International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685; Impact Factor: RJIF 5.28
Received: 06-08-2019; Accepted: 08-09-2019
www.pharmacyjournal.net
Volume 4; Issue 4; October 2019; Page No. 38-40

Assessment of microbiological quality and anti-bacterial traits of oral liquid drugs used in Bangladesh

Md Aminul Islam Apu[1], Shamsun Nahar Jolly[2], Mohammad Minnatul Karim[3*]
[1-3] Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Islamic University, Kushtia, Bangladesh

Abstract
Microbial contamination into the pharmaceutical products is not unlikely due to a number of parameters including the imperfect good manufacturing practice as well as the imperfect total quality management. This study aimed to assess the microbiological quality and anti-bacterial traits of oral liquid drug used in Bangladesh. Nine (9) different types of liquid oral drugs were selected to determine the microbial load, presence of common pathogenic bacteria and antibacterial trait. Microbiological analysis was carried out using spread plate technique on different culture media including nutrient agar, Potato dextrose agar, MacConkey agar, EMB agar, Pseudomonas agar, SS agar and Mannitol salt agar for the determination of total viable bacteria, total fungi, total coliform, fecal coliform, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* respectively. Anti-bacterial traits were tested against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* spp. using well diffusion method. The results revealed that all of the tested samples showed compliance with the official requirement of microbial quality as they did not show any common pathogenic organisms or did not show any growth or their count is within the USP permissible limit ($<10^2$ CFU/ml). Five (5) samples are exhibited the anti-bacterial activity as they showed zone of inhibition against almost all pathogens tested.

Keywords: oral liquid drug, antibacterial trait, Bangladesh

Introduction
Pharmaceutical drug have long been used to fight different disease in human [1]. Varieties of liquid drug are used such as the aqueous solutions, suspension, emulsions and syrups for the pediatric patient's. Liquid drugs can serve as substrate for microorganisms. Microbiological contamination of pharmaceutical liquid products may ultimately play role in secondary bacterial infections [1]. Due to the presence of high amount of sugars and moisture, oral liquid drugs may provide suitable environment and even may serve as nutrients for the growth and survival of both pathogenic and nonpathogenic microorganisms [2].The pharmaceutical manufacturing and packaging environment, raw materials as well as the manufacturing water may contribute to the microbiological spoilage of the finished products. To reduce the public health risk, it is very much essential to monitor microbial quality of the finished products [3]. Different microorganisms can be present in oral liquid drugs including the harmful ones. Children who are comparatively of weak immune system may be further affected upon consumption of such drug, especially due to the existence of drug resistant bacteria in the consumed drugs [2]. In this context, determination of the microbiological contamination level in oral liquid drug including presence of pathogenic microorganisms and antibacterial trait was attempted

Materials and methods
Collection of samples
Nine different liquid oral drugs samples (Table 1) were collected from different retailer drug stores in the local market Shekhpara, Garagonj, Jinaedha and Kushtia during April 2016 – June 2016, and were subjected to microbiological examination. Collected Oral drugs were kept safely to avoid the microbial contamination.

Table 1: List of collected oral liquid drugs used in the study

Sample type	Sample code	Active Compound
Anti-Diarrheal drug	D1	Metronidazole
	D2	Metronidazole
	D3	Metronidazole
	D4	Metronidazole
	D5	Nitazoxanide
Antipyretic syrup	O1	Paracetamol
	O2	Paracetamol
Cough syrup	O3	Guaiphenasine
Eye drop	O4	Chloramphenicol

Enumeration of total viable bacterial and fungal count
Spread plate technique was used to enumerate the microbial contaminant from the collected syrup samples. 100 µl from each sample was withdrawn aseptically, spread onto Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media, incubated at 37ºC for 24 hours to enumerate the total viable bacteria (TVB) and total fungal load.

Enumeration of specific pathogens
100 µl of each sample was spread onto MacConkey agar, EMB agar, Pseudomonas agar, SS agar and Mannitol salt agar for the determination of total viable bacteria, total fungi, total coliform, fecal coliform, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* respectively. All the plates were incubated at 37ºC for 72 hours to enumerate the specific pathogen.

Assay of antimicrobial activity

Antimicrobial activity of oral liquid was determined by the well diffusion technique [2] against *E. coli*, *Pseudomonas aeruginosa*, *and Salmonella* spp. Single colony of each bacterium were grown in Luria Bertani (LB) broth separately for 18-24 h and then used to prepare the bacterial inoculums with the turbidity of 0.5 McFarland standard (equal to 1.5×10^8 CFU)/ml). Turbidity of the bacterial suspension was measured at 600 nm. The bacterial inoculum was spread onto Mueller-Hinton agar (MHA) plates (150 mm diameter) using sterile cotton swabs as a lawn culture. The wells (9 mm diameter) were made by using cork borer in MHA plates. Each well was loaded with different volume of oral liquid drug and Plates were incubated at 37ºC for 24 hours. The diameters of zones of inhibition around the wells were measured in millimeter (mm). The experiment was performed in triplicates.

Results

Prevalence of total viable bacteria, fungi and specific pathogens

From the current study, the tested samples were found free any specific pathogens as well as from bacteria and fungi (Table 2) though the prevalence of *Staphylococcus spp. and Pseudomonas spp.* is possible during the preparation of drugs and hence indicates that the microbial quality of the products is in line with the recommended standard [1,3,4,5,6,7,8].

Antimicrobial trait of oral liquid drugs

Five samples were tested and exhibited the anti-bacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *and Salmonella* spp. All of the tested samples had shown the antibacterial activity (Table 3).

Table 2: Prevalence of Microorganisms in Liquid Oral Drugs

Samples Code	Total Count CFU/ml	Total Fungal Count CFU/ml	Total Coliform Count CFU/ml	Fecal Coliform Count CFU/ml	*Peudomonas* spp. CFU/ml	*Salmonella* spp. CFU/ml	*Shigella* spp. CFU/ml	*Staphylococcus aureus* CFU/ml
D1	No	No	No	No	No	No	No	No
D2	No	No	No	No	No	No	No	No
D3	No	No	No	No	No	No	No	No
D4	No	No	No	No	No	No	No	No
D5	No	No	No	No	No	No	No	No
O1	No	No	No	No	No	No	No	No
O2	No	No	No	No	No	No	No	No
O3	No	No	No	No	No	No	No	No
O4	No	No	No	No	No	No	No	No

Table 3: Anti-bacterial activity of the tested oral liquid drugs

Bacteria	Sample Code	Zone of Inhibition (mm) against different dose				
		25µl	50µl	75µl	100µl	125µl
Escherichia coli	D1	20	60	80	100	100
	D2	30	50	35	30	40
	D3	100	100	80	75	100
	D4	20	35	100	60	90
	D5	60	100	100	80	90
Pseudomonas aereginosa	D1	20	10	10	30	30
	D2	20	30	20	60	80
	D3	10	10	15	65	100
	D4	40	20	30	40	80
	D5	20	10	10	30	30
Salmonella spp.	D1	10	20	30	10	30
	D2	42	30	25	80	100
	D3	30	30	50	65	80
	D4	50	20	60	55	100
	D5	105	130	200	200	200

Discussion

Pharmaceutical products like liquid drugs are commonly used in many different ways in the prevention, treatment and diagnosis of diseases. In recent years, quality of the pharmaceutical products has been improved in Bangladesh by many manufacturers to minimize the bio-burden. Administration of contaminated oral liquid drugs can be harmful to the recipients such as, young and elderly patients. Survival and growth of microorganisms can deteriorate the product quality and production of metabolites/toxins may be harmful to the patient even they are present in minute quantities [9] Presence of such microbial contaminants become major health concern when their number exceeds the acceptable limit (10^2 CFU/ml) recommended by the USP [10]. Microbial contamination in non-sterile oral drugs rather claimed more significance as the patients, who are taking the drug, are already diseased. Therefore, it is very necessary to examine the efficacy and/or potency of some drugs those are very commonly used for the diseases medication. However, microbiological studies on oral liquid drugs in Bangladesh are still in infancy. There are several reports [10, 11, 12] concerning the microbiological contamination of the pharmaceuticals products like liquid oral drugs etc. have been published though all the syrup samples used in this study were still within their shelf lives when the analysis was carried out. The study findings revealed that all oral syrup samples were found free from pathogenic microorganisms including *E. coli*, *Salmonella*

spp. and *Pseudomonas aereginosa* (Table 2). From the current study, all of the tested samples were found to be highly free from bacteria and fungi (Table 2). The presence of total viable bacteria in liquid oral drug samples showed the bacterial prevalence in the samples was within the limit specified by USP or BP. Examination for the presence of Gram negative pathogens showed the complete absence of fecal coliforms and other pathogens. All anti-diarrheal syrups exhibited the anti-bacterial activity against *E. coli, Salmonella spp.* and *Pseudomonas aereginosa* (Table 3).

Conclusion

In Bangladesh many drugs are produced largely, some of them are solid and some of the drugs are in liquid form. Assessment of microbiological quality and the anti-bacterial traits of liquid oral drugs used in local market of Bangladesh were carried out in this work. It might conclude that tested drugs were not contaminated with any kinds of bacteria or pathogen and these drugs don't have any adverse effect on health.

References

1. Khanom S, Das KK, Banik S, Noor R. Microbiological analysis of liquid oral drugs available in Bangladesh. International journal of pharmacy and pharmaceutical sciences. 2013; 5(4):479-482.
2. Fatema K, Chakraborty SR, Sultana T, Rahman MM, Kamali NM, Das KK, Noor R. Assessment of microbiological quality of the pediatric oral liquid drugs. Journal of Pharmacognosy and Phytochemistry, 2014; 3(1):165-171.
3. Urmi NJ, Noor R. Microbiological profile and the anti-bacterial traits of commonly available antacid suspensions in Dhaka metropolis. Int J Pharm Pharm Sci. 2014; 6(4):174-6.
4. Hugo WB, Russel AD. Pharmaceutical Microbiology. 6th ed. Oxford, UK: Blackwell Scientific Publications; 1998
5. Gad GFM, Aly RAI, Ashour MSI Microbial Evaluation of Some Non-sterile Pharmaceutical Preparations Commonly Used in the Egyptian Market. Trop. J. Pharm. Res., 2011; 10(4): 437-445.
6. Braide W, Nwosu IL, Offor EIU, Popgbara LB, Awurum IN. Microbial quality of some topical pharmaceutical products sold in abia state, Nigeria. International journal of pharmacology and therapeutics, 2012; 2 (4): 26-37.
7. Dabbah R, Knapp J, Sutton S. The role of USP in the assessment of microbiological quality of pharmaceuticals. Pharmaceutical Technology, 2001; 25: 54–60.
8. Parker MS. Microbiological contamination and preservation of pharmaceutical preparations. In: Aulton ME, editor. Pharmaceutics: The Science of Dosage from Design. Hong Kong, China: Churchill Livingstone; 2000.
9. Nester MT, Anderson DG, Roberts CE, Jr, Pearsall NN. Microbiology – A Human Perspective. Genitourinary Infections and Antimicrobial Medications. Edn 3, Madrid, Spain: MacGraw Hill, 2002.
10. Carstersen JT, Rhodes CT. Drug Stability: Principles and Practices. Edn 3, New York: Marcel Dekker, 2000.
11. Abdullah Al Mamun, Tapash Kumar Shaha, Md. Murad Khan, Md. Shahidul Kabir. Determination of Microbial Load in Multivitamin and Cough Syrups Sold in Dhaka City. International Journal of Pharmaceutical Sciences and Drug Research, 2014; 6(3): 235-238.
12. Mohammad Moniruzzaman, Md. Farukh Faisal Ashraf and Zakaria mia. Qualitative and quantitative microbiological studies of antacid and paracetamol suspensions from different drugstores of Dhaka, Dhaka Univ. J. Biol. Sci. 2012; 21(1):105107.