Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

MICROBIOLOGICAL ANALYSIS OF LIQUID ORAL DRUGS AVAILABLE IN BANGLADESH

SADIA KHANOM[1], KAMAL KANTA DAS[1], SOURAV BANIK[1] AND RASHED NOOR[1]*

[1]Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh.
Email: noor.rashed@yahoo.com

Received: 29 July 2013, Revised and Accepted: 30 Aug 2013

ABSTRACT

Objective: Present study attempted to determine the microbiological quality of the drugs commonly used for disease medication in Bangladesh.

Methods: Forty (40) different types of oral liquid drugs (26 syrups and 14 suspensions) manufactured in different pharmaceutical industries of Bangladesh were microbiologically examined using standard cultural and biochemical methods.

Results: All the samples except one syrup were found to be contaminated with total viable bacteria and fungi with a maximum load of 10^3 cfu/ml among which 4 syrup samples exceeded the United States Pharmacopeia (USP) limit (<10^2 cfu/ml). While the Gram negative bacteria were found to be completely absent in all samples, the prevalence of Gram positive bacteria including *Staphylococcus* spp. and *Bacillus* spp. were significant in both types of samples (the former in 24 syrups and 11 suspension samples, and the later species in 7 syrup and 4 suspension samples).

Conclusion: Existence of microorganisms in the oral liquid samples might explain the treatment complicacy of the diseased children. A routine microbiological study of such drugs is thus suggested.

Keywords: Oral drugs, Syrup, Suspension, Microbiological quality.

INTRODUCTION

Pharmaceutical drugs have long been used to eradicate an array of diseases in human [1]. Oral drugs, being non-sterile, are not unlikely to contain difference types of microbial species due to the impaired manufacturing condition [2-8]. Contamination (*Clostridium tetani*, *Pseudomonas aeruginosa*, fungi, viruses, etc.) from raw materials as well as the water used for the manufacturing of oral drugs may be associated with the unexplained treatment complications in patients [9, 10]. Thus the presence of contaminating microflora, especially when exceeding the acceptable limit of <10^2 cfu/ml in oral drugs, brings a major threat in public health measures [11].

Some of the dosage forms of oral drugs, if stored in favorable environment, can serve as substrates for microorganisms [4, 12, 13-14]. Moisture and high amount of sugar in the oral liquid drugs in particular can support the microbial growth. Oral liquid drug formulations such as aqueous solutions, suspensions, emulsions and syrups used for pediatrics are at a greater risk of microbial contamination during consumption due to sweetening agents, reconstitution methods, improper storage and handling defects [1, 15,16]. Microbial contaminations may ultimately contribute to secondary bacterial infections in pediatric patients [17].

Although pharmaceuticals is one of the dynamically growing and expanding sectors in Bangladesh, the quality of drugs available domestically varies significantly as they are mostly retail oriented. An anarchic situation prevails in the marketing and sales of drugs, due to a large number of illegal and unlicensed drug stores selling poorly-manufactured pharmaceuticals [18]. Therefore, regular examination of microbiological quality of the pharmaceutical products especially of the oral drugs which are administered mostly by the children is of significant demand regarding consumer safety. Unfortunately, a few studies have been carried out in Bangladesh in this perspective [4, 5] which is not proved to be sufficient in predicting the safety of the sold oral liquid drugs. Present study thus attempted to determine the microbiological quality of different types and categories of oral drug samples manufactured by different reputed pharmaceuticals, collected from retailer shops in Dhaka metropolis.

MATERIALS AND METHODS

Study area, sampling and sample processing

Forty samples of 14 different categories of oral liquid drugs (26 out of 7 categories of syrups and 14 out of 7 categories of suspensions) with appropriate dates of manufacturing and expiry were collected from different retailer drug stores in Dhaka city during January 2013 to June 2013. All samples were transported to the Microbiology Laboratory in order to assess their microbiological quality. The total bacterial and fungal load were estimated, and the presence (if any) of specific pathogens was detected [19, 20]. Membrane filtration techniques (MF) were used for assessing the filterable drugs [21]. In case of non-filterable concentrated drugs, serial dilutions were prepared up to 10^{-2} following the standard methods [19-21].

Enumeration of total viable bacteria and fungal count

Membrane filtration technique: Eighty ml of liquid filterable drugs (analgesic and antipyretic syrups and suspensions) were passed through a membrane filter (0.4 µm), which was then aseptically transferred on to nutrient agar and Sabouraud dextrose agar plates to determine the bacterial load and fungal load, respectively. Then the nutrient agar and Sabouraud dextrose agar plates containing the filter paper were incubated at 37°C for 18 to 24 hours and at 25°C for 48 to 72 hours, respectively.

Spread plate technique: An aliquot of 0.1 ml of each non-filterable suspension (samples other than antipyretic and analgesic drugs) from the dilution 10^{-2} was spread onto NA plate for enumerating total viable count (TVC) and on SDA plate for the estimation of fungal load [19-21]. The plates were incubated as previously stated.

Enumeration of total fecal coliform (TFC), *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp.

From the dilution of 10^{-2} of each sample, 0.1 ml of suspension was spread onto membrane fecal coliform (MFC), MacConkey agar, mannitol salt agar (MSA), cetrimide agar and phenol red egg yolk polymyxin (MYP) agar base media for the enumeration of total fecal coliform, *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. consecutively. All the plates were incubated at 37 ˚C for 24 hours except MFC agar which was incubated at 44.5 ˚C for 18-24 hours. Presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with green metallic sheen on the eosine-methylene blue (EMB) agar [21, 22].

Enumeration of *Salmonella* spp., *Shigella* spp., *Vibrio* spp. *and Clostridium* spp.

Initially no growth was observed for *Salmonella* spp., *Shigella* spp., and *V. cholerae* probably due to some endogenous and exogenous stressed or in viable but non-culturable state therefore, enrichment was performed for *Salmonella* spp. and *Shigella* spp. (in selenite cystine broth), and for *Vibrio* spp. (in alkaline peptone water) [20, 23-25]. After enrichment, the samples were serially diluted up to 10^{-2} from

each of the enriched broth (4-6 hour old, 37 °C), and 0.1 ml of suspension were spread from the dilution of 10^{-2} onto thiosulfate citrate bile salt sucrose agar (TCBS) agar and *Salmonella-Shigella* (SS) agar, for the isolation of *Vibrio* spp. and *Salmonella- Shigella spp.* respectively and incubated at 37 ˚C for 24 hours. In order to isolate *Clostridium* spp., each sample was mixed in sterile normal saline in a ratio of 1:8 and was heated at 80 °C for 15 minutes. Then 1 ml of heated suspension was allowed to grow in 9 ml fluid thioglycolate broth for 4 hours at 37 °C. Subsequently, 10-fold dilution was performed from 1 ml of enriched broth for pouring on *Clostridium* isolation agar plates, and was incubated at 37 °C within the anaerobic jar (2.5 L Anaero Jar, Oxoid Ltd., UK) for 48 hours [23]. For the final identification, the biochemistry of the isolates was tested following standard biochemical methods [19-21].

RESULTS AND DISCUSSION

In developing countries, the possibility of the disease incidence is very high due to the unstable environmental condition, poor hygienic practices, and consumption of contaminated food and water [26]. Smaller numbers of opportunistic pathogens become infectious when resistance mechanisms are impaired, either by severe underlying disease, or by use of immunosuppressive drugs [27, 28]. 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.

From the current study, all the tested samples were found to be highly contaminated with fungi (Table 1 & 2). The presence of total viable bacteria in 6 (23.7%) out of 26 syrup drug samples exceeded the USP limit (<10^2 cfu/ml); on the other hand, total aerobic plate count of all suspension samples did not cross the USP limit (<10^2 cfu/ml) (Table 1 & 2). *S. areues* were found to be present in 24 (92.3%) out of 26 syrup samples. Seven (26.9%) syrup samples were found to harbor *Bacillus* spp.

Examination for the presence of Gram negative pathogens including *E. coli, Vibrio* spp., *Salmonella* spp., *Shigella* spp. *Clostridium* spp. and *Pseudomonus* spp. were carried out, but fortunately they were completely absent from all the syrup samples (Table 1). Eleven (78.8%) and 4 (28.6%) samples out of total 14 suspension drugs were contaminated with *Staphylococcus* spp. and *Bacillus* spp. Total aerobic count and total fungal count of the oral suspensions were found to be in the range from 10^1 to 10^2. The highest total fungal load of ~10^3 cfu/ml was detected in cough syrup X-cold.

Raw materials, ingredients, unhygienic environmental condition and lack of aseptic handling would be the main factors for the observed microbial growths in the samples studies [13, 29-32]. To minimize the load of microbes and the possibility of spoilage during the preparation of liquid drugs, different antimicrobial agent or chemical preservatives (parabens, quarternary ammonium compounds, sorbic acids, formic acids etc.) may be used [33]. *Staphylococcus* spp. and *Bacillus* spp. might transmit from soil and hands of handler during the preparation of drugs, their incidence does not always mean that the consumption of drugs are potentially be hazardous to users as not all the strain of *Staphylococcus* spp. can necessarily produce enterotoxin and higher infectious dose (10^5-10^6 cfu/ml) of *Bacillus* spp. is required [30, 34]. Absence of coliform and pathogenic bacteria indicated that fecal contamination of water might not occur. Unhygienic environmental condition and improper handling of raw materials, ingredients and products might be the cause of contamination.

Table 1: Microbial load in the oral syrups tested

Sample Type	Sample name	Total aerobic plate count (cfu/ml)	Total Fungal Count (cfu/ml)	*E.coli*	*vibrio* spp.	*Salmonella & Shigella* spp.	*Clostridium* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.	*Bacillus* spp.
Analgesic & Antipyretic syrup	Ace	3.0×10^1	1.6×10^1	-	-	-	-	-	+	-
	Reset syrup	3.6×10^1	1.2×10^1	-	-	-	-	-	+	-
	Napa	3.5×10^1	2.0×10^1	-	-	-	-	-	+	-
	Xcel	1.3×10^3	2.4×10^2	-	-	-	-	-	+	-
	Renova	5.0×10^1	1.0×10^1	-	-	-	-	-	+	-
	Tamen	2.6×10^3	1.7×10^2	-	-	-	-	-	+	+
Antithistamine syrup	Alatrol	2.5×10^1	1.0×10^1	-	-	-	-	-	+	-
	Histacin	4.3×10^2	9.5×10^1	-	-	-	-	-	+	+
	Atrizin syrup	1.2×10^3	3.0×10^1	-	-	-	-	-	+	-
Antitussive syrup	Brofex	1.3×10^1	1.0×10^1	-	-	-	-	-	+	-
	Dextromethorphan	1.6×10^1	1.0×10^1	-	-	-	-	-	-	-
	Dexpoten syrup	3.3×10^2	2.0×10^1	-	-	-	-	-	+	-
	Ofkof syrup	1.8×10^1	0	-	-	-	-	-	+	-
Cough syrup	Tofen syrup	2.4×10^1	1.3×10^1	-	-	-	-	-	-	-
	X–cold	3.5×10^2	1.01×10^3	-	-	-	-	-	+	+
	Tusca	3.9×10^3	2.5×10^2	-	-	-	-	-	+	+
	Brodil syrup	7.0×10^1	1.6×10^1	-	-	-	-	-	+	-
	Mucolyt syrup	0	0	-	-	-	-	-	+	-
	Deslor syrup (Desloratadine)	3.3×10^3	1.7×10^2	-	-	-	-	-	+	-
H2 blocker	Neoceptin R Syrup	5.1×10^1	3.7×10^1	-	-	-	-	-	+	-
	Gepin Syrup	4.5×10^1	3.1×10^1	-	-	-	-	-	+	-
	Neotack	6.0×10^1	3.0×10^1	-	-	-	-	-	+	-
Antipasmodic Syrup	Colicon	5.5×10^2	1×10^1	-	-	-	-	-	+	-
Vitamin	Nine Seas	5.8×10^1	1.7×10^1	-	-	-	-	-	+	-
Syrup	Zinc B (Zinc and vitamin B-complex)	$3.7^1\times10^3$	2.8×10^2	-	-	-	-	-	+	+
	Nid (Elemental Zinc)	2.0×10^1	1.5×10^3	-	-	-	-	-	+	+

*USP limit- <10^2 cfu/ml

\+ Presence of bacteria

\- Absence of bacteria

Table 2: Microbial load in the oral suspensions tested

Sample Type	Sample name	Total aerobic plate count (cfu/ml)	Total Fungal Count (cfu/ml)	*E. coli*	*vibrio* spp.	*Salmonella* & *Shigella* spp.	*Clostridium* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.	*Bacillus* spp.
Analgesic & Antipyretic suspension	Zerin	4.9×10^2	1.0×10^2	-	-	-	-	-	+	-
	Feva suspension	7.4×10^2	4.0×10^1	-	-	-	-	-	+	+
	Parapyrol	2.1×10^2	7.0×10^1	-	-	-	-	-	+	+
Antihistamine Suspension	Lorfast	3.9×10^2	8.0×10^1	-	-	-	-	-	+	-
	Ordain	6.5×10^2	1.4×10^2	-	-	-	-	-	+	-
Antipyretic Suspension	Aceta	5.3×10^1	1.0×10^1	-	-	-	-	-	+	-
Antihelminthic Suspension	Azole	1.1×10^2	6×10^1	-	-	-	-	-	-	-
	Alben syryp(Albendazoe)	1.7×10^2	4.6×10^2	-	-	-	-	-	+	-
	Delentin (Pyrantel pamoate)	1.6×10^2	6.8×10^3	-	-	-	-	-	+	+
	Sintel (Albendazole)	8.7×10^2	1.4×10^2	-	-	-	-	-	-	-
Antiemetic Suspension	Motigut	5.3×10^2	3.5×10^2	-	-	-	-	-	-	-
Antiemetic Oral solution	Zofra	7.1×10^2	4.2×10^2	-	-	-	-	-	+	-
Anti-amoebic susoension	Amodis (Metronidazole BP)	9.6×10^2	5.0×10^1	-	-	-	-	-	+	-
	Filmet (Metronidazole)	7.6×10^2	3.0×10^1	-	-	-	-	-	+	+

*USP limit- $<10^2$ cfu/ml

\+ Presence of bacteria

\- Absence of bacteria

CONCLUSION

Usually most patients are supposed to be immune-compromised when they taking drugs which accelerate the chances of diseases acquired by opportunistic pathogens. Therefore, presence of any microorganism should be considered undesirable for all drugs. Although specific Gram negative enteric bacteria were not found in the tested samples, presence of viable bacteria especially Gram positive ones along with fungi claimed a sort of public health risk associated with the consumption of those drugs. The compliance sectors among the Bangladeshi Pharmaceuticals should strictly deal with microbial stringency within the manufacturing, packaging, distribution and storage of pharmaceutical products. Present situation might be of global significance in terms of public health measure and hence, a regular microbiological examination of oral drugs is suggested, especially in the developing countries.

ACKNOWLEDGEMENTS

The work was financially supported by the Department of Microbiology, Stamford University Bangladesh.

Conflict of Interest

Authors have no potential conflict of interests.

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