Breif Reports

TIPS Trends in Phramaceutical Sciences 2016: 2(3): 177-180.

Application of dose response model for infection on selected cases of oral mouth wash products

Mostafa Essam Eissa[1,*]

[1]Quality Control Department, Microbiology Laboratory Division, HIKMA Pharma pharmaceutical company, Giza, Egypt.

Abstract

Microbial contamination of pharmaceutical products constitutes a great concern for stakeholders and professionals in the field. Special attention is brought to multidose medicinal products with considerable water activities (a_w). Such pharmaceutical products are prone to microbial spoilage with serious consequences on patients' health and even their lives. The current investigation aimed to study a new approach in the risk assessment of the contamination of oral antiseptic mouthwash in a quantitative manner using dose-response model of microbial infection. The present study combines both preservative efficacy test (PET) results with specific dose-response model of indicator bacteria. The risk was assessed at its maximum level using the worst case scenario of repeated contamination of the medicine bottle with each use. The indicator microbe selected was *Escherichia coli* with two models: exponential and beta-Poisson based on antimicrobial efficacy test (AET) results. The mouthwash met the acceptance criteria of USP<51> PET, with notably strong effect on bacteria and yeast (not recovered from culture media) at any testing point (14 and 28 days). On the other hand, *Aspergillus brasiliensis* showed significant reduction after only 28 days. The current investigation showed that repeated product contamination with each use increased the risk of infection and different contaminating varieties of the same microbial species constituted various hazard levels, although the antimicrobial properties of the product were sufficiently strong against the dedicated microbe (>3.00 log reduction (LR)). The current study provided new insight for the conventional pharmacopeial AET and demonstrated the limitation of it.

Keywords: *Aspergillus brasiliensis*, *Escherichia coli*, Mouthwash, PET, USP<51>, Water Activity.

1. Introduction

Microbial contamination of pharmaceutical products constitutes a health hazard to the final consumers and this matter leads to their withdrawal from the drug market. According to Sutton and Jimenez (2011), 72% of the non-sterile products recalls were related to the contamination with objectionable microbes. The contamination of the products with Enterobacteriaceae alone constituted 11% of the total screened items (1).

The study of preservative efficacy test of the product was conducted as detailed by Eissa and Mahmoud, 2015 (2). The parameters applied for dose response models of infection are detailed in Table 1 with their references (3, 4) using *Escherichia coli* as an indicator microbe. Alcohol-based mouth washes are the major contributors in the market of oral rinse products (5).

The maximum risk in the current simulation study was taken into account to determine the probability of infection from the contaminated product on repeated exposure recontamination cycle. The greatest hazard from oral mouth wash is considered when accidental ingestion occurs. This incident is not uncommon especially in young age individuals. Children younger than 6 years of age comprised 52.5% of the 27361 reported exposures

Corresponding Author: Mostafa Essam Eissa, HIKMA Pharma pharmaceutical company, P.O. Box 1913, 2nd Industrial Zone, Plot no.1, 6th of October City, Giza, Egypt.
Email: mostafaessameissa@yahoo.com

Recieved:28/02/2016; Accepted:11/06/2016

Table 1. Dose-Response infection model parameters per route of administration using critical or indicator microorganisms.

Microorganism	Best fit model	Optimized parameter(s)	LD_{50}/ID_{50}	Route	Dose units	Reference
Escherichia coli entero-hemorrhagic (EHEC): Dose Response Models	exponential	k=2.18E-04	3.18E+03	oral (in food)	CFU	Cornick & Helgerson (2004)
Escherichia coli: Dose Response Models	beta-Poisson	α=1.55E-01, N50=2.11E+06	2.11E+06	oral (in milk)	CFU	DuPont *et al.* (1971)

attributed to mouth rinses from 1989 to 1994 (6-11). Outcomes were reported for 1322 of the 2237 children younger than 6 years of age in 1992 (9).

The results in Table 2 show that the product met the pharmacopoeial acceptance criteria for preservative efficacy test (PET) (12). However, Aspergillus brasiliensis showed greatest tolerance to the product while significant effect was observed after 28 days contact time. Table 3 and 4 demonstrates the probability risk of infections from two different types of models for *E. coli*, if the product was assumed to be

Table 2. Preservative efficacy test (PET) results of selected pharmaceutical products based on USP<51>, 2015.

Dosage Form	Test Microorganisms	Testing Days		Antimicrobial Components
Antiseptic mouth wash solution	*S. aureus**	>3.40	>3.40	Hexetidine and Ethanol 96%
	*E. coli**	>3.00	>3.00	
	*P. aeruginosa**	>3.30	>3.30	
	*B. cepacia**,ψ	>3.07	>3.07	
	*C. albicans**	>3.70	>3.70	
	A. brasiliensis	0.25	1.50	

*=Microorganisms that have not been recovered at any stage of the test from the recovery medium.

ψ=Non-pharmacopeial, water-borne isolate was included in the study of the products from facilities from which this microorganism was found.

contaminated with successive doses of the bacteria at different contamination levels (CFU).

The current study provided novel approach to the true validity of the preservation system of the pharmaceutical products. Interestingly, the risk evaluation study used the antimicrobial efficacy test (AET) as the starting baseline for product assessment but it showed the drawback of the stan-

Table 3. Infection risk expressed as percent from repeated contamination with each maximum dose/frequency administration, using exponential dose-response model of *Escherichia coli*.

Number of Doses	Contamination Dose (D) CFU/15 ml (Single Administration)					
	500	200	100	50	25	1
1	<0.2783%	<0.1120%	<0.0561%	<0.0281%	<0.0141%	<0.0006%
2	<0.5199%	<0.2104%	<0.1056%	<0.0529%	<0.0265%	<0.0011%
3	<0.7301%	<0.2968%	<0.1492%	<0.0748%	<0.0375%	<0.0015%
4	<0.9134%	<0.3729%	<0.1877%	<0.0942%	<0.0472%	<0.0019%
5	<1.0735%	<0.4398%	<0.2217%	<0.1113%	<0.0558%	<0.0022%
6	<1.2136%	<0.4988%	<0.2517%	<0.1264%	<0.0634%	<0.0025%
7	<1.3364%	<0.5507%	<0.2782%	<0.1398%	<0.0701%	<0.0028%
8	<1.4441%	<0.5965%	<0.3016%	<0.1516%	<0.0760%	<0.0031%

Table 4. Infection risk expressed as percent from repeated contamination with each maximum dose/ frequency administration using beta-Poisson dose-response model of enterohemorrhagic (EHEC) *Escherichia coli*.

Number of Doses	Contamination Dose (D) CFU/15 ml (Single Administration)					
	500	200	100	50	25	1
1	<8.8734%	<3.6486%	<1.8412%	<0.9249%	<0.4635%	<0.0186%
2	<16.0670%	<6.7662%	<3.4424%	<1.7363%	<0.8719%	<0.0350%
3	<21.9578%	<9.4410%	<4.8375%	<2.4487%	<1.2320%	<0.0496%
4	<26.8252%	<11.7439%	<6.0553%	<3.0749%	<1.5495%	<0.0624%
5	<30.8788%	<13.7331%	<7.1200%	<3.6257%	<1.8296%	<0.0738%
6	<34.2784%	<15.4559%	<8.0521%	<4.1106%	<2.0768%	<0.0839%
7	<37.1472%	<16.9519%	<8.8692%	<4.5376%	<2.2951%	<0.0928%
8	<39.5813%	<18.2536%	<9.5863%	<4.9139%	<2.4879%	<0.1007%

dard test. The "overkill" approach with very long intervals of the sampling and testing frequency did not mimic the actual use and did not provide the information regarding the "wash out rate" of the microbial cells against rebuilding of the contamination during the drug consumption. Thus, it is recommended to modify the procedure to include the interval hours of the medicine use. As in the current situation of the mouth wash (125 ml bottle, with maximum volume of 15 ml per single use, at maximum six hours interval per day) i.e. the product will be used up after about 8 times of use.

Acknowledgment

Thanks to Ahmed Saber Nouby for his technical assistance with work in AET. Results interpretation and issuing was conducted by HIKMA microbiology laboratory staff. Reference and writing style review was done by Dr. Engy Refaat Rashed.

Conflict of Interest

None declared.

References

1. Sutton S, Jimenez L. A Review of Reported Recalls Involving Microbiological Control 2004-2011 with Emphasis on FDA Considerations of "Objectionable Organisms". *American Pharmaceutical Review*.2012:1;42-57.
2. Eissa ME, Mahmoud AM. Assessment of Modified Preservation Formulae after Optimizing Preliminary Neutralization Evaluation Study. *International Journal of Multidisciplinary Research, Studies and Developments*. 2015;1(2): 5-11.
3. Cornick NA, Helgerson AF. Transmission and infectious dose of *Escherichia coli* O157: H7 in swine. *Appl Environ Microbiol.* 2004;70:5331-5.
4. DuPont HL, Formal SB, Hornick RB, Snyder MJ, Libonati JP, Sheahan DG, LaBrec EH, Kalas JP. Pathogenesis of *Escherichia coli* diarrhea. *N Engl J Med*. 1971;285:1-9.
5. Shulman JD, Wells LM. Acute ethanol toxicity from ingesting mouthwash in children younger than 6-years of age. *Pediatr Dent.* 1997;19:404-8.
6. Litovitz TL, Schmitz BF, Bailey KM. 1989 annual report of the American Association of Poison Control Centers national data collection system. *Am J Emerg Med*. 1990;8:394-442.
7. Litovitz TL, Bailey KM, Schmitz BF, Holm KC, Klein-Schwartz W. 1990 annual report of the American Association of Poison Control Centers national data collection system. *Am J Emerg Med.* 1991;9:461-509.
8. Litovitz TL, Felberg L, White S, Klein-Schwartz W. 1995 annual report of the American Association of Poison Control Centers toxic exposure surveillance system. *Am J Emerg Med.* 1996;14:487-537.
9. Litovitz TL, Holm KC, Clancy C, Schmitz BF, Clark LR, Oderda GM. 1992 annual report of the American Association of Poison Control Centers toxic exposure surveillance system. *Am J Emerg Med.* 1993 Sep;11(5):494-555.
10. Litovitz TL, Clark LR, Soloway RA. 1993 Annual report of the American Association of Poison Control Centers toxic exposure surveillance

system. *Am J Emerg Med.* 1994;12:546-84.
11. Litovitz T, Felberg L, Soloway R, Ford M, Geller R. 1994 Annual report of the American association of poison control centers toxic exposure surveillance system. *Am J Emerg Med*. 1995;13:551-97.
12. United States Pharmacopeia General Chapter <51> Antimicrobial Effectiveness Testing, USP 34-NF29, US Pharmacopeia, Rockville, Maryland, USA, 2010.
13. David P. Elder P, Crowley. Antimicrobial Preservatives Part One: Choosing a Preservative System [Internet]. Americanpharmaceuticalreview.com. 2016 [cited 20 February 2016]. Available from: http://www.americanpharmaceuticalreview.com/Featured-Articles/38886-Antimicrobial-Preservatives-Part-One-Choosing-a-Preservative-System/