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Research Article

A Case of Rapidly Declining Contamination of Antimalarial Tablet by *Stenotrophomonas maltophilia*

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Abstract: A case of an oral antimalarial tablet was analyzed microbiologically for microbial contamination immediately on the same manufacturing day (zero time) and was found to be contaminated by an exceptionally out-of-trend (OOT) bioburden of total viable aerobic count (TVAC) equals to 1233 ± 275 colony forming unit (CFU) ± SD/g of the medicinal product. The result of the test was observed only after two days. Hence, resampling and retesting processes were conducted promptly and interestingly all culture plates showed no signs of growth even after five days of incubation. Thus, the outcome was recorded as <100 CFU/g. Thus, the logarithmic (log_{10}) reduction (LR) within a couple of days was 1.08 ± 0.09. The test was repeated again after that 15 times from different locations from the batch and again no sign of microbial presence was detected. The contamination was from single species of Gram-negative rods which were identified using miniaturized biochemical identification kits. A bacterium was identified as *Stenotrophomonas maltophilia*. The case highlighted the importance of the test conduction time after manufacturing to detect the rapidly fading bioburden from the product otherwise misinterpretation of drug cleanliness and good manufacturing practice (GMP) will result. Another annoying issue also raised which is related to the possibility that the microbial cells may be still present but not able to grow. Thus, the microorganism may become viable-but-not-culturable (VBNC). This fact might allow for objectionable microbes to pass silently to the patients unnoticed.
Keywords: antimalarial tablet, out-of-trend, total viable aerobic count, *Stenotrophomonas maltophilia*, good manufacturing practice, viable-but-not-culturable.

INTRODUCTION

Substantial improvements in the microbial quality of manufactured products have been seen in the last 30 years. Despite these improvements, however, there is no room for complacency because outbreaks of infection continue to be reported from time to time [1-5]. Microbial contamination has been one of the prime reasons for regulatory product recalls for the recent years with the most frequently detected organisms of pseudomonads and other Gram-negative organisms [6,7].

Product quality is evaluated through analysis of the finish dosage forms. Products that are found to be contaminated with microorganisms are recalled from the market. A product can also be recalled if there is evidence that a deviation occurred during its manufacture or distribution, resulting in a possible risk to public health. Such incidences typically occur in small numbers of batches. However, if a product is found to be unsafe for continued marketing, it must be withdrawn completely. The microbial contamination of pharmaceuticals has been studied extensively during the past decades [6].

Microbiological quality of the pharmaceutical formulation is crucial attribute as small numbers of organisms (yeast, mold and bacteria, including *Pseudomonas* spp. and members of *Enterobacteriaceae*) introduced into a product during manufacture or use may be of little consequence to the patient, where these organisms undergo subsequent multiplication within the product the infection hazard will be significantly increased [8,9]. Certain manufactured goods, of which foodstuffs, cosmetics, and pharmaceutical products are the prime examples, can be contaminated with microorganisms during manufacture; this contamination can, at the best, cause spoilage and consequent rejection of the contaminated material and, at the worst, harm or even bring death to the consumer. The culprits are usually bacteria or fungi [8].

The currently reported case showed the significance of microbial contamination on the quality

of the pharmaceutical product and the potential microbiological health risk that may be carried silently to the final customer.

MATERIAL AND METHODS

All culture media and reagents were obtained from OXOID (Basingstoke, Hampshire) and Sigma-Alrich (St. Louis, MO 63103), respectively. Plastic 9 mm sterile plastic plates and RODAC (contact) plastic plates were purchased from Sterilin Limited (solaar house, 19 mercers row, Cambridge, UK). Microbial enumeration was done using digital colony counter (Digital Colony Counter Model: 361, Laxman Mahtre Rd. Navagaon, Dahisar West, Mumbai). All media were sterilized by autoclaving in steam sterilizer (FEDEGARI FOB3, Fedegari Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). All culture media used in the current study were subjected to growth promotion test as described in standard methods in USP, 2015 [10]. Moreover, microbiological environmental monitoring (EM) samples from surfaces and air in the work area were taken according to Eissa, 2014 with every test group performed in biological safety cabinet (BSC) (Jouan MSC 9 Class II A2 BioSafety Cabinet, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, California 95134) to ensure appropriate cleaning, sanitization and aseptic attitude under laminar air flow (LAF) conditions [11]. Incubation of samples was done in Series BD 115 Incubators with natural convection (BINDER GmbH, Im Mittleren, Ösch 5, 78532 Tuttlingen, Germany). Microbial cells examination and morphology was observed under optical microscope Leica DM2000 LED (Leica Microsystems Inc., 1700 Leider Lane Buffalo Grove, IL 60089 United States). Bacterial visualization was enhanced using colorless Triphenyltetrazolium Chloride dye which turns red by viable cells. Culture isolation and identification was done according to methods stated by some investigators [12]. The bacterial isolates were identified to genus and species using miniaturized biochemical identifications kits BBL™ Crystal™ enteric/non fermenter (E/NF) and Gram-stain reagents purchased from BD (Becton Dickinson Microbiology Systems, Cockeysville, Md.). The case of product is an oral antimalarial tablet based on the components shown in Table (1) and indicated in the treatment of acute, uncomplicated malaria infection due to *Plasmodium falciparum.*

Table-1: The composition and general information about the antimalarial product of the case study.

Tablet* Ingradient Class	Name of The Component	Chemical Nature	Pharmaceutical Function
Active Pharmaceutical Ingradients (API)	Artemether 20 mg/tab.	Semisynthetic chiral acetal derivative of Artemisinin, isolated from the plant Artemisia anua	fixed-dose combination artemisinin-based combination therapy (ACT)
	Lumefantrine 120 mg/tab.	Racemic mixture of synthetic Fluorene derivative	
Inactive Additives	Tween 80	Polyoxyethylene (20) sorbitan monooleate	Solubilizer
	HPMC	Propylated and methylated Glucose units in Cellulose	Controlled-delivery component and binder in oral medicaments
	MCC	Carbohydrate of Cellulose mainly	Disintegrant and direct compression
	Colloidal Silicone Dioxide	SiO_2	Anti-caking agent, adsorbent, disintegrant, or glidant
	CCS	Internally cross-linked sodium Carboxymethylcellulose	Disintegrant
	Mg Stearate	Octadecanoic acid Magnesium salt	Lubricant

HPMC = Hydroxypropyl methylcellulose (Hypromellose). MCC = Microcrystalline cellulose. CCS= Croscarmellose Sodium.

* = Data obtained from the manufacturer of the product and its pamphlet.

RESULTS AND DISCUSSION

All culture media used in the current study passed growth promotion test. EM samples which were taken during the study passed the acceptance criteria. All negative control samples did not show any signs of microbial growth. The medicinal product under investigation was found to be contaminated by an exceptionally out-of-trend (OOT) bioburden of total viable aerobic count (TVAC) equals to 1233 ± 275 colony forming unit (CFU) ± SD/g of the medicinal product. The result of the test was observed only after two days. Hence, resampling and retesting processes were conducted promptly and interestingly all culture plates showed no signs of growth even after five days of incubation. Thus, the outcome was recorded as <100 CFU/g. Thus, the logarithmic (log_{10}) reduction (LR) within a couple of days was 1.08 ± 0.09. The test was repeated again after that 15 times from different locations from the batch in the warehouse and again no sign of microbial presence was detected.

Pharmaceutical products are subject to microbiological contamination that can represent a health hazard to the consumer and cause product spoilage, aesthetic changes, and possible loss of drug efficacy. Microbial contamination may originate from the raw materials and excipients or may be introduced during manufacture (operators and contaminated equipment, environment, and packaging materials), storage and use. Most raw materials used in pharmaceutical manufacturing, including water, may contain several types of microorganisms. Depending on the type of the manufacturing process, these contaminants may be reduced or eliminated. However, care must be taken not to further increase the potential for introducing microorganisms during an uncontrolled manufacturing process [6,13].

The contamination was from single species of Gram-negative rods which were identified using miniaturized biochemical identification kits. Bacteria were identified as *Stenotrophomonas* (formerly known as *Xanthomonas or Pseudomonas) maltophilia*. *S. maltophilia* is ubiquitous in aqueous environments, soil, and plants[4]. Thus, the contamination of the drug product from environment in the clean area in the pharmaceutical plant is the prime cause either directly or indirectly especially that it was reported frequently in water distribution system and has previous history of forming strong biofilm through one of the processing stages in the treatment station. However, It is an uncommon pathogen in humans. *S. maltophilia* is an organism of low virulence and frequently colonizes fluids used in the hospital setting (e.g, irrigation solutions, intravenous fluids) and patient secretions (e.g, respiratory secretions, urine, wound exudates). *S. maltophilia* usually must bypass normal host defenses to cause human infection. For example, if an irrigation solution becomes colonized with this organism, irrigating an open wound can cause colonization or infection of the wound. *S. maltophilia* is usually incapable of causing disease in healthy hosts without the assistance of invasive medical devices that bypass normal host defenses [14]. On the other hand, *S. maltophilia* is naturally resistant to many broad-spectrum antibiotics [15]. Hence, it may contribute to the dissemination of antibiotic resistance to environment or at worst, to the patients through consumed medicines.

The results of the study of Lane and Brooke 2014 showed that *S. maltophilia* also has the ability to persist for up to 2 days on dry filter paper. This is in agreement with the current finding. However, there are two possible reasons for the deterioration of microbial growth. The first explanation is that the dryness condition in the tablet (water activity a_w<0.5) put the bacterial cells under a considerable amount of stress; this has been observed for *Pseudomonas aeruginosa* [16], a bacterium related to *S. maltophilia*. The second explanation is that the cells entered into what is known as a 'viable, but non-culturable' (VBNC) state. Studies have shown that many pathogenic bacteria are capable of entering such a state. These bacteria have the ability to become dormant without undergoing changes to physical cell structure; once the cells are reintroduced to appropriate conditions, VBNC microbes will resume growth [17].

CONCLUSION

The case highlighted the importance of the time of test execution after manufacturing to detect the rapidly fading bioburden from the product otherwise misinterpretation of drug cleanliness and good manufacturing practice (GMP) will result. Another annoying challenge also raised which is related to the possibility that the microbial cells may be still present but not able to grow. Thus, the microorganism may become injured, stressed and/or viable, but non-culturable (VBNC). This fact might allow for objectionable microbes to pass silently to the patients unnoticed. In addition the resistance to antibiotics may be acquired unintentionally which aggravates health risk concern issues.

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