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Vulnerability of cough syrups marketed in Palestine to microbial challenge test

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ABSTRACT

Microbial contamination of cough syrups can bring clinical hazards as well as physical changes in the product. The objective of the current investigation was to assess and compare the ability of imported and locally manufactured cough syrups to maintain minimum or no microbial growth after being challenged with different types of microbes. The growth of five microorganisms of known quanta of S. aureus, E. coli, P. aeruginosa and C. albicans was compared among five different cough products designated A through E. Two of the products (A and E) were locally manufactured while three (B, C and D) were imported products which contained different preservatives. Both A and E did not indicate the type of preservative used. Normal saline was used as a positive growth control. Growth of microorganisms into syrups was compared by counting the colony forming units (CFUs) from a subculture of inoculated syrups at zero, 3, 6, 24 and 48 hr intervals. 1) at time zero, growth of S. aureus was seen in all products except product B; 2) little or no growth of C. albicans, P. aeroginosa and E. coli was observed at time zero; 3) no growth of any of the tested microbes was seen when subcultures were done after 6 hours of inoculation; and 4) imported products showed lesser or no microbial growth compared to locally manufactured ones. Normal saline showed heavy growth of all tested microbes while unchallenged syrupsof the tested products showed no signs of microbial growth at all tested times.Despite the noticeable growth of S. aureus at time zero, all tested cough syrups passed the pharmacopeal guidelines regarding microbial challenge. Good manufacturing and packaging practices need to be implemented and maintained by local pharmaceutical companies. The Palestinian general public needs to be educated on the proper handling and storage of oral liquid pharmaceuticals to eliminate or reduce microbial contamination.

Key Words: Cough syrups; Microbial growth; Preservatives; Palestine.

INTRODUCTION

Liquid formulations are very commonly dispensed and administered to pediatric patients. Respiratory medications, particularly cough syrups are one of the most commonly used medications among pediatrics. Analysis of prescriptions dispensed in community pharmacies in Palestine had shown that 7% of the prescribed medications were cough syrups [1]. In a

survey study of medications stored in Palestinian households, respiratory medications, particularly cough syrups, were present and stored in 13.6% of the surveyed households [2]. Furthermore, the study indicated that more than one third of these medications were stored inappropriately. Cough and other liquid preparations are not manufactured under aseptic conditions because they are intended for oral use. However, manufacturers usually add preservatives as well as other formulation materials such as alcohol to maintain minimum or no accidental microbial growth upon use and storage of oral solutions. Despite that, microbial contamination of liquid pediatric preparations might happen. A report on May 2010 in Los Angeles Times (http://articles.latimes.com) indicated that Food and Drug Administration founds bacterial contamination in children's medicine ingredients which led to massive recall of these medicines from the market.

Manufacturers usually test the ability of liquid preparation to maintain minimum microbial growth by deliberately inoculating the final product with a suitable micro-organism such as *Satphylococus aurues, Escherichia coli, Psuedomonas aeroginosae* and *Candida albicans* then monitoring the level of contamination at several time intervals [3 – 7].

The aim of this study was to evaluate the ability of different cough syrups manufactured by different pharmaceutical companies to maintain minimum or no microbial growth after being challenged with different types of microbes.

EXPERIMENTAL SECTION

2.1 Selected Cough Products

A phone survey was made to community pharmacies in Nablus district to list the most commonly prescribed cough syrups. The most common five were selected for the study and labeled A through E. Two of the products (A and E) were manufactured locally by Palestinian pharmaceutical companies while the other three products (B, C and D) were imported by Palestinian agents. The type of package and inactive materials present in each of the products are listed in table 1. The researchers bought the products from a community pharmacy and made sure that the expiration date of each of the product was still valid for at least one year. The whole experimental design and procedures were carried out at the department of pharmacology/ toxicology at An-Najah national university, Nablus, Palestine. The five products were challenged with four types of clinical isolates, *E. coli*, *S. aureus P. aeroginosae* and *C. albicans*. Normal 0.9% sterile saline was used as a positive growth control. All isolates were obtained from the medical laboratory of the Specialized Arab Hospital. The overall methodology of this study was similar to that followed by Wachowski *et al*. (1999) and Crowther *et al*. (1996). [8, 9]

2.2. Microbial cultures

Overnight cultures of the clinical isolates of *E. coli*, *S. aureus*, *P. aeroginosae*, and *C. albicans* were diluted to a density of 0.5 McFarland units with 0.9% sterile saline. A 1:50 dilution was prepared from each isolate**.**

2.3. Microbiological assay

Four 10 ml aliquots of each cough syrup were challenged with 100 µl of each isolate. Following the challenge with the microbial loads, each aliquot was vortexed for one minute and then sub-cultured on three Mueller Hinton plates and incubated at 37 ^{o}C for 24 hours.

Subcultures were carried out at times 0, 3, 6 and 24 hours. The contaminated syrups were kept at room temperature throughout the duration of the experiments. At the end of the incubation time, the number of colony forming units (CFUs) were counted and recorded for each plate. The numbers of CFUs is presented as the mean of each microorganism per plate.

RESULTS

3.1 Product A

Product A is a locally manufactured cough syrup with the inactive ingredients shown in table 1. Microbial testing of a sample from product A (negative control) indicated no growth of any of the tested microbes. On the other hand, microbial growth in a normal saline 0.9% challenged with the various microbes showed heavy growth of all tested microbes (Figure 1). Upon challenge of product A with different microbes, heavy growth of *S. aureus* (184 CFU) and scattered growth of *E. coli* (5 CFU), *P. aureogenosa* (2 CFU), and *C. albicans* (6 CFU) were seen at time 0. After 3 hours of microbial challenge, the microbial growth was substantially reduced to 0 CFU while that of normal saline slightly declined or remained the same for all tested microbes (Figure 2). Similar results were obtained after 6 and 24 hours of microbial challenge.

3.2. Product B

Product B is an imported cough syrup. Negative control of product B showed no microbial growth while normal saline showed heavy microbial growth of all tested microbes. At all tested times (0, 3, 6 and 24 hours), no colonial growth of any of the tested microbes was seen (Figure 2).

3.3. Product C

Product C is an imported cough syrup with the inactive ingredients shown in table 1. Negative control of product C showed no microbial growth of any of the tested microbes. Upon challenge of product C with different microbes, there was a heavy growth of *S. aureus* (21 CFU) at time 0 (Figure 2). Scattered microbial growth of *E. coli* (1 CFU), and no growth of *P. aureogenosa* of *C. albicans* was seen at time 0. After 3 hours of microbial challenge, the microbial growth was substantially reduced to 1 CFU for *S aureus* and 0 for all other microbes. The microbial growth after 3 hours in normal saline slightly declined or remained the same for all tested microbes. Results obtained after 6 and 24 hours of microbial challenge were 0 for all tested microbes inoculated into product C.

3.4. Product D

Product D is an imported cough syrup with the ingredients shown in table 1. Negative control of product D showed no microbial growth of any of the tested microbes. Upon challenge of product D with different microbes, there was little growth of *S. aureus* (3 CFU) at time 0 (Figure 2). Scattered microbial growth of *P. aureogenosa* (1 CFU), and no growth of *E. coli* or *C. albicans* was seen at time 0. After 3 hours of microbial challenge, the microbial growth was slightly increased to 6 CFU for *S aureus* and 0 for all other microbes. The microbial growth after 3 hours in normal saline slightly declined or remained the same for all tested microbes. Results obtained after 6 and 24 hours of microbial challenge were 0 for all tested microbes inoculated into product D.

Table 1. Tested cough syrups and their inactive ingredients and nature of packaging

Product	Inactive	Type of package
A	Not indicated in the leaflet	Plastic
B	Sucrose, Sorbitol solution 70%, Saccharin sodium, Methyl hydroxybenzoate, Liquid glucose, Citric acid, Disodium hydrogen phosphate, Ethanol 95%, Propyl hydroxy benzoate, Ascorbic acid, Sunset yellow FCF, Orange oil 926, Purified water monosodium glutamate.	Glass
C	Purified Water, Sucrose, Glycerol, strawberry flavor, Saccharin Sodium, Citric acid anhydrous, Propyl Hydroxy Benzoate, Methyl Hydroxybenzoate Sodium	Glass
D	Ethanolum 250 mg (6%v/v), Aromatic Vanillinum, Saccharin, Sorbitolum 1.4 g equivalent to 1.4 g carbohydrate	Glass
E	Not indicated in the leaflet	Plastic

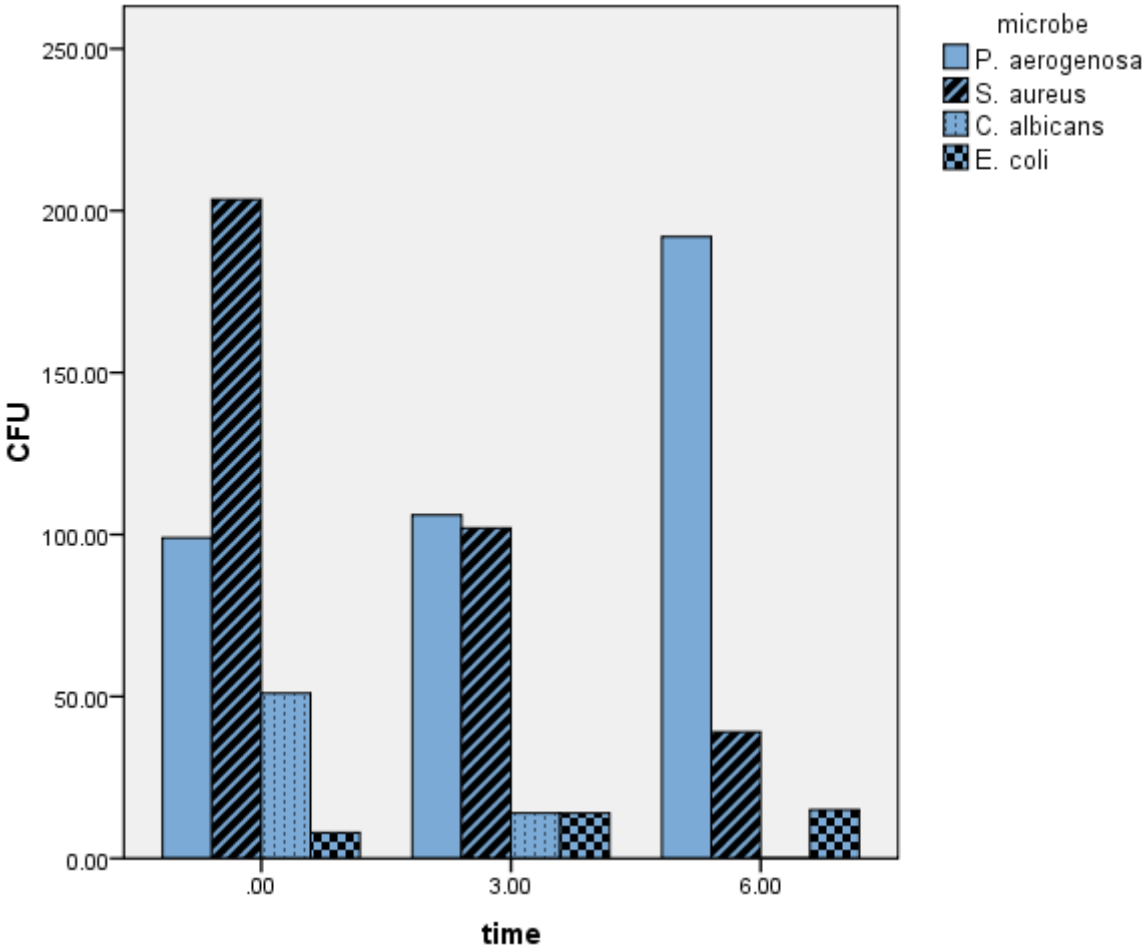

Figure 1. Number of colony forming units (CFU) of various microbes versus time after inoculation into normal saline

3.5. Product E

Product D is a locally manufactured cough syrup and its inactive ingredients are shown in table 1. Microbial testing of a sample from product E (negative control) indicated no growth of any of the tested microbes. On the other hand, microbial growth of a normal saline 0.9% challenged with the various microbes showed heavy growth of all tested microbes (Figure 1).

Upon challenge of product E with different microbes, there was a heavy growth of *S. aureus* (53 CFU) at time 0 (Figure 2). No microbial growth of *E. coli*, *P. aureogenosa*, and *C. albicans* was seen at time 0. After 3 hours of microbial challenge, the microbial growth of *S. aureus* was substantially reduced to 0 CFU while that of normal saline slightly declined or remained the same for all tested microbes. No growth of any tested microbe was obtained after 6 and 24 hours of the microbial challenge.

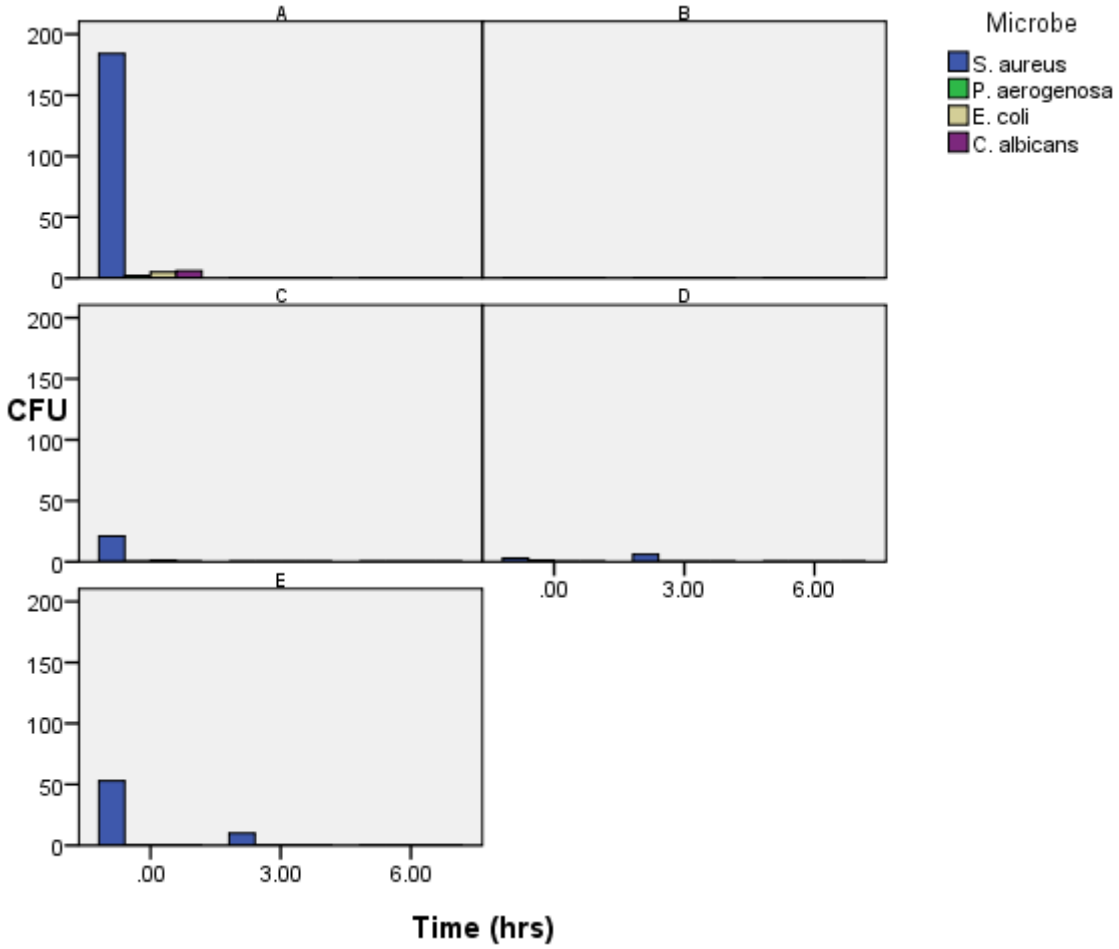

Figure 2. Number of colony forming units (CFU) of various microbes versus time after inoculation in various cough syrups. Results obtained from cultures at 24 and 48 hrs were all negative and therefore not presented

DISCUSSION

Liquid preparations intended for oral use, like cough syrups, are not required to be sterile. However, the accidental contamination of such products while in use might adversely affect the health of the patient. Therefore, manufacturers have to ensure and to implement certain measures that will prevent and minimize microbial growth of liquid preparations during use and home storage. In this study, we investigated growth of *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* inoculated into 5 different cough syrups: two were manufactured by different local pharmaceutical companies and three were imported by Palestinian agents. The results showed that 1) at time zero, growth of *S. aureus* was seen in all products except product B; 2) little or no growth of *C. albicans*, *P. aeroginosa* and *E. coli* were observed at time zero; 3) no

growth of any of the tested microbes was seen when subcultures were done after 6 hours of inoculation; 4) imported products showed lesser or no microbial growth compared to locally manufactured ones; and finally 5) despite the noticeable growth of *S. aureus* at time zero, all tested cough syrups passed the guidelines of British Pharmacopea regarding microbial challenge. [10]

It was noticeable that product B showed no microbial growth of any type at any time. Product B contained methyl and propyl paraben as well as 95% ethanol as preservatives. This combination of preservatives seem to be most effective in inhibiting microbial growth compared to methyl and propyl paraben with glycerol present in product C or ethanolum (6% v/v) present in product D. It is noteworthy that at time zero the greatest growth of *P. aerogenosa* was seen in product D. This might suggest that cough syrups having only ethanol as a preservative might not be capable of suppressing *P. aerogenosa* growth. Unfortunately, products A and E, which are locally, manufactured did not indicate in the leaflet the type of preservatives or other inactive materials present in the product. The Palestinian legislation does not obligate local pharmaceutical companies to indicate the inactive materials used in the product formulation. Nevertheless, all tested products, regardless of the nature of preservative used, were capable of suppressing growth of all types of microbes after 6 hours of inoculation. Based on these results, regulatory bodies should recommend pharmaceutical companies to use preservatives that are proven most effective and to state the nature and type of preservatives used in package inserts. It is alarming that locally manufactured pharmaceutical cough syrups do not have preservative efficacy compared to that of imported ones. Other published studies that tested microbial contamination of liquid oral preparations showed considerably antimicrobial activity of various preservative combinations against infectious bacteria during 48 hr studied period [11]. Nature and type of preservatives are not the only factors that influence microbial growth in liquid preparations. Other factors like nature of packaging material and type of cap used might influence microbial growth as well. Traditional glass containers do not interact with the preservatives. If the closure is airtight there is no problem of contamination. But plastic containers pose problems such as permeation through the container or interaction with it [12]. In this study, it was noteworthy that cough syrups which showed heavier microbial growth had plastic containers while products with little microbial growth had glass containers.

As a conclusion, the results of the microbiological study of five brands of cough preparations marketed in Palestine revealed that locally manufactured cough preparations were more vulnerable to microbial contamination compared to imported ones. However, the degree of microbial vulnerability and contamination was less than the official permissible limits for non sterile oral pharmaceutical preparations. It is therefore suggested that GMP and good packaging practices be enforced by the Palestinian ministry of health and to be implemented and maintained by local pharmaceutical companies. Also, the Palestinian general public needs to be educated on the proper handling and storage of these products to eliminate or reduce microbial contamination.

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