ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 10, pp.74464-74469, October, 2018

DOI: https://doi.org/10.24941/ijcr.32728.10.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

MICROBIOLOGICAL QUALITY ANALYSIS OF ORAL LIQUID ANTIBIOTICS AND ASSESSMENT OF THE ANTIMICROBIAL EFFICACY

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ARTICLE INFO

Article History:
Received 20th July, 2018
Received in revised form
15th August, 2018
Accepted 28th September, 2018
Published online 31st October, 2018

Key words:
Efficacy of Flucoxacillin,
Antibiotic Quality,
Oral liquid Suspension Efficacy.

ABSTRACT

The current study was designed to demonstrate the microbiological quality of oral liquid antibiotics. The *in vitro* antibacterial potential of the antibiotics was checked. In this regard three samples of oral liquid suspension of Flucoxacillin collected from different drug stores of Dhaka city were tested. All the samples were found to be loaded with total viable bacteria and fungi in the average of 10^4 cfu/ml and the bacterial and fungal load exceeded United States Pharmacopeia (USP) or British Pharmacopeia (BP) limit ($\leq 10^2$ cfu/ml for bacteria and 10 cfu/ml for fungi). Pathogenic bacteria were also encountered among which *Pseudomonas* spp. was predominant as they found in all samples. *Escherichia coli* and *Staphylococcus* spp. were found in majority of the samples. All the samples ofantibiotic exhibited substantial killing effect on *E. coli*, *Staphylococcus* spp. and fungi. Flucoxacillin samples had effect on them. *E. coli*, *Klebsiella spp., Staphylococcus* spp. and fungi. Flucoxacillin had the elevated antimicrobial effect on *Staphylococcus* spp.

Citation: Md. Tanjidul Hasan, Dr. Tarana Tabashum, S.M. Yasir Arafat, Nilufar Banu and Md. Zahidul Islam. 2018. "Microbiological quality analysis of oral liquid antibiotics and assessment of the antimicrobial efficacy", *International Journal of Current Research*, 10, (10), 74464-74469.

INTRODUCTION

Bacterial infections are among the important infectious diseases. Hence, over 5 decades of extensive researches have been launched for achieving new antimicrobial medicines isolated from different sources for combating bacterial infections that once ravaged humankind (Moat et al., 2002, Kamat et al., 2006, Lovine et at., 2008). Different antibiotics exercise their inhibitory activity on different pathogenic organisms. Despite progress in development of antibacterial agents, there are still special needs to find new antibacterial agents due to development of multidrug resistant bacteria (Sayeed *et al*., 2014).The increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs (Usha *et al*., 2010). Today, clinically important bacteria are characterized not only by single drug resistance, but also by multiple antibiotic resistance - the legacy of past decades of antimicrobial use and misuse. Drug resistance presents an ever increasing global health threat that involves all major microbial pathogens and antimicrobial drugs. Antibiotics that work today may not work tomorrow. It is essential to investigate newer drugs to which there is lesser resistance (Levy, 2002). Beside the resistance concern, presence of microorganism in oral suspention liquid (syrup) antibiotic preparation is a great public health concern globally (Cundell 2005a, 2005b, Prescott *et al*., 2005). Contaminations in pharaceutical preparations with microorganisms irrespective of being pathogenic and non-pathogenic can bring about changes in their physical characteristics, breaking of emulsion, fermentation appearance of turbidity or deposit and producing off odors and color changes (Hugo and Russel,1988, Squadritoa *et al*, 1998, Cabiscol *et al*., 2000, Schutterm *et al*., 2000).

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Moreover, paediatric oral liquid drug formulations may introduce pathogenic microorganisms to infants. Further, these pathogenic organisms may be highly detrimental for immuno-compromised infants. Therefore, microbiological quality of such oral liquid medicines is a very important factor for the above mentioned patients (Hossain *et al*., 2009, Sykes *et al*., 1971). By considering the facts discussed above, the present study was undertaken to investigate the microbiological attributes of commonly available antibiotics Flucoxacillin.

MATERIALS AND METHODS

Sample Collection and processing: Liquid suspension of antibiotics such Flucoxacillin of the three different companies (Phylopen –Square, Flux- Opsonin, Revistar- Biopharma) were collected from different drug store of Dhaka City. A total of 250 mg suspension powder of both types of antibiotic were mixed with 10 ml autoclaved distilled water. All the suspensions were then diluted up to 10^{-2} following standard guidelines (Choudhury *et al*. 2015; Cappuccino and Sherman 1996; Acharjee *et al*. 2013; Ahmed *et al*. 2014).

Microbiological Analysis

Estimation of Total Viable Bacteria and Fungi: For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 ml of each sample from the dilutions 10^{-1} and 10^{-2} was introduced onto the nutrient agar (NA) plate (Hi media laboratories Pvt. Ltd Mumbai, India) and Sabouraud's dextrose agar (SDA) plates (Bhiwadi- 301019, Rajasthan India), respectively, by means of spread plate technique .Plates were incubated at 37 °C for 24 hours and at 25 °C for 48 hours for total viable bacteria and fungi, respectively (Sharmin *et al*., 2015; Cappuccino and Sherman, 1996; Acharjee *et al*., 2013; Acharjee *et al*., 2014; Ahmed *et al*., 2014).

Estimation of Escherichia coli, Klebsiella spp., Staphylococcus spp. and Pseudomonas spp., Bacillus spp: From the dilutions 10^{-1} and 10^{-2}, 0.1 ml of each sample was spread onto the Mac Conkey agar plate (Hi media laboratories Pvt. Ltd Mumbai, India) for the enumeration of coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), respectively. Plates were incubated for 24 hours at 37 °C for coliforms, correspondingly. Likewise, *Staphylococcus spp., Pseudomonas spp., Bacillus spp.* were isolated onto Mannitol Salt Agar (MSA) plate (Hi media laboratories Pvt. Ltd Mumbai, India) and *Pseudomonas* agar plate, Starch agar (Hi media laboratories Pvt. Ltd Mumbai, India) respectively by adding 0.1 ml of diluted sample each, and all the plates were then incubated at 37 °C for 24 hours (Sharmin *et al*., 2015; Cappuccino and Sherman, 1996; Acharjee *et al*., 2013; Acharjee *et al*., 2014; Ahmed *et al*., 2014).

Biochemical identification of the isolates: All the isolates were biochemically examined following standard procedures as described earlier (Sharmin *et al*., 2015; Cappuccino and Sherman, 1996; Acharjee *et al*., 2013; Acharjee *et al*., 2014; Ahmed *et al*., 2014). Biochemical testing was performed for selection specific microorganisms such as Triple Sugar Iron (TSI) Slants (Hi media laboratories Pvt. Ltd Mumbai, India) , Methyl-Red (MR), Voges-Proskauer (VP), Motility Indole Urease (MIU) semisolid medium (Hi media laboratories Pvt. Ltd Mumbai, India), Citrate Utilization Slants (Becton Dickinson and company, France), Catalase test and Oxidase test.

Assay of determining anti-bacterial properties of samples through agar well diffusion method: Agar well diffusion method was performed to determine the antimicrobial activity of the Antibiotic samples (Jagessar *et al*., 2008). Individual bacterial pathogens (*Pseudomonas spp, Klebsiella spp, E.coli, Staphylococcus aureus* and *Bacillus* spp) were spreaded properly over Muller Hinton Agar (Oxoid, Ltd Hampshire England) plates using sterile cotton swabs (Ahmed *et al*., 2013). Wells were made in MHA by cork borer. Each of the antibiotics were used directly on MHA, separately. Sample solutions were added in wells along with a positive control (Gentamycin disc: 10µg) and a negative control (Normal Saline). The presence of antimicrobial activity was determined by the production of a clear zone around the wells and the diameters of these zone s were then measured. (Hussain *et al*., 2010).

RESULTS AND DISCUSSIONS

Microbiological quality of antibiotic samples: Many factors can increase microbial contamination during consumption includes improper storage conditions, unhygienic handling of the product, not following aseptic procedures when opening of the bottles and reconstituting. Air, water, reconstituting equipment's, reconstituting personnel and the consumer can be taken as the major sources of microbial contamination of oral liquid drug formulations. There have been an increasing number of reports of infections due to above mentioned reasons (Adeshina *et al*., 2009). In present study microbial contaminations were evident. All the samples were found to harbor viable bacteria and fungi in average of 10^{-4} cfu/ml irrespective of the type of antibiotic tested (Table I). *Pseudomonas* spp. was predominantly present in all the samples in a range of $10^{2}$to 10^{4} cfu/ml. However *E.coli* and *Bacillus* spp. were found in six samples in a range of 10^{2} to 10^{3} cfu/ml; whereas, *Staphylococcus* spp. were encountered in five samples. Three samples found to harbor *Klebsiella* spp. (Table II). Presence of pathogenic bacteria was confirmed by biochemical identification test (Table II).According to USP or BP, the finished products of the oral aqueous preparation should not go over the limit of10^{2} cfu/ml for total aerobic microbial count and 10 cfu/ml for total yeast mold count. *E. coli* must be absent from both categories of oral preparations (Noor *et al*., 2015). The microbial load found in our study clearly exceeded the USP or BP recommended microbial limit. Presence of microorganisms in oral liquid was also previously evident in different formulation of oral liquid drugs in Bangladesh (Noor *et al*., 2015, Khanom *et al*., 2013, Urmi and Noor, 2014).

Antimicrobial activity of antibiotics: Antimicrobial activities of non-antibiotic drugs have been demonstrated in several Studies previously (Noor *et al*., 2015, Akon *et al*., 2015, Quaiyum *et al*., 2014, Sharmin *et al*., 2014,Sultana *et al* 2014).Our current study aimed to investigate the antimicrobial effect of locally available oral suspension of antibiotics. All the samples of both Flucoxacillin were effictive in killing of *E. Coli*, *Staphylococcus* spp., *Klebsiella* spp. and fungi. as large zone of inhibition representing sensitivity was found (Table III). *Bacillus* spp. were found to acquire resistance against all the samples of Flucoxacillin.

Table 1. Microbial proliferation in Flucoxicillin

Antibiotic	Micobial load (cfu/ml)						
	TVB	TFC	*E. coli*	*Klebsiella spp.*	*Bacillus* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.
Flucoxacillin							
1	4.5×10^4	8.0×10^3	2.3×10^3	2.3×10^3	0	2.1×10^3	0
2	1.3×10^4	7.5×10^3	1.2×10^3	0	1.8×10^2	1.7×10^3	3.0×10^3
3	1.6×10^4	6.0×10^3	9.0×10^3	9.0×10^3	1.2×10^2	1.1×10^4	2.5×10^3

TVB= Total viable bacteria; TFC= Total fungal count
The experiments were in triplicates. Average count (cfu/ml or g) from all samples have been shown here.
*Bacterial load after enrichment (Prior to enrichment, the recovery was nil).
Vibrio spp. and *Shigella* spp. were absent in all samples.
USP or BP microbial limit: TVMC $\leq10^2$ cfu/ml; not more than 10 cfu/ml TYMC; *E. coli* should be absent.

Table 2. Biochemical identification of pathogenic isolates

Organisms	TSI				Motility	Indole	MR	VP	Citrate	Catalase	Oxidase
	Slant	Butt	Gas	H_2S							
E.coli	Y	Y	+	_	+	+	+	_	+	+	_
Klebseilla spp.	Y	Y	+	_	+	+	+	_	+	+	_
Staphylococcus spp.	Y	R	_	_	_	_	+	+	_	+	_
Pseudomonas spp.	R	R	_	_	_	_	_	_	_	+	+
Bacillus spp.	Y	R	_	_	+	_	_	+	+	+	_

Table 3. Antimicrobial activity of antibiotics against laboratory microbial isolates

Antibiotic	Zone of inhibition (mm) against test bacteriaand fungus					
	E. coli	Fungi	*Klebsiella* spp.	*Bacillus*spp.	*Pseudomonas* spp.	*Staphylococcus* spp.
Flucoxacillin						
1	40mm (S)	19 mm (S)	23mm (S)	0mm (S)	8mm (R)	43mm (S)
2	42mm (S)	20 mm (S)	21mm (S)	0 mm (S)	0mm (R)	40mm (S)
3	38mm (S)	24mm (S)	18mm (S)	0 mm (S)	0mm (R)	42mm (S)

Conclusion

All the oral liquid antibiotic samples contained a significantly higher number of microorganisms including the pathogenic ones. The microbial load exceeded the USP or BP recommended microbial limit to an extent which accounts for high public health concern. Thus, the local pharmaceutical industries need to be more careful and attentive about following the safety rules and standard regulations in all stages of manufacturing, packaging and distribution of the products. The local stores should maintain the appropriate conditions for the storage of the pharmaceutical products. Presence of some resistant pathogenic isolates against the tested antibiotics was also of a major concern.

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