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Original Article

MICROBIOLOGICAL PROFILE AND THE ANTI-BACTERIAL TRAITS OF COMMONLY AVAILABLE ANTACID SUSPENSIONS IN DHAKA METROPOLIS

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ABSTRACT

Objective: Loss of anti-bacterial activity with a concomitant access of pathogenic microorganisms renders pharmaceutical products ineffective in course of medication. Present study attempted to assess the microbiological quality and the anti-bacterial activity of the common antacid suspensions.

Methods: Eight samples collected from different drugstores in Dhaka metropolis were microbiologically tested to isolate and quantify the contaminating bacterial- and fungal population employing conventional cultural and biochemical methods. The anti-bacterial activity of the samples was determined by the agar-well diffusion method.

Results: All samples were found to be populated with a huge number of bacteria (~10^5 cfu/ml) and fungi (10^4-10^5 cfu/ml). Staphylococcal load was significant while *Pseudomonas* spp., *Escherichia coli,* and *Salmonella* spp. and *Salmonella* spp. were absent. Study of anti-bacterial activity revealed 4 samples to exhibit the anti-bacterial activity against *Bacillus* spp., among which one was also active against *Pseudomonas* spp., *Vibrio* spp. and *Klebsiella* spp.

Conclusion : The results of the current investigation attribute to the overall microbiological management of the antacid suspensions together with the routine examination of the active ingredients in respect to their potency.

Keywords: Antacid suspensions; Microorganisms; Anti-bacterial activity; Public Health

INTRODUCTION

Microbiological contamination of non-sterile pharmaceutical liquid products has long been a major concern in context of public health risks and drug efficacy [1, 2]. As the microbiological quality of the non-sterile oral drugs like syrups or suspensions often reaches the threshold, routine assessment of bacteria and fungi populating within these drugs is required to ensure consumer safety [3, 4]. Syrups and suspensions are non-acidic with organic constituents, and usually consist of sweetening agents which in turn tend the products suitable for supporting microbial growth and proliferation [5, 6].

Contaminated raw materials, inactive or outdated preservatives prone to microbiological attack with the subsequent loss of antimicrobial activity, and finally the malpractice of hygiene during manufacturing, packaging, distribution and storage may result in drug incompetency and treatment complicacy [1, 3-5].

In Bangladesh, the commonly used antacid suspensions contain either aluminium hydroxide, magnesium hydroxide & simethicone or magaldrate preparation as basic ingredients which may subject to microbial spoilage due to low-cost production procedure as well as improper storage and distribution systems [6]. The pharmaceutical manufacturing and packaging environment, raw materials as well as the manufacturing water may attribute to the microbiological spoilage of the finished products [7-11]. 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 [12, 13]. 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 [4-6, 14-16]. Along these lines, the current study was designed to determine the status of these non-prescribing antacid suspensions manufactured by different national pharmaceuticals to improve the present scenario.

METHODS AND MATERIALS

Study area, sampling and sample processing

Eight samples of antacid liquid drugs (coded A1-A8) with valid manufacturing and expiry dates were collected during November 2013 to December 2013. These oral suspensions were collected from different retailer drug stores in Dhaka city, Bangladesh and then transported to the laboratory to determine the microbiological quality of the respective drugs. The total bacterial and fungal load as well as presence of specific pathogens was determined following the standard methods. [3, 16, 17]

Enumeration of total viable bacterial and fungal count

For enumerating the total viable bacterial and fungal load, 0.1 ml of each antacid suspensions was spread onto Nutrient agar (NA) and Sabouraud Dextrose agar (SDA) consecutively [18, 19]. The NA plates were then incubated at 37°C for 24 hours whereas the SDA plates were incubated at 25°C for 48 to 72 hours.

Enumeration of specific pathogens

From each sample, 0.1 ml of suspension was spread onto MacConkey agar, mannitol salt agar (MSA), pseudomonas agar media for the isolation and enumeration of *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., respectively. For the isolation of *Salmonella* spp. and *Shigella* spp. the samples were at first enriched in selenite cystine broth and then 0.1 ml of enriched suspension from each sample was spread onto Salmonella- Shigella (SS) agar [20, 21]. All the plates were incubated at 37°C for 24 hours. The isolated strains were further identified by following confirmative biochemical tests [17, 18].

Antimicrobial activity against pathogens

Eight antacid liquid drug samples were checked for antimicrobial activity against eight pathogenic laboratory isolates *E. coli*, *Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Vibrio* spp., *Listeria* spp.,

Klebsiella spp. and *Salmonella* spp. Bacterial lawns were prepared on Muller-Hinton agar plates by adding 100 µL of the suspensions of the test microorganisms and spreading with sterile cotton swabs. To assess the antimicrobial activity of antacid drugs, 0.1 ml (11µg//µL) of each sample was loaded in the wells [3]. Streptomycin 10 µg and normal saline were used as positive control and negative control, respectively.

RESULTS AND DISCUSSION

Undesirable access of microorganisms into the pharmaceutical products is a global health concern [1, 3, 5, 15, 17, 22-24]. Commencement of microbiological contamination in non-sterile pharmaceutical liquid drugs is a matter of concern in perspective of Bangladesh due to low cost drug manufacturing process, use of contaminated water & raw materials as well as inadequate care during storage and distribution [1, 12, 15, 16, 22]. As a result microbial quality assessment of these commonly used oral suspensions has become very much crucial. While several reports for microbiological assessment of the finished products of the topicals, oral liquids and sterile liquids manufactured in Bangladesh exist, work on the common antacid suspensions has not been done to the significant extent [4, 6, 15, 16].

However, a study revealed the existence of bacteria in the antacid suspension exceeding the limit in 5 out of 8 samples [25]. In our study, 8 liquid antacid suspensions of 7 different pharmaceutical companies were tested, and surprisingly all the samples were found to be heavily contaminated with bacteria and fungi (Table 1) exceeding USP limit ($<10^2$ cfu/ml) [11].

Table 1: Microbial load in the antacid suspensions available in Dhaka city, Bangladesh

Sample code	Active Ingredients	Total Viable Bacterial Count	Total Fungal Count	*E. coli*	*Staphylococcus* spp.	*Pseudomonas* spp.	*Salmonella* spp.	*Shigella* spp.
A-1	$Al(OH)_3$, $Mg(OH)_2$ & Simethicone	$9.3x10^5$	$2x10^4$	0	$2x10^4$	0	0	0
A-2	$Al(OH)_3$, $Mg(OH)_2$ & Simethicone	$2.9x10^5$	$2x10^4$	0	$3x10^4$	0	0	0
A-3	Magaldrate & Simethicone	$2.8x10^5$	$9.3x10^5$	0	$1.5x10^5$	0	0	0
A-4	$Al(OH)_3$, $Mg(OH)_2$ & Simethicone	$2x10^5$	$2x10^4$	0	$4x10^4$	0	0	0
A-5	Magaldrate preparation	$7.2x10^5$	$2x10^4$	0	$2x10^4$	0	0	0
A-6	$Al(OH)_3$, $Mg(OH)_2$ & Simethicone	$8.6x10^5$	$4x10^4$	0	$3x10^4$	0	0	0
A-7	$Al(OH)_3$, $Mg(OH)_2$ & Simethicone	$4x10^5$	$6x10^4$	0	$9x10^4$	0	0	0
A-8	$Al(OH)_3$ & $Mg(OH)_2$	$5x10^5$	$1x10^4$	0	$4x10^5$	0	0	0

Table 2: Antibacterial activity of antacid suspensions against specific pathogens

Samples	Zone of inhibition (mm) against pathogenic microorganisms							
	E. coli	*Bacillus* spp.	*Staphylococcus* spp.	*Pseudomonas* spp.	*Vibrio* spp.	*Listeria* spp.	*Klebsiella* spp.	*Salmonella* spp.
A-1	0	23	0	0	0	0	0	0
A-2	0	20	0	0	0	0	0	0
A-3	0	17	0	0	0	0	0	0
A-4	0	0	0	0	0	0	0	0
A-5	0	0	0	0	0	0	0	0
A-6	0	0	0	0	0	0	0	0
A-7	0	0	0	0	0	0	0	0
A-8	0	19	0	19	15	0	8	0
Positive control (Streptomycin 10 µg)	22	21	21	30	19	17	0	18
Negative control (Saline)	0	0	0	0	0	0	0	0

USP specifications

Total viable bacteria: $<10^2$ cfu/ ml

Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and *Salmonella* spp. were absent.

Presence of pathogenic microorgisms were also checked out and *E. coli*, *Salmonella* spp., *Shigella* spp. and *Pseudomonas* spp. were found to be absent in all antacid suspensions where as *Staphylococcus* spp. remained present in all samples. The load of *Staphylococcus* spp. was quite high (up to 10^5 cfu/ml) in the tested samples indicating poor handling of raw materials, ingredients and final products; however, absence of coliforms in all 8 samples was a sign of lacking fecal contamination. Lack of manufacturing environmental control and sanitation, insufficient equipment cleaning, unhygienic practices might be responsible for such commencement of contaminating microorganisms. The high microbial load in the antacid suspensions suggests the condition for antacid drug preparation in the respective pharmaceutical industries or the performance of the preservatives used in these drugs was not satisfactory. As antacid suspensions contain organic ingredients which support the rapid microbial growth, addition of effective antimicrobial preservatives is expected to maintain the product quality [1, 2].

Though antacid drugs are mainly used to neutralize the acidic pH condition in the gastroenteric system, preservatives used in these drugs may play role to inhibit the growth of several pathogenic microorganisms as well [26]. In the current study, 50% (4 out of 8 samples) tested antacid suspensions exhibited antimicrobial activity against *Bacillus* spp. The surprising result observed in case of the antacid suspension A-8 which showed inhibitory action against 4 types of pathogens including *Klebsiella* spp. against which the positive control (Streptomycin 10 µg) itself failed to show any antimicrobial activity (Table 2). All these sampled showed no inhibitory activity against *Staphylococcus* spp. which appeared to be the most prevailing species among these drugs.

CONCLUSION

The results of our study indicated a substantial degree of microbiological contamination of all the samples which might further lead to medication complicacy among the patients. Besides, the complete absence of anti-bacterial traits in half of the samples tested is also indicative of the probable lack of the potency of the active ingredient. Therefore, appropriate chemical examination of the raw materials with preservative efficacy testing is required prior to manufacturing. Moreover, besides the proper storage of the ingredients, monitoring of antacid spoiling microorganisms through regular surveillance may attribute to the betterment of the overall product quality.

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CONFLICT OF INTEREST

Authors have declared no conflict of interest.

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