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(RESEARCH ARTICLE)

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Microbial contamination of selected nonsterile pharmaceuticals in OPD pharmacy of a teaching hospital in Sri Lanka

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Abstract

A pharmaceutical product must be effective, safe and should be of good quality to assure their sustainability in the competitive pharmaceutical industry. The quality of the pharmaceutical products can be influenced by the presence of microorganisms (MO). In addition to sterile preparation contamination of non-sterile pharmaceuticals (NSP) should be monitored according to British Pharmacopeia (BP) microbial limits for the preparations. The objective of this study was to investigate the contamination of MOs of NSPs. Total 05 drug samples out of bulk opened containers were collected from OPD unit of a teaching hospital, Sri Lanka. The total aerobic bacterial count and fungal contamination was tested by surface spread plate method and checked with the specified limits in BP. The contaminated microorganisms were identified using microbial identification methods. According to the results 1/5 (20%) of the tested samples were contaminated with microorganisms and 4/5 (80%) of the samples were free from contamination. The identified microorganisms were Aspergillus species, gram negative Spore forming Bacilli and Staphylococcus aureus.

Keywords: Microbial contaminations; Non-Sterile Pharmaceuticals; Teaching Hospital; Sri-Lanka; Total Microbial Count

1. Introduction

The pharmaceutical industry is growing day by day to meet the demands of the patients [1]. This is primarily due to pharmaceutical products that adds up in several dosage forms and in different formulations to suit the route of administration and to bring near the most effective therapeutic outcome [2]. A pharmaceutical product must be effective, safe, and good quality to assure their sustainability in the competitive pharmaceutical industry [2]. The quality of the pharmaceutical products can be influenced by the presence of microorganisms. Microorganisms have ability to influence the efficacy and safety of a pharmaceutical product [3,4].

Pharmaceutical products based on its sterility are of two types; sterile pharmaceuticals and non-sterile pharmaceuticals [5]. Sterile pharmaceuticals are the products that must be free from microorganisms. In sterile pharmaceuticals, it is an absolute prerequisite to be free from bioburden [6]. Thus, all the sterile pharmaceuticals must be free from viable bacteria, fungi, yeast, protozoa, viruses, and other microorganisms such as rickets, Mycoplasma [2].

Non-sterile pharmaceuticals are the products that may contain microorganisms within the limits of the type and concentration [2]. Usually, the oral medicines and topical medicines are non-sterile [7]. The acidity of the stomach may act as a barrier to ingested microorganisms in the oral route and inactivation of the biological activities of the

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microorganism occurs [8]. In the topical route, skin act as a functional barrier, preventing the entry of the microorganisms into the body [2]. Good Manufacturing Practices are applied in the production of non-sterile pharmaceuticals to minimize the bioburden [9,10].

Although non-sterile pharmaceuticals can contain microorganisms, they must satisfy the microbial limit testes specified in relevant pharmacopeias [11]. Various pharmacopeias including British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP) and United States Pharmacopoeia (USP) have specified the microbial limit test [12]. This is a test that allows the determination of the absence or limited occurrence of specified microorganisms that is relevant to the conditions and the dosage forms. Microbial limit test allows to determine whether the product or the substance that is tested is according to the specified conditions regarding the microbial quality [13]. This test explains about which microorganism should be absent in a particular product, maximum bioburden of the product, number of samples to be taken for the test and interpretation of the outcomes [14].

According to pharmacopeias, the non-sterile pharmaceuticals should not exceed certain amount of total viable bacterial count and fungi (Table 1). The BP specifies the types of pathogenic microorganisms that should not be exhibited in these products [15].

2. Methodology

Five drug samples were collected from the bulk dispensing drugs in the OPD pharmacy of a Teaching hospital in Sri Lanka. The samples were collected from various counters in the OPD pharmacy. The drug samples from each counter were obtained in the upper, middle and lower compartments. The same drug samples were collected from the unopened containers in the pharmacy.

The Tryptone Soy agar (TSA), and *Sabouraud* dextrose agar (SDA) (HiMedia Laboratories, India) were used as the media. The medias were prepared according to the manufacturers' instructions.

Analysis of the collected drug samples were done according to the method specified in British Pharmacopiea, 2013 [14].

2.1. Test sample preparation

Tablet samples were crushed using sterilized motor and pestle. Then 10vg of the sample was weighed to a sterilized watch glass using electronic balance. The powder was suspended in 90ml of the stock buffer solution (phosphate buffer solution pH 7.2). Then a dilution series was prepared aseptically.

2.2. Surface Spread Method

Petri dishes 9cm in diameter were used in this experiment. 15-20 ml of the TSA or SDA at 45 ^{0}C was added and it was allowed to solidify. For each sample two plates were used. The plates were dried. A measured volume of the sample not less than 0.1ml is spread over the dried medium. Then it was incubated. The microbial colony growth was observed. The arithmetic mean of the counts per medium were taken. Then the number of CFU in the original inoculum was calculated.

2.3. Calculation of the Results

The incubated plates corresponding to a given dilution and highest number of colonies less than 250 for the total aerobic microbial count (TAMC) and 50 for the total combined yeasts/mould count (TYMC) were selected. The arithmetic mean per culture medium of the counts were taken. Then the number of Colony Forming Units (CFU) per gram of the product was calculated.

2.4. Interpretation of the results

Interpretation of the results shown in Table 1.

2.5. Microbial identification

The microbial colony growth was subjected to further identification according to the procedure in Figure 1 [17].

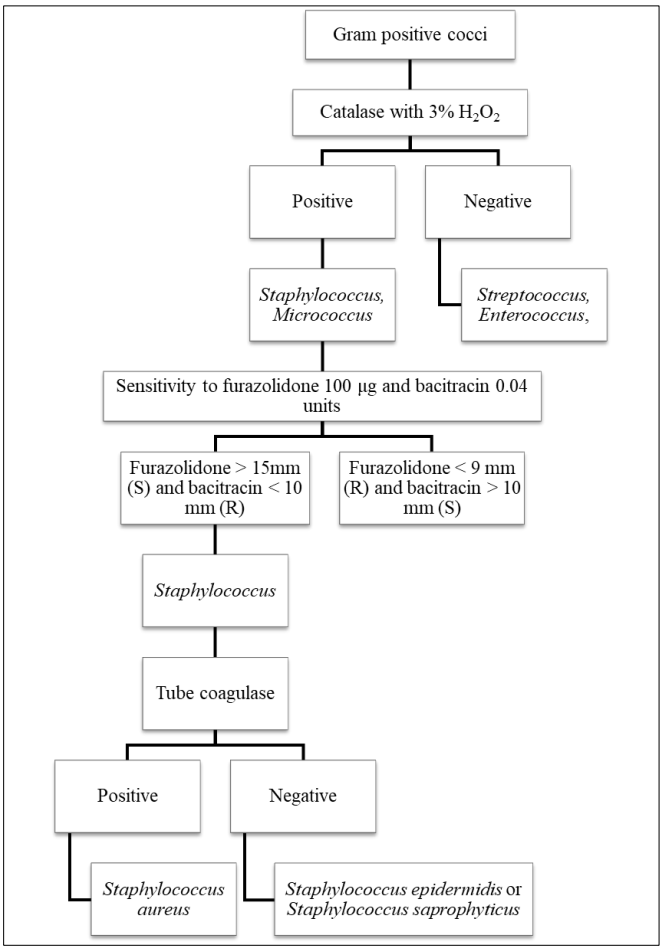

Figure 1 Test procedure for the identification of microorganisms

Table 1 Acceptance criteria for microbiological quality of non-sterile dosage form Adapted and reproduced from British Pharmacopeia 2016 [15]

Route of administration	**TAMC (CFU/g or CFU/ml)**	**TYMC (CFU/g or CFU/ml)**	**Specified microorganisms**
Non aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1g or 1 ml)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1g or 1 ml)
Rectal use	10^3	10^2	-
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1g or 1 ml) Absence of *Pseudomonas aeruginosa* (1g or 1 ml)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1g or 1 ml) Absence of *Staphylococcus aureus* (1g or 1 ml) Absence of *Candida albicans*(1g or 1 ml)

Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulization)	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1g or 1 ml) Absence of *Staphylococcus aureus* (1g or 1 ml) Absence of bile tolerant gram negative bacteria (1g or 1 ml)
Special Ph. Eur. provisions for oral dosage form containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 10^3 CFU/g or CFU/ml	10^4	10^2	Not more than 10^2 CFU of bile tolerant gram negative bacteria(1g or 1 ml) Absence of *Salmonella (*10g or 10ml) Absence of *Escherichia coli* (1g or 1 ml) Absence of *Staphylococcus aureus* (1g or 1 ml)

3. Results

The dilution series of five drug samples collected from opened containers were inoculated in TSA media. Results of the total bacterial count in each dilution series of tablets were shown in the table 2.

Table 2 Dilution series of the tablets from opened containers - TSA media (S)- sample microbial count, (N)-negative control microbial count and (P)-positive control microbial count

	CFU			**CFU**			**CFU**			**CFU**			**CFU**		
Dilution Series	**10-1**			**10-2**			**10-3**			**10-4**			**10-5**		
Name of the tablet	S	N	P	S	N	P	S	N	P	S	N	P	S	N	P
Salbutamol Sulphate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Calcium Lactate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Chlorpheniramine	<100	0	<100	2	0	<100	0	0	<100	0	0	<100	0	0	<100
Domperidone	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Diclofenac Sodium	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100

The figures below show the images of the Petri dishes which contained the microbial colony growth due to presence of the contamination of the tablets.

The dilution series prepared from the tablet samples obtained from unopened containers were inoculated in TSA media. The results of the microbial colony count of each dilution series were shown in the table 3.

The dilution series prepared from the drug samples obtained from opened containers were inoculated in the SDA media. The table 4 show the results of the microbial colony count of each dilution series of five drug samples.

Figure 2 Chlorpheniramine TSA 10^{-1} Plate

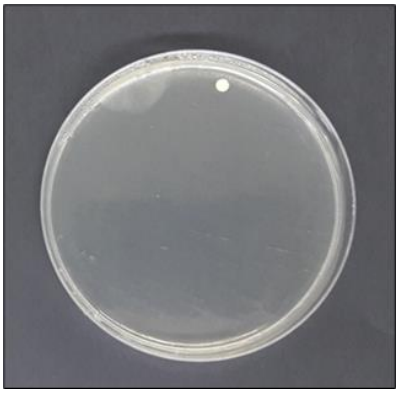

Figure 3 Chlorpheniramine TSA 10-2 Plate

Table 3 Dilution series of tablets from unopened containers – TSA media (S)- sample microbial count, (N)-negative control microbial count and (P)-positive control microbial count

Dilution Series	**CFU**			**CFU**			**CFU**			**CFU**			**CFU**		
	10-1			**10-2**			**10-3**			**10-4**			**10-5**		
	S	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**
Salbutamol Sulphate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Calcium Lactate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Chlorpheniramine	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Domperidone	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Diclofenac Sodium	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100

Table 4 Dilution series of tablets from opened containers – SDA media (S)- sample microbial count, (N)-negative control microbial count and (P)-positive control microbial count

Dilution Series	**CFU**			**CFU**			**CFU**			**CFU**			**CFU**		
	10-1			**10-2**			**10-3**			**10-4**			**10-5**		
	S	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**
Salbutamol Sulphate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Calcium Lactate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Chlorpheniramine	1	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Domperidone	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Diclofenac Sodium	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100

The dilution series prepared from the drug samples obtained from unopened containers were inoculated in the SDA media. The table 5 show the results of the microbial colony count of each dilution series of five drug samples.

Table 5 Dilution series of tablets from unopened containers – SDA media (S)- sample microbial count, (N)-negative control microbial count and (P)-positive control microbial count

Dilution Series	**CFU**			**CFU**			**CFU**			**CFU**			**CFU**		
	10-1			**10-2**			**10-3**			**10-4**			**10-5**		
	S	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**	**S**	**N**	**P**
Salbutamol Sulphate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Calcium Lactate	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Chlorpheniramine	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Domperidone	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100
Diclofenac Sodium	0	0	<100	0	0	<100	0	0	<100	0	0	<100	0	0	<100

Out of the five drug samples tested only Chloramphenicol tablets were contaminated. According to the method specified in British Pharmacopeia Chloramphenicol inoculated in the TSA media was analyzed.

$$\text{Maximum number of CFU in 1g of the diluted drug sample} = \frac{1\ \text{CFU} * 1\text{g}}{0.01\text{g/ml}} = 100\ \text{CFU/ml}$$

According to British Pharmacopoeia 2013 the acceptance level of TAMC for Non aqueous preparations for oral use are 1000 CFU/ml. The maximum number of CFU does not exceed the acceptance level specified in British Pharmacopeia. Therefore, Chlorpheniramine does not fail the microbial quality test.

The microbial contaminations in the Chlorpheniramine TSA 10^{-1}, 10^{-2} and SDA 10^{-1} plates were also subjected to further microbial identification. The gram stain results showed that the Chlorpheniramine TSA 10^{-1} plate was contaminated with Gram positive spore forming bacilli and Chlorpheniramine TSA 10^{-2} plate Gram positive cocci.

Catalase Test and the coagulase test was performed to identify Gram positive cocci. The results revealed that gram positive cocci is *Staphylococcus aureus.*

Further identification of fungi in Chloramphenicol 10^{-1} SDA plate was done by Lactophenol cotton blue test. This is used as a staining procedure for fungi. The results showed that contaminated fungi belong to *Aspergillus* Species.

4. Discussion

Contamination of non-sterile pharmaceuticals can cause grave health hazard to the user. The ultimate consequence of microbial contamination of non-sterile pharmaceuticals is the secondary infections in patients who are consuming the contaminated product [18]. Apart from that microbial contamination change the physical, chemical and organoleptic properties of the drug, thus losing or diminishing its therapeutic activities [16].

The scope of this study was to investigate the microbial contamination of five commonly used tablets in the OPD pharmacy of teaching hospital setting. These drugs come in bulk containers and the necessary amount is withdrawn from the container at the time of dispensing. The required amount of drugs were counted manually and they are dispensed in envelops to the patient. Thus, a microbial contamination can occur from the environment due to opening to the surrounding and through the mishandling techniques of the dispenser. The risk of contaminating non-sterile pharmaceuticals within this environment was assessed in this study. To ensure that the drugs were not contaminated

during the manufacturing procedure the drugs samples from the unopened containers were also tested for their microbial contamination.

It is disclosed from this study, that only 20% of the tested samples in opened containers were contaminated with microorganisms. The isolated bacteria were gram positive bacilli and gram-positive cocci. The fungal contaminants isolated were *Aspergillus* spp. All these contaminations were only seen in Chlorpheniramine tablets.

Although the contaminated samples did not contain the *E. coli,* the specified microorganism that should not be present in non-aqueous preparations for oral use with reference to BP, the tested samples were contaminated with pathogenic organisms like *Aspergillus* spp. It is not desirable to have *Aspergillus* spp. within a pharmaceutical product as these pathogenic microorganisms can cause health hazards to patients whose immunity is already impaired by the presenting disease condition [16,18]. If these contaminated products are consumed by an immune compromised patient, it can yet have more serious health hazards [16].

Gram positive cocci isolated were identified as *Staphylococcus aureus. Staphylococcus aureus* is found as normal human flora in anatomical sites such as upper respiratory tract [19]. This gram positive cocci is an opportunistic microorganism which means that this organism is capable of causing infections when the immunity of a person is lowered [19]. As *Staphylococcus* is found as normal flora, the transmission of this organism might be from the hands of the dispenser. This concludes the improper handling techniques used in the dispensing processes for bulk drug dispensing. Although *Staphylococcus aureus* is an opportunistic microorganism, it is not hazardous to contaminate oral tablets [20]. The reason is that not all the strains *Staphylococcus aureus* produce endotoxins, the preliminary cause of intoxication [20].

Staphylococcus aureus is a major cause in community-associated bloodstream infections, and it causes morbidity and mortality. *Staphylococcus aureus* can cause infective endocarditis or thrombophlebitis if they enter the cardiovascular system. So, contamination of drugs with *Staphylococcus aureus* increases the risk of developing endovascular complications. This concludes that improper handling techniques during dispensing procedure need to be corrected since contamination of pathogenic organisms like *Staphylococcus aureus* may cause serious health hazards to the patient [21].

The Gram-positive bacilli are present abundant in the environment [19]. The environmental factors such as temperature and relative humidity affects for the growth of microorganisms [16]. If favorable environmental conditions are present within the environment, high growth of microorganisms can be detected. The relative humidity and temperature in Sri Lanka are favorable for microbial growth. Sporulating bacilli can show a high growth in these environmental conditions. Therefore, it suggests that these drug samples were contaminated with sporulating Gram positive bacteria during handling procedures and storage. All these were environmental contaminations due to opening of bulk containers to the environment. Furthermore, it is evidenced by the absence of microbial contamination in the samples taken from the unopened packs of the same drugs.

According to British Pharmacopeia the TAMC counts of the contaminated samples should be within the acceptable criteria. Since the TAMC count in the drug samples of opened containers exceed the acceptable criteria for the TAMC and the drug samples in the unopened containers were not contaminated it proves that drugs were contaminated due to improper handling techniques in the hospital setting. Since the contaminated tablets exceed the acceptable criteria, it indicates the failure of drug for consumption.

Although this study showed less bioburden, it is necessary to take steps to prevent contaminations with pathogenic microorganisms. This is essential to improve the quality of life of the patient who is consuming pharmaceutical products. In other hand, this will reduce the health care cost of the government, by reducing readmissions to hospitals. In advance it will tend to the trustworthiness of the people towards the government hospitals.

5. Conclusion

This study proves that 20% of the tablets were heavily contaminated with microorganisms. The contaminated bacteria were gram positive bacilli and *Staphylococcus aureus*; gram positive cocci. The fungal contaminants isolated were *Aspergillus* spp. It was confirmed that tablets were free from microorganisms in the manufacturing process. The contamination tablets with microorganisms may be due to improper handling of drugs during the dispensing process or repackaging.

Therefore, it is important to educate the dispensers about the personal hygiene and correct handling procedures of drugs during the dispensing process. As well as it is important to maintain the cleanliness in the pharmaceutical area to

prevent the environmental contamination of drug samples. The dispensers and pharmacists should also educate about the correct storage practices of drugs. The health care staff should be informed about the importance of prevention and control of microbial contamination of pharmaceuticals and prevention of medicine related infections.

Compliance with ethical standards

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Colombo South Teaching Hospital, Kalubowila, Colombo, Sri Lanka and Department of Pharmacy and Pharmaceutical Sciences, Faculty of Allied Health Sciences, University of Sri Jayewardenepura.

Disclosure of conflict of interest

There are no conflicts of interest regarding the publication of this paper.

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