Iranian Journal of Pharmaceutical Research (2004) 3: 47-50
Received: June 2003
Accepted: January 2004

Original Article

Bioburden of Pharmacy Prepared Eucerin-Urea Ointments

Mohammad Reza Fazeli*[a], Nasrin Samadi[b], Mina Fattahi[a]

[a]*Department of Pharmaceutical Biotechnology, School of Pharmacy, Tehran University of Medical Sciences and Health Services, Tehran, Iran.* [b]*Department of pharmaceutics, School of Pharmacy,Tehran University of medical Sicences and Health Services, Tehran, Iran.*

Abstract

Pharmaceutical products prepared in pharmacies have the potential of contamination with different microorganisms. This is in part due to the unhygienic environment and also lack of a suitable preservative system. In this study, microbial quality of Eucerin-Urea ointments prepared at different pharmacies in Tehran, at the point of sale and also after two weeks storage at room temperature was examined. All the samples examined immediately after purchase found to have total viable counts of lower than 10^2 cfu/g. The objectionable organisms e.g. *Staphylococcus aureus, Candida albicans, Escherichia coli* and *Pseudomonas aeruginosa*, however, were found in about 77%, 45.5%, 9.1% and 4.5% of the samples, respectively. After two weeks storage, contamination levels increased such that about 36.4% of samples were found to have the total viable counts greater than 10^2 cfu/g and *Staphylococcus aureus, Candida albicans, Escherichia coli* and *Salmonella* sp. were isolated from 86%, 59%, 18.2% and 9.1% of the samples. Depicted results show that significant microbial contamination of unhygienically produced or poorly formulated products in pharmacies can occur and because of lacking a suitable preservative system, the microbial population will increase during storage which may be harmful to the consumers or patients.

Keywords: Microbial contamination; Pharmaceutical products; Bioburden; Indicator microorganisms.

Introduction

Pharmaceutical or hygienic products prepared in pharmacies are susceptible to contamination with different microorganisms. The incidence of microorganisms in these preparations is dictated by the quality of raw materials, containers, instruments applied, environment and the care and attitude of the personnel involved. In 1971, the working party of the pharmaceutical society of Great Britain reported the contamination of hospital or community pharmacies prepared products (1). Other investigations showed that application of contaminated topical products had been associated with the development of infections (2-5). It is also a practice at the pharmacies to dilute commercially available topical preparations by addition of bases like Eucerin or Paraffin with no preserrative adjustment or incorporating suitable preservative systems. This would negatively affect the preservative concentration and consequently its antimicrobial activity which could prone the product to further contamination during repeated patient use. Nobel and Savin (6) have also reported the contamination of a diluted cream containing Chlorcresol.

In this study, the microbial quality of a pharmacy-prepared Eucerin-based urea ointment was assessed. It was also important to

* Corresponding author:
E-mail: fazelimo@sina.tums.ac.ir

realize that the initial microbial count in a freshly prepared product could be increased during storage, particularly in formulations containing no preservative.

Experimental

Materials

Chemicals

All culture media and chemicals were obtained from the Merck Chemical Co. Sabouraud chloramphenicol agar was purchased from the Scharlau Chemie S. A.

Microorganisms

The indicator microorganisms used in this study were all from the Collection of School of Pharmacy, Tehran University of Medical Sciences and include: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella* sp. and *Candida albicans* ATCC 10231.

Methods

Collection and preparation of the samples

Twenty two 5% Eucerin-Urea ointments containing 10% distilled water packed in 100 g multi-dose plastic pots collected from 10 pharmacies at five different locations in Tehran city and also a Tehran university affiliated pharmacy were evaluated in this study. Two samples were taken from each pharmacy. The possible antimicrobial effects of formulation ingredients were eliminated by dilution, ascertained by examining the bacterio-static or fungi-static properties of test materials against microorganisms (7).

Bioburden determination

Samples were examined immediately after purchase and also after two weeks storage at room temperature. According to the microbiological requirements for non-sterile pharmaceutical products, the samples were subjected to the following examinations: total aerobic viable count (TAVC) by multiple-tube method and the presence or absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* sp. and *Candida albicans*. 10 g of each sample was mixed with 1 g Tween 80 and warmed to 45^{o}C for a maximum of 30 min to dislodge possible microbial cells. The total volume was adjusted to 100 ml by adding trypticase soy broth (TSB) or lactose broth (LB) for detection of bacteria, and Sabouraud dextrose broth (SDB) for detection of yeasts. The mixtures were incubated for 24 h at 32.5± 2.5˚C and for 14 days at 22.5± 2.5˚C for bacterial and yeast growth respectively (7).

Isolation and identification of the indicator bacteria

For the detection of *E. coli* contamination, LB enrichments were streaked onto differential Mac-Conkey and eosine methylene blue agar plates while one-ml aliquots of the LB cultures were transferred into 9 ml fluid selenite cystine media to detect *Salmonella* sp. The fluid selenite cystine cultures were incubated at 32.5± 2.5˚C for 12-24 h, and were further subcultured on the surface of bismuth sulfite and brilliant green agar media. TSB enrichments were streaked onto cetrimide and Pseudomonas isolation agars to detect any *Pseudomonas aeruginosa* contaminant. Vogel-Johnson and mannitol salt agar plates were used to detect *Staphylococcus aureus* presence. The identities of the isolated bacteria on selected media were confirmed by gram staining, colonial appearance and biochemical tests (7).

Isolation and identification of the indicator yeasts

SDB enrichments were streaked onto Sabouraud chloramphenicol agar and incubated at 22.5± 2.5^{o}C for 7 days. Resulting colonies were identified by germ-tube test and morphological characteristics were examined microscopically (8).

Results and Discussion

In this study, the bioburden of 22 unpreserved Eucerin-Urea ointments prepared in 11 pharmacies were examined.

Table 1 shows the TAVCs per g of the samples immediately after purchase (day 1), indicating that the TAVCs in all samples tested were lower than 10^2 cfu/g. The inhouse microbiological standards for non-sterile products made in Iran, stipulate that the TAVC should be less than 10^2 cfu/g or ml of the product and certain indicator microorganisms including *Pseudomonas aeruginosa*,

Table 1. Total aerobic viable counts of Eucerin-Urea ointments prepared at different pharmacies immediately after purchase (day 1) and after two weeks storage at room temperature.

Pharmacies	TAVCs* (cfu/g)-day 1		TAVCs (cfu/g)-day 14	
	Sample 1	Sample 2	Sample 1	Sample 2
1	<23	<23	40	40
2	90	23	200	200
3	23	23	200	200
4	40	40	200	40
5	90	90	500	500
6	<23	<23	23	90
7	<23	<23	23	23
8	<23	<23	90	23
9	<23	<23	23	40
10	<23	<23	90	40
11	<23	<23	23	200

* Total aerobic viable counts

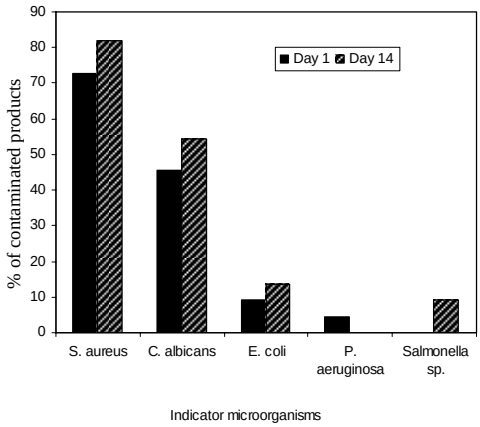

Figure. 1 Comparison of rates of objectionable and harmful organisms found in Eucerin-Urea ointments on days 1 (purchase day) and 14 (after two weeks storage at room temperature).

Staphylococcus aureus, *Escherichia coli*, *Salmonella* sp. and *Candida albicans* should be absent.

All samples showed higher counts on day 14 compared to counts obtained on day 1. 36.4% of the samples collected from 4 pharmacies had also bioburdens significantly beyond the acceptable in-house standards.

The presence of indicator organisms in the samples is reported in table 2. These results indicate that nearly 86% of the samples (19 out of 22) were contaminated at least with one objectionable or harmful organism on day 1. Table 2 also shows that in all samples, the indicator organisms were detected after two weeks.

The objectionable and harmful organisms detected on days 1 and 14 are compared in figure 1. Products collected from some pharmacies contained higher rates of the indicator organisms on day 14 compared to corresponding samples on day 1. High proportion (77%) of the samples (17 out of 22) was found to be contaminated with *Staphylococcus aureus* on day 1. This figure was increased to 86% after two weeks storage at room temperature. About half of the samples (45%) tested were *Candida albicans* positive on day 1, which this rate was increased to 59% on day 14. *E. coli* was found in 9.1% and 18.2% of the samples (2 and 4 out of 22) on day 1 and 14, respectively. *Pseudomonas aeruginosa* was detected in 4.5% of the samples on day 1, while it was not detected on day 14. Also two identical samples taken from pharmacy 2 showed *Salmonella* contamination on day 14, whereas this organism was not detected in both samples on the purchase day. Other contaminating organisms were Gram-positive bacilli.

Depicted results show that incidence of contamination varried between pharmacies. The contaminant Gram-positive air-borne bacilli might be originated from the pharmacy environment. The normal flora of the unhygienic pharmacy staff are the likelihood source for *Staphylococcus aureus* (9*)*. Gram-negative isolates of *Pseudomonas aeruginosa* and *E. coli* are usually water-borne and may have been appeared in the products from the water supplies (10, 11). Water is usually part of the ingredients or used in processing of the products. Contamination may also arise from raw materials supplied in open container exposed to the air and dust and could be contaminated more during repeated use.

The results of this study are similar with previous reports on the contamination of hospital pharmacies prepared products by *Pseudomonas aeruginosa*, aerobic spore bearers, Gram-positive coagulase-negative cocci and other Gram-negative rods (12, 13) and address a need for microbiological control of the pharmacy prepared products on all steps of remedies preparation. Raw materials, containers, water supplies should be checked routinely for microbial presence. Specifying a carefully microbial free environment e.g. a laminar air flow hood for the preparation of

Table 2. Microorganisms found in Eucerin-Urea ointments prepared at different pharmacies immediately after purchase (day 1) and also after two weeks storage at room temperature.

Pharmacies	Day 1		Day 14	
	Sample 1	Sample 2	Sample 1	Sample 2
1	[a]*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
2	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
	[b]*C. albicans*		*C. albicans*	
3	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
		[c]*P. aeruginosa*	[d]G+ bacilli	G+ bacilli
4	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
	C. albicans	*C. albicans*	*C. albicans*	*C. albicans*
			Salmonella sp.	*Salmonella* sp.
				[e]*E. coli*
5	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
	C. albicans		*C. albicans*	*C. albicans*
				E. coli
6	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
	C. albicans	*C. albicans*	*C. albicans*	*C. albicans*
	E. coli	*E. coli*	*E. coli*	*E. coli*
7	*S. aureus*	_	*S. aureus*	*S. aureus*
8	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
	C. albicans	*C. albicans*	*C. albicans*	
9	_	*S. aureus*	*C. albicans*	*S. aureus*
			G+ bacilli	
10	*S. aureus*	*C. albicans*	*S. aureus*	*C. albicans*
			C. albicans	
11	*C. albicans*	_	*C. albicans*	*C. albicans*
			G+ bacilli	G+ bacilli

[a]*Staphylococcus aureus;* [b] *Candida albicans;* [c]*Pseudomonas aeruginosa;* [d]*Gram- positive bacilli;* [e]*Escherichia coli*

these products would efficiently exclude the air-borne organisms which are usually destructive to the products ingredients and may also be harmful to the patients. The incorporation of a suitable preservative system into these multiple-dose non-sterile products seems to be necessary in order to prevent any subsequent microbial growth during use.

References

(1) Report by a public health laboratory service working party. Microbial contamination of medicines administered to hospital patients. *Pharm. J.* (1971) 207: 96-99

(2) Morse LJ, Williams HL, Grenn FP, Eldridge EE and Rotta JR. Septicemia due to *Klebsiella pneumoniae* originating from a hand-cream dispenser. *New Eng. J. Med.* (1967) 277: 472-473

(3) Bassett DCJ, Stokes KJ and Thomas WRG. Wound infection with *Pseudomonas multivorans*, a water-born contaminant of disinfectant solutions. *Lancet* (1970) i: 1188-1191

(4) Baird RM, Awad ZA and Shooter RA. Contaminated medicaments in use in a hospital for diseases of the skin. *J. Hyg. Camb.* (1980) 84: 103-108

(5) Parrott PL, Terry PM, Whitworth EN, Frawley LW, Coble RS, Wachsmuth IK and McGowan JE. *Pseudomonas aeruginosa* peritonitis associated with contaminated poloxamer-iodine solution. *Lancet* (1982) ii: 683-685

(6) Noble WC and Savin JA. Steroid cream contaminated with *Pseusomonas aeruginosa. Lancet* (1966) i: 347-349

(7) *United States Pharmacopeia.* XXV. US Pharmacopeial Convention. Maryland (2002) 1873-1878

(8) Domsch KH, Gams W and Anderson TH. *Compendium of Soil Fungi*. Academic Press. New York (1980) 1156-1160

(9) Luppens SBI, Reij MW, van der Heijden RWL, Rombouts FM and Abee T. Development of a standard test to assess the resistance of *Staphylococcus aureus* biofilm cells to disinfectants. *Appl. Env. Microb.* (2002) 68: 4194-4200

(10) Ayliffe GA, Barry DR, Lowbury EJL, Roper-Hall MJ and Walker WM. Postoperative infection with *Pseudomonas aeruginosa* in an eye hospital. *Lancet* (1966) i: 1113-1117

(11) Baird RM, Parks A and Awad ZA. Control of *Pseudomonas aeruginosa* in pharmacy environment and medicaments. *Pharm. J.* (1977) 119: 164-165

(12) Baird RM, Brown WRL and Shooter RA. *Pseudomonas aeruginosa* in hospital pharmacies. *Br. Med. J.* (1976) 1: 511-512

(13) Baird RM. Microbial contamination of non-sterile pharmaceutical products made in hospitals in the North East Thames Regional Health Authority. *J. Clin. Hosp. Pharm.* (1985) 10: 95-100