ORIGINAL ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Assessment of Microbiological Quality of Non- Sterile Multivitamin and General tonic Pharmaceutical Drops Produced in Egypt

Mona Hamido Mohamed*, Salah Abd Allah Galal, Ali A. Abdelrahman

Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Ismailia, 41522, Egypt

Received on: 11. 12. 2022

Revised on: 09. 01. 2023

Accepted on: 19. 01. 2023

*Correspondence Author:

Tel: +201008333562

E-mail address:

monahamido12@gmail.c0m

Abstract

The present study was carried out to demonstrate the microbiological quality of nonsterile pharmaceutical drops produced by Egyptian companies. For this purpose, commercially available non-sterile pharmaceutical drops products were obtained from different Egyptian manufacturers, which are currently purchased sporadically from various retail pharmacies in Egypt. They were unopened before testing. The non-sterile pharmaceutical drops were of Multivitamin and General Tonic Drops. Quantitively, there was no viable bacterial growth in 291(80.83%) of the tested samples and 315(87.50%) of the tested samples were free from fungi. Qualitative, non-sterile pharmaceutical drops were including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus* species, *Pneumonia*, *Pseudomonas aeruginosa*, *Salmonella* species, *Aspergillus niger*, *Saccharomyces* species, *Penicillium* species, and *Aspergillus flavus*. *Several measures*, including equipment automation, monitoring programs and post-marketing surveillance are required to reduce the level of microbial contamination.

Keywords: Microbial quality, non-sterile, pharmaceutical drops.

1. Introduction:

The use of contaminated pharmaceutical preparations has proved hazardous to the health of the users. There have been reports of drug-borne human infections worldwide **(Coker, 2005).** The extent of the hazard will vary from product to product and patient to patient, depending on the types and numbers of organisms present, the route of administration, and the resistance of the patient to infection **(Bloomfield, 2007).** Contamination of pharmaceutical products with microorganisms can also bring about changes in their physical characteristics, including the breaking of emulsions, the thinning of creams, fermentation of syrups, and appearance of turbidity or deposit, besides producing possible off odors and color changes **(Shaikh *et al.*, 1988).** These changes will not only make the product aesthetically unacceptable but can also affect the therapeutic potency and dosage delivery **(Bloomfield, 2007).** The incidence of microflora in non-sterile pharmaceutical preparations generally is influenced by the nature of the ingredients (whether natural or synthetic), the quality of the vehicle and the care and attitude of personnel involved in their handling **(Parker, 2013 and USA, 2018).**

Microbiological contamination remains a critical focus for the pharmaceutical industry that might affect their safety, efficacy, or acceptability to the patient. Microbiological quality control of pharmaceutical products can be found throughout the manufacturing process, but not limited to raw materials, equipment, cleanroom environments, finished product manufacturing, storage and

shipping procedures (**Sandle, 2016).** Bioburden control programs are also essential for both sterile and non-sterile manufacturing and classified as a necessary part of pharmaceutical microbiology. The manufacturing environment and processes are very essential to be controlled well. The costs of resolving product quality problems and Pharmaceutical Current Good Manufacturing Practices (cGMPs) compliance issues arising from poor microbial control or recurring microbial contamination should eclipse concerns about operational costs (**Sandle, 2016).**

The present study aimed to evaluate the non-sterile pharmaceutical drops products available in the Egyptian Market with respect to their microbial contamination, which may affect their safety, efficiency and acceptability to consumer or patient.

2. Materials and Methods

Collection and specimens processing:

Sample preparation was conducted according to the United States Pharmacopeia (USP 2018). The method for sample preparation depended on the physical characteristics of the product to be tested. Samples were analyzed as soon as possible after purchasing them or stored as specified on the label. Samples then inspected carefully before opening to note any irregularities. Three random samples of each product batch were taken. Before opening and removing sample contents, the surface of each sample bottle or container was disinfected with aqueous mixture of 70% ethanol (v/v) and 1% HCl (v/v). Leave the cleaned surface to dry before opening. The selected samples then mixed in a sterile container under laminar flow hood.

Total bacterial viable count (TVC) & total fungal count (TFC):

Pour plate method

Using a sterile pipette, serial tenfold dilutions of the treated sample were done in TSB to obtain 10^1, 10^2, 10^3, 10^4, 10^5dilutions. 1 ml of each dilution was added into each of 5 sterile Petri dishes. To each of 3 dishes of every dilution, 15 ml to 20 ml of TSA medium was added that previously has been melted & cooled to 45°C for TVC. 15 ml to 20 ml of SDA medium also has been melted & cooled to 45°C previously was added to each of 3 dishes for TFC. The sample with the agar was mixed by tilting or rotating the dishes, and the contents were allowed to solidify at room temperature. The petri dishes were inverted. TSA & SDA plates were incubated at 32.5± 2.5 ° C for 48-72 hours and at 22.5± 2.5° C for 5-7 days respectively. Following incubation, the plates were examined and the number of colony forming unit (CPU) per ml of sample was determined.

Total coliform count (BP, 1988 & USP 2018):

One ml of the prepared sample was mixed with 9 ml of tween peptone and then ten –fold serial dilution was applied. Five levels spacing one logarithmic unit were investigated by pipetting one ml from each level in 3 parallel plates. Promptly 13-15 ml of MacConkey agar were added then mixed well, the contents allowed to solidify, inverted plates were incubated at 37°C for 2 days and the number of bacterial colonies developed were counted.

3. Results

Total Viable Aerobic Bacterial, Coliform and Fungal Count of Drops

Multivitamin and General Tonic Drops

A total of 120 samples representing ten brand products of non-sterile pharmaceutical multivitamin and general tonic drop samples were investigated. For each product, four batches were selected and three containers from each batch were examined microbiologically for their total aerobic bacterial, coliform and fungal counts. The samples were also examined for the presence of potential pathogens. Out of 120 non-sterile pharmaceutical multivitamin and general tonic drop samples, 30 (25%) were microbially contaminated.

Twenty isolates were of bacterial origin; including three different bacterial species as *Staphylococcus epidermidis, Bacillus subtilis,* and *Micrococcus* species. On the other hand, ten isolates were of fungi including three different fungal species as *Aspergillus* species, *Saccharomyces* species and *Penicillium* species.

Qualitative tests showed that 9 samples contained *Staphylococcus epidermidis*, 7 samples contained *Bacillus subtilis and* 4 samples contained *Micrococcus* species while all samples gave zero coliform count and negative tests for *S. aureus, E. coli, P. aeruginosa* and *Salmonella* species. With respect to fungi, 7 samples contained *Aspergillus niger,* 2 samples contaminated by *Saccharomyces* species and one samples were contaminated by *Penicillium* species.

The number and percentage of fungal contents of different batches and containers of multivitamin and general tonic of non-sterile pharmaceutical drop samples with colony count within the range zero represented 110(91.67), while colony count within the range **10- <10^2** were 10(8.33) and there were no colony count within the range >10^2-10^3

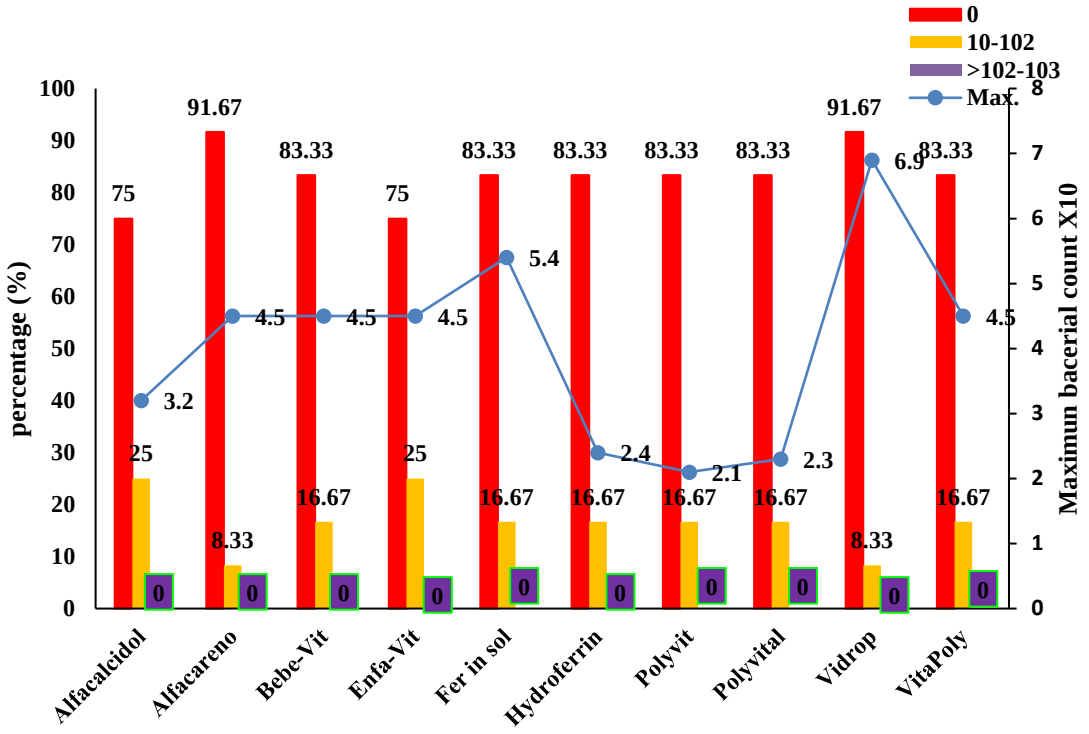

Figure 1: Bacterial contents of different batches and containers of non-sterile pharmaceutical multivitamin and general tonic drop samples

Table 1: Fungal contents of different batches and containers of non-sterile pharmaceutical multivitamin and general tonic drop samples

Pharmaceutical Drops	No. of tested Sample	Fungal count/ml		No. and % of items with colony count within the range		
				0	10- <10^2	>10^2-10^3
		Min.	Max.			
Alfacalcidol	12	0	0.6×10	11 (91.67)	1 (8.33)	0 (0)
Alfacareno	12	0	0	12 (100.00)	0 (0.0)	0 (0)
Bebe-Vit	12	0	0	12 (100.00)	0 (0.0)	0 (0)
Enfa-Vit	12	0	0.4×10	10 (83.33)	2 (16.67)	0 (0)
Fer in sol	12	0	1.5×10	11 (91.67)	1 (8.33)	0 (0)
Hydroferrin	12	0	0	12 (100.00)	0 (0.0)	0 (0)
Polyvit	12	0	10×1.5	10 (83.33)	2 (16.67)	0 (0)
Polyvital	12	0	0.4×10	11 (91.67)	1 (8.33)	0 (0)
Vidrop	12	0	10×0.7	10 (83.33)	2 (16.67)	0 (0)
VitaPoly	12	0	0.8×10	11 (91.67)	1 (8.33)	0 (0)
Total	120	0	1.5×10	110 (91.67)	10 (8.33)	0 (0.00)

The present study showed that *Staphylococcus epidermidis* was the most predominant bacterial isolate recovered from multivitamin and general tonic oral drop samples representing 30.0% of isolates followed by *Bacillus subtilis* (23.33 %), and *Micrococcus* species (13.33). On the other hand *A. niger* was the most predominant fungal isolate represented (23.33% for each) of isolates followed by *Saccharomyces* species (6.66%) and *Penicillium* species (3.33%).

4. Discussion

All analyzed non-sterile pharmaceutical multivitamin and general tonic drop samples in the present study were within (USP, 2018) bacterial and fungal limits except (2/120) samples were out of the fungal limit (≤ 10 CFU/ ml) for Fer in sol and polyvit drpops. Our study revealed that the most predominant isolates recovered from all multivitamin and general tonic drops were (9) of *Staphylococcus epidermidis* for bacteria and (7) of *Aspergillus niger* for fungi.

In Egypt, comprehensive surveys were done on the microbial quality of non-sterile pharmaceutical preparations **Abdelaziz (1975); Salama (1977); Khatibi (1978); Ashour *et al.*, (1987); Hefni (1987); Awad (1993); Radwan (1999); Hefni (2004) AbdElalim,M.(2011)** and **Khalaf (2020)** tell now. Several reports have also been published describing clinical hazards that are attributable to microbiologically contaminated pharmaceutical preparations **(USP 2003, USP 2018** and **USP 2020).** The major health concern is when such microbial contaminants exceed acceptable limits (10^2 cfu/ml) **(Carstersen and Rhodes, 2000).** It must be stressed, however, that the majority of cases of medicine-related infections are probably not recognized or reported as such **(Denyer and Baird, 2006).** Microbial infections are not only the result of the physical presence of microorganisms, but also their metabolites/toxins that become harmful even if they are found in minute quantities **(Shukla *et al.*, 2004).** Previous studies have demonstrated microbiologic quality concerns with regard to both commercially available and extemporaneously prepared pharmaceuticals, storage, and sale of expired liquid disinfectants **(Denyer and Baird, 2006).** It was clearly established that a significant microbial contamination of non- sterile pharmaceutical preparations can and do occur.

Contamination of pharmaceutical products with microorganisms can bring about changes in their physical characteristics, including breaking of emulsions, thinning of creams, fermentation of syrups, appearance of turbidity or deposit and changes in odor and color **(Shaikh *et al.*, 1988),** and the presence of pathogenic or opportunistic microorganisms or bacterial toxic metabolites and enzymatic activity in pharmaceutical products can result in the inactivation of the product **(Smith and Ross 1991; Hugo, 1998; Gimenez-Bastida *et al.*, 2019).**

5. Conclusion

The holder of a manufacturing authorization must drug so as to ensure that they are fit for their intended use, comply with the requirements of the Marketing Authorization and do not place patients at risk due to inadequate safety, quality or efficacy. To achieve the quality objective, it is necessary to control all stages of drugs, which covers all matters, which individually or collectively influence the quality of a product, including raw materials, the manufacturing process and the evaluation of finished product.

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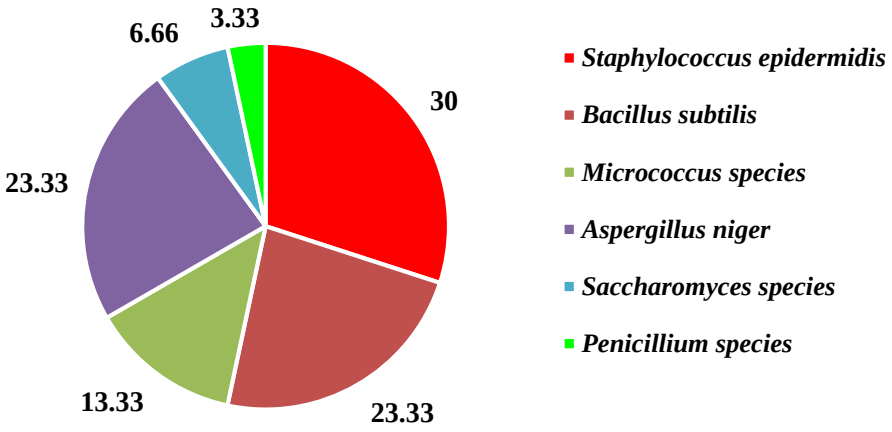

Figure 4: Types of microbial contaminants and their percentage appearance in non-sterile pharmaceutical multivitamin and general tonic drop samples

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