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QUALITATIVE AND QUANTITATIVE MICROBIOLOGICAL STUDIES OF ANTACID AND PARACETAMOL SUSPENSIONS FROM DIFFERENT DRUGSTORES OF DHAKA

MOHAMMAD MONIRUZZAMAN, MD. FARUKH FAISAL ASHRAFI[1] AND ZAKARIA MIA*[2]

Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh

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At the beginning of the 21st century, microbial contamination of non-sterile products is one of the major reasons for product recalls and production shutdowns(1). Contamination of oral liquid pharmaceuticals with microorganisms can bring about changes in their physical characteristics, including phase separation in emulsions, the thinning and discoloration of creams, fermentation and precipitation of syrups, flocculation in suspension and color-odor changes(2). The presence of certain microorganisms in non-sterile pharmaceutical products adversely affect the therapeutic activity of the product or even harmful for the health of the patient.

As antacid and paracetamol are two widely used drugs in Bangladesh among the general population, the microbiological safety of these drugs is an important public health concern. Liquid antacids often contain ingredients of high basic pH which readily support the growth of a variety of microorganisms if appropriate precautions are not taken(3,4). A research carried out in 2004 indicated the aerobic microbial count exceeding the USP limit in 75% of the antacid samples checked(5). From 1995 to 2002, pharmaceutical products with acetaminophen as the active ingredient have been recalled time to time by FDA of USA from markets due to contamination with aerobic bacteria exceeding the acceptable limits(1). This research elucidates the necessity of improved microbiological quality control of paracetamol and antacid suspensions from various pharmaceutical companies of Bangladesh.

One ml of the sample was taken in 9 ml sterile normal saline to have 10 ml of 10^{-1} dilution, and was mixed well. Fifty ml nutrient broth in each conical flask (triplicate) was inoculated with 1 ml diluted sample (10^{-1}). Incubation was done at 30ºC for 24 to 48 hrs in a shaker incubator (for aerobic growth). In case of positive growth, 1 - 2 loop full of culture was transferred aseptically to nutrient and MacConky agar plates. Following a four way streak inoculation, the plates were incubated overnight at 30^0C and then examined for presence of discrete colonies. Each isolated colony from the incubated plates was subjected to staining and various biochemical tests for identification at genera

*Corresponding author: <mmzakaria@yahoo.com>.

[1]Department of Microbiology, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka-1344, Bangladesh.

[2]Department of Microbiology and Biotechnology, Jagannath University, Dhaka-1100, Bangladesh.

level. The biochemical tests used were oxidase, catalase, methyl red (MR), voges-proskaur (VP), motility indole ornithin decarboxylate (MIO), triple sugar iron (TSI), citrate utilization, urea and mannitol fermentation.

Ten ml of samples diluted with buffered sodium chloride peptone solution were added to 90 ml sterile buffered NaCl peptone solution (pH 7.0) and thus 10^{-2} dilutions were made. Then 10^{-3} and 10^{-4} dilutions from 10^{-2} dilution were prepared. Plate count method was used to determine the total viable microbial count in the samples. Solidified nutrient agar Petri plates were used for the cultivation of bacteria. Two hundred µl of sample was spread over the surface of the medium from $10^{-2,}$ $10^{-3,}$ and 10^{-4} dilution of the samples. After incubation at 35°C for five days, colony forming units (cfu) were counted from each of the plates. Arithmetic average of the counts was taken and the number of colony forming units per milliliter was calculated.

The mean bacterial counts for the antacid and paracetamol suspensions varied between 1.5×10^2 to 2.3×10^6 cfu/ml and 2×10^2 to 1.7×10^3 cfu/ml, respectively, as shown in Table 1. Twelve bacterial strains were isolated from the preparations, listed in Table 1.

Table 1. Total microbial count and isolated microorganisms from the collected oral liquid drug samples.

Sample No.	Total viable count (cfu/ml)	Isolated microorganisms
Antacid		
A-01	3.5×10^4	*Klebsiella* spp., *Bacillus* spp.
A-02	01×10^3	*Bacillus* spp.
A-03	1.2×10^4	*Klebsiella* spp.
A-04	×	
A-05	1.5×10^2	*Proteus* spp., *Pseudomonas aeruginosa*
A-06	2.3×10^6	*Proteus* spp., *Pseudomonas aeruginosa*
A-07	03×10^3	*Staphylococcus aureus*
A-08	×	
Paracetamol		
P-01	02×10^2	*Proteus* spp.
P-02	×	
P-03	4.5×10^2	*Staphylococcus aureus*
P-04	×	
P-05	×	
P-06	6.8×10^2	*Pseudomonas aeruginosa*
P-07	1.3×10^3	*Pseudomonas aeruginosa, Proteus* spp., *Klesiella* spp.
P-08	1.7×10^3	*Staphylococcus* spp., *Pseudomonas aeruginosa*
P-09	×	
P-10	09×10^2	*Pseudomonas aeruginosa*

*USP limit: Not more than 10^2 cfu/ ml

In this work microbiological quality of 18 samples of 18 pharmaceutical industries was investigated. Seventy five per cent of the antacid samples (6 out of 8) showed microbial count exceeding the USP limit of 10^2/ml. Among the paracetamol samples, 60% were contaminated above the USP limit. Two antacid samples and 4 paracetamol samples showed no growth at all after proper enrichment and plating procedure. This might be due to addition of higher amount of preservative in the liquid samples. Although inadequate addition of preservative might lead to development of resistant variants of microorganisms[6], adding preservatives beyond the approved limit is harmful for human health[5].

Absence of indicator microorganisms (*P. aeruginosa, E. coli, S. aureus* and *Salmonella* spp.) is an absolute requirement[7-8]. In this study, 2 samples of antacid and 4 samples of paracetamol showed presence of *P. aeruginosa* whereas 1 sample of antacid and 2 samples of paracetamol were contaminated with *S. aureus*, which indicates the possibility of contamination with other pathogens. However, none of the samples showed presence of *E. coli* or *Salmonella* spp. The organisms detected in the remaining samples are not pathogenic but objectionable as they can deteriorate active ingredients and thus can interfere with the action of the product.

Results indicate that the antacid and paracetamol manufacturing conditions and the type of preservative(s) should be investigated with more number of samples to find out the real status of present pharmaceutical practices in Bangladesh for manufacturing oral liquid preparations.

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