Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 3 (1): 165-171
Received: 12-04-2014
Accepted: 30-04-2014

Kaniz Fatema
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh.
Email: kanizema@yahoo.com,
Tel: +8801681625732.

Sowmitra Ranjan Chakraborty
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh.
Email: sowmitra.bd@gmail.com,
Tel: +8801735351435.

Tohora Sultana
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh.
Email: esheta_10@yahoo.com,
Tel: +8801683755322.

Md. Mokhlesur Rahman
Department of Pathology and Microbiology, Pioneer Dental College, Baridhara, Dhaka 1229, Bangladesh.
Email: bd.mokhlesurrahman@gmail.com,
Tel: +8801742095047

Niaz Muhammad Kamali
Family Planning Directorate, Ministry of Health & Family Welfare, Saturia, Manikgonj, Bangladesh.
Email: niaz.kamali64@gmail.com,
Tel: +8801715017127

Kamal Kanta Das
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road Dhaka, Bangladesh.
Email: kkanta_36@yahoo.com,
Tel: +8801740563446

Rashed Noor
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road Dhaka, Bangladesh.
Email: noor.rashed@yahoo.com,
Tel: +8801749401451

Correspondence
Rashed Noor
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road Dhaka, Bangladesh.
Email: noor.rashed@yahoo.com,
Tel: +8801749401451.

Assessment of microbiological quality of the pediatric oral liquid drugs

Kaniz Fatema, Sowmitra Ranjan Chakraborty, Tohora Sultana, Md. Mokhlesur Rahman, Niaz Muhammad Kamali, Kamal Kanta Das, Rashed Noor*

ABSTRACT
Present study performed a time course analysis of microbiological contamination level within the pediatric oral liquid drugs commonly available in Dhaka metropolis. Employing cultural and biochemical methods of microbial isolation, the contaminating microorganisms were quantified both from the fresh drugs in sealed bottles and also after 21 days of drug usage. All 20 samples were found to be populated with huge bacteria which were further found to be heightened after 21 days. While fungal presence was nominal during the initial assessment, most of the samples were found to support the fungal growth after 21 days. Staphylococcal growth was observed to be the most predominant after 21 days, followed by the growth of *Klebsiella* spp., *Pseudomonas* spp. and *E. coli*. The study of antibiogram revealed that most of the bacterial isolates were found to be resistant against ampicillin 10 μg, ciprofloxacin 5 μg, amoxicillin 10 μg and piperacillin 10 μg. Other drugs, including ciprofloxacin 5 μg, gentamicin 10 μg, nalidixic acid 30 μg, ceftriazone 30 μg, chloramphenicol 10 μg, erythromycin 15 μg, trimethoprim-sulfamethoxazole 25 μg and erythromycin 15 μg were also found to be ineffective against the bacterial isolates to a moderate extent. Based on the findings of the increase in microorganisms along with the time of usage as well as the proliferation of drug-resistant bacteria with the pediatric drug samples tested, a stringent regulation on the regular microbiological assessment of the drugs is of significance for the sake of consumer safety as well as for the overall public health management.

Keywords: Pediatric oral liquid drugs; Microorganisms; Drug-resistance; Consumer safety; Hygiene; Public health.

1. Introduction
Microbial propagation into the pharmaceutical products is not unlikely due to a number of microbial growth influencing parameters including the imperfect good manufacturing practice (GMP) together with the casualty in the total quality management (TQM) especially in the local pharmaceutical industries in the developing countries [1-5]. A huge number of children in the developing countries suffer from the fatality which is unfortunately related to the medical complications, mostly due to lack of unhygienic handling of drugs in oral dosage forms and due to the inappropriate storage condition which in turn accelerates the growth of a range of bacteria and fungi within these non-sterile drugs [1, 3, 6-9]. Oral liquids such as the aqueous solutions, suspensions, emulsions and syrups are used for the pediatric patients [4-5]. Such drugs may provide suitable environment and even may serve as nutrients for the growth and survival of both pathogenic and non-pathogenic microorganisms as they consist of high amount of sugars and moisture [3, 8-10].
In Bangladesh, the quality of drugs in local pharmaceuticals varies significantly and most of them are poorly manufactured [1, 11-13]. Contaminated raw materials and manufacturing water consisting of microbial bio-burden or pyrogens could be a virtuous source of bacterial and fungal contaminations in the pharmaceutical products [1, 3, 4, 5, 14, 15]. Besides, due to the Governmental legislative regulations, especially in the developing countries, a large number of unlicensed and illegal drug stores are selling poorly manufactured pharmaceutical products [1, 3, 16]. For the consumer safety issues, regular examination of the microbiological quality of the products purchased from such stores is of essence.
Oral liquids are usually non-sterile and therefore are likely to harbor different microorganisms, including the harmful ones [3, 4, 12, 17-21]. In this context, children who are comparatively of weak immune system may be further affected upon consumption of such drugs, especially due to the existence of drug-resistant bacteria in the consumed drugs [22].

Another vital issue of the propagation of drug contaminating bacteria lies on the hygienic usage of drugs. Along these lines, present study attempted (1) to determine the initial microbiological contamination level in the fresh pediatric drugs commonly used, (2) to demonstrate the extent of contamination during usage up to 21 days in order to project on the hygienic handling of the drugs, and finally (3) to detect the existence of drug-resistant bacteria in the samples.

2. Materials and Methods

2.1. Study area, sampling and sample processing

Twenty (20) types of oral liquid drugs with appropriate dates of manufacturing and expiry were collected from different retailer drug stores in Dhaka city, and were transported to the Microbiology Laboratory to assess their microbiological quality. In this context, serial dilution were prepared up to 10^{-3} following the standard methods as described earlier [1, 3, 23]. Spread plate techniques were used for the estimation of total bacterial and fungal load and also to detect the presence of pathogenic microorganisms [1-3, 23].

2.2. Enumeration of total viable bacteria and fungi

An aliquot of 0.1 ml of each sample from the dilution 10^{-3} was spread onto the nutrient agar (NA) plates for enumerating total viable bacteria count (TVB) and on Sabourad's dextrose agar (SDA) plates for the estimation of fungal load [1-3, 23]. Plates were incubated at 37 ºC for 18 to 24 hours and at 25 ºC for 48 to 72 hours, respectively.

2.3. Enumeration of specific bacterial pathogens

From the dilution of 10^{-3} of each sample, 0.1 ml of suspension was spread onto the membrane fecal coliform (MFC) agar, MacConkey agar, mannitol salt agar (MSA), and cetrimide agar for the isolation and quantification of total fecal coliform, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., and *Pseudomonas* spp., consecutively [1-3, 23-25]. MFC agar plates were incubated at 44.5 ºC for 18-24 hours, while the other plates were incubated at 37 ºC for 24 hours. Presence of *E. coli* was further detected on the eosine-methylene blue (EMB) agar as described previously [2, 23].

2.3.1. Enrichment for *Salmonella* spp. and *Shigella* spp.

Since the cells of *Salmonella* spp. and *Shigella* spp. could be in stressed condition or in viable but non-culturable condition probably due to some endogenous and exogenous factors, enrichment was performed for *Salmonella* spp. and *Shigella* spp. in the selenite cystine broth (SCB) [1, 11, 26-28]. After enrichment, samples were serially diluted up to 10^{-3} from each of the enriched broth (4-6 hour old, 37 ºC), and 0.1 ml of suspension were spread from the dilution of 10^{-3} onto *Salmonella-Shigella* (SS) agar, for the isolation of S*almonella* spp. and *Shigella* spp., and incubated at 37 ^{0}C for 24 hours.

2.4. Study of antibiogram

To demonstrate the susceptibility pattern of the bacterial isolates to the common antibiotics, the study of antibiogram was performed using on Mueller-Hinton (MH) agar (Difco, Detroit, MI) against ampicillin (10 µg), amoxicillin (30 µg), ciprofloxacin (5 µg), ceftriazone (30 µg), nalidixic acid (30 µg), imipenem (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), trimethoprim/sulfamethoxazole (25 µg), gentamicin (10 µg), and piperacilin (10 µg) by modified Kirby-Bauer method [29, 30]. Cell suspensions of the bacterial isolates were prepared using MH broth, and after a brief incubation for 4 hours (with the appearance of the turbidity equivalent to the 0.5 McFarland standard), bacterial lawns were prepared over the MH agar, and the antibiotic discs were placed aseptically over the surface. After 12-18 hours of incubation, plates were examined and the diameters of the zones of inhibition were measured (in mm) as described by Ferraro *et al*., 2001 [31].

3. Results and Discussion

Diseased infants and children are often immune-compromised and can be easily affected by the spoilage microorganisms prevailing in the oral liquid drugs used for their medication purposes. The immune-compromised children consuming the microbiologically contaminated drugs might be subject to potential life risk as well as to the heightening of the chance of opportunistic infections. Current investigation thus assessed the growth and proliferation of microorganisms in oral liquid drugs commonly used by the children, and also emphasized upon the extent of microbial contamination upon usage of the drugs in a time course manner.

3.1. Prevalence of microorganisms in the pediatric oral drugs tested

Initially all the samples were populated with the total bacterial load of 10^3-10^4 cfu/ml while after 21 days, the bacterial prevalence was noticed to be increased up to 10^6-10^7 cfu/ml revealing an increase by approximately 3-log in average (Tables 1 and 2). Fungal presence at the initial stage of microbiological assessment was noticed only in one sample, while after 21 days, 16 samples got contaminated with fungi with a bio-burden of 10^2-10^3 cfu/ml.

Among the pathogenic bacteria, staphylococcal growth was the most predominant (Tables 1-3). While the initial staphylococcal load was observed in 11 samples (10^2-10^3 cfu/ml), after 21 days, except one sample, all were found to be populated with *Staphylococcus* spp. within a range 10^2-10^5 cfu/ml (Tables 1-3). The contamination observed by *Staphylococcus* spp. might occur due to the lack of aseptic handling of the products as well as due to the personnel hygiene; however, their presence is indeed not always regarded as harmful [32-37]. Nevertheless, the staphylococcal presence could be regarded a very sufficient indication of unhygienic handling of products which in turn may trigger the propagation of other bacteria into the oral drugs.

In case of *Klebsiella* spp., initially the prevalence was observed in 6 samples while the prevalence of this bacterium was found to be extended in 10 samples after 21 days (10^2 cfu/ml in 7 samples and 10^3 cfu/ml in 3 samples). *Pseudomonas* spp. was found to be present in 4 samples during the initial assessment (10^2 cfu/ml); however, after 21 days, 10 samples were found to harbor this pathogenic bacterium with a load of 10^3-10^4 cfu/ml. The initial load of *E. coli* was observed only in one sample while the frequency widened in 9 samples after 21 days with a bio-bio-burden of 10^2 cfu/ml in 8 samples and 10^3 cfu/ml in one sample. *Klebsiella* spp. is capable of producing pneumonia and gastroenteritis disease like diarrhea, and hence a careful handling of products during consumption is needed. The possible sources of *Klebsiella* spp. might be due to the untreated raw materials and the unhygienic storage facilities [5].

As found in our study, the presence of *E.* and *Pseudomonas* spp. in the samples tested could be a major health risk concern [32, 35, 37-39]. Being an enteric microorganism, *E. coli* may be responsible for the gastroenteritis like bloody and watery diarrhea, followed by the onset of post-diarrheal hemolytic uremic syndrome (HUS), a type of kidney failure especially in children under 5 years old. *Pseudomonas* spp. is an opportunistic human pathogen, most commonly affecting immune-compromised patients, such as those with cystic fibrosis or AIDS [33, 34, 36]. The source of these two bacteria might be the contaminated raw ingredients, manufacturing water, lack of hygienic manufacturing environment, and finally due to the propagation during drug usage as has been observed through our time course study of microbial increase (Tables 1and 2).

Table 1: Initial microbiological load of the oral pediatric drugs.

Samples	TVB (cfu/ml)	Total fungal count	*Escherichia coli* (cfu/ml)	*Klebsiella* spp. (cfu/ml)	*Staphylococcus* spp. (cfu/ml)	*Pseudomonas* spp. (cfu/ml)
Sample 1	2.3×10^3	0	0	0	0	0
Sample 2	5.3×10^3	0	0	0	0	0
Sample 3	1.1×10^3	0	0	0	0	0
Sample 4	1.5×10^4	0	0	0	5.0×10^3	0
Sample 5	2.1×10^4	0	0	8.0×10^2	1.7×10^2	1.2×10^2
Sample 6	1.0×10^4	0	0	1.0×10^2	5.0×10^2	0
Sample 7	17×10^4	0	0	1.1×10^2	3.0×10^2	0
Sample 8	11×10^4	0	0	1.1×10^2	1.1×10^3	0
Sample 9	9.2×10^3	0	0	6.0×10^2	2.0×10^2	0
Sample 10	1.9×10^4	0	0	2.7×10^2	0	0
Sample 11	7.5×10^4	0	4.0×10^2	0	2.0×10^2	0
Sample 12	9.9×10^4	0	0	0	0	0
Sample 13	9.2×10^4	0	0	0	0	0
Sample 14	1.2×10^5	0	0	0	1.0×10^2	2.3×10^2
Sample 15	2.2×10^4	0	0	0	1.0×10^2	0
Sample 16	2.6×10^4	1.2×10^1	0	0	2.2×10^2	7.0×10^2
Sample 17	7.2×10^3	0	0	0	0	0
Sample 18	3.6×10^3	0	0	0	0	0
Sample 19	4.6×10^4	0	0	0	1.0×10^2	1.2×10^2
Sample 20	5.6×10^3	0	0	0	0	0

The experiment has been done in triplicate and the results were reproducible. One representative data has been shown.
*TVB = Total viable bacteria
Salmonella spp. and *Shigella* spp. were completely absent.

USP limit:
TVB $<10^2$ cfu/ml
Absence of specific bacterial pathogens.

Table 2: Microbiological load of the oral pediatric drugs after 21 days.

Samples	TVB (cfu/ml)	Total fungal count	*Escherichia coli* (cfu/ml)	*Klebsiella* spp.(cfu/ml)	*Staphylococcus* spp. (cfu/ml)	*Pseudomonas* spp. (cfu/ml)
Sample 1	1.2×10^7	5.5×10^3	0	0	5.0×10^5	0
Sample 2	7.5×10^6	4.5×10^3	0	0	1.1×10^4	0
Sample 3	1.0×10^7	9.0×10^3	0	0	2.2×10^5	0
Sample 4	2.0×10^6	7.5×10^2	0	0	1.9×10^4	0
Sample 5	4.0×10^6	1.0×10^2	0	2.4×10^2	1.2×10^4	1.4×10^3
Sample 6	1.4×10^6	4.6×10^2	2.0×10^2	5.0×10^2	2.2×10^3	2.7×10^3
Sample 7	2.3×10^6	7.0×10^2	1.3×10^2	1.0×10^2	4.5×10^2	2.2×10^3
Sample 8	1.2×10^6	0	0	4.5×10^2	8.8×10^4	0
Sample 9	8.0×10^7	3.5×10^2	1.0×10^2	1.0×10^2	6.5×10^2	8.0×10^3
Sample 10	5.0×10^7	1.5×10^2	1.2×10^2	2.0×10^3	3.0×10^4	0
Sample 11	1.2×10^6	2.0×10^2	5.0×10^2	7.0×10^3	1.4×10^4	3.5×10^3
Sample 12	6.0×10^7	6.5×10^2	0	6.0×10^2	1.8×10^2	2.3×10^3
Sample 13	4.0×10^6	2.0×10^3	0	0	0	7.0×10^4
Sample 14	2.7×10^6	0	1.0×10^2	0	2.9×10^2	3.8×10^3
Sample 15	1.4×10^6	0	0	0	2.8×10^4	0
Sample 16	2.0×10^6	0	0	0	1.0×10^3	2.6×10^3
Sample 17	9.0×10^7	6.4×10^2	1.0×10^2	0	2.0×10^3	0
Sample 18	1.0×10^7	7.0×10^2	2.0×10^3	1.0×10^2	1.0×10^3	0
Sample 19	2.0×10^7	2.4×10^2	4.0×10^2	1.0×10^3	1.5×10^3	1.7×10^3
Sample 20	2.0×10^6	1.3×10^2	0	0	1.0×10^3	0

The experiment has been done in triplicate and the results were reproducible. One representative data has been shown. *Salmonella* spp. and *Shigella* spp. were completely absent.
*TVB = Total viable bacteria

USP limit:
TVB $<10^2$ cfu/ml
Absence of specific bacterial pathogens.

Table 3: Confirmative biochemical identification of the isolates.

Assumed microorganisms	TSI			H_2S creation	Indole test	MR test	VP test	Citrate utilization	Motility	Oxidase test
	Slant	Butt	Gas							
E. coli	Y	Y	+	-	+	+	-	-	+	
Klebsiella spp.	Y	Y	+	-	-	-	+	+	-	
Pseudomonas spp.	R	R	-	-	-	-	-	+	+	+
Staphylococcus spp *sss*spp.	Y	R	+	+	-	+	-	+	+	-

Y Yellow (Acid)
R Red (Alkaline)
MR Methyl red
VP Voges-Proskauer

3.2. Drug-resistance traits of the pathogenic bacterial isolates

Bacterial resistance against the commonly used antibiotics has recently been reported to raise complications in effective medication [22, 40-42]. Such drug resistance trait of microorganisms is a stern hindrance in treating diseases. Among the pathogenic isolates found in our study, the *Pseudomonas* ones were found to be completely resistant against ampicillin 10 µg, ciprofloxacin 5 µg, amoxicillin 10 µg, and moderately resistant against gentamicin 10 µg (Table 4). Like the *Pseudomonas* isolates, the staphylococcal isolates also exhibited 100% resistance against ampicillin 10 µg and amoxicillin 10 µg; and such a complete resistance was also scored against piperacillin 10 µg. Besides, a moderate drug-resistance attribute of *Staphylococcus* spp. was noticed against ciprofloxacin 5 µg and gentamicin 10 µg. *Klebsiella* isolates were found to be moderately resistant against nalidixic acid 30 µg, ciprofloxacin 5 µg, ceftriazone 30 µg, chloramphenicol 10 µg and erythromycin 15 µg. *E. coli* isolates were exhibited a moderate degree of drug-resistance against ceftriazone 30 µg, and nalidixic acid 30 µg; while a weak extent of resistance was scored against ciprofloxacin 5 µg, chloramphenicol 10 µg, trimethoprim-sulfamethoxazole 25 µg, gentamicin 10 µg and erythromycin 15 µg (Table 4). Thus an array of common drugs was found to be ineffective against the pathogenic isolates, and in other words, almost all the isolates were multi-drug resistant (MDR) [43].

Table 4. Antimicrobial susceptibility pattern of the pathogenic isolates.

Isolates Antibiotics	*Escherichia coli* (n=9)		*Klebsiella* spp (n=8)		*Staphylococcus* spp. (n=16)		*Pseudomonas* spp. (n=7)	
	R	S	R	S	R	S	R	S
AMP (10 µg)	20%	80%	25%	75%	100%	0%	100%	0%
CIP (5 µg)	35%	65%	50%	50%	50%	50%	100%	0%
PIP (10 µg)	20%	80%	25%	75%	100%	0%	0%	100%
CEF (30 µg)	55%	45%	50%	50%	ND	ND	0%	100%
AMO (10 µg)	20%	80%	25%	75%	100%	1%	100%	0%
IPM (30 µg)	10%	90%	0%	100%	25%	75%	0%	100%
CHL (10 µg)	40%	60%	50%	50%	ND	ND	ND	ND
TMP-SUL (25 µg)	40%	60%	25%	75%	25%	75%	25%	75%
GEN (10 µg)	35%	65%	0%	100%	50%	50%	50%	50%
NALI (30 µg)	55%	45%	75%	25%	ND	ND	0%	ND
ERY (15 µg)	30%	70%	50%	50%	25%	75%	ND	ND

All the experiments have been done three times and the results were reproducible. One representative data have been shown.
AMP= Ampicillin, AMO= Amoxicillin, CIP= Ciprofloxacin, CEF= Ceftriazone, NALI= Nalidixic acid, IPM= Imipenem, ERY= Erythromycin, CHL= Chloramphenicol, TMP/SUL= Trimethoprim Trimethoprim- sulfamethoxazole sulfomethoxazole, GEN= Gentamicin, PIP= Piperaciline

ND Not done
N Number of isolates
R Resistant
S Sensitive

Overall, as resulted from our investigation, in order to combat against the microbial contamination in the oral drugs, proper implementation of good manufacturing practice (GMP) together with the total quality management (TQM) during product manufacturing in a microbiologically controlled environment, regular quality assessment during distribution and storage of the finished products, and finally the appropriate aseptic handling of the drugs during consumption would be effectual [4, 5, 8, 19, 20].

4. Conclusion

Pediatrics oral liquid drugs are for infants and children and therefore the presence of pathogenic microorganisms is undesirable. The current investigation revealed a huge extent of microbial contamination including the drug-resistant ones within the tested samples especially in course of their exposure during usage. Such an incidence is very much likely to lead the infants and the children to be easily infected by microorganisms propagated from these drugs during medication. Therefore, stringent regulatory actions on the microbiological quality control together with the personal hygienic improvement would be effective for the better management of the overall public health situation not only in Bangladesh but also in the other developing countries.

5. Acknowledgments

Authors are thankful to Stamford University Bangladesh for the logistic and financial supports.

6. Conflicts of Interest

Authors have declared no conflict of interest

7. References

1. Rana J, Sultana T, Das KK, Noor R. Microbiological analysis of topicals available in Bangladesh. Int J 2014; 6(2):330-332.
2. Das KK. Pharm and Pharma Sci Fatema KK, Nur IT, Noor R. Prevalence of microorganisms in commonly used cosmetics samples in Dhaka Metropolis. J Pharm Sci Innovat 2013; 2(6):7-9.
3. Khanom S, Das KK, Banik S, Noor R. Microbiological analysis of liquid oral drugs available in Bangladesh. Int J Pharm and Pharmaceutical Sci 2013; 5(4):479-482.
4. United States Pharmacopeia (USP). Microbiological examination of nonsterile products: Tests for specified microorganisms. Pharm Forum 2003; 29(5):1722–1733.
5. Hugo WB, Russell AD. Pharmaceutical microbiology. 6th ed. Oxford, UK: Blackwell Scientific Publications; 1998.
6. Hossain J. Importance of the bioburden test in pharmaceutical quality control. Pharmaceutical Microbiology Forum 2009; 15(1):2-14.
7. Shaikh D, Jamshed TA, Shaikh R. Microbial contamination of pharmaceuticals preparation. Pakistan J Pharm Sci 1988; 1(1):61-66.
8. Lund W. The Pharmaceutical Codex. Ed 12, London: The Pharmaceutical Press, 1994.
9. Lowe RA, Shaw RJS. Storage, stability and in-use shelf-life guidelines for non-sterile medicines. Eastern and South East London: Quality Assurance Service; 2001.
10. Adeshina GO, Ajayi S, ,Onaolapo JA. Microbiological quality of some commercially available paediatric anti-malarial and cough preparations in Ilorin, Nigeria. Niger J Pharm Sci 2009; 8(1):109–117.
11. Raton KA, Hossain MA, Acharjee M, Noor R. Assessment of microbiological quality and the anti-bacterial traits of sterile liquids used for medication of eye and ear infections in Bangladesh. Ame J Pharm Health Res 2013; 1(9):67-75.
12. Mugoyela V, Mwambete KD. Microbial contamination of nonsterile pharmaceuticals in public hospital settings. Therapeutics and Clinical Risk Management 2010; 6:443–448.
13. Nirmala MJ, Chandrasekaran N, Mukherjee A. Enhanced solubilization of aqueous insoluble anti-hypertension drug. Int J Pharm Pharma Sci 2012; 4(5):366-368.
14. Mwambete KD, Justin-Temu M, Fazleabbas SF. Microbiological assessment of commercially available quinine syrups and water for injections in Dar es Salaam, Tanzania. Trop J Pharm Res 2009; 8(5):441–447.
15. Kallings LO, Ringertz O, Silverstolpe L, Ernerfeldt F. Microbial contamination of medical preparation. Acta Phamaca Succica 1996; 3:219-228.
16. Espicom. The pharmaceutical market: Bangladesh, opportunities and challenges. Available at: http://www.espicom.com/prodcat2.nsf/Product_ID_Lookup /00000311? Open Document. 24 March, 2013.
17. Aulton ME. Pharmaceutics: The science of dosage form design. Ed 2, London, UK: Churchill Livingstone; 2002.
18. Moniruzzaman M, Ashrafi MFF, Mia Z. Qualitative and quantitative microbiological studies of antacid and paraceutamol suspensions from different drugstores of Dhaka. Dhaka Uni J Biolog Sci 2012; 21(1):105-107.
19. Halls N. Microbiological contamination control in pharmaceutical clean rooms. CRC Press, 2004.
20. Coker M. An assessment of microbial contamination during drug manufacturing in Ibadan, Nigeria. Eur J Scientific Res 2005; 7:19-23.
21. Bajaj S, Singla D, Sakhuja S. Stability Testing of Pharmaceutical Products. J Appl Pharma Sci 2012; 2(3):129-138.
22. Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ. A ten year analysis of multi-drug resistant blood stream infections caused by *Escherichia coli* & *Klebsiella pneumoniae* in a tertiary care hospital. Indian J Med Res 2012; 135(6):907-12.
23. Cappuccino JG, Sherman N. Microbiology- A laboratory manual. California: The Benjamin/Cummings Publishing Co. Inc.; 1996.
24. Acharjee M, Rahman F, Jahan F, Noor R. Bacterial proliferation in municipal water supplied in Mirpur locality of Dhaka city, Bangladesh. CLEAN- Soil, Air, Water, 2013. doi: 10.1002/clen.201200618.
25. Noor R, Acharjee M, Ahmed T, Das KK, Paul L, Munshi SK *et al*. Microbiological study of major sea fish available in local markets of Dhaka city, Bangladesh. J Microbiol Biotech Food Sci 2013; 2(4):2420-2430.
26. Rahman F, Noor R. Prevalence of pathogenic bacteria in common salad vegetables of Dhaka metropolis. Bang J Bot 2012; 41(2):159-162.
27. Oliver JD. The viable but non culturable state in bacteria. J Microbiol 2005; 43:93-100.
28. Colwell RR. Bacterial death revisited. In: Colwel RR, Grimes DJ, editors. Non-culturable microorganisms in the environment. Washington DC, USA: American Society of Microbiology, Washington DC; 2000, 325-342.
29. Munshi SK, Rahman MM, Noor R. Detection of virulence potential of diarrheagenic *Escherichia coli* isolated from surface water rivers surrounding Dhaka city. Journal of Bangladesh Academy of Sciences 2012; 36(1):109-122.
30. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45(4):493-6.
31. Ferraro MJ, Craig WA, Dudley MN. Performance standards for antimicrobial susceptibility testing, Ed 11, NCCLS, Pensylvania, USA, 2001.
32. 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.
33. Noor R, Saha SR, Rahman F, Munshi SK, Uddin MA, Rahman MM. Frequency of opportunistic and other intestinal parasitic infections among the HIV infected patients in Bangladesh. Tzu Chi Medical Journal 2012; 24(4):191-195.
34. Prüss-Üstün A, Corvalán C. Preventing disease through healthy environments: Towards an estimate of the environmental burden of disease. Geneva, Switzerland:

WHO Press, 2006.
35. Denyer SP, Hodges NA, Gorman SP, Hugo W, Russell A. Pharmaceutical microbiology. Ed 7, London, U.K., Blackwell Science, 2004.
36. Manu-Tawiah W, Brescia BA, Montgomery ER. Setting threshold limits for the significance of objectionable microorganisms in oral pharmaceutical products, PDA. J Pharm Sci Technol 2001; 55:171-175.
37. Jawetz E. Review on medicinal microbiology. Ed 17, Norwalk: CT, Appleton & Larpe, 1987.
38. Sarker N, Islam S, Hasan M, Kabir F, Uddin MA, Noor R. Use of multiplex PCR assay for detection of diarrheagenic *Escherichia coli* in street vended food items. American Journal of Life Sciences 2013; 1(6):267-272.
39. Beys L, Hoest B. Investigation for staphylococci in foods, dietetic products and oral drugs. Rev J Food Protec 1971; 25:26-33.
40. Khan Md. Anik A, Sutradhar P, Islam MM, Ojha RK, Biswas GC. Screening of antibiotic resistant gram negative bacteria and plasmid profiling of multi-drug resistant isolates present in sewage associated with health care centers. International Journal of Medical Research & Health Sciences 2013; 2(4):2319-5886.
41. Dutta S, Hasan MR, Rahman F, Jilani MSA, Noor R. Study of antimicrobial susceptibility of clinically significant microorganisms isolated from selected areas of Dhaka, Bangladesh. Bangladesh Journal of Medical Science 2013; 12(1):34-42.
42. Khan SA, Feroz F, Noor R. Study of extended spectrum β-lactamase producing bacteria from urinary tract infection in Dhaka city, Bangladesh. *Tzu Chi Medical Journal* 2013; 25(1):39-42.
43. Noor R, Hossain A, Munshi SK, Rahman F, Kamal SMM. Slide drug susceptibility test for the detection of multi-drug resistant tuberculosis in Bangladesh. Journal of Infection and Chemotherapy 2013; 19(5):818-824.