifty (1,550) bags were divided into two groups of 775 each, and sampled. In one set, the bags were opened using mechanical means, by squeezing the loose end by hand, and in the other set by blowing open with the mouth. These two methods of opening the bags were adopted as they constitute the normal in-use practice by the end users. Five volunteers (three males and two

females) were selected as blowers.

Sterile physiological saline was added in 10ml amount into each bag, tied at the loose ends and agitated by several inversions. Thereafter, 1ml was pipetted and transferred into sterile McCartney bottles containing 19ml molten agar 45°C. The content was mixed thoroughly and poured aseptically into plates. On solidifying, the plates were incubated aerobically at 37°C for 48h. Cultures on Sabouraud dextrose agar plates were incubated at room

temperature ($30 \pm 0^\circ\text{C}$) for 7 days. All formed colonies were counted using New Brunswick colony counter. The different isolates were identified using standard microbiological methods^(8, 9).

CONTROL EXPERIMENT:

In order to determine the effect of mouth blowing on the character of the microbial isolates, the volunteer blowers were asked to blow into McCartney bottles containing 10ml of physiological saline or directly onto the surface of Blood agar plates. All

inoculated plates were incubated as described earlier. Each blower was sampled on four different occasions and the isolates and their frequencies recorded.

In another experiment, Blood agar and Sabouraud destrose agar plates were opened for 30 minutes in the laboratory and the cottage factory producing the polythene bags to confirm that the isolates were not from these sources. This was done to determine the likely source of organisms isolated from the sampled polythene bags. All data were subjected to statistical

analysis using Chi-squared with priori level of significance at $p < 0.05$.

RESULTS

The gross contamination rate of the squeezed polythene bags was found to be 71.90% (Table 1.0). All the bags sampled in this set showed small numbers of contaminants with a mean count of 7cfu/ml. The distribution of viable numbers showed 33.03% of isolates having between 1 – 3 colonies per plate; while 16.77% had more than 10 colonies (Table 2.0).

Table 2.0: Distribution of Viable Numbers of Isolates Recovered from Contaminated Nylon Bags

VIALE NUMBER	SQUEEZED	SAMPLES	MOUTH BLOWN	SAMPLES
	Number of bags Contaminated	% Contamination	Number of Bags Contaminated	% Contamination
1 – 3 Colonies	256	33.03	120	15.48
4 – 10 Colonies	171	22.07	245	31.62
> 10 Colonies	130	16.71	325	41.94
Total	557	71.87	690	89.03

Calculated $X^2 = 113.085$

Tabular X^2 at 2df = 7.38; $113.085 > 7.38$, which showed a significantly strong association between mouth blown samples of the bags and the distribution of viable numbers of the contaminants ($p < 0.05$).

In comparison, 89.0% of the polythene bags blown open by mouth was found to be contaminated; the data showed a significantly strong association between the blowing of the bags and the contamination rate of the bag ($p < 0.05$). Equally affected was the distribution of the viable numbers of contaminants recovered from

this set of bags, which increased significantly ($p < 0.05$) over the values obtained from the squeezed bags. The mean colony count for this group was found to be 12cfu/ml (Table 2.0). There was also a significantly higher rate ($p < 0.05$) of isolation of bacterial contaminants from the mouth blown bags compared to the squeezed bags, a situation which maybe attributed to the release of

oral microbes into the bags during blowing. Oral isolates were found to constitute about 52% of the total microorganisms recovered from the mouth blown bags.

Table 3.0 gives the list of microorganisms isolated from the polythene bags; of the 31 genera of microorganisms isolated, the moulds

Table 3.0: Microorganisms Isolated from Polythene Bags

S/NO	FUNGI	S/NO	BACTERIA
	<u>Moulds</u>		
1.	<u>Penicillium</u> spp	1.	<u>Micrococcus</u> spp
2.	<u>Aspergillus</u> spp	2.	<u>Staphylococcus</u> spp
3.	<u>Rhizopus</u> spp	3.	<u>Staphylococcus aureus</u> *
4.	<u>Mucor</u> spp	4.	<u>Corynebacterium</u> spp
5.	<u>Helminthosporium</u> spp	5.	<u>Bacillus</u> spp
6.	<u>Absidia</u> spp	6.	<u>Flavobacterium</u> spp
7.	<u>Trycophyton</u> spp	7.	<u>Pseudomonas</u> spp
8.	<u>Microsporium</u> spp	8.	<u>Klebsiella</u> spp
9.	<u>Fusarium</u> spp	9.	<u>Escherichia coli</u>
10.	<u>Cladosporium</u> spp	10.	<u>Chromobacterium</u> spp
11.	<u>Altermaria</u> spp	11.	<u>Hafnia</u> spp
	<u>Yeasts</u>	12.	<u>Proteus</u> spp
1.	<u>Candida</u> spp	13.	<u>Acinetobacter</u> spp
2.	<u>Torulopsis</u> spp	14.	<u>Streptococcus</u> spp*
3.	<u>Trichosporon</u> spp	15.	<u>Moraxella catarrhalis</u> *
4.	<u>Kluyveromyces</u> spp		
5.	<u>Saccharomyces</u> spp		

constituted 35.50%, yeast 16.12%, while bacterial isolates were 49.30%. All the organisms listed were environmental contaminants and were common to bags sampled by the two methods except for Staphylococcus aureus, Streptococcus spp, and Moraxella catarrhalis which came from mouth-blown bags. They formed 28.71% of the total isolates recovered from all the contaminated bags. The data obtained from the control experiments (Table 4.0) showed that most of the contaminants came from the environment, with the exception of those isolated from droplets discharge from the mouth.

The rate of isolation of the oral organisms from droplet

discharge from the mouths of the volunteer blowers gave a value of 50% for Streptococci, while Staph. aureus was 30%. Moraxella and Corynebacterium spp were the least isolated with a value of 10% each.

DISCUSSION

The data obtained from this study showed a high incidence of microbial contamination of the nylon bags. A contamination rate of over 70% was observed with the squeezed bags; one third of which consisted of mixed cultures of bacteria and fungi while 10% yielded only fungi. This high rate of contamination maybe attributed to environmental contaminants introduced into the bags during manufacture, packing, storage or even during transportation. It is possible that air-borne contamination arising from influx of air carrying microbe-laden dusts would have contributed to these

observed contamination⁽¹⁰⁾. It implies that the contamination of the bags is not transient but wide spread..

Mendie et al 1993⁽¹¹⁾ reported that the high incidence of microbial contamination of non-sterile products maybe due to non-adherence to good manufacturing practice and unhygienic handling of such products during packing, storage and use.

When the bags were blown open with the mouth, the rate of contamination increased significantly ($p < 0.05$) to 89.03%. This higher value compared to that obtained from squeezed bags maybe linked with the influx of air from the mouth carrying droplets discharge into the bags. Thus, oral and throat microorganisms were released into these bags to produce the observed contamination. It is pertinent to stress that the strong association observed in this study ($p < 0.05$) between contamination and mouth blowing of the bags portends serious danger. It also exposes the potential health risk inherent in the consumption of products packaged in such bags^(6, 7).

With increase in the rate of isolation and the significantly higher ($p < 0.05$) proportion of isolates yielding more than 10 colonies, there is a greater propensity for such in-use practice of mouth-blowing of bags to transmit pathogenic organisms to consumers. The isolation of oral flora such as Strep. Pyogenes, Strep.

Table 4.0: Isolates from Droplets Discharge from the Mouth of Volunteer Blowers

ISOLATES	FREQUENCY OF ISOLATION	RATE OF ISOLATION (%)
<u>Staph. aureus</u>	6	30.0
<u>Strep. pyogenes</u>	3	15.0
<u>Strep. viridans</u>	7	35.0
<u>Corynebacterium spp</u>	2	10.0
<u>Moraxella spp</u>	2	10.0
Total	20	100

Viridans, Moraxella catarrhalis and Corynebacterium diphtheriae is a confirmation of their indictment in product contamination and possible infection hazards^(3, 5, 12, 13)

The clinical significance of microorganisms in pharmaceuticals have been reviewed by Ringertz and Ringertz⁽¹⁴⁾. Isolates such as pseudomonas, enterobacteria, and staphylococci, etc, can be regarded as opportunist pathogens. They are not generally harmful to normal healthy individuals, but readily becomes infectious where

resistance mechanisms are impaired⁽¹⁵⁾. However, the pathogenic roles of Strep. pyogenes, flavobacteria, Corynebacterium diphtheriae and Moraxella catarrhalis are well documented^(16, 17). Fungal isolates such as Aspergillus, Penicillium, Rizopus, and Mucor which have been implicated in food spoilage as well as being agents of opportunistic mycotic infections^(18, 19, 20), while Candida and Torulopsis are frequently involved in cutaneous and localized infections^(21, 22).

In practice many users of nylon bags particularly the petty traders exhibit very low sanitary and

hygienic habits. Though not isolated in this study, there is a strong likelihood that such in-use practice of mouth blowing of the bags may enhance the spread of Mycobacterium tuberculosis, Bordetella pertusis, Haemophilus influenzae or other pathogenic infections.

CONCLUSION

This study has aptly demonstrated the contamination potentials and the health hazards inherent in use of polythene bags as packaging materials for food. Such hazards become highly exaggerated when the bags are blown open by mouth. With the spectrum and number of contaminants recovered particularly from the mouth blown bags, nylon bags must therefore be sanitized appropriately before use, and the users educated on the public health menace of blowing-open the bags by mouth during product packaging.

REFERENCES

- Negretti, F. (1981). Findings on the microbiological characteristics of Pharmaceutical containers. *Bull. Chim-Farm.* 120: 193 - 201
- Hugbo, PG and Akpan, UE (1989). Microbial contamination of intravenous infusions during clinical use at Lagos University Teaching Hospital. *Nig. J. Pharm.* 20: 75 - 79.
- Umoh, JU, Adesiyun, AA and Adekeye JO (1983). Epidemiological features of an outbreak of gastroenteritis/choleera in Katsina, Northern Nigeria. *J. Hyg. Camb.* 91: 101 - 111.
- Niemogha, MT; Alabi, SA; Uzoma, KC; Odugbemi, TO; Adegbola, RA and Coker, AO (1995). The incidence of Salmonella shigella and other enteric bacterial pathogens in stool specimens of diarrhoeic patients. *Nig. Med. J.* 28(2): 70 - 74.
- Shulman, ST (1993). Severe group A streptococcal infections. *Crit. Care Med.* 21(95): 5314 - 315.
- Baird RM and Shooter, RA (1976). Pseudomonas infection associated with the use of contaminated medicaments. *Br. Med. J.* 2: 349 - 350.
- Grigo J. (1976). Microorganisms in drugs and cosmetics: Occurrence, harms and consequences in hygienic manufacturing. *Zentbl. Bakt. Hyg. Abt. I. Orig. B.* 162: 233 - 287.
- Cowan, ST (1974). In Cowan and Steel manual for the identification of medical bacteria. 2nd ed. Cambridge University Press, London.
- Rippon JW (1988). The pathogenic fungi and pathogenic actinomycetes. *Medical Mycology* 3rd ed. W. B. Saunders Company, Philadelphia.
- Hansen IS and Helper CD (1973). Contamination of intravenous solutions by air-borne microbes. *Am. J. Hosp. Pharm.* 30: 326 - 331.
- Mendie, UE; Ifudu, ND; and Brown SA (1993). How safe are non-sterile liquid preparations. *J. West Afr. Pharm.* 7(1): 8 - 11.
- Baird RM; Crowden, CA; O'Farrell, SM; and Shooter, RA (1979^b). Microbial contamination of Pharmaceutical products in the home. *J. Hyg. Camb.* 83: 277 - 283.
- Wieneke, AA, Roberts D; and Dilbert RJ (1993). Staphylococcal food poisoning in the United Kingdom 1969 - 90. *Epidemiol. Infection.* 110(3): 519 - 531.
- Ringertz, O. and Ringertz S. (1982). The clinical significance of microbial contamination in pharmaceutical and allied products. *Adv. Pharm. Sci.* 5: 201 - 226.
- Parker, MT (1972). The clinical significance of the presence of microorganisms in pharmaceutical and cosmetic preparations. *J. Soc. Cosmet. Chem.* 23: 415 - 426.
- Baum, KF; MacFarlane DL; Cupidore, L. and Serjeant, GR (1985). Corynebacterium diphtheriae in sickle cell leg ulcers in Jamaica. *West Indian Med. J.* 34(1): 24 - 28.
- Hafiz S; Hafiz T. and Yazdani I. (1993). Significance, isolation, identification and sensitivity of Branhamella Moraxella catarrhalis. *J. Pak. Med. Assoc.* 43(9): 178 - 179.
- Vrabec, DP (1993). Fungal infection of the larynx. *Otolaryngol. Clin. North. Am.* 26(6): 1091 - 1114.
- Wills, BA (1958). Fungal growth in syrup of Tolu. *J. Pharm. Pharmacol.* 10: 302 - 305
- Slotman GJ; Shapiro E. and Moffa SM (1994). Fungal sepsis: Multisite colonization versus fungemia. *Am. Surg.* 60(120): 107 - 113.
- Dupont B and Drauhet E. (1985). Cutaneous, ocular and osteoarticular candidiasis in heroin addicts: new clinical and therapeutic aspects in 38 patients. *J. Infect. Dis.* 152(3): 577 - 591.
- Neeman A. and Kadish U. (1984). Candidal infection of gastric ulcer. *Gastroenterol.* 87(6): 1407.

