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EFFECT OF PRIMARY PACKAGING ON MICROBIOLOGICAL STATUS OF ORAL SOLID DOSAGE FORM

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ABSTRACT

The emergence of microbial contaminants in non-sterile drugs caused not only the degradation of many products, but also proved to be a potential risk to consumer health. The aim of this study was to test microbial load of non sterile solid pharmaceutical product and investigate the effects of different packaging system on microbial status of pharmaceutical product. A total of 18 sample of solid dosage form packaged in different packaging were procured from market. All samples have been tested for the presence of specific microorganisms, Total aerobic microbial counts (TAMC) and Total yeast and mold counts (TYMC) using compendial procedures. Out of 18 sample 72.22 % (n=13) had shown microbial growth and only 16 % (n=3) of samples were non-compliant. Sample containing herbal ingredients, were the most heavily contaminated, showing a bacterial load > 104 CFU/g. The result showed that all the tested samples were free from *E. coli*. There was no significant difference ($p>0.05$) in microbial load of product packaged in different primary packaging.

INTRODUCTION

Microbial contamination is a common quality issue with pharmaceutical product, which has been raised by regulatory authorities. Products that are contaminated with microorganisms are withdrawal from the market and cause massive financial losses to the manufacturer. A product may also be withdrawn if it is proven that a discrepancy has occurred during its manufacture or distribution, which poses a potential risk to public health [1]. The US Food and Drug Administration had announced a recall of 642 products because of microbial contamination, from 2004 to 2011 [2]. Recently, microbial burden of non-sterile pharmaceutical product get an

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attention because of change in formulations, manufacturing and marketing of non sterile drugs, and the introduction of many new ingredients in these types of pharmaceutical preparations [3]. The microbial contamination of the product not only makes them dangerous from the infectious point of view, it can also change the chemical, physical and organoleptic properties of the medicines or change the content of the active ingredients. In addition, microorganisms can convert medicines into toxic products [4].

The microbiological specifications, the criteria & methodology for microbial examination of non-sterile products are established and updated in the continuous editions of the European Pharmacopoeia (EP). Manufacturers should assure that the microbiological status of finished products is meet with acceptable criteria and free from potentially harmful organisms. This is achieved through the implementation of the current guidelines for Good Manufacturing Practices (GMPs) during the production, storage and distribution of these products [3]. The methods used and the results obtained must meet the specifications and criteria set out in the relevant pharmacopoeia. Tests for total antimicrobial counts (TAMC) and total counts of yeasts and molds (TYMC), as well as identification tests for the different microorganisms were performed on both ingredients and end products [5].

The Indian pharmaceutical market is the third largest in terms of volume and thirteenth in terms of value, and represents 20% in volume and 1.4% in value of the global pharmaceutical industry, according to a report by Equity Master. India is the world's largest supplier of generic drugs and account for 20% of world output by volume. The Indian manufacturer is often confronted with problems in the context of market complaints or the adverse effects of oral drug use [6].

Packaging is an important factor in maintaining the quality of the product and maintaining its properties until the end of its shelf life. Considering the variety of the packaging available today, it is of great interest the study of the relationship between the drug itself and the pack-aging material used, with the target to the unveil potential microorganism contamination [7]. The aim of this study was to evaluate microbiological status of non sterile solid pharmaceutical product and investigate the effects of different packaging system on microbial status of pharmaceutical product.

Table 1: Microbiological Quality of Non Sterile Solid Pharmaceutical Product

Route of administration	TAMC (CFU/g)	TYMC (CFU/g)	Specified micro-organism
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g)
Oral dosage forms containing raw materials of natural origin	10^4	10^2	Absence of *Escherichia coli* (1 g) Absence of *Staphylococcus aureus* (1 g) Absence of *Salmonella* (10 g) Not more than 102 *Enterobacteriaceae* and certain other gram-negative bacteria (1 g)
Cutaneous use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g) Absence of *Pseudomonas aeruginosa* (1 g)
Vaginal use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g) Absence of *Pseudomonas aeruginosa* (1 g) Absence of *Candida albicans* (1 g)

TAMC= Total aerobic microbial counts, TYMC= Total yeast and mold counts

MATERIALS & METHODS

Sample Collection

A total of 18 pharmaceutical samples were obtained from the market and analyzed for microbial limit test and qualitative evaluation of objectionable microorganism. The samples included were different brands of nine active pharmaceutical ingredients and had different form of packaging. All purchased items were manufactured by companies registered in India and their drugs approved by the Central Drugs Standard Control Organization (CDSCO). For each sample (brand), 3 packs with different batch numbers were obtained. Each obtained sample was examined for the details specified on the package label,

including the quantity of active ingredients, the date of manufacture, the expiry date and the lot number.

Sample preparation

Ten gm of sample to be examined was taken aseptically and dissolved or diluted in 100 mL of soybean casein digest medium (SCDM).

Determination of total viable bacteria and fungi

The prepared samples were immediately filtered in the 0.45 µm double membrane filtration assembly. The membrane filters was then rinsed with 200 mL of buffer sodium chloride peptone solution pH 7.0. One of the membrane filters was kept at the surface of Soyabean casein agar (SCDA) for the enumeration of total aerobic microbial count (TAMC) and another to the surface of Sabouraud dextrose agar (SDA) with an antibiotic for determination of fungal number and yeasts. The agar medium for bacteria incubated at 32^0C for 18-24 hr, and the plate of agar medium for fungi at 25^0C for five days. Arithmetic average of the count was taken and the number of colony forming unit (CFU) per g was calculated [8].

Isolation of Microorganism

From the dilution of 10^{-3} of each sample, 0.1 ml of suspension was spread onto the membrane fecal coliform (MFC) agar, MacConkey agar, mannitol salt agar (MSA), and cetrimide agar for the isolation and quantification of total fecal coliform, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., and *Pseudomonas* spp., consecutively. MFC agar plates were incubated at 44.5 ºC for 18-24 hours, while the other plates were incubated at 37 ºC for 24 hours [8].

Table 2: Microbial count of studied non sterile solid dosage form

Sample	Active ingredients	Packaging	TAMC (CFU/g)	TYMC(CFU/g)
1	Paracetamol	Blister	ND	ND
2	Paracetamol	Alu-Alu	1.0×10^1	ND
3	Nimesulide + Paracetamol	Blister	ND	ND
4	Nimesulide + Paracetamol	Alu-Alu	ND	ND
5	Aceclofenac + Paracetamol	Blister	3.0×10^1	1.0
6	Aceclofenac + Paracetamol	Alu-Alu	8.0×10^1	2.5
7	Metformin	Blister	1.5×10^1	ND
8	Metformin	Alu-Alu	ND	ND
9	Lovastatin	Blister	ND	ND
10	Lovastatin	Alu-Alu	ND	6.0
11	Multivitamin with minerals	Blister	1.35×10^2	7.0
12	Multivitamin with minerals	Alu-Alu	8.0×10^1	4.0
13	Multivitamin with herbal ingredient	Blister	1.05×10^4	8.0
14	Multivitamin with herbal ingredient	Alu-Alu	1.78×10^4	1.8×10^1
15	Calcium with vit.D3	Blister	5.1×10^1	2.6×10^1
16	Calcium with vit.D3	Alu-Alu	3.5×10^1	ND
17	Antacid capsule	Blister	1.2×10^2	3.0
18	Antacid capsule	Alu-Alu	9.0×10^1	5.0

TAMC= Total aerobic microbial counts, TYMC= Total yeast and mold counts

Identification of isolates

Purified bacterial colonies recovered from contaminated samples were identified according to the diagnostic tables given by Barrow & Feltham. Tests performed for this purpose include Gram reaction, shape, carbohydrate utilization, catalase production, oxidase test, Indole production, spore formation, methyl red, Voges Proskauer, nitrate reduction, starch hydrolysis, tryptophan hydrolysis, hydrogen sulfide production, and citrate utilization [9].

Statistical analysis

The data were analyzed by GraphPad version 7.0 statistical software. According to the nature of data, two independent samples T-test or paired samples were used. T-test was used for

statistical comparisons. The differences among the mean values were found to be significant at $P \leq 0.05$.

RESULTS & DISCUSSIONS

Non sterile pharmaceuticals, regardless of their dosage form and route of administration, must meet the microbiological purity criteria set out in an appropriate edition of the pharmacopeia. The control of pharmaceuticals is a preventive method to prevent the release of harmful products into the consumer market. Many microorganism or, more specifically, the metabolites they produce, have the ability to degrade or inactivate the active ingredients. In addition, the drugs are administered by people whose immunity is compromised, so that to avoid drug-induced infections, consecutive editions of the Pharmacopoeia impose limits on microbial contamination [1].

Table 3: Microbial contaminants isolated from studied samples.

S. No	Active ingredients	Packaging	*E. coli*	*P. aeruginosa*	*S. aureus*	*Salmonella spp*	*Clostridia spp*
1	Paracetamol	Blister	N	N	N	N	N
2	Paracetamol	Alu-Alu	N	N	N	Y	N
3	Nimesulide + Paracetamol	Blister	N	N	N	N	N
4	Nimesulide + Paracetamol	Alu-Alu	N	N	N	N	N
5	Aceclofenac + Paracetamol	Blister	N	N	Y	N	N
6	Aceclofenac + Paracetamol	Alu-Alu	N	Y	Y	N	y
7	Metformin	Blister	N	N	N	Y	N
8	Metformin	Alu-Alu	N	N	N	N	N
9	Lovastatin	Blister	N	N	N	N	N
10	Lovastatin	Alu-Alu	N	N	N	N	N
11	Multivitamin with minerals	Blister	N	Y	N	N	N
12	Multivitamin with minerals	Alu-Alu	N	N	N	Y	N
13	Multivitamin with herbal ingredient	Blister	N	Y	N	N	Y
14	Multivitamin with herbal ingredient	Alu-Alu	N	Y	N	N	Y
15	Vitamin D3 Soft gel Capsule	Blister	N	N	N	Y	N
16	Vitamin D3 Soft gel Capsule	Bottle	N	N	N	N	Y
17	Antacid granule	Sachet	N	N	Y	N	N
18	Antacid granule	Bottle	N	N	N	N	Y

Y= Present, N= Absent

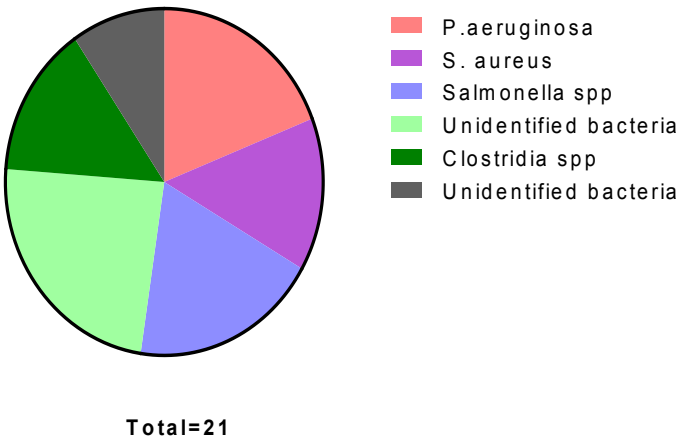

Figure 1: Extent of bacterial isolate from solid dosage form

A total of 18 samples of solid pharmaceutical dosage form packaged in different primary packaging system were tested. The sub groups of the primary packaging system were blister and Alu-Alu. Formulation containing raw materials of natural source also included: accounted for 11.11% (n=2) of tested sample. The detailed analysis of the outcome obtained, non-compliance and their prevalence is shown in missiles 2 and 3. The results indicated that 72.22 % (n=13) of samples had shown microbial growth. The obtain outcome showed that 16 % (n=3) of samples were non-compliant. The samples presented no complaints with the EP criteria because of: excessive microbial criteria.Sample containing herbal ingredients were the most heavily contaminated, showing a

bacterial load > 10^4 CFU/g. A study by Yasir Mehmood et. al (2017) showed that 76 % of sample had microbial count out of the normal range, during microbial count of tablets in blister pack sold in Pakistan [10]. Qasem M Abu Shaqra et al (2014) conducted a study on the microbial load of blister pack tablets in community pharmacies in Jordan. They acquired a total of 66 samples of 22 different brands of tablets packed in blister packs from community pharmacies in Amman. Out of 66 items, forty eight (72.7 %) products were free from microbial contamination, while 11 (16.7 %) harbored bacteria in counts < 10^2 CFU/g. The remaining 7 (10.6 %) items contained counts between 10^2 and < 10^3 CFU/g [11].

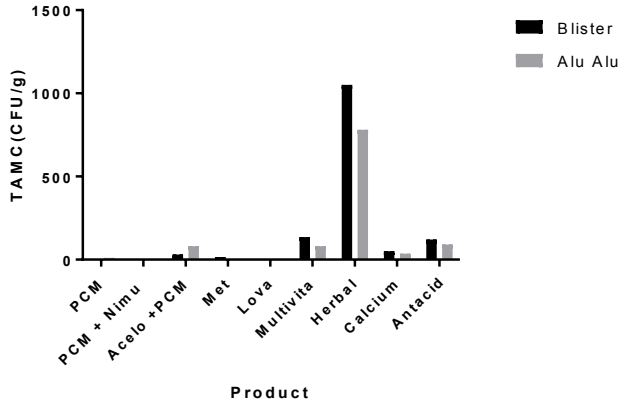

Figure 2: TAMC of solid dosage form in two different packages

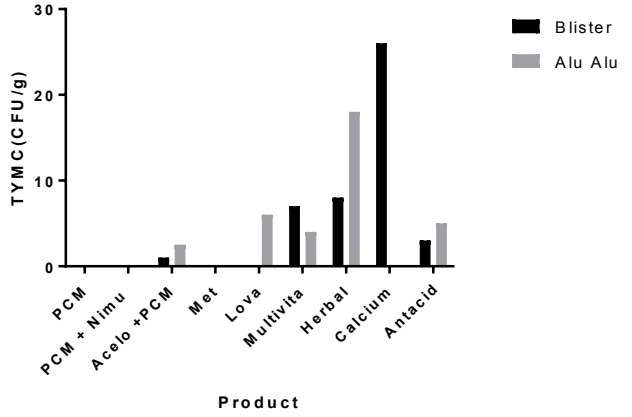

Figure 3: TYMC of solid dosage form in two different packages

Likewise for yeast and mould, about 88.88 % (n=16) of samples contained less than 10 CFU/g viable fungi. TYMC outcome indicated that only 5.55 % (n=1) of sample had count more than 10^2 CFU/g. According to specification of EU (Table 1), only one sample exceeded the specified limits.

The results of detailed microbial evaluation are shown in table 3 and figure 1. The result showed that all the tested samples were free from *E.Coli*. The microorganism *P.aeruginosa, Staph. aureus, Salmonella spp* and *Clostridia spp* were found in about 22.22 % (n=4), 16.66 % (n=3), 22.22 % (n=4) and 27.77 % (n=5) respectively. But these microorganisms were within acceptable criteria. Some studies on the microbial count of tablet confirmed presence of *E.Coli*. Various microorganism such as *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus species and Penicillium species* were isolated from solid dosage form in different studies [5,10,12]. The most common causes of contamination may be water, person handling the product, improper handling, surroundings and the storage procedures [4].

The comparison between two primary blister and Alu-Alu packaging system for solid dosage form was presented in figure 2. There was no significant difference ($p>0.05$) in microbial load of product, packaged in blister and Alu-Alu. The same observation was noted for yeast count in blister and Alu-Alu packaging system ($p>0.05$).

CONCLUSION

This work has demonstrated the acceptable quality of solid dosage form manufactured by Indian pharmaceutical companies in relation to microbial count and the isolation of specified microorganisms. Quality of solid dosage form manufactured by Indian pharmaceutical companies revealed good adherence to GMP in the country. The microbiological quality of tested products was almost similar and within acceptable limit, for both studied packs (Alu-Alu and Blister) .Hence, effectiveness of the protection provided by the two primary packaging was not significantly different.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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