Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 suppl 2, 2014

Research Article

MICROBIOLOGICAL ANALYSIS OF TOPICALS AVAILABLE IN BANGLADESH

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Received: 15 Nov 2013, Revised and Accepted: 29 Jan 2014

ABSTRACT

Objective: Present study was carried out to demonstrate the microbiological quality of the pharmaceutical topical products commonly used for the medication of skin diseases in Bangladesh.

Methods: In this context, 30 different types of topical products (15 creams and 15 ointments) manufactured in different pharmaceutical industries of Bangladesh were microbiologically examined using standard cultural and biochemical methods.

Results: All the samples were found to be contaminated with total viable bacteria and fungi, and the bacterial load exceeded United States Pharmacopeia (USP) or British Pharmacopeia (BP) limit (<10^2 cfu/g) in 50% cases ranged between 10^3 - 10^5 cfu/g. While fecal coliforms were absent in all samples, *Escherichia coli* and *Klebsiella* spp. were found to be present only in four cases. Prevalence of *Staphylococcus* spp. and *Pseudomonas* spp. were scored in <50% and 80% samples, respectively.

Conclusion: The study revealed a bacterial contamination above the safety limit in most of the samples, especially more in ointments than that in cream samples, which may impart the treatment complicacy. A routine microbiological assessment of such pharmaceutical medicaments is thus suggested.

Keywords: Creams, Ointments, Microorganisms, Quality, Consumer safety.

INTRODUCTION

Pharmaceutical topical products including creams and ointments have long been employed to combat the skin and soft tissue bacterial and fungal infections [1]. However, they may undergo microbial spoilage due to the impaired manufacturing and packaging condition or defective distribution and storage [2-5].

Therefore, microbiological assessment of the product and the knowledge of pathogen-specific antibiotic resistance are important [6].Skin and soft tissue infections can be caused by an array of bacteria including *Staphylococcus aureus, Streptococcus pyogenes*, and the normal flora [6]. The most common skin infections are caused by or the normal skin flora [7, 8].

Individuals acquiring compromised epidermis, lacking hygiene, living in dense environment, and having close contact with people having skin diseases tend to be at high risk of acquiring a skin and soft tissue infection themselves [7, 8]. Hence, this is of highly significance that a product formulated and manufactured for the purpose of eradicating a specific skin disease, must be microbiologically sound. Otherwise, microbiological contamination of the medicament itself could lead to the treatment complication [9-14].

Pharmaceutical industries are expanding day by day in Bangladesh. However, the chemical and microbiological quality of drugs locally available varies significantly as a number of market complaints raise for a number of sold products [15]. To minimize the risk of such quality compromised product usage, routine monitoring and microbiological examination of the pharmaceutical products claim a significant demand regarding consumer safety. Besides, microbiological quality examination of topical drugs in Bangladesh are still in infancy.

Based on these facts, present study assessed the bacterial and fungal load of the topical products commonly used and available in the general drug store. Enumeration of specific pathogens was also furnished.

MATERIALS AND METHODS

Study area, sampling and sample processing

Thirty finished samples of topical products (15 creams and 15 ointments) with appropriate dates of manufacturing and expiry were collected from different retailer drug stores in Dhaka city during June 2013 to June September 2013, and were subjected to microbiological examination. Enumeration of total bacterial and fungal load was performed as well as the presence (if any) of specific pathogens was detected and quantified [16, 17].

Enumeration of total viable bacteria and fungal count

Ten grams of samples were homogeneously mixed with 90 ml of buffer peptone water (BPW), and serial dilutions were prepared up to 10^{-2} following the standard protocols [16-18].

An aliquot of 0.1 ml of each non-filterable suspension from the dilution 10^{-2} was spread onto nutrient agar (NA) plate to enumerate the total bacteria (TVC) and on SDA plate for the estimation of fungal load [16-18]. Then the NA plate and Sabouraud dextrose agar plates were incubated at 37 ^{o}C for 18 to 24 hours and at 25 ^{o}C for 48 to 72 hours, respectively.

Enumeration of specific pathogens

0.1 ml from the dilution of 10^{-2} of each sample was spread onto membrane fecal coliform (MFC), MacConkey agar, mannitol salt agar (MSA), and cetrimide agar for the enumeration of total fecal coliform, *Escherichia coli*, *Staphylococcus* spp., and *Pseudomonas* spp., consecutively. All the plates were incubated at 37 ⁰C for 24 hours except MFC agar which was incubated at 44.5 ⁰C for 18-24 hours.

Presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with the production of green metallic sheen on the eosine-methylene blue (EMB) agar [18, 19]. Confirmative biochemical tests revealed the identity of the specific pathogens [18].

RESULTS AND DISCUSSION

Topical medication is the usual form of mitigating most skin disorders. In developing countries the onset of such disease is a bit frequent due to the unhygienic dense environment, improper sanitation, and the usage of microbiologically contaminated water[20-22].

In cohort with this suggestive data, results from our study revealed a huge microbial contamination of some cream products and to a greater extent in case of the ointment samples studied (Tables 1, 2).

Microbial prevalence in cream samples

Bacterial prevalence; however Out of 15 cream samples, 5 were found to harbor the total viable bacteria within a range of 10^3-10^5 cfu/g (Table 1). Rest of the samples also showed the, *S.*

aureus and *P. aeruginosa* should not be present according to USP or BP specification, within the USP or BP limit (10^2 cfu/g).Study of Gram negative bacteria showed the complete absence of fecal coliforms, with the presence of *E. coli* and *Klebsiella* spp. samples 1 and 4, respectively. Around 50% of the samples studied were found to be contaminated either with *Pseudomonas* spp. or *Staphylococcus* spp. Two samples were found to be contaminated with both the species, revealing the risk of product usage to public health.

Microbial prevalence in ointment samples

Out of 15 ointment samples, 4 were found to harbor the total viable bacteria up to10^5 cfu/g, 1 with 10^4 cfu/g, and 6 with 10^3 cfu/g (Table 2). Rest 4 samples also showed the bacterial prevalence within the USP or BP limit (10^2 cfu/g). the fungal load in all cream samples. Within the limit specified by USP or BP, 1 sample in ointments showed excess load of fungi. As in the cream samples, fecal coliforms was totally absent.

Table 1: Prevalence of pathogenic microorganisms in creams

Sample name	TVB (cfu/g)	Total fungal count	*E. coli* (cfu/g)	*Klebsiella* spp. (cfu/g)	Fecal Coliform count (cfu/g)	*Pseudomonas* spp. (cfu/g)	*Staphylococcus* spp. (cfu/g)
Cutivate	3.5×10^2	2.5×10^1	0	0	0	5.8×10^1	0
Dermovate	5.5×10^2	4.6×10^1	0	0	0	7.6×10^1	0
Betnovate-N	2.0×10^2	3.8×10^1	0	0	0	1.5×10^1	0
Tinatrim	1.0×10^3	3.8×10^1	0	0	0	1.8×10^1	2.6×10^1
Micosone	1.8×10^2	1.6×10^1	0	0	0	3.0×10^1	0
Pevisone	1.6×10^2	3.3×10^1	0	0	0	6.0×10^1	0
Avison	2.7×10^3	4.2×10^1	0	2.5×10^1	0	0	2.5×10^1
Econate	1.3×10^3	2.1×10^1	0	5.0×10^1	0	0	4.0×10^1
Apalene	1.8×10^2	2.2×10^1	0	0	0	0	1.1×10^1
Eczena	2.2×10^4	1.1×10^1	1×10^2	7.0×10^1	0	0	2.6×10^1
Xfin	1.0×10^2	1.1×10^1	0	0	0	0	6.4×10^1
Virux	9.2×10^4	2.9×10^1	0	2.34×10^1	0	1.2×10^1	2.2×10^1
Toget	2.5×10^2	1.3×10^1	0	0	0	0	0
Cosmotrin	1.2×10^2	6.0×10^1	0	0	0	0	0
Skinalar-N	6.8×10^2	5.2×10^1	0	0	0	0	0

USP or BP Microbial Limit: TVC-10^2 cfu/g and TFC-10^1 cfu/g.

Table 2: Prevalence of pathogenic microorganisms in ointments

Sample name	TVB (cfu/g)	Total fungal count (cfu/g)	*E. coli* (cfu/g)	*Klebsiella* spp. (cfu/g)	Fecal Coliform count (cfu/g)	*Pseudomonas* spp. (cfu/g)	*Staphylococcus* spp. (cfu/g)
Eumovate	8.5×10^2	6.1×10^1	0	0	0	0	0
Betnovate-N	1.2×10^2	5.6×10^1	0	0	0	0	0
Clovate	3.8×10^2	1.2×10^1	0	4.0×10^1	0	0	0
Nebanol	3.2×10^3	3.0×10^1	0	2.9×10^1	0	0	2.8×10^1
dermex	3.0×10^5	2.1×10^1	3.0×10^1	4.4×10^1	0	2.5×10^1	2.9×10^1
Bet-cl	1.1×10^3	0	0	0	0	4.8×10^1	5.7×10^1
Bactrocin	2.0×10^3	5.0×10^1	0	0	0	1.2×10^1	1.6×10^1
Aristocort	1.3×10^3	4.2×10^1	0	2.4×10^1	0	0	9.3×10^1
Fusidic plus	2.0×10^5	7.5×10^2	0	5.1×10^1	0	4.6×10^1	8.5×10^1
Halobet	2.4×10^3	2.7×10^1	0	2.5×10^1	0	0	7.8×10^1
Topidin	2.9×10^5	8.5×10^2	0	1.6×10^1	0	1.2×10^1	6.5×10^1
Dermovate	4.5×10^3	1.0×10^1	0	0	0	1.8×10^1	7.0×10^1
Exovate	1.8×10^4	2.1×10^1	0	3.0×10^1	0	5.5×10^1	6.0×10^1
Miclo	1.5×10^5	1.4×10^1	0	1.5×10^1	0	1.7×10^1	3.6×10^1
Lutisone	1.0×10^5	1.0×10^1	0	3.5×10^1	0	1.2×10^1	4.2×10^1

USP or BP Microbial Limit: TVC-10^2 cfu/g and TFC-10^1 cfu/g

S. aureus and *P. aeruginosa* should not be present according to USP or BP limit.

While the presence of *E. coli* and *Klebsiella* spp. was encountered samples 1 and 9, respectively. Around 60% and 80% of the ointment samples were found to be contaminated either with *Pseudomonas* spp. or *Staphylococcus* spp., respectively. samples (60%) were found to be contaminated with both *Pseudomonas* spp. and *Staphylococcus* spp., which indeed poses a serious threat to public health on such type of product usage. The origin or sources of such contamination in the samples studied might be due to (1) inappropriate manufacturing condition, i.e., uncontrolled microbial and particle rich manufacturing zone, (2) insufficient microbial compliance in case of raw materials and other ingredients, (3) lack of aseptic handling of all materials, during filling and sealing or even in the packaging belt, and (4) improper storage condition during distribution [23-25]. The prevalence of *Staphylococcus* spp. and *Pseudomonas* spp. in the samples is assumptive of bacterial shedding from floor and hands of handler during the preparation of drugs [3, 23, 24, 26, 27]. Complete absence of fecal coliform and presence of *E. coli* only in 4 samples revealed the microbial clarity of the water used for manufacturing. Consistently, the load of another enteric bacterium *Klebsiella* spp. was also found to be within the limit specified by USP or BP. However, as revealed from the study, ointments were found to be more prone to microbial attack than those of creams, and the dominance of *Staphylococcus*

spp. and *Pseudomonas* spp. especially in the ointment samples pose a serious menace to public health care.

CONCLUSION

Overall, the data presented in this study imposed on the betterment in manufacturing, packaging and storage condition of topical products. Besides the presented work, further microbial examination of the other creams and ointments would increment the actual scenario of microbial safety. However, the implication of this study lies on the simplicity of the experiments conducted here. Thus our study is highly suggestive of routine microbiological testing of topical products sold in the drug store in order to ensure consumer safety.

ACKNOWLEDGEMENT

The work was financially supported by the department of Microbiology, Stamford University Bangladesh.

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