Surface Microbial Contamination in Some Commonly Available Tablet Dosage Forms

C.O. Obuekwe^a I.F. Obuekwe^c M. Rafiq^b

^aDepartment of Biological Sciences, and ^bEM Unit, SAF, Faculty of Science, Kuwait University, Kuwait; ^cDepartment of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Key Words

Tablets · Microbial contamination

Abstract

Objective: Nonprescription drugs are subject to unrestricted handling and are, therefore, potentially susceptible to postproduction contamination by microorganisms from both the handlers and the environment. The aim of this work is to investigate the occurrence of contamination of certain tablet surfaces by microorganisms. **Methods:** Twenty-two samples of commercially available analgesic and vitamin preparations in tablet form were obtained as sold or dispensed from retail pharmacies and clinical pharmacies in Nigeria and Kuwait. Sample surfaces of tablets were investigated by scanning electron microscopy and augmented by streaking and superficial implantation on agar media for culture development. **Results:** Of 22 samples tested, 14 (64%) were visually found to have surface microbial contamination. However,

only 4 samples (18%) of the total samples investigated yielded positive microbial cultures on growth media. The most commonly observed surface contaminants were yeast cells. The microorganisms isolated included *Saccharomyces* sp., *Rhodotorula rubra*, coagulase-negative staphylococci and *Penicillium* sp. **Conclusion:** Commonly available nonprescription drugs in tablet dosage form have been shown to be frequently contaminated by microorganisms. However, cases of microscopic visualization of microbial contamination of drugs do not often result in recoverable cultures on growth media.

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Introduction

Analgesics and vitamin products are among the most commonly available non-prescription drugs and are, therefore, subject to unrestricted postproduction handling. The microbiological quality of pharmaceutical

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C.O. Obuekwe
Department of Biological Sciences, Kuwait University
PO Box 5969, 13060 Safat (Kuwait)
Tel. +965 481 1188, ext. 5662, Fax +965 484 7054
E-Mail okey@kuc01.edu.kw

products not only depends on the quality of raw materials and production practices, but also on storage conditions [1, 2]. Moreover, the common practice of pharmacies repackaging and dispensing bulk products into smaller dosage units will increase product handling and the risk of microbial contamination from handlers and the environment.

Different types of pharmaceutical products have been routinely contaminated right after manufacture [3, 4] and several fungi and bacteria have been identified with such contamination. Under tropical conditions, the loss of microbiological quality can be extensive with such contamination [2, 5]. While most of the microorganisms contaminating nonsterile pharmaceutical products may be nonpathogenic commensals of environmental origin, they pose problems as agents of spoilage [6, 7]. Many of these organisms may also become opportunistic pathogens in compromised individuals. In the hospital environment, higher contamination rates with pathogens would be expected simply because greater opportunities for cross-contamination with pathogens exist in the hospital environment.

Apart from health problems of microbial contamination of pharmaceuticals [8, 9], the deteriorating effects on the products are various, ranging from introduction of toxic metabolites and cell fractions to chemical and physical modifications [7, 10]. It has been shown that surface contamination and growth of *Klebsiella aerogenes* on acetylsalicylic acid tablets will prolong the tablets' disintegration times [10]. Disintegration properties of tablets are important as they affect solubilities [11, 12] and may ultimately affect bioavailability of the drugs [13].

The importance of and potential for microbial contamination of pharmaceuticals is widely recognized in the pharmaceutical industry and attempts to safeguard products from contamination in the manufacturing

process include, among others, good manufacturing practices and packaging of products in individual waterproof, tamperproof wrappings. Available information on medicine-borne contamination is poor and such reports are portrayed as episodic, being associated with specific isolated incidents [6].

While culture methods can determine contamination of tablets by viable microorganisms, they may fail to delineate contamination originating during manufacturing and postmanufacturing. On the other hand, since scanning electron microscopy (SEM) is restricted to the visualization of surfaces only, it can provide some evidence of postproduction contamination of tablets and other solid dosage forms, as this would be expected to be restricted to surfaces. The objective of this investigation was, therefore, to assess the surface microbial contamination, which may be indicative of postproduction contamination of commonly available nonprescription pharmaceutical products in tablet dosage form from dispensing outlets.

Materials and Methods

Samples

Twenty-two samples from 20 brands of commonly available nonprescription drugs in tablet dosage form were obtained from pharmacy outlets which included retail pharmacies, patent medicine stores and clinical pharmacies in Nigeria and Kuwait. The drugs included analgesics, antispasmodics and vitamin preparations. Samples from retail pharmacies were procured as retailed, either as multidose packs (boxes) containing up to 100 tablets or as single dosage packs in waterproof/tamperproof wrappings. Samples from clinical pharmacies were obtained as repacked multidoses in waterproof sleeves, forms in which the tablets are conventionally dispensed to patients.

Scanning Electron Microscopy

Preliminary investigations had shown that the conventional methods of sample preparation for SEM as described previously [14], involving fixations in glu-

Table 1. Microbial contamination on the surfaces of different brands of commonly available nonprescription drugs in tablet dosage form

Sample	Drug type	Packaging	Surface finish	Source	Microbial contamination	
					microscopy	culture
1	Analgesic ¹	tamperproof single dose (aluminium foil/plastic film wrap)	uncoated	retail pharmacy (Nigeria)	yeast	none
2	Analgesic ²	tamperproof single dose (aluminium foil/plastic film wrap)	uncoated	retail pharmacy (Nigeria)	not detected	none
3	Analgesic ¹	tamper proof single dose (aluminium foil/plastic film wrap)	uncoated	retail pharmacy (Nigeria)	cocci	none
4	Analgesic ¹	multidose box	uncoated	retail pharmacy (Nigeria)	bacterial rods, yeast	none
5	Analgesic ¹	tamperproof single dose (aluminium foil wrap)	uncoated	retail pharmacy (Nigeria)	yeast, bacterial rods	none
6	Analgesic ²	tamperproof single dose (plastic film wrap)	uncoated	retail pharmacy (Nigeria)	yeast	yeast cells
7	Multivite	multidose box	uncoated	retail pharmacy (Nigeria)	yeast, mold fragment	none
8	Vitamin C (colored)	multidose box	uncoated	retail pharmacy (Nigeria)	mold fragment	none
9	Vitamin C (not colored)	multidose box	uncoated	retail pharmacy (Nigeria)	mold fragment	none
10	Analgesic ¹	multidose box	uncoated	retail pharmacy (Nigeria)	bacterial rods, cocci	none
11	Vitamin C	multidose box	uncoated	retail pharmacy (Nigeria)	mold	none
12	Analgesic ²	single-dose, tamperproof (aluminium foil wrap)	uncoated	retail pharmacy (Nigeria)	yeast, bacterial rods	<i>Rhodotorula</i>
13	Multivite-mineral mix	multidose box	coated	retail pharmacy (Kuwait)	bacterial rods, mold, yeast	gram-negative rods
14	B-complex vitamin	multidose box	uncoated	retail pharmacy (Nigeria)	not detected	none
15	Multivite	tamperproof single dose (aluminium foil/plastic film wrap)	coated	retail pharmacy (Kuwait)	not detected	none
16	Analgesic ²	prepack in plastic sleeve, from multidose box	uncoated	clinical pharmacy (Kuwait)	bacterial rods, cocci, yeast	none
17	Analgesic ¹	tamperproof (aluminium foil/plastic film wrap)	uncoated	clinical pharmacy (Kuwait)	not detected	none
18	Multivite-mineral mix	prepack in plastic sleeves from multidose box	coated	clinical pharmacy (Kuwait)	mold, bacteria, yeast	staphylococci
19	Analgesic ¹	tamperproof (aluminium foil/plastic film)	uncoated	clinical pharmacy (Kuwait)	not detected	none

Table 1 (continued)

Sample	Drug type	Packaging	Surface finish	Source	Microbial contamination	
					microscopy	culture
20	Antispasmodic	tamperproof (aluminium foil/plastic film)	coated	clinical pharmacy (Kuwait)	not detected	none
21	Antispasmodic	tamperproof (aluminium foil/plastic film)	coated	clinical pharmacy (Kuwait)	not detected	none
22	Analgesic ¹	prepack in plastic sleeves, from multidose box	uncoated	clinical pharmacy (Kuwait)	not detected	none

¹ Acetoamido phenol base.

² ASA base.

taraldehyde and OsO₄ and dehydration in gradient concentrations of desiccants, caused the disintegration and/or dissolution of the samples. Also, the technique of subjecting the tablets to only critical-point drying in acetone/liquid CO₂ as a desiccant [14] and avoiding fixation preliminaries resulted in selective leaching of the surface components of the tablets tested. Consequently, it was adjudged that the technique was capable of introducing artifacts or could result in the loss of relevant information, although it provided very sharp micrographs.

Because of the above preliminary experimental observations, all tablets for SEM were simply coated 'as is' without any other treatment. For coating, each sample was mounted on a brass stud by means of silver gum. Before mounting, each tablet sample was aseptically and carefully broken into two halves and each half mounted to expose each of the two major surfaces. Duplicate samples of tablets were observed (i.e., a total of four specimens per sample). The mounted specimens were then sputter-coated in gold in a Balzer SCD-050 sputter-coater for 30 s. The prepared samples were subsequently observed in a JOEL ISM-6300 scanning electron microscope at accelerating voltage of 20 kV or less, depending on the stability of the tablet's surface.

Culture Methods

For the surface-contaminating organisms, each tablet was aseptically streaked (smeared by means of sterile forceps) across the surface of nutrient agar (NA for bacteria) and potato dextrose agar (PDA for fungi).

Also, each tablet was separately placed (superficial implantation) on NA and PDA. Plates were subsequently incubated at 25 °C for up to 7 days. Colonies that grew were selected and subcultured on NA or PDA for purification and isolation of pure cultures. The isolates were identified on the basis of their biochemical and morphological characteristics.

Results

The results of the investigation of the surface contamination for different brands of commonly available nonprescription tablet dosage forms are summarized in table 1. Evidently, visual indications of surface microbial contamination were obtained in samples irrespective of packaging or coating. Fourteen (64%) of the 22 samples of the brands investigated showed visual evidence of microbial contamination. However, only 4 (18%) of the total samples and 27% of the visually positive microbially contaminated ones resulted in positive microbial cultures. Of the 7 vitamin preparations, 6 were visually contaminated, of which 2 were gross with various microbial forms – filamentous fungi, bacteria and morphological yeast forms. Three of the 4 acetyl-

Fig. 1. SEM of a vitamin C tablet showing extensive ramification of the surface by hyphae of filamentous fungi. The sample was obtained from a multidose box from a retail pharmacy.

Fig. 2. SEM showing coccoid and rod morphological forms in the surface coating of a multivite preparation. Dumbbell-shaped cells (DBC) suggest evidence of cell division. The breach on the lower left corner exposes the tablet material, showing that the organisms were associated with the surface coat.

salicylic acid (ASA)-based analgesics showed visual microbial contamination, but only 2 of the samples supported viable microorganisms which were identified as coagulase-negative *Staphylococcus* and *Penicillium* spp. In the

other ASA sample, an organism with purplish red-colored colonies on PDA, identified as *Rhodotorula rubra*, and another coagulase-negative *Staphylococcus* were isolated.

Fig. 3. SEM showing yeast cell scattered superficially over the surface of an ASA-base product obtained from a clinical pharmacy.

Figures 1–5 represent typical scanning electron micrographs of microbially contaminated surfaces of the different tablets investigated. Figure 1 shows the surface of an uncoated brand of vitamin C extensively ramified by fungal hyphae. Such extensive fungal colonizations were found in some other brands of vitamin C. In another vitamin preparation, a multivite-mineral mix, where fungal growth was also observed, bacterial contamination was associated with the coating (fig. 2). Although most of the contamination of tablets observed was superficial, as seen in figure 3, in others the surface contaminants appeared to have penetrated into the solid matrix of the tablets (fig. 4). Figure 3 shows chains of yeast cells with buds (possibly *Saccharomyces* sp.) on the surface of an aspirin tablet, while figures 4a and b show ASA-based analgesic samples where extensive developments of yeast cells of different morphological forms have penetrated or emanated from the tablets through fissures on the surface of the

preparation. Such a growth pattern may be facilitated by poor compaction of the tablet which leaves interparticulate spaces on the surface as was the case in an ASA-based analgesic sample studied (figure not shown). The yeast cells in figures 3 and 4a, b clearly bear buds which are indicative of some active growth activity on the tablets some time after the contamination. The presence of buds and pseudomycelium in figure 4b suggests *Candida* contamination. Unlike the samples shown in figures 3 and 4a, which were obtained from repackaged tablets of a multidose box from a clinical pharmacy, the sample shown in figure 4b was a single-dose preparation packaged in a tamperproof, waterproof wrapping. In some of the samples investigated, the contaminating organisms appeared to be embedded in a network of capsular materials. Multiple microbial contamination is clearly indicated in figure 5 where yeast cells and grapelike clumps of cocci are superimposed on the surface of tablets obtained from a clinical phar-

Fig. 4. SEM showing different types of budding yeast cells in the fissures on the surfaces of an aspirin tablet obtained from a clinical pharmacy (**a**) and a retail pharmacy (**b**).

macy. Such superimposed and multiple contamination could be encouraged by the production of exopolysaccharides observed earlier in some organisms. Surface contaminants of a different sort may also occur: a long fibrous inanimate material was found embed-

ded in the coating of a multivitamin tablet obtained from a retail pharmacy. The significance of this observation is that such inanimate contaminants may be sources of microbial contaminants for the pharmaceutical products and may serve as fomites.

Fig. 5. SEM showing multiple microbial contamination of the surface of an ASA-base product by yeast cells (large cells with buds) and grapelike clumps of cocci cells. The sample was obtained from a clinical pharmacy.

Discussion

In this work, SEM was used to study the incidence of surface contamination in several brands of commonly available nonprescription drugs in tablet form. Microscopy, because of its inability to differentiate viable and nonviable cells, has been augmented with culture techniques that were intended to limit observation to the surfaces of the products. There was not attempt to quantify or assess the level of microbial load of the entire sample of the tablet.

Results so obtained have indicated a rather common incidence of microscopically detectable microorganisms on the tablet surfaces, and the superficiality of the occurrence of some of these contaminants suggests postproduction contamination. Although the actual number of samples ($n = 22$) or brands ($n = 20$) investigated was low compared to commercially available brands, the results show that microscopically observable contamination is

common and underscores the need for a high level of environmental hygiene and good manufacturing practices in pharmaceutical processing, especially for nonsterile products. Also, the visualization and in a few cases, culture demonstration of surface microbial contaminants from pharmaceutical products (including tamperproof, waterproof packaging) obtained from a humid, tropical environment (Nigeria) and a hot, dry subtropical region (Kuwait) point to the immense potential for surface contamination in tablets in all climates. The higher level of visualized contamination (70%) in samples from Nigeria, compared to 33% observed in samples from Kuwait, may be attributable to the generally higher humidity and lower but favorable ambient temperatures which would support microbial viability and occurrence, as reflected in the observations in this work. In this investigation, only very low levels of viable microorganisms were demonstrated in these tablets and none of the organisms isolated is a

known pathogen. Even so, in immunologically compromised individuals common environmental microorganisms (commensals) may pose a threat if ingestion and infection occur.

Yeast cells were evidently the most commonly observed surface contaminants. Surprisingly, hardly any of the ubiquitous yeast cells (*Saccharomyces* sp.) observed microscopically were recovered by culture. As seen on most of the samples, some of these cells showed evidence of cell damage, probably from processing. If this were so, it would suggest contamination that occurred before or during production. However, the superficiality of the occurrence of some of these contaminants suggests otherwise (i.e., postproduction contamination).

The observations made by microscopy in this investigation have provided some evidence to support the view that these surface contaminants were not just dormant, inert contaminants, but actually exhibited some growth process postcontamination. As commonly seen, many of the yeast cells occurred in chains with buds. Buds are the young offspring of yeast reproduction in some yeast genera. Also, as seen in some samples, some of the microbial cells appeared embedded in capsular materials. Capsular materials are anionic exo-polysaccharides formed by several microorganisms during growth to aid anchorage to surfaces [15]. The observed or isolated organisms such as *Candida* sp. coagulase-negative staphylococci and *Penicillium* sp. are recognized opportunistic pathogens which can infect immunocompromised individuals such as AIDS victims. Riederer et al. [16] reported that opportunistic *Candida* infections constitute one of the commonest disorders (>50%) following HIV infections in 250 patients investigated. In their review, Weber et al. [17] highlighted the prevalence of opportunistic microsporidial infection in man.

However, susceptibility to opportunistic infections is not restricted to HIV-infected cases only, since it was reported [18] that immunosuppression arising from therapy resulted in opportunistic pneumocystis pneumonia as a complication. Moreover, in the absence of viable cells, microbial metabolites may be toxic and cell wall fractions pyrogenic [6, 7]. The poor demonstration of viable contaminants in the tablet samples could be due, among other factors, to the inability of the media used to recover stressed organisms present and also the restriction of the sampling to the surface only. Obviously, the use of liquid extraction procedures (enrichment in both cultures) would have demonstrated organisms trapped within the solid matrix of the tablet, a procedure that would be more in consonance with production-stage contamination than postproduction contamination.

Conclusion

Commonly available nonprescription drugs in tablet dosage form have been shown microscopically to be frequently contaminated by microorganisms. However, such incidence of microscopic visualization of microbial contamination very poorly translated into recoverable (viable) microbial cultures.

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