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NOVEL APPROACH TO THE ASSESSMENT OF PRESERVATION EFFECT FOR ORAL LIQUID PHARMACEUTICAL PRODUCTS

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Abstract

Preservation of multidose pharmaceutical products is essential criterion for both the efficacy and the safety of medicinal products for human consumption. Yet, there are still several reports of contamination of several products either for treatment of hospitalized or outpatients. This current study aims to provide new approach for assessing the preservation of medicinal product using dose-response model of infection. This will involve the most infective bacteria for the route of administration and the application of repeated recontamination of the product using simulation study as a method for risk evaluation. Three different non-sterile oral liquid formulae were subjected to this study. All products passed the preservative efficacy test (PET) with the iron supplement syrup showing the highest rate of microbial count reduction followed by antitussive syrup then antidiarrheal suspension – especially after 14 days of the test - when using Escherichia coli as an indicator microorganism. The application of simulated multi-spot contamination model integrated with both PET and dose-response model of infection showed the reverse order of descending risk of microbial infection. The relative probabilities values of the geometric means for both types of the infection models were approximately 1 : 1.2 : 1.4 for iron supplement : antitussive : antidiarrheal products, respectively. However, these products means did not differ significantly from each other using One-way ANOVA at 95 % confidence interval. On the other hand, the exponential model of enterohemorrhagic (EHEC) E. coli showed from 20 to 34 times higher risk of infection than Beta-Poisson model depending on the level of contamination of the liquid product during in-use application. The study offered new approach of assessing the risk of infection from consumption of contaminated multidose product from in-use application quantitatively.

Keywords: Multidose pharmaceutical; Dose-response model; Preservative efficacy test; Antidiarrheal; Antitussive; Iron supplement.

INTRODUCTION

Pharmaceutical preparations have a wide spectrum of applications including in the prophylaxis, treatment and diagnosis of diseases. Recently, the pharmaceutical manufacturing industry has witnessed improvement in the quality of non-sterile pharmaceuticals in such a way that has reduced the amount of bioburden. [1] The issue of microbiologically-polluted products has been documented by several researchers, and contaminants range from genuine pathogens to opportunistic pathogens. [2] A few reports have also been distributed showing the clinical implications that have been attributed to microbiologically contaminated pharmaceuticals. [3-5] The main risk to the health should be considered when the contamination density exceeds (100 CFU/mL). [6] This is made worse by the fact that most cases of drug-related infections are not well reported or documented. [7]

The misuse of medicinal products containers may lead to the health hazard complications following the intake of exceedingly contaminated dosage forms by consumers whose immunity is already affected by their disease conditions. Microbial presence in medicinal dosage form may be hazardous by virtue of their infectivity, altering physical, chemical, organoleptic properties, changing the product components composition or even transforming them to harmful byproducts. [8] Thus, a medicinal dosage form may be regarded unacceptable due to microbiological spoilage in this situation, depending on its intended use. The pharmaceutical product efficacy and safety is affected by very low number of pathogenic microbes, high number of opportunistic. Microbial growth causes changes in physicochemical of pharmaceutical products and hence they become unsafe for human consumption. [8]

Pharmaceutical products become harmful not only due to microbial contamination but also due to the toxic metabolites which may be harmful from even very minute quantities in the products that have been used as substrates for microbial assimilation. [9] Some of these toxin-linked sicknesses embody different signs of gastrointestinal (GIT) diseases. Symptoms range from simple gastric problems to death, according to person to person difference in vulnerability to the toxic substance, intake concentration of toxin, and the general health conditions of the affected individuals. [9,10] There have been reports of severe microbial infections of immunocompromised patients due to Gram positive and negative bacteria. [10] Several cases observed of

nosocomial and community-related infections have been attributed to one genus of *Enterobacteriaceae*. [11]

Pharmaceutical products are liable to microbial spoilage, deterioration or degradation. Another critical concern that could be emerged from microbiological intrusion of dosage forms is the undetected signs of any spoilage of ingested medicine. Therefore, the bioburden content of medicinal products either sterile or non-sterile should not be overlooked. [12] Former researches have illustrated microbiological quality issues with account to marketed and in-house prepared pharmaceuticals in addition to stocked liquid antimicrobial commercial products. [5,13,14]

Due to the previously discussed challenges, the current study aimed to simulate the process of contamination and recontamination of multidose pharmaceutical non-sterile products – being highly susceptible products to microbial spoilage due to high water activity (a_w) - in attempt to associate both preservative efficacy test (PET) and dose-response infection model of indicator microorganism to determine the risk at its maximum value of infection probability from product contamination. The study focused on providing new insight for assessment of the safety multi-dose medicinal product during in-use consumption of the drug.

MATERIALS AND METHODS

Standard strains were purchased from ATCC (American Type of Culture Collection**,** Manassas, Virginia) and processed according to the stated procedure by the supplier. All microbiological media for culturing and reagents were obtained from OXOID (Basingstoke, Hampshire) and Sigma-Alrich (St. Louis, MO 63103), respectively. Plastic 9 mm sterile plates were purchased from Sterilin Limited (solaar house, 19 mercers row, Cambridge, UK). Microbial suspensions were quantified by making serial dilutions and plating using conditions and media suitable for each organism and selecting dilutions of suitable microbial concentration as working suspensions. Appropriate inoculums from the prepared serial dilution tubes were selected after enumeration using digital colony counter (Digital Colony Counter Model: 361, Laxman Mahtre Rd. Navagaon, Dahisar West, Mumbai). All media were sterilized by autoclaving in steam sterilizer (FEDEGARI FOB3, Fedegari Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). All pH measurements and weighing procedures were done using Mettler-Toledo S20 SevenEasy™ pH Meter and XPE Analytical Balance respectively (Mettler-

Toledo, LLC 1900 Polaris Parkway Columbus, OH 43240). Incubation of cultures was done in BD 115 incubator (BINDER GmbH, ImMittlerenÖsch 5 D-78532 Tuttlingen). Microbial tests of pharmaceutical products were done using culture media that passed growth promotion tests according to the methods and specifications by USP, 2015. [15] Bacterial visualization was facilitated using colorless Triphenyltetrazolium Chloride dye (TTC) which becomes red-colored by viable bacterial cells. Negative control samples were included concurrently with the test. Identification of the frequently water-borne bacteria and the verification of standard strains culture purity was done according to Ashour et al., 2011. [16] Environmental monitoring (EM) specimens from working area and air were taken in the working area under safety cabinet as described by Eissa, 2014 to ensure appropriate cleaning, disinfection and aseptic behavior under laminar air flow conditions. [17] The purity of standard strains and the identification of water-borne bacterial isolate was conducted using miniaturized biochemical identifications kits BBL™ Crystal™ enteric/non fermenter (E/NF) and Gram-positive (GP) Identification System Identification System and Gram-stain reagents purchased from BD (Becton Dickinson Microbiology Systems, Cockeysville, Md.). PET study was conducted based on the method and criteria of pharmacopeial guide. [18] Preliminary neutralization study was conducted to ensure neutralization of antimicrobial effect during PET. Neutralization procedures were done according to Eissa and Mahmoud, 2015. [19,20] Products were tested for low-inoculum level recovery from the products.

The composition of the three non-sterile oral pharmaceutical products as indicated by leaflet of each medicine: 1- Antidiarrheal suspension: Nifuroxazide, sorbitol, glycerin, microcrystalline cellulose, sodium carboxy methyl cellulose, sodium benzoate, saccharin sodium, citric acid, sodium hydroxide, flavor and purified water. 2- Antitussive syrup: Guaiphensin, ephedrine hydrochloride, diphendydramine hydrochloride, citric acid, sodium benzoate, sorbitol, sucrose, sunset yellow, aspartame, flavor, sodium hydroxide and purified water. 3- Iron supplement syrup: Sodium feredetate, sorbitol, glycerin, saccharin sodium, citric acid, sodium hydroxide, flavor, ponceau 4R, ethanol 96%, methyl hydroxybenzoate, propyl hydroxybenzoate and purified water. All microbial processing was made under validated and calibrated biological safety cabinet (Jouan MSC 9 Class II A2 BioSafety Cabinet, Thermo Fisher Scientific Inc. 81 Wyman Street, Waltham, MA, USA 02451). Illustrations of generated data and calculations were

performed using Microsoft Office Excel 2007. GraphPad Prism v6.01 for windows was used for statistical analysis using to One-way ANOVA followed by Tukey's multiple comparisons test at $\alpha = 0.05$ and for constructing box and whisker diagram.

Theory/Calculation

The principle theory for assessing risk of infection from repeated recontamination of multidose liquid product depends on the following facts:

1- Only pharmaceutical products that pass PET test will be subjected for further simulated study analysis. Those drugs that failed to achieve acceptance criteria will require reformulation and should be considered high risk products that must not be distributed in the market.

2- From the microorganisms tested spectrum in PET study, the most probable one as a causative agent for infection per the route of administration of the medicinal product should be selected as reference or indicator microbe for the maximum assessment of the potential risk for health hazard.

3- Microorganism of the lowest infective dose per route of administration can be used as supportive and/or alternative criterion for selection of the indicator microorganism. [21,22] Contamination was assumed to occur with the second administered dose of the medicine, where

4- The most applicable dose-response model of infection for specific route of administration should be selected to be applied in correlation with dosage form size, maximum application frequency and largest size of the dose administered in order to assess the risk at its highest value. In the current case a model done by Cornick and Helgerson, 2004 and DuPont et al., 1971 were found to be the most appropriate. [23,24]

5- The choice of the maximum contamination risk model using multiple spots contamination i.e. repeat contamination with each opening and use of the product package with non-sanitary behavior till the primary drug package is used up. The equation derived for this model is:

$$D\,f = Y.\ \frac{n.\ (X^{f-2} + X^{f-3} + \cdots\cdots + 1)}{X^{f-2}.\ (V - Z)} \quad \text{.....eq. 1}$$

Where: f = Dose rank number and should be ≥ 2. X = Reduction factor number between successive doses (obtained from transforming log reduction). Y = Maximum administered volume of the single dose (ml). V = Bulk volume of the product in

bottle (ml). *Z* = Cumulative consumed volume of the product (ml) during consumption of the volume *Y*. *n* = Bioburden delivered to the product (CFU). *Df* = Dose in CFU ingested by the administered liquid product into the body of the patient.

6- Depending on the contamination density selected for the simulation study and PET results, the selected portion of the kinetics of microbial death will be selected i.e. from zero to 14 days, 14 to 28 days or there is no significant difference in the curve slope so the whole curve will be chosen.

7- The dose response model of infection of *Escherichia coli* in the current cases was used which follows Beta Poisson and exponential (equation 2 and 3, respectively) models with data of the indicator microbe demonstrated in Table 1 as the following:

$$P = 1 - \left[1 + Df.\frac{\left(2^{\frac{1}{\alpha}} - 1\right)}{\text{N50}}\right]^{-\alpha} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq. 2}$$

$$P = 1 - \exp(-\text{k}\,.Df) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq. 3}$$

8- The maximum recommended dose, its frequency and the size of the unit for each product was obtained from the leaflet (pamphlet) with each medicinal product as demonstrated in Table 2.

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

Microorganism	Best fit model	Optimized parameter(s)	LD50/ID50	Route	Dose units	Reference
Escherichia coli enterohemorrhagic (EHEC): Dose Response Models	exponential	k=2.18E-04	3.18E+03	oral (in food)	CFU	Cornick and 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)

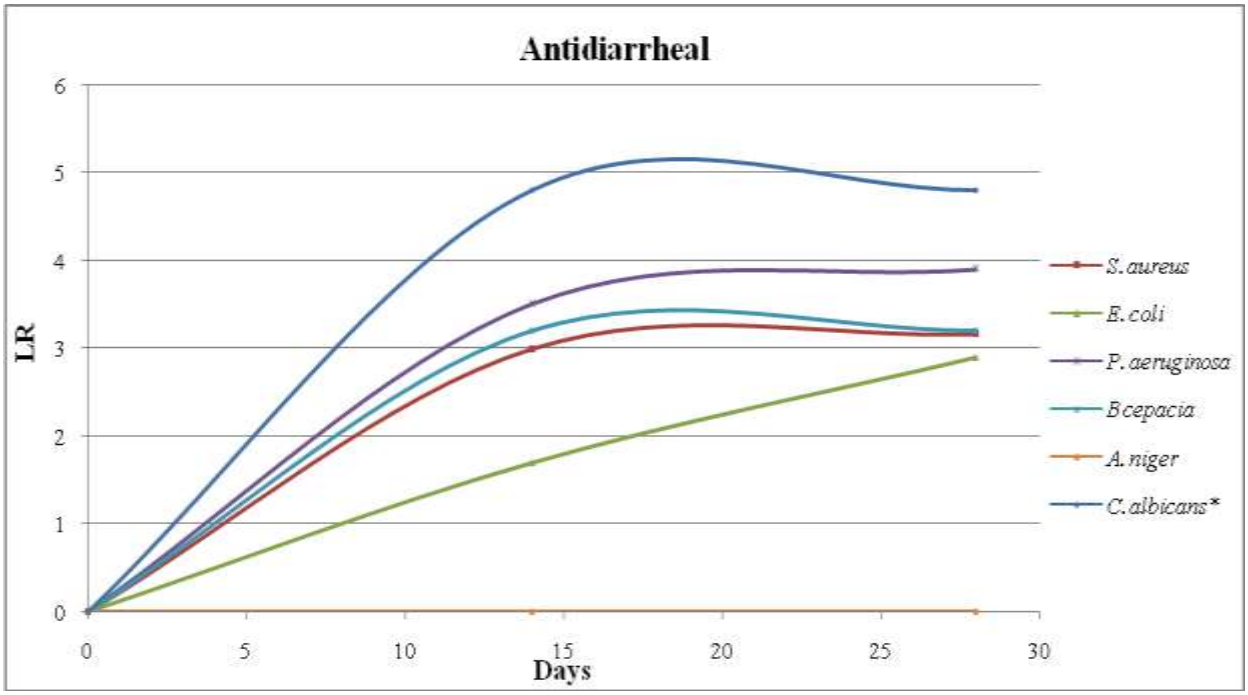
Table 2: Non-sterile oral pharmaceutical products characteristics.

Product Form	Package	Maximum Dose Size	Maximum Dose Frequency Per Day	Route of Administration	Storage	Use
Syrup	Glass bottle of 100 ml	10 ml	2	Oral	≤ 30 °C	Iron Supplement
		10 ml	4		≤ 25 °C	Antitussive Syrup
Suspension	Glass bottle of 60 ml	5 ml	3		≤ 30 °C	Antidiarrheal

RESULTS

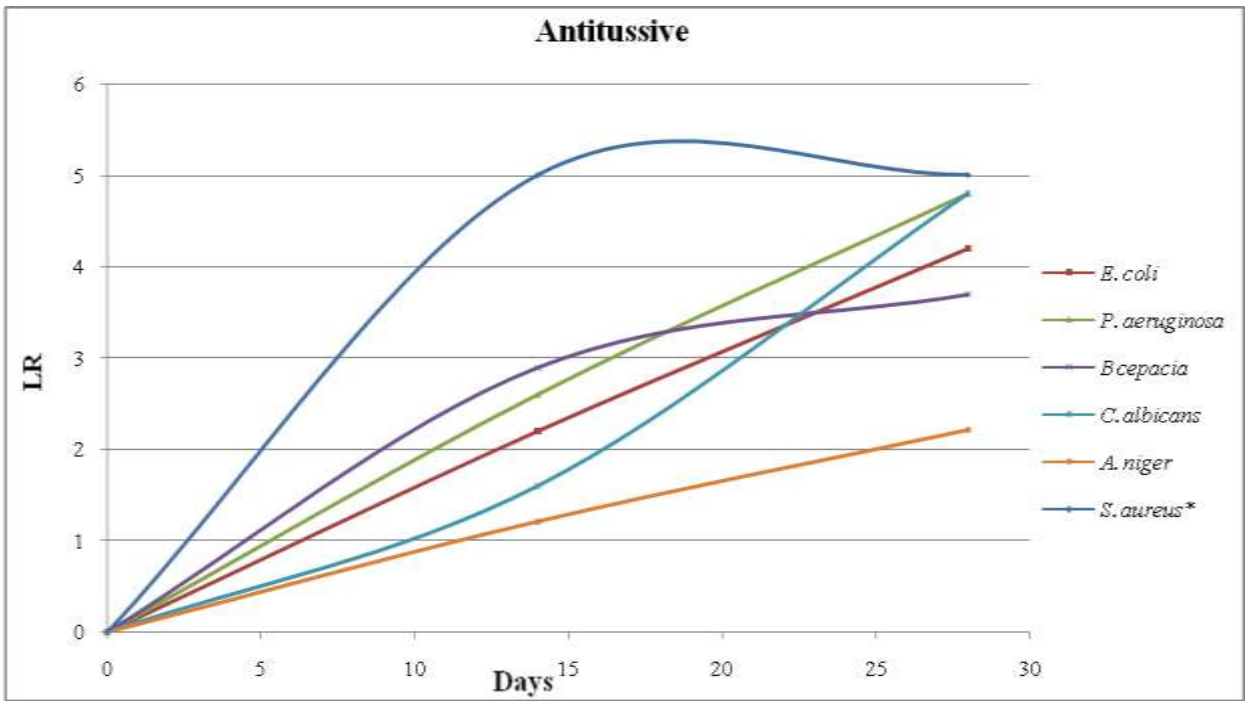
Suitability of neutralization of the antimicrobial properties of the products was verified and demonstrated validity of the neutralization procedure. The kinetics of microbial reduction was demonstrated for antidiarrheal, antitussive and iron supplement pharmaceutical products in Fig.1, 2 and 3, respectively. The selected indicator microorganism (*E. coli*) had LR kinetics (Y) for antidiarrheal, antitussive and iron supplement drugs as the following: = 0.10 x (contact time) + 0.08, = 0.15 x (contact time) + 0.03 and = 0.20 x (contact time), respectively. From these equations, the reduction factor (X) between successively administered doses could be theoretically calculated. Iron supplement drug had the highest rate of microbial reduction (>2.8), followed by antitussive (≈2.2) then antidiarrheal (≈1.7). When applying multiple spots contamination simulation, the risk of infection after each maximum dose was as shown in Table 3 and 4. This analysis showed agreement with the previously observed finding of antimicrobial efficacy test (AET), with the product of the highest killing rate showing the lowest risk of infection. This could be explained in view that simulation study encompass in addition to PET effect, the dosage form size, frequency and dose magnitude. Thus, the risk of infection from certain consumed multidose drug is an outcome event of several interacting and influencing factors on the product infectivity.

Figure 1: Logarithmic reduction (LR) from antidiarrheal product: PET kinetics study for oral antidiarrheal suspension.



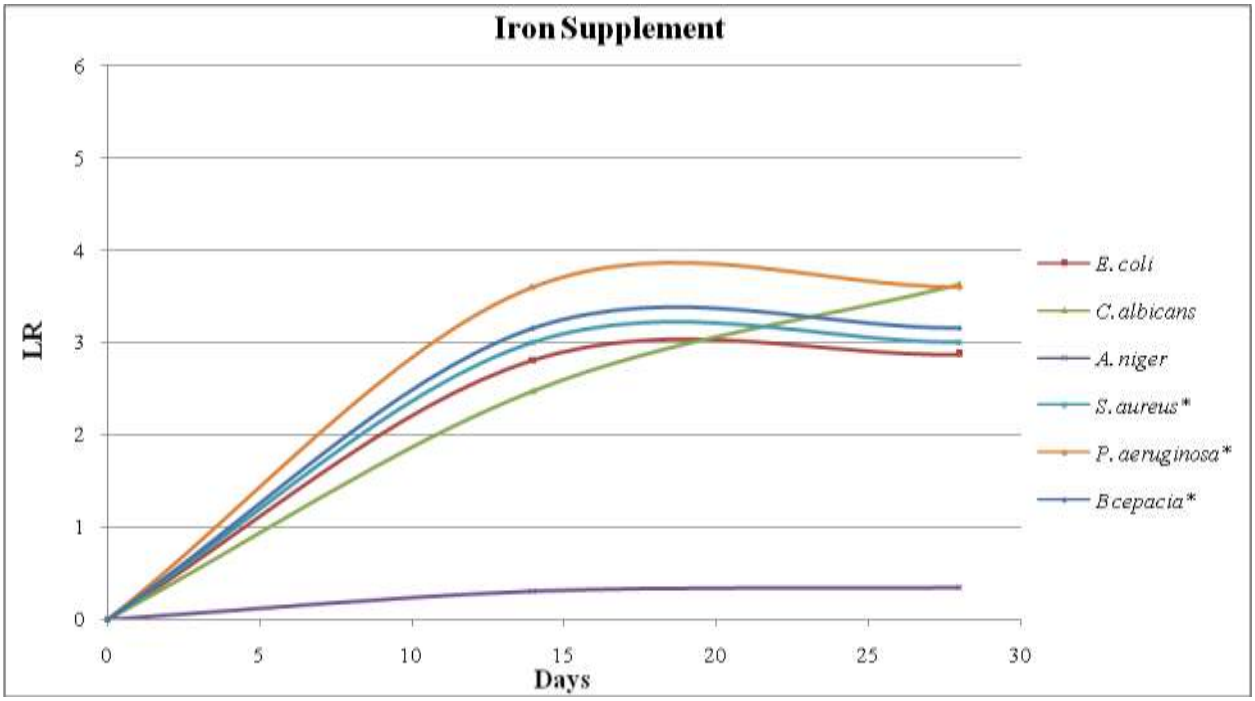
*= Microbial species that failed to be recovered after exposure to product.

Figure 2: Logarithmic reduction (LR) from antitussive product: PET kinetics study for oral antitussive syrup.



*= Microbial species that failed to be recovered after exposure to product.

Figure 3: Logarithmic reduction (LR) from iron supplement product: PET kinetics study for oral iron supplement syrup.



*= Microbial species that failed to be recovered after exposure to product.

Table 3: Probability of infection (expressed as percent) from exponential model of dose-response model of infection.

Product		Antidiarrheal Suspension			Antitussive Syrup			Iron Supplement Syrup		
Contamination (CFU)*		10	100	1000	10	100	1000	10	100	1000
Dose Rank	2	0.218%	2.156%	19.587%	0.218%	2.156%	19.587%	0.218%	2.156%	19.587%
	3	0.416%	4.080%	34.072%	0.416%	4.086%	34.108%	0.390%	3.835%	32.363%
	4	0.596%	5.800%	44.983%	0.597%	5.815%	45.068%	0.527%	5.146%	41.042%
	5	0.759%	7.340%	53.343%	0.762%	7.367%	53.478%	0.635%	6.174%	47.130%
	6	0.908%	8.721%	59.849%	0.913%	8.763%	60.031%	0.721%	6.983%	51.511%
	7	1.044%	9.961%	64.982%	1.050%	10.019%	65.207%	0.789%	7.619%	54.728%
	8	1.167%	11.076%	69.085%	1.175%	11.152%	69.347%	0.843%	8.121%	57.129%
	9	1.279%	12.080%	72.403%	1.290%	12.174%	72.697%	0.886%	8.518%	58.944%
	10	1.381%	12.985%	75.115%	1.394%	13.098%	75.436%	0.920%	8.831%	60.329%
	11	1.474%	13.801%	77.353%						
	12	1.559%	14.538%	79.216%						

* = Contamination delivered to product at each use during application.

Figure 4 demonstrates the pattern of the infection risk that was theoretically aroused from the assumed repeated contamination model with ten CFU using box-plot diagram. However, statistically they were not significantly different at 95 % confidence interval using One-way ANOVA. The relative values of the geometric means for both types of the

infection models were approximately 1 : 1.2 : 1.4 for iron supplement : antitussive : antidiarrheal products, respectively. On the other hand, the exponential model of enterohemorrhagic (EHEC) *E. coli* showed from 20 to 34 times higher risk of infection than Beta-Poisson model depending on the level of contamination of the liquid product during in-use application.

Figure 4: Box Plot diagram showing the pattern of the infection risk that occurs from each product type using repeated contamination model with ten CFU: Beta-Poisson (upper figure) and exponential (lower figure).

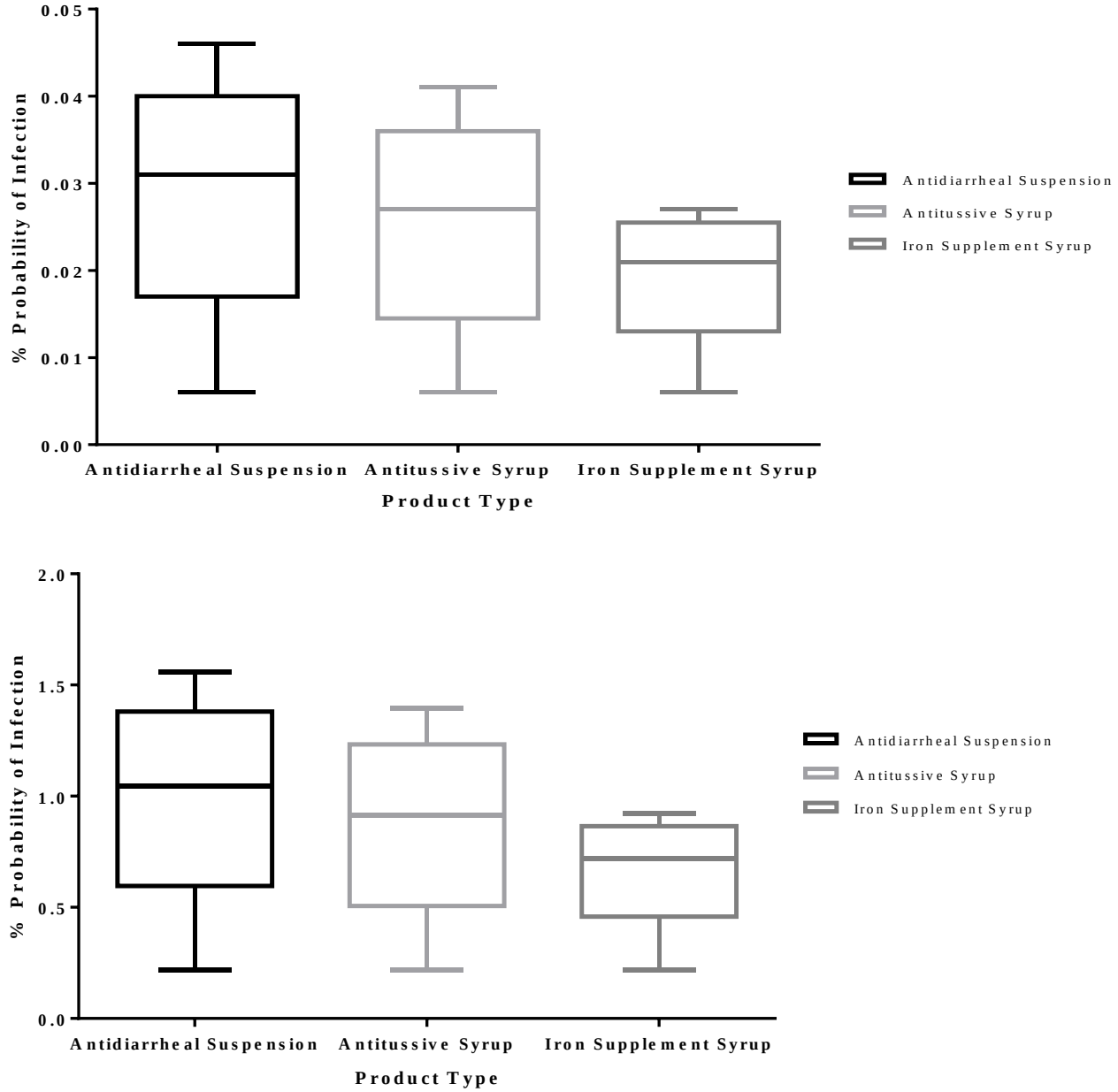


Table 4: Probability of infection (expressed as percent) from Beta-Poisson model of dose-response model of infection.

Product		Antidiarrheal Suspension			Antitussive Syrup			Iron Supplement Syrup		
Contamination (CFU)*		10	100	1000	10	100	1000	10	100	1000
Dose Rank	2	0.006%	0.063%	0.621%	0.006%	0.063%	0.621%	0.006%	0.063%	0.621%
	3	0.012%	0.121%	1.163%	0.012%	0.121%	1.164%	0.011%	0.114%	1.094%
	4	0.017%	0.173%	1.637%	0.017%	0.174%	1.641%	0.015%	0.153%	1.458%
	5	0.022%	0.220%	2.056%	0.022%	0.221%	2.063%	0.019%	0.185%	1.740%
	6	0.027%	0.263%	2.425%	0.027%	0.265%	2.437%	0.021%	0.209%	1.959%
	7	0.031%	0.303%	2.753%	0.031%	0.304%	2.769%	0.023%	0.229%	2.131%
	8	0.034%	0.338%	3.045%	0.034%	0.340%	3.065%	0.025%	0.245%	2.265%
	9	0.037%	0.370%	3.306%	0.038%	0.373%	3.330%	0.026%	0.257%	2.371%
	10	0.040%	0.400%	3.538%	0.041%	0.403%	3.567%	0.027%	0.267%	2.455%
	11	0.043%	0.426%	3.747%						
	12	0.046%	0.450%	3.934%						

* = Contamination delivered to product at each use during application.

Discussion

The in-use period where the patients consume the medicine still remains the bottle neck, when the pharmaceutical item integrity is breached so that a dose can be withdrawn and more importantly where there is potential for microbial intrusion. There is no globally approved method that this can be achieved for each and every product type. Getting it wrong at any stage of manufacture and storage or in-use will almost certainly result in a return to health problems of patients caused by microbial contamination of multidose medicines. [25] The use of indicator microorganisms for each product category is supported by other investigators. [26] Each of the three products demonstrated significant different and unique behavior toward repeated successive contamination in the same order of the rate of cessation of *E. coli*. Interestingly, the applied multi-spots contamination study showed progressive increase of the probability risk of infection with continuous successive contamination of the product.

The preliminary PET study showed that products did not differ significantly in the efficacy after 14 days but microbial species differed in the level of LR. A Gram-negative rod bacterium which was identified using miniaturized biochemical identification system was *Burkholderia cepacia* which has been included in the routine study of preservation efficacy study of multidose products because it is listed as an objectionable microbe. [27,28]

The approach done in the current study rather than relying on a single limited test to decide the microbiological safety of multidose pharmaceuticals with high water activity i.e. PET, was supported by other investigators. Elder and Crowley, 2012 stated that "evaluation specifications and assessment methodologies, on the basis of product type, dose, environmental history in manufacture and the knowledge acquired by the application of medicine by the patient might be more appropriate than applying a single quality standard defined in pharmacopoeias that may show “overkill” in a microbiological and commonsense context for many products". [28]

CONCLUSION

The currently applied methodology for assessing product safety for consumption presented reasonable approach to actual risk of infection from multidose drug consumption. It is a simple tool that can be designed and adapted to a wide range of new dosage forms. The technique is a quantitative tool for risk assessment in pharmaceutical industry that provides evidence for the product efficacy, improvement (in case of changing composition) and safety for the patient consumption. On the other hand, the commonly used other risk evaluation techniques in the drug manufacturing industry are qualitative or semi quantitative at the best and are subjective in nature and depend solely on the view of many experts (number may reach 10 to 15 expertise is specific field) to resolve challenging issues. Thus, the present method of risk analysis is fast, effective and time-saving to manage decisions to ensure consumer safety.

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