http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (11): 2014-2017, 2004
ISSN 1028-8880
© 2004 Asian Network for Scientific Information

Quantitative Examination of Aerobic Bacteria and Fungi in Locally Available Antacid Suspension and Possible Contamination by Specified Bacteria

Mahboob Hossain, Shamim Ara and Mohammed Zakiur Rahman
Department of Pharmacy, The University of Asia Pacific, Dhaka-1209, Bangladesh

Abstract: The microbiological status of liquid antacid suspension of 20 pharmaceutical industries of Bangladesh were studied. Aerobic viable microbial count of 27 (75%) out of 36 samples studied were found to exceed the USP limit. Viable microbial count varied between less than 10^2 CFU mL^{-1} and greater than 10^6 CFU mL^{-1}. In 11 (30%) samples of 8 companies the total aerobic microbial count was more than 10^3 CFU mL^{-1}. Microbial counts of two large reputed pharmaceutical companies were 1000 fold higher than those of some small companies. None of the samples contained *Escherichia coli* and *Salmonella* spp. Out of 36 samples 1 (2.5%) of the 2 samples of one company having different manufacturing date contained *Pseudomonas aeruginosa* and both samples (5%) having different manufacturing date of another large company contained *Staphylococcus aureus*. No significant correlation between pH deviation and contamination by microbes was observed.

Key words: Antacid suspension, microbial contamination, colony forming unit

INTRODUCTION

Microbial contamination of non-sterile pharmaceutical liquid products is rapidly becoming a matter of worldwide concern[1,2]. Some pharmaceutical manufacturers of our country consider liquid antacid to be a low cost item, as a result insufficient care is taken to ensure maximum control of microbiological aspects of manufacture. Since wrong or inadequate preservatives used in medicine cannot control the contamination, it is imperative that correct preservatives should be used in appropriate amount. Liquid antacids often contain ingredients which readily support the growth of a variety of microorganisms if appropriate precautions are not taken[2,3]. Antacid suspension containing organic components and being of neutral pH are processed under neutral appropriate preservation conditions, is highly susceptible to microbial contamination. As the product is available as multidosed medicament there is further risk of contamination during the intake period. As a result inclusion of antimicrobial preservatives is a necessary part of the formulation process.

Usually foreign literatures are followed by the pharmaceutical manufacturers of our country for the formulation of the medicines. Hence, the antimicrobial agents used as preservatives may not be effective against the microbes of varied or subtropical habitat. In Bangladesh very little work has been carried out on microbial contamination and preservation of liquid antacid. Study on the quality of antacid done earlier[4,5] gave a gloomy picture and in this study a comparison was made between the present and the previous situation. The main objective of this study was to grow awareness among the concerned persons so that microbial quality of liquid antacid preparation improves further.

MATERIALS AND METHODS

Antacid suspension: Liquid antacid preparation of 20 companies having different manufacturing date were purchased from various drug stores of Dhaka city, Tongi, Gazipur, Borishal and Bogra. Samples were checked for their batch number, production and expiry date. Samples were randomly coded for and stored under appropriate conditions.

Culture media: In this study, before culturing in selective media, the microorganisms were grown in Casein soybean digest broth or lactose monohydrate broth depending on the type of bacteria to be detected. Enterobacteria enrichment broth-mossel (EE broth) was used to enhance the growth of bacteria belong to Enterobacteriaceae such as *Escherichia coli, Salmonella* spp. and *Pseudomonas aeruginosa* etc. Violet Red Bile Glucose agar (VRBG) was used to detect the presence of gram negative bacteria. MacConkey broth and MacConkey agar were used for the

Corresponding Author: Dr. Mahboob Hossain, Assistant Professor, Department of Pharmacy, The University of Asia Pacific, House No. 73, Road No. 5A, Dhanmondi, Dhaka-1209, Bangladesh
Tel: +880 2 8629367 Fax: +880 2 9664950 E-mail: mahboob987@yahoo.com

detection of E*scherichia coli*. Xylose Lysine Deoxycholate agar, cetrimide agar and Baired Parker media were used to detect the presence of *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. Triple sugar iron agar and Motility Indole Urea (MIU) agar media were used to identify all the above mentioned microorganisms except *Staphylococcus aureus*. All the culture media were purchased from HiMedia Laboratories Limited, Mumbai, India.

Organisms: Standard organisms such as *Salmonella typhi., Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* were supplied by the Microbiology Laboratory of Bangladesh Institute of Research Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM).

Quantitative enumeration of mesophilic bacteria and fungi

Preparation of the sample suspension: Prior to bacteriological study, the inhibitory effects of antimicrobial agents used as preservatives were removed by dilution of the antacids with buffered sodium chloride peptone solution[6]. Ten milliliter of liquid antacid was added to 90 mL of sterile buffered sodium chloride peptone solution pH 7.0 in 100 mL conical flasks.

Detection of total microbial count: In the present study total microbial count was performed by pour plate method. Using petridishes of 9 cm diameter 1 mL of the diluted sample (prepared as described above) was added to each petridish previously sterilized in hot-air oven. For each sample plating was done in duplicate. The average value obtained was multiplied by the dilution factor to get the total microbial count. Approximately 15 to 20 mL of the liquefied Soyabean Casein Digest (SCD) agar and Saboured-dextrose Agar (SDA) with antibiotic were added to each petridishes at 46°C. Samples with SCD agar and SDA were incubated at 35 and at 28°C, respectively. Both the media were incubated for 5 days. Two petri dishes for each level of dilution were prepared for each medium. Samples were diluted 100 or 1000 or more times depending on number of Colony Forming Units (CFU) observed in the plate. Arithmetic average of the counts was taken and the number of colony forming units per milliliter was calculated.

Detection of specified bacteria: Ten milliliter of liquid antacid preparation was added to 90 mL of lactose monohydrate broth, homogenized and was incubated at 35°C to revive the bacteria for about 3 h. After incubation the conical flask was shaken well and 1 mL of the product was transferred to 100 mL of Enterobacteria enrichment broth-mossel (EE broth) and was incubated at 35°C for 48 h. After incubation, it was subcultured on crystal Violet Neutral Red Bile agar with glucose (VRBG) plate and was incubated at 35°C for 24 h. Negative growth indicated the product is not contaminated by gram-negative bacteria.

Test for the detection of specific bacteria: Tests for the detection of *Escherichia coli, Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done according to the standard procedures[7].

Interpretation of results: The bacterial number were considered to be equal to the average number of Colony Forming Units (CFU) found on Soyabean Casein Digest agar (SCD). The fungal counts were considered to be equal to the average number of colony forming units on Sabouraud-Dextrose agar. The total viable aerobic count was the sum of the bacterial count and the fungal count. The growth of identical microorganisms in different media was counted once.

RESULTS AND DISCUSSION

In this study microbiological quality of 36 samples of 20 pharmaceutical industries was investigated. Proper sealing of containers is essential from the microbiological point of view and sealing of the containers of all the companies were satisfactory. Manufacturing and expire date were printed in case of all the companies. Changes in product pH can occur depending on whether acidic or basic microbial metabolites are released and modification causes secondary attack by microbes previously inhibited by the product initial pH[8] and since relationship was observed between microbial contamination and pH deviation[4], the pH of the samples were checked. However, present study revealed no convincing data which indicates the relationship between pH deviations with microbial contamination (data not shown).

The microbial quality of pharmaceutical products is influenced by the environment in which they are manufactured and by the materials used in their formulations[8]. Marked spoiled pharmaceuticals can often yield surprisingly low numbers of spoilage organism[9]. To get the real picture of the antacid quality of Bangladesh, samples were collected from various parts of the country and from large reputed, medium and small companies.

Aerobic viable microbial count of 27 (75%) out of 36 samples studied were found to exceed the USP limit. Viable microbial counts varied between less than 10^2 CFU mL^{-1} and greater than 10^6 CFU mL^{-1} (Table 1). In 11 (30%) samples of 8 companies, the total aerobic microbial count was more than 10^3 CFU mL^{-1} (Table 1).

Table 1: Total aerobic viable microbial count and presence of specific bacteria in antacid suspension

Brand codes	No. of samples	Total Aerobic viable count		1) *Pseudomonas aeruginosa* 2) *Escherichia coli* 3) *Salmonella* spp. 4) *Staphylococcus aureus*		Exceeding *USP limit
		Sample 1	Sample 2	Sample 1	Sample 2	
MS-1	2	$2.0x10^2$	$6.0x10^1$	Nil	Nil	1
MS-2	2	$5.0x10^2$	$4.1x10^2$	Nil	Nil	2
MS-3	2	$3.0x10^2$	$4.4x10^2$	Nil	Nil	2
MS-4	2	$4.8x10^2$	$1.8x10^2$	Nil	Nil	2
MS-5	2	$1.5x10^5$	$6.8x10^2$	*Pseudomonas aeruginosa*	Nil	2
MS-6	2	$1.3x10^2$	$3x10^1$	Nil	Nil	1
MS-7	2	$7.5x10^4$	$7.9x10^2$	Nil	Nil	2
MS-8	2	$2.6x10^5$	$1.2x10^4$	Nil	Nil	2
MS-9	2	$1.5x10^2$	$6.0x10^1$	Nil	Nil	1
MS-10	1	$11.0x10^6$	----------	Nil	Nil	1
MS-11	1	$5.0x10^1$	----------	Nil	Nil	0
MS-12	2	$1.3x10^2$	$9.0x10^1$	Nil	Nil	1
MS-13	2	$4.9x10^2$	Nil	Nil	Nil	0
MS-14	2	$2.0x10^2$	$1.2x10^2$	Nil	Nil	2
MS-15	2	$1.0x10^5$	$6.2x10^4$	*Staphylococcus aureus*	*Staphylococcus aureus*	2
MS-16	1	$4.0x10^1$	----------	Nil	Nil	0
MS-17	2	$8.0x10^3$	$4.1x10^3$	Nil	Nil	2
MS-18	2	$6.9x10^2$	$1.3x10^3$	Nil	Nil	2
MS-19	2	$2.8x10^3$	$9.0x10^1$	Nil	Nil	1
MS-20	1	$2.0x10^1$	--------	Nil	Nil	0
Total no. of samples:	36		Total number of samples exceeding USP limit:			27

*USP limit: Not more than 10^2 CFU mL^{-1}

To avoid any false reading both positive (medium inoculated with standard microorganisms) and negative control (medium only) were used. Since media composition and incubation temperature greatly influence the resultant microbial counts, media used to detect the total microbial count were nutritious. It is not possible to detect 100% microorganisms present in the sample because considerable variation in estimated microbial count may be obtained from the same sample using different media or even different batches of the same medium.

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[2,3]. Particularly, liquid antacid containing neutral pH which is processed under partial aseptic condition, is highly susceptible to microbial contamination. In this study it was observed that the number of fungal colonies were much lower than those of bacterial colonies. This may be due to less number of fungal contamination in the samples studied. Another reason could be the preservative commonly used in antacid such as methyl paraben and propyl paraben act against wide range of fungi but less effective against bacteria. Moreover, parabens are active at pH between 7 and 9[9], inappropriate use of preservatives and formulation of the drug may be responsible for this high microbial count. In this study the microbial status of large companies were no better than small companies. Absence of indicator microorganisms (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Salmonella* spp.) is an absolute requirement[7,10]. However, the study of samples of two leading pharmaceutical companies were heavily contaminated by microbes and both of the samples of one company contained coagulase positive *Staphylococcus aureus* and one of the sample of another company was found to contain *Pseudomonas aeruginosa* (Table 1). None of the 36 samples were found to contain *Escherichia coli* and *Salmonella* spp. In other two studies[4,5] on the qualitative analysis of Antacid suspensions of Bangladesh absence of *Salmonella* spp. were confirmed. However, in one of the studies, significant number of the samples contained *Escherichia coli* (12.3%) and *Pseudomonas aeruginosa* (12%)[4]. In the present study one sample (2.5%) contained *Pseudomonas aeruginosa* which was much lower than the previous findings. It appears from these three studies that among the specific pathogenic microorganisms *Pseudomonas aeruginosa* is more common. In a different study a high incidence of *Pseudomonas aeruginosa* in liquid antacids was found[11]. Absence of *Escherichia coli* and *Salmonella* spp. in all the 36 samples studied indicates that the water used by the pharmaceutical industries of our country may not be contaminated by coliform bacteria. The organisms of these types are water-borne and frequently contaminate liquid pharmaceutical product[1]. Moderate to heavy contamination by microbes in the liquid preparation of the 75% of the samples studied indicate that the excipients used might be contaminated or the antacid preparation room were not satisfactorily cleaned.

Antacid suspension acts as weak bases and as it contains organic components, it is highly susceptible to microbial contamination. As a result, it is necessary to add anti-microbial preservatives in the formulation process. Inadequate preservation may lead to the microbes getting exposed to sub-lethal concentration of preservatives and develop resistant variants[12]. 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 the antacid samples of 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 which preservatives they use. Use of toxic preservatives to reduce the microbial load is not acceptable. Rather to reduce microbial load in the antacid suspension, the ingredients must be examined to determine whether there is any pathogenic microbe and the microbial count within 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[9].

Maintenance of sterility in liquid antacid preparation is a common problem in many settings. In this study also it appears to be a problem for our products. Although fewer numbers of samples have been analyzed without recourse of statistical design, the results indicate the antacid manufacturing conditions and or the preservative(s) used were not effective. Study can be extended in the laboratory setting to determine appropriate preservative(s), its level and conditions that may be required under our conditions of production.

ACKNOWLEDGMENTS

The author would like to thank Dr. Md. Selim Reza, Mohiuddin Abdul Quadir and Mirza Mohammad Ali Reza for their continuous support during the study. The author would also like to thank Dr. Jalaluddin Ashraf, of the Department of Microbiology of BIRDEM for providing standard microorganisms.

REFERENCES

1. Sykes, G., 1971. *Pseudomonas aeruginosa* contamination of liquid antacids: A survey. J. Pharma. Sci., 60: 604-605.
2. Baird, R.M. and P.S. Petris, 1981. A study of microbiological contamination of oral medicaments. Pharm. J., 266: 10-11.
3. Kallings, L.O., O. Ringertz, L. Silverstolpe and F. Ernerfeldt, 1996. Microbial contamination of medical preparations. Acta Pharmaca Succica., 3: 219-228.
4. Hoq, M.M., M.B. Syeda and D.J. Gomes, 1991. Development of appropriate preservative system for liquid antacid I. Bacterial contaminants in antacid samples. Bangladesh J. Microbiol., 8: 5-10.
5. Malik, A.K.M., A.S.M. Alam, S.K. Gosh, M.A. Hossain and A.B. Siddique, 1991. Analytical evaluation of locally available pharmaceutical products, Part 1. Analysis of some antacid preparations for their active ingredients and for possible contamination by trace metals and microorganisms. J. Bangladesh Chem. Soc., 4: 87-92.
6. Rawlins, E.A., 1996. Microbial Contamination, Control and Sterility Testing. Bentley's Textbook of Pharmaceutics, 8th Edn., Bailleere Tindall. London, pp: 546.
7. Anonymous, 1998. British Pharmacopoeia. Test for Microbial Contamination, 2: A243-A253.
8. Hugo, W.B. and A.D. Rusell, 1998. Pharmaceutical Microbiology. 6th Edn., Blackwell Scientific Publications, pp: 339, 360.
9. Hugo, W.B and A.D. Rusell, 1983. Pharmaceutical Microbiology, 3rd Edn., Blackwell Scientific Publications, pp: 223, 345, 347.
10. Anonymous, 1980. United States Pharmacopoeia, 20th Edn., pp: 872-873.
11. Robinson, E.P., 1971. *Pseudomonas aeruginosa* contamination of liquid antacids: A survey. J. Pharma. Sci., 60: 604-605.
12. Gilbert, P. and N. Wright, 1987. Non-plasmidic Resistance Towards Preservatives of Pharmaceutical Products. In: Preservatives in the Food, Pharmaceutical and Environmental Industries. Eds. Board, R.G., M.C. Allwood and J.G. Banks. Blackwell Scientific Publication, Oxford, pp: 255.