Qualitative and quantitative microbial load in oral liquid drugs in Bangladesh

Md. Shahidul Islam[1,2], Md. Nura Alam[1], Md. Al Arif Kabir[1], Tanzinah Nasrin[3], Zakaria Mia[1]

[1]Department of Microbiology, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka 1344, Bangladesh
[2]Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh
[3]Department of Microbiology Stampford University, Dhaka-1217, Bangladesh

ABSTRACT

A microbiological quality control study was performed on oral liquid drugs (antacid amoxyciline and paracetamol) in Bangladesh. Nine (9) syrup preparations collected from different drug stores and were assayed for their bacterial load using the spread plate technique. The microbial contaminants in the liquid drugs were characterized to species level. All the oral liquid preparations are heavily contaminated with bacteria at levels far above the officially stipulated limit for non-sterile oral pharmaceutical preparations. Six bacterial genus (*Alcaligenes, Pseudomonas*, *Staphylococcus, Bacillus, Proteous, Klebsiella)* were identified from all suspension. Total viable microbial count varied between less than 10^2 CFU ml^{-1} and greater than 10^6 CFU ml^{-1}. Microbial counts in liquid preparation from big pharmaceutical companies were higher than those of some small companies. The result of this study highlights the necessity for strict control of non-sterile oral liquid pharmaceutical products. Considering the fact that a great proportion of people in Bangladesh consume non-sterile oral liquid medicines, the public health impact of this gross microbial contamination of the non-sterile oral samples must be considered. It is very necessary to maintain Good Manufacturing Practice (GMP) for non-sterile oral liquid pharmaceutical product preparation.

Key words: Microbial contamination, bacteria, oral suspension, non-sterile, Bangladesh.

*Corresponding author.
E-mail address: shahiduldhamrai@gmail.com (MS Islam)

INTRODUCTION

Presence of microbes in non sterile pharmaceutical liquid preparations (syrup, suspension) is a great public health concern globally. Contamination of pharmaceutical preparations with microorganisms irrespective of being pathogenic and nonpathogenic can bring about changes in their physical characteristics, including the breaking of emulsions, fermentation of syrups, and appearance of turbidity or deposit, besides producing possible off odors and color changes. The presence of certain microorganisms in non-sterile pharmaceutical products may adversely affect the therapeutic activity of the product or even harmful for the health of the consumer. The pharmaceutical manufacturing and packaging environment, raw materials as well as the manufacturing water may attribute to the microbiological spoilage of the finished products (Cundell 2005a, 2005b; Prescott 2005). Manufacturers are required to ensure that the bioburden of finished products falls within acceptable limits, and that they are free from potentially harmful organisms. This is achieved by implementing current Good Manufacturing Practice (cGMP) guidelines during the manufacture, storage and distribution of such products.

Tests for microbial limits are designed for the determination of the level of bioburden and the absence of specific pathogens in pharmaceutical raw materials and finished products, which are not absolutely sterile. The bioburden limit expressed as total aerobic microbial count and absence of specific pathogens are stipulated in some of the USP/BP monographs of the non-sterile pharmaceutical products such as ointments,

creams, lotions, tapes, aerosols, gels, gauze, pastes, powders, oral suspensions and syrup preparations.

There is evidence for presence of bacteria in liquid pharmaceutical product including *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus,* Gram-positive cocci. Microbial examination is performed by the manufacturers of non-sterile pharmaceutical products to demonstrate microbiological control during the manufacturing process. The methods used and results obtained should comply with the specifications and criteria outlined in the appropriate pharmacopoeia. Testing, which is performed on both raw materials and finished products, involves microbial enumeration tests for total aerobic microbial counts (TAMC) and total yeast and mould counts (TYMC), in addition to tests for the following specified microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella*, *Candida albicans* and *Clostridium sporogenes*.

Microbial examination of non-sterile products has a quantitative and a qualitative phase of testing. The quantitative phase is microbial enumeration, where the total number of viable aerobic microorganisms, yeasts and moulds are counted. The qualitative phase searches for specific microorganisms that could be potentially pathogenic to the consumer, to ensure that products are free from such risks.

Bangladesh, with a blooming sector in pharmaceutical industries, often faces trouble in context of market complaints or with the adverse effects of oral drug consumption (Das et al., 2013; Khasru, 2007). Besides the in process quality control or the microbiological regulation of the raw materials and finished products, a routine monitoring of microbiological contamination of pharmaceutical products is required to assess the product quality thereby reducing the public health risk (Hossain 2009; Sykes 1971; Hossain 2004; Raton et al., 2013; Khanom 2013). The current study was aimed to determine the total viable aerobic microbial count in antacid amoxyciline and paracetamol suspension and to determine the pharmaceutical objectionable organisms in these products for assuring public health.

MATERIALS AND METHODS

Sample collection

Samples were collected from Dhaka city and Dhamrai bazar in Bangladesh. To get the real picture of the quality of these oral suspension manufactured in Bangladesh, samples different brands were collected from different location of the country. A total of 9 non-sterile oral pharmaceutical samples were used in this study. Three (3) of these samples were liquid antacid suspension, 3 were liquid paracetamol suspension and 3 were liquid Amoxyciline suspension. Antacid suspensions were coded as An1, An2, An3; Paracetamol suspensions were coded as Pa1, Pa2, Pa3 and Amoxyciline suspension are coded as Am1, Am2, Am3 in this study. Samples were checked for their batch number, production and expiry date. Samples were randomly collected and stored under appropriate conditions. The study was carried out in the Gonoshasthaya Vaccine Research Laboratory (GVRL), the Department of Microbiology, Gono Bishwabidyalay (Gono University), Dhaka, Bangladesh.

Qualitative analysis of bacteria

Enrichment culture

One ml of the sample was taken in 9ml sterile normal saline to have 10ml of 10^{-1} dilution of sample, and mixed well by vortex. Fifty ml nutrient broth in each conical flask (triplicate) was inoculated by 1ml diluted sample (10^{-1}) in nutrient broth containing each conical flask. Incubation was done at 30^0C for 24 h to 48 h in shaker incubator (for aerobic growth). After incubation, turbidity of the medium was observed as the indication of growth of microbe in the sample.

Sub culture

In case of positive growth, 1-2 loop full of culture was transferred aseptically to an appropriately labeled Nutrient agar and MacConky agar plates. Then a four way streak inoculation on each medium was followed. Incubation of the agar plate culture in an inverted position was done for 24 hours at 30^0C. After incubation the plates cultures were examined for the presence of discrete colony.

Stock culture from different isolates

Each isolated colony from subculture was aseptically transferred and streaked in nutrient agar slants (1.5ml vial). Then the agar slant cultures were incubated for 24 hours at 30^0C. After incubation the cultures were stocked for biochemical tests.

Bacterial colonies in each medium were then characterized on the basis of staining colonial, cellular morphology, and biochemical characteristics using standard microbiological technique (Barrow and Feltham, 1993).

Quantitative analysis of bacteria

Sample preparation

Before doing the quantitative analysis of contaminated bacteria, the inhibitory effects of antimicrobial agents used as preservatives in the suspension were removed by serial dilution of the sample suspension with buffered sodium chloride peptone solution (p^H 7.0) to make 10^{-2}.

Determination of total microbial count

The total microbial count was carried out by plate count method. Petri dishes containing 15ml to 20ml of nutrient agar medium was allowed to solidify at above 45° C overnight and checked the media whether contaminated or not. A 500µl sample from each dilution (10^{-1}, 10^{-2}, 10^{-3}, dilution) was spread over the surface of the medium with at least two replicates. The plate was incubated at 37°C for one day and the number of colony forming units per milliliter was calculated to count the total viable bacteria present in antacid amoxyciline and paracetamol samples. To avoid any false reading both positive (medium inoculated standard microorganisms) and negative control (medium only) were used.

Total Viable Count:
Number of cell per ml = Total colony × Dilution factor
X = Total colony
Y = Dilution factor
Z = Inoculum size **/** Spread amount

RESULT AND DISCUSSION

In this study microbiological quality of 9 samples (9 brands) from different pharmaceutical industries was investigated. Proper sealing of containers of all companies were satisfactory. Manufacturing and expire dates were recorded for all products. Since relationship was observed between microbial contamination and pH deviation, the pH of the samples were checked. However, present study revealed no convincing data which indicate the relationship between pH deviations with microbial contamination (data not shown).

Microbial contaminants isolated from the antacid amoxyciline and paracetamol preparations are shown in table no.1. The mean bacteria counts for the antacid amoxyciline and paracetamol samples varied between 1.5×10^2 to 2.3×10^6 CFU ml^{-1} and 2×10^2 to 1.7×10^3 CFU ml^{-1} respectively. Of the 9 preparations examined, the organisms most commonly isolated were gram negative rods. There are 12 bacterial strains were isolated from the antacid amoxyciline and paracetamol preparations. Of them 4 *Klebsiella sp.*, 2 *Staphylococcus sp.*, 1 *Bacillus sp.*, 2 *Proteus sp.* and 3 *Peudomonas sp.*

Aerobic viable microbial count of 8 out of 9 samples studies were found to be exceeded the USP recommended limit. Viable microbial counts varied between less than 10^2 CFU ml^{-1} and greater than 10^6 CFU ml^{-1} (Table 1). In 7 (38.9%) samples of 7 companies, the total aerobic microbial count was more than 10^3 CFU ml^{-1} (Table 1).

Identification of the isolated organisms

The staining results and biochemical properties provided a list of isolated organism from amoxyciline, paracetamol and antacid suspension (Table 1).

Most oral liquid medicines are prepared as mixtures, suspensions, syrups or emulsions. These often contain ingredients, which readily support the growth of a variety of microorganisms if appropriate precautions are not taken. Particularly, liquid antacid with neutral pH value is processed under partial aseptic condition, is highly susceptible to microbial contamination.

Table 1
Identification of the isolated organisms from antacid amoxyciline and paracetamol preparations.

Microorganism	Antacid	Paracetamol	Amoxyciline	Total	Percentage
Alcaligenes	0	1	1	2	10
Peudomonas	3	1	1	5	25
Klaebsiella	1	0	0	1	5
Bacillus	0	0	1	1	5
Proteus	0	1	0	1	5
Staphylococcus	4	4	2	10	50
Total	8	7	5	20	100

Table 2
Quantitative analysis of bacteria in oral liquid drugs

Oral preparation	Codes	Average total count ($CFU\ ml^{-1}$)
Antacid suspension	An1	6.3×10^2
	An2	8.6×10^2
	An3	1.6×10^3
Paracetamol Suspension	Pa1	1.6×10^3
	Pa2	11.4×10^2
	Pa3	1.6×10^3
Amoxyciline Suspension	Am1	10.2×10^2
	Am2	8.4×10^2
	Am3	10.4×10^3

*USP limit: Not more than $10^2\ CFU\ ml^{-1}$

Preservative commonly used in antacid and paracetamol such as methyl paraben which support the bacterial growth. Moreover, parabens are active at pH between 7 and 9; inappropriate use of preservatives and formulation of the drug may be responsible for this high microbial count. In this study the microbial contamination of suspension from large companies were more than small companies. However, the study demonstrated the presence of microbial contamination in oral suspensions. In the present study three samples (16.67%) contained *Pseudomonas aeruginosa*. However, the organisms found in this study are water borne and frequently contaminate liquid pharmaceutical products. Moderate to heavy contamination by microbes in the liquid preparation of the 75% of the samples studied indicates poor hygienic condition in the manufacturing process. Inadequate preservation may lead to the microbes getting exposed to sub-lethal concentration of preservatives and develop resistance variants. However, high concentration may prove to be toxic for consumer's health. Therefore, it is necessary to determine the appropriate preservation conditions for efficacy and usefulness of the product during its storage period.

Lower microbial count in samples of the some small companies than those of many large reputed companies does not necessarily indicate that the samples of the small companies with less microbial count are better. It is important to note types of preservatives they used. Use of the toxic preservatives to reduce the microbial load is not acceptable. Rather to reduce microbial load in the suspension, the ingredients must be examined to determine whether there is any pathogenic microbe and the microbial count with acceptable limit. Preservative should not be expected to sterilize formulations that are heavily contaminated as a result of low quality raw materials and poor manufacturing procedures.

Maintenance of sterility in liquid suspension preparation is a common problem in many pharmaceutical settings. In this study it also appears to be a problem in our country. Although fewer numbers of samples have been analyzed without resources of statistical design, the results indicate the suspension manufacturing conditions and the type of preservative(s) should be investigated with a broader number of samples to find out the real scenario of present pharmaceutical practices in Bangladesh for manufacturing oral liquid preparations.

CONCLUSION

Sterility is not a requirement in official compendia for oral Pharmaceutical dosage forms. However contamination may occur during manufacturing, packaging and handling by the consumer. This causes concern, since some dosage forms if stored in favorable environment, can serve as substrates for microorganisms. Furthermore the contaminated drugs can mediate infection in man and hence harmful organisms should be absent from non-sterile Pharmaceutical preparations. This exercise was carried out to obtain general information on the microbial content of non-sterile liquid preparations produced by different local Pharmaceutical firms of Bangladesh.

The findings of the investigation for testing samples of antacids and paracetamol in the present study showed the growth of microbial contaminants that was not supposed to be for non-sterile products. Some "pathogenic" and "objectionable" organisms are detected in some samples. The organisms which are detected in the remaining samples are not "pathogenic" but they are "objectionable" as they can bring about the destruction of active ingredients and thus can interfere with the function of the therapeutic product.

REFERENCES

Barrow GH and Feltham RKA (1993). Cowan and Steel's Manual for Identification of Medical Bacteria. Third edition. Cambridge University Press, Cambridge. pp. 331.

Cundell AM (2005a). Environmental Monitoring in Non-Sterile Product Manufacturing. In Environment Monitoring. Edited J. Moldenhauser Davis Horwood/PDA.

Cundell AM (2005b). Managing the microbiological quality of pharmaceutical excipients. PDA Journal of Pharmaceutical Science and Technology; 59(6): 381–395.

Das KK, Fatema KK, Nur IT and Noor R (2013). Prevalence of microorganisms in commonly used cosmetics samples in Dhaka Metropolis. Journal of Pharmaceutical Science and Innovation, 2(6): 7-9.

Hossain J (2009). Importance of the bioburden test in pharmaceutical quality control. Pharmaceutical Microbiology Forum, 15(1): 2-14.

Hossain M, Ara S and Rahman MZ (2004). Quantitative examination of aerobic bacteria and fungi in locally available antacid suspension and possible contamination by specified bacteria. Pakistan Journal of Biological Science, 4: 7(11): 2014-2017.

Khanom S, Das KK, Banik S and Noor R (2013). Microbiological analysis of liquid oral drugs available in Bangladesh. International Journal of Pharmacy and Pharmaceutical Sciences, 5 (4): 479-482.

Khasru SM (2007). SME sector in Bangladesh: a critical overview. The Cost and Management, 35 (6): 73-84.

Prescott LM, Harley JP and Klein DA (2005). Microbiology. 6th edition.

Raton KA, Hossain MA, Acharjee M and Noor R (2013). Assessment of microbiological quality and the anti-bacterial traits of sterile liquids used for medication of eye and ear infections in Bangladesh. American Journal of Pharmacy and Health Research, 1(9): 67-75.

Sykes G (1971). *Pseudomonas aeruginosa* contamination of liquid antacids: A survey. Journal of Pharmacological Science, 60: 604- 605.