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Case Study

BURKHOLDERIA CEPACIA GROWTH IN PRESERVED PHARMACEUTICAL ANTIHISTAMINIC ORAL SUSPENSION

MOSTAFA ESSAM EISSA

Microbiological Quality Control Section Head in HIKMA Pharma Pharmaceutical Company, Egypt
Email: mostafaessameissa@yahoo.com

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ABSTRACT

A chemically-preserved oral antihistaminic suspension based on fexofenadine as an active pharmaceutical ingredient and preserved with aminobenzoic acid esters (parabens) was found contaminated with *Burkholderia cepacia (B. cepacia*). This finding was detected only after six months from manufacturing. The bacterial count increased from 10, after six months, to 1475 Colony Forming Unit (CFU)/ml after nine months. The organism constituted continually increasing the hazard to the users long after passing undetected to the market. The current finding highlighted the importance of both appropriate neutralization method of the preservatives in the preservative efficacy test and the sensitivity of the method of bioburden enumeration and detection.

Keywords: Antihistaminic suspension, Fexofenadine, Parabens, *Burkholderia cepacia*, Chemically-preserved.

INTRODUCTION

Pharmaceutical product preservation is an important aspect for drugs with high water activity, in order to protect them from microbial spoilage. Aminobenzoic acid esters (parabens) are very common preservatives that are used in the field of non-sterile dosage forms. Elder and Crowley [1] discussed that parabens are more active against Gram-positive, than Gram-negative bacteria and more active against yeasts and molds than bacteria. The activity increases with increased alkyl chain length (butyl>propyl> ethyl>methyl), but the aqueous solubility commensurately decreases, and consequently the parabens are also often used in combination, e. g. methyl and propyl paraben. Parabens also show some synergy with ethylene diamine tetraacetic acid (EDTA)[2].

CASE REPORT

A pharmaceutical liquid oral dosage form containing non-sedating antihistaminic compound fexofenadine as an active pharmaceutical ingredient (API) and a mixture of methyl and propyl parabens as preservatives. The finished product was found to be clean microbiologically when examined directly after manufacturing. The product was packaged in 100 ml dark amber colored glass containers with white screw caps. Other ingredients included Xanthan gum, Disodium Edetate, Sucrose, Sorbitol solution, Poloxamer, Sodium Phosphate Monobasic, Sodium Phosphate Dibasic and Raspberry Flavor. The product showed no growth in Tryptone Soya Agar (TSA) when tested for the total viable aerobic count (TVAC) according to the United States Pharmacopeia (USP)<61>[3] with results less than ten Colony Forming Unit (CFU)/ml.

The used culture media were verified for sterility and growth promotion ability as per USP<61>[3]. The laminar air flow (LAF) hood in which all tests were performed had passed all the engineering tests for air velocity, particulate and integrity tests using verified and calibrated measuring instruments. In addition, all the culture media incubators were calibrated, validated and continuously monitored by a validated chart recorder.

Appropriate training and knowledge of operators were insured and verified for each test procedure. The sterility of the used media and aseptic handling was confirmed by the concurrent inclusion of negative control plates with test ones. Quality control (QC) of activity in LAF unit was performed by taking passive air sample through the whole activity and surface samples by contact plates from the bench as well as the two side walls of LAF unit to verify surface cleanliness.

Samples from the manufactured batch were retained in calibrated and validated stability cabinets for both on-going and accelerated product stability testing with conditions of 30±2 °C/Relative Humidity (RH) % 65±5 and 40±2 °C/RH% 75±5; respectively [4]. All the chemical tests were within the accepted laboratory limits. The range of pH was 6.2-6.3, and the concentrations of Methyl and Propyl Parabens were 97.7-105.7% and 94.6-104.4 %, respectively, during the course of study. The microbiological count test was acceptable either accelerated or on-going with TVAC less than and ten Colony Forming Unit (CFU)/ml at three and six months stability points, respectively. However, at nine months of on-going stability point the microbiological count raised at a relatively higher rate with TVAC of 1475 CFU/ml. The breaking point of the generation time for the bacteria was expected to be between 3-6 mo with an slow initial rate from 0-3 mo and fast one from 6-9 mo. Since no count was detected till the point of 6 mo, the generation time was estimated to be between 1.89 to 41.28 mo (56.67 to 1238.49 d) with the average middle point (5 CFU/ml) to be 6.28 mo (188.25 d). The initial lag phase length could not be determined. Meanwhile, the higher rate section was determined to have a generation time of 1.49 mo (44.73 d). A simulated XY data study for less than ten CFU/ml count was performed using GraphPad Prism v 6.01 for Windows, and the result is illustrated in fig. 1. Based on a simulation study, the initial count was between 2 and 7 CFU/ml. The microorganism was identified by Gram stain and miniaturized biochemical identification system BBL CRYSTAL Enteric/Non-Fermenter (E/NF) as described by Eissa [5]. Its identification tests showed Gram-negative rods with negative Indole and Oxidase reactions; the identification (ID) profile referred to *Burkholderia cepacia (B. cepacia*).

However, the organism passed the test of the specified microorganisms as described by USP<62>[6] because it was not included in the routine testing. The presence of *B. cepacia* was detected once in the water system of the manufacturing plant used for liquid products and identified among the routine tests of water but was not considered as an objectionable threat. The interesting behavior of this microorganism in the product required further investigation. A procedure that was performed by testing another microbiologically clean batch of the same product to perform antimicrobial efficacy test (AET) using an isolated *B. cepacia* and comparing it with a benchmark product of another company after testing its microbiological cleanliness. The formula of the benchmark included Propylene Glycol, Disodium Edetate, Propylparaben, Butylparaben, Xanthan Gum, Poloxamer 407, Titanium Dioxide, Sodium Phosphate Monobasic, Sodium Phosphate Dibasic.

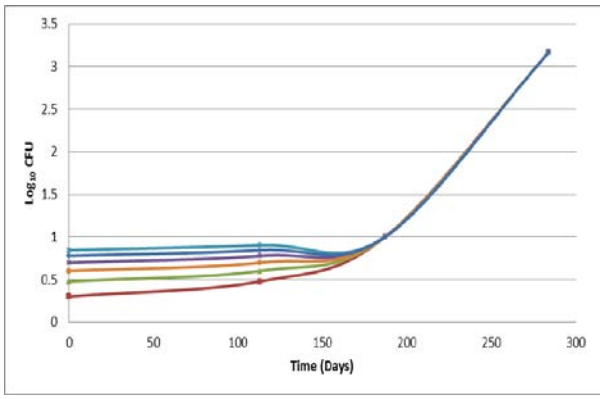

Fig. 1: Microbial growth profile of *Burkholderia cepacia* observed in the non-sedating oral antihistaminic suspension medicinal product with initial half portion of the curve generated with different values of possible starting contamination from two to seven CFU/ml at zero time. (Graph generated by Microsoft Office Excel 2007)

Artificial Raspberry Cream Flavor and Xylitol in amber glass bottle with plastic screw cap. Preliminary neutralization study was conducted first to ensure that the processing and dilution techniques could recover the low level of the microbial count from the tested products as shown in table 1. Results showed that all the tested organisms passed the test according to USP<61>[3] and Eissa and Norby criteria [7]. Interestingly, both formulae failed to pass the preservative efficacy test (PET) which was performed according to USP<51>[8] with the studied product showing greater failure rate than the benchmark product. The results showed that Gram-negative bacteria were the most resistant to the preservation of both formulae notably *B. cepacia* and *Escherichia coli*.

Both organisms actually increased in number after 14 ds to high to be count (HTBC) values indicating that these microorganisms got nutritional benefits from products rather than being suppressed or killed during the proposed period of testing. However, benchmark product showed greater activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the new oral suspension product viz.>1.65 vs. 0.69 and>0.61 vs. HTBC log reduction (LR); respectively. Interestingly both formulae passed the AET for fungi with the new product being more active than the benchmark viz. 3.6 vs. 1.2 LR for *Candida albicans* and 0.37 vs. 0.00 LR for *Aspergillus brasiliensis*.

Table 1: Preliminary neutralization study for the tested microorganisms against the two liquid non-sedating antihistaminic products

Oral antihistaminic microorganisms	Incubation conditions	Relative microbial recovery ratio [(b)]	
		New product suspension	Benchmark suspension
Staphylococcus aureus	30-35 °C	1.13	0.85
Pseudomonas aeruginosa	in	1.56	0.64
Escherichia coli	TSA	1.08	0.60
Burkholderia cepacia[(a)]		1.13	0.57
Candida albicans	20-25 °C in	1.13	0.55
Aspergillus brasiliensis	SDA	0.88	0.86

(a)= Non pharmacopoeial microorganism included in the study from the isolated Gram-negative rod found in the new product. (b) = 0.3 Log_{10} variation (0.5) is the criteria of passing the test to account for plating variability.

DISCUSSION

The current case highlighted several critical situations in relation to medicinal product safety delivered to the final customer. First, the sensitivity of test should be reviewed by pharmaceutical firms to ensure maximum possible recovery and detection of specific microorganisms. This should not allow to overlook the criticality of the next point; PET. Secondly, the regulatory agencies are required to enforce pharmaceutical companies not to release drugs with significant water activity a_w>0.6 except after providing a scientific justification for their products efficacy against test microorganisms in AET test [9]. In addition, each firm should include specific annoying bugs that are resistant to antimicrobials, isolated from their environment and water of the facility in their test microorganisms' spectrum. Such a procedure would confirm the product ability to stop these organisms from being a source of a hazard to users [10]. The observed growth after appreciably long lag phase provided an evidence of the ability of microorganisms to not only survive but proliferate in a hostile environment, causing harm to the final consumer upon contact especially those with specific disease conditions and with weak immune system populations. Thirdly, PET should be accompanied by a proper neutralization method in order not to exaggerate the potency of the antimicrobials in the tested pharmaceutical formulae [7].

Otherwise, an overestimated activity and safety will be incorrectly addressed with the result of the release of potentially unsafe drugs to the market. If the product did not catch the contamination from the manufacturing environment and/or the pharmaceutical water, it would do so from the final consumer. Consequently, it may turn harmful at the following administration as most of the non-sterile products are packaged in containers that lose physical integrity and separation from the surrounding environment and individuals once opened in the first time use. Finally, the possibility of the microorganism to be viable but not cultivable should not be underestimated. Thus it may be useful to use advanced technologies and techniques to detect its presence in the medicinal product.

CONCLUSION

The current case demonstrated the ability of very low level of contamination of *B. cepacia* to survive and proliferate in harsh environmental conditions in the presence of chemical antimicrobial compounds. In addition, it highlighted the importance of deploying an effective preservative efficacy program in complementation with sensitive bioburden detection techniques in order to not allow of skipping unwanted contamination to the final consumers.

However, the annoying possibility of that microorganism may have been viable but not cultivable (VBNC) submitted warning alarm signal about the need to switch to rapid microbiological methods (RMM).

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CONFLICT OF INTERESTS

Declared None

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