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Antibiogram of bacterial isolates and fungi associated with some creams and lotions sold in Zaria, Nigeria

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Skin moisturizing creams and lotions are external preparations that contain special additives that could serve as nutrients for microorganisms; it is possible that such products may get contaminated and serves as vehicles for pathogen transfer. Eight cosmetic products, consisting of 4 body creams and 4 lotions were randomly purchased from the local markets within Zaria and analyzed for their microbiological quality. Five out of the eight products examined were found to be contaminated with bacteria, which had counts ranging from 6.0×10^4 to 5.3×10^5 cfu/g. Three of the products showed evidence of fungal contamination, with counts ranging between 1.3×10^4 and 2.8×10^8 propagules/g. However, three of the products were free from both bacterial and fungal contaminants. Organisms isolated from the creams include *Staphylococcus aureus, Bacillus* sp., *Micrococcus* sp., *Escherichia coli, Pseudomonas aeruginosa, Aspergillus* spp., *Mucor*and *Penicillium* spp. The organisms showed a high level of antibiotic resistance. However, erythromycin had the highest activity against the Gram-positive isolates while gentamycin had the highest activity against the Gram-negative isolates. There was significant correlation between microbial contamination and package orifice diameter, with highest bacterial and fungal count observed in products with wider orifices. Based on the U.S. Food and Drug Administration (FDA) and U.S. Pharmacopeial Convention (USP) standard of 10^3 cfu g^{-1} for cosmetic products, it was evident that five products had counts above this level; thereby representing serious health hazard. As a result, manufacturers should adhere to good manufacturing practices.

Key words: Moisturizing cream, body lotions, microbiological quality, Zaria, Nigeria.

INTRODUCTION

The Food and Drug Administration (FDA) regulates cosmetics, which they define as products that are used for cleansing, beautifying, promoting attractiveness or altering the appearance without affecting the body's structure or function (Perry, 2011; Siegert, 2012; Noor et al., 2015). Included in this definition are products such as body creams, lotions, perfumes, lipsticks, fingernail polishes, eye and facial make-up preparations, shampoos, permanent waves, hair colors, toothpastes, deodorants and any material intended for use as a component of a cosmetic product (Özalp, 1998; USP, 2003; FDA, 2007).

Skin moisturizing creams and lotions are external preparations with different rheological properties that

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contain special additives like plant extracts, fatty acids and vitamins (Crowshow, 1997). As these additives could serve as nutrients for microorganisms, it is possible that such products may get contaminated and be vehicles for pathogen transfer (Gopalkrishna et al., 2010; Noor et al., 2015). They are therefore liable to microbial contaminations either in the course of their preparation, transportation and/or use by the consumers which may lead to their spoilage (Osungunna et al., 2010).

The warm humid conditions that are characteristic of a tropical environment are conducive for the growth of micro-organisms, which are responsible for a number of infectious diseases and the spoilage of food, cosmetic and pharmaceutical product (Ballereau et al., 1997; Okeke and Lamikanra, 2001).

Moulds, yeasts and pathogenic bacteria have been isolated from cosmetics (FDA, 2007; Okeke and Lamikanra, 2001; Gopalkrishna et al., 2010; Osungunna et al., 2010; Qasem et al., 2012; Aminu and Umar, 2012). According to FDA (2007), most cases of contamination are due to manufacturers using poorly designed, ineffective preservative systems and not testing the stability of the preservatives during the product's customary shelf-life and under normal use conditions.

The practice of controlling the microbial levels of industrially processed food and other products is an age-old one. For these products, microbial standards have since been established. Unfortunately, such standards appear to be non-existent with regards to cosmetics in general. However, as a result of this, some infectious diseases arise due to usage of contaminated moisturizing creams, body lotion and other cosmetic products. Some of these diseases include scabies, acne, eczemaand dyschromia (Parker, 1972; Brannan and Dille, 1990; Becks and Lorenzoni, 1995; Pollack, 2000; Mahé et al., 2003; Behravan et al., 2005). Contamination with *Clostridium tetani* and infection of neonates with *Pseudomonas aeruginosa* from contaminated cleansing solutions have been reported (Perry, 2001). It has also been shown that a contaminated hand cream could be a source of septicemia in intense therapy unit (Baird, 1977).

The incidence of skin diseases is likely to be frequent in the developing countries due to the unhygienic environment, dense population favoring contagious diseases, lack of awareness on cleanliness, improper sanitation practices, and finally the massive use of contaminated processing water (USP, 2003; Denyer et al., 2004; Cundell, 2005a, b; Prüss-Üstün and Corvalán, 2006).

Reports on the microbial quality evaluations of cosmetics and toiletries have mainly been from temperate countries (Morse and Schonbeck, 1968; Becks and Lorenzoni, 1995; Itin et al., 1998; Sánchez-Carrillo et al., 2009). As there is great difference in conditions between the temperate and the tropics, these results cannot be extrapolated to Nigeria as a tropical country. Some studies have however examined cosmetic creams and lotion marketed in Nigeria with the identification of potential pathogens such as *Escherichia coli, Salmonella* spp., *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* spp., *Staphylococcus aureus, Klebsiella* spp., *Enterobacter gergoviae, Aspergillus flavus* and *Penicillin* spp. (Okore, 1992; Onwunali, 2000; Okeke and Lamikanra, 2001; Anelich, 2007; Osungunna et al., 2010). Only one of such study was conducted in Northern Nigeria (Aminu and Umar, 2012). The present study therefore aimed at evaluating the microbiological quality of some moisturizing creams and body lotions sold in cosmetic stores in Zaria, north-western Nigeria, where there is dearth of data.

MATERIALS AND METHODS

Sample collection

A total of eight commercially available products, consisting of four body creams and four lotions most commonly used were employed in this study. The products were purchased from different market stalls within Zaria, Kaduna State Nigeria. The samples ranged from moisturizing creams to toning creams commonly used by people in this part of the country, and all were within their shelf life.

Media preparation

Plate count agar was used for bacterial count, nutrient broth was used for enrichment and nutrient agar was used for preservation of isolates in slants. Simmon citrate agar,mannitolsalt agar, MacConkey agar, eosin methylene blue agar and *Salmonella-Shigella* agarwere used for bacteria isolation and Sabouraud dextrose broth and Sabouraud dextrose agar were used for fungi isolation. All media used were prepared according to the manufacturer's instruction.

Analysis of sample

Enumeration of microorganisms

Ten grams of each sample was transferred into 90 ml distilled water and stirred thoroughly. Serial dilutions of the stock solution were made and appropriately labeled. 1 ml of the diluent 10^3 was inoculated on plate count agar, and incubated at 37°C for 24 h. Bacterial counts were determined by spread plate method. Also, 0.1ml of the 10^2 diluent was transferred each unto Sabouraud dextrose agar, and the plate was incubated at ambient temperature for 3 days after which observation was made and counting carried out upon the appearance of growth. The fungi count was carried out using the pour plate method. Sterility test was carried out by incubating freshly prepared media at room temp for 24h (Cheesbrough, 2005).

Cultural and morphological characterization of the bacterial and fungi isolates

For bacterial isolation, 1 g of each body cream was weighed and inoculated unto 9 ml of nutrientbroth, and incubated over night at 37°C for enrichment. After which, one loopful each was again taken from the enrichment culture and streaked on Mannitol salt agarand

Table 1. Microbial loads of the products analyzed.

Product analyzed	Orifice diameter	Bacterial count (cfu/g)	Fungal count (propagules/gram)	Manufacturing date	Expire date
P1	4mm	-	-	11/2011	11/2014
P2	Wide opening (60 mm)	3.5×10^5	2.7×10^4	04/2012	04/2015
P3	Wide cream opening (65 mm)	4.0×10^4	-	10/2011	10/2013
P4	3 mm	-	-	02/2012	02/2014
P5	6 mm	6.0×10^4	-	11/2011	11/2013
P6	5 mm	1.6×10^5	1.3×10^5	12/2011	12/2013
P7	Wide cream opening (70 mm)	5.3×10^5	2.8×10^4	12/2011	12/2014
P8	Wide cream opening (50 mm)	-	-	02/2012	02/2014

P = Product, - = no growth.

Mac Conkey agar and then incubated at 37°C for 24 h. The plates were examined for evidence of growth, colonies were sub-cultured unto nutrient agar slants, Gram stained and examined under 100x objective according to the methods outlined by Cheesbrough (2005) as described by Aminu and Umar (2012). Thereafter, isolates were identified by their colonial morphology and Gram reaction.

For fungi isolation, 1 g each of the body creams were weighed, transferred into 9ml of Sabouraud dextrose broth andincubated over night at 37°C for enrichment, this was to selectively favor fungi growth. After incubation, one loopful each was inoculated unto Sabouraud dextrose agar and the plates incubated at ambient temperature for 3 days. The plates were observed for evidence of growth and colonies were identified according to the methods outlined by Cheesbrough (2005) as described by Onwunali (2000).

Biochemical identification of the bacterial isolates

The bacterial isolates were further identified using conventional biochemical methods. Isolates that were Gram-positive cocci were subjected to coagulase, catalase and mannitol fermentation test, Gram-positive rods were subjected to indole, citrate, motility, VP,etc tests. While Gram-negative organisms were subjected to indole, citrate, MRVP and oxidase tests. Other biochemical test such as swelling of bacilli body, citrate, methyl red and growth on Cetrimide agar were also employed in bacteria identification.

Antimicrobial susceptibility test

Antimicrobial susceptibility pattern of each isolate was done using conventional disc diffusion method according to NCCLS standard. This was carried out using multiple antibiotic discs. A turbid suspension of the isolates was made in distilled water using McFarland Standard, prepared as a comparator. A sterile swab was dipped into the bacteria suspension, pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar. Sterile forceps were then used to place the multiple antibiotic discs in a circular pattern on the media. The process was carried out for all the presumptively identified isolates, and the plates incubated at 37°C for 24h. After incubation, the zone of inhibition for each antibiotic was measured from the center of the disc to the point where clearing stopped.

Analysis of data

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test using SPSS version16. All data were expressed as mean ± SDand p value < 0.05 was considered significant at 95% confidence interval.

RESULTS

Out of the eight products examined for bacterial contaminant, 5 (62.5%) yielded bacterial growth, with counts ranging between 6.0 x 10^4 and 5.3 x 10^5 cfu/g (Table 1). Both Gram-positive and negative bacterial were isolated from the products. Gram-positive organisms such as *Staphylococcus aureus* (3/5: 60%), *Bacillus* spp. (3/5: 60%) *and Micrococcus* spp. (1/5: 20%)were isolated from the products, while Gram-negative organisms such as *Escherichia coli* (2/5: 40%) and *Pseudomonas* spp.(1/5: 20%)were isolated (Table 2). One of the products from which Gram-negative organisms were isolated had the highest count.

Out of the eight products examined for fungal contamination, 3 (37.5%) showed evidence of fungal contamination, with counts ranging from 1.3 x 10^4 to 2.8 x 10^4 propagules/g (Table 1). Fungal contaminants isolated from the products include *Aspergillus* spp., *Penicillium* spp. and *Mucor* (Table 3). Table 4 shows the biochemical identification of the bacterial isolates.

Erythromycin had the highest activity against the Gram-positive isolates (Table 5), while Gentamycin had the highest activity against the Gram-negative isolates (Table 6). There was significant correlation between microbial contamination and package orifice diameter. Product six and seven with highest bacterial and fungal count had wider orifices.

DISCUSSION

Five out of the eight samples analyzed had evidence of microbial contamination. Generally, it is desirable that cosmetics and toiletries including body creams should contain <10^3cfu/g or cfu/ml of bacteria at the time they reach the consumer and they should also be free of

Table 2. Morphological characteristic of bacterial isolates.

Products analyzed	Colonial morphology	Gram reaction	Cellular morphology
P2 (A)	Creamy large macerated colonies.	+	Spherical, Scattered organisms (Cocci)
P2 (B)	Large, flat pale colonies	+	Rod like cells in single, pairs and clusters
P3	Large, flat pale colonies	+	Rod like cells in single, pairs and clusters
P5	Creamy large macerated colonies.	+	Spherical, clustered organisms (Cocci)
P6 (A)	Creamy large macerated colonies.	+	Spherically clustered organisms (Cocci)
P6 (B)	Pinkish smooth colonies	-	Rods in clusters and pairs
P6 (C)	Pale colonies	-	Rods in clusters and scattered
P7 (A)	Creamy large macerated colonies.	+	Spherical, clustered organisms (Cocci)
P7 (B)	Large, flat pale colonies	+	Rod like cells in single, pairs and clusters
P7 (C)	Pinkish smooth colonies	-	Rod like cells in Chain

+ = Positive - = negative, P = product.

Table 3. Morphological characteristic of fungal isolates.

Products	Colonial morphology	Microscopic morphology	Presumptive identification
P2 (A)	Black fluffy colony; Reverse: Creamy	Conidial head splits into columns	*Aspergillus* spp.
P2 (B)	White cottony colony Reverse: Cream/yellow, becomes grey on getting older	Non-septate hyphae, branched sporangiophore, with round sporangiospores	*Mucor* spp.
P6	Black fluffy colonies Reverse: Creamy	Conidial head splits into columns	*Aspergillus* spp.
P7 (A)	Blue green fluffy colony Reverse: Dark green	Branched conidia, resembling bushes	*Penicillium* spp.
P7 (B)	White cottony colony; Reverse: Cream/yellow, becomes grey on getting older.	Non-septate hyphae, branched sporangiophore, with round sporangiospores	*Mucor* spp.

P = Product.

Table 4. Biochemical identification of the bacterial isolates.

Products	Cat	Cog	FM	Mot	Cit	VP	SBB	SH	Ind	MR	OX	GCA	Presumptive Identification
P2 (A)	+	+	+	-	-	ND	ND	ND	ND	ND	ND	ND	*S. aureus*
P2 (B)	+	ND	ND	+	+	+	-	+	-	ND	ND	ND	*Bacillus* spp.
P3	+	ND	ND	+	+	+	-	+	-	ND	ND	ND	*Bacillus* spp.
P5	+	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	*Micrococcus* spp.
P6 (A)	+	+	+	-	-	ND	ND	ND	ND	ND	ND	ND	*S. aureus*
P6 (B)	ND	ND	ND	ND	-	-	ND	ND	+	+	-	-	*E. coli*
P (C)	ND	ND	ND	ND	+	+	ND	ND	-	-	+	+	*Pseudomonas* spp.
P7 (A)	+	+	+	-	-	ND	ND	ND	ND	ND	ND	ND	*S. aureus*
P7 (B)	+	ND	ND	+	+	+	-	+	-	ND	ND	ND	*Bacillus spp.*
P7 (C)	ND	ND	ND	ND	-	-	ND	ND	+	+	-	-	*E. coli*

P = Product, ND = not determined, Cat = catalase, Cog = coagulase, FM = fermentation of Mannitol, Mot = motility, Cit = citrate, VP = Voges-Proskauer, SBB = swelling of bacillary body, SH = starch hydrolysis, Ind = indole, MR = methyl red, OX = oxidase test, GCA = growth on Cetrimide agar.

Table 5. Antibiotic susceptibility patterns of Gram positive bacterial isolates.

Sample	AU	FX	AP	AM	CO	CX	GN	CD	CP	E
P2 (A)	R	R	S	S	S	S	S	S	S	S
P2 (B)	S	R	R	S	R	R	S	S*	R	S
Product 3	R	R	R	S	R	S	R	R	S	R
Product 5	S	S	R	R	S	S	S	R	S	S
Product 6 (A)	S	R	R	S*	R	R	R	S	S	S
Product 7 (A)	R	S*	R	S	R	S	S	R	S	R
Product 7 (B)	S*	R	R	R	R	R	S	R	R	S
Percentage sensitive (%)	57	28	14	72	28	57	72	43	72	72
Percentage Resistant (%)	43	72	86	28	72	43	28	57	28	28

R = Resistant, S = sensitive, * = intermediate resistance, AU = Augmentin, FX = Ceftriazone, AP = Cloxacillin, AM = Ampicillin, CO = Cotrimoxazole, CX = Cephalexin, GN = Gentamycin, CD = Clindamycin, CP = Ciprofloxacin, E = Erythromycin.

Table 6. Antimicrobial susceptibility patterns of Gram negative isolates.

Sample	N	CIP	TE	NF	AX	OF	C	CF	AM	GN
product 6 (B)	S	S	S	S	S*	S	R	S	S*	S
product 6 (C)	R	R	R	S*	R	R	S	S	R	S
Product 7 (C)	S	S*	S	R	S	R	R	S	S*	S
Susceptible (%)	67	67	67	67	67	33	33	100	67	100
Resistant (%)	33	33	33	33	33	67	67	0	33	0

R = Resistance, S = sensitive, * = intermediate sensitivity, AM=Ampicillin, CIP = Ciprofloxacin, TE = Tetracycline, NF = Norfloxacin, AX = Amoxicillin, OF = Ofloxacin, C = Chloramphenicol, CF = Cefuroxime, AM = Ampicillin, GN = Gentamycin N = Nitrofurantoin. Zone of Inhibition: 0 -13 mm = resistance; 14 -17 mm = intermediate sensitivity; 18 mm and above = sensitivity.

potentially pathogenic organisms (FDA, 1995; USP, 2003). The microbial count varied with the products. The bacteria counts ranged from 6.0×10^4 to 5.3×10^5 cfu/g. The bacteria count obtained in this study is slightly higher than that previously reported in Nigeria (Okeke and Laminaka, 2001) and similar to a study in same area where count of 5.4×10^3 to 1.7×10^5cfu/g was obtained (Aminu and Umar, 2012). The count contrast results of other studies with higher contamination rates (10^4 to 10^9cfu/ml) were reported (Okore, 1992; Onwunali, 2000). Similar rates of 10^5cfu/g (Gopalkrishna et al., 2010) and $>10^5$cfu/g have also been reported from other countries and in outbreaks where counts of facultative pathogens were in levels capable of infecting the immuno-compromised (Baird, 1977; Becks and Lorenzoni, 1995; Itin et al., 1998).

The variation of the counts may be due to non-uniformity in the processing of raw materials and procedures as well as handling and distributions by the various companies. Poor personal hygiene and level of awareness to maintain good manufacturing practice may also contribute to the total viable counts obtained. Although, the bacterial counts of the moisturizing creams and body lotions examined in this study were not higher than counts $>10^5$cfu/g previously reported in some studies, the counts were higher than the recommended level for cosmetic products by FDA and BP/USP. For example, in the case where cosmetic items are applied within the non-eye area, the total aerobic microbial count (TAMC) should notbe more than 10^3cfu g^{-1}; and for the items used within the eye area, the limit should not exceed 10^2cfu g^{-1} (Pollack, 2000; Behravan et al., 2005; Onurdağ et al., 2010).Therefore, there is the possibility that these products could be vehicles for pathogen transmission when shared.This is in view of the facts that, the preservative content of these products were apparently incapable of dealing with the organisms isolated and the counts obtained. Furthermore, bacterial contamination may cause spoilage of the product as earlier observed by Aminu and Umar (2012).

Both Gram-positive and negative bacteria were isolated in the present study. This result contrast the finding of Anelich and Korsten (1996) where only Gram-negative bacteria were isolated and that of Aminu and Umar (2012) where only Gram-positive bacteria were isolated. The present report is however similar to the findings of Okeke and Lamikanra (2001), Gopalkrishna et al. (2010), Osungunna et al. (2010) and Qasem et al. (2012) where both Gram-negative and Gram-positive bacteria were isolated.

Gram positive organisms predominated in the products examined in this study as previously reported (Onmunali,

2000), with *S. aureus* and *Bacillus* spp. isolated from three products each and micrococcus from one product. *S. aureus* was also one of the predominant bacteria isolated recently from body creams and lotions by Osungunnaet al. (2010). *S. aureus* being a normal flora of the skin easily contaminate products during handling and processing; and the heat resistance nature of *S.*spp. and *Micrococcus* spp. also contribute to their survival in processed products (Efiuvwevwere, 1988). The presence of *Bacillus* spp. which are spore formers is due to their resistance to moist heat even at temperatures as high as 120°C (Moedenhauer et al., 1995).

The products from which *S. aureus* were isolated can be considered unsuitable because the bacterium is an opportunistic pathogen and it was isolated from the product before use. *S. aureus* is associated with skin infections such as folliculitis (pimples) and furuncles (boils) (Arora and Arora, 2008). Although not many investigations have examined cosmetics as potential sources of infections, there are documented cases of eye infection such as conjunctivitis and blepharitis from cosmetics and toiletries particularly in immunocom-promised (Becks and Lorenzoni, 1995; Itin et al., 1998).

The Gram negative organisms identified were *E. coli* isolated from two products and *Pseudomonas* spp. isolated from one product. This result is similar to an earlier report by Okeke and Lamikanra (2001). *Pseudomonas* spp. was the predominant species isolated by Gopalkrishna et al. (2010). The presence of *E. coli* indicates the presence of faecal contaminants, while *Pseudomonas* spp. is an opportunistic pathogen that contaminate wound hence can serve as a source of wound infection, which could result in serious complications when such creams are applied.

The source of contaminants detected in these products are likely due to the raw materials used, conditions prevalent in the environment in which the products are manufactured and packaged as well as the storage conditions they are subjected to (Balsam, 1974). Water employed in the manufacture has been described as the most likely source of contaminants in cosmetics (Crowshow, 1997).

In this study, there was significant correlation between bacterial count and package orifice diameter and pack size as earlier observed (Brannan and Dille, 1990; Gopalkrishna et al., 2010; Aminu and Paul, 2012). People usually use their fingers to take the creams present in wide mouthed containers. Lack of growth seen in Product 1 may be probably due to the nature of its packaging and its narrow orifice, which prevented the contamination of the cream by microorganisms. None of the products packaged in narrow orifice containers and tubes were contaminated in the study conducted by Gopalkrishna et al. (2010). It has been earlier observed that the use of non-invasive packages (tubes, pumps or narrow orifice containers) and adequate preservation increase the chances that contamination level will remain low during storage and use of products (Brannan and Dille, 1990).

It has been shown that every time a bottle of cosmetic is opened, microorganisms in the air have the opportunity to rush in, but narrow orifices reduces the chances of exposure to air and contamination. In addition, adequately preserved products can kill a lot of the microorganism to keep the product safe. Indeed the presence of micro-organisms in body creams coming directly from the industries does not only indicate a possible threat to health but also possibility of economic losses due to spoilage during storage (Onmunali, 2000). The use of water and raw materials of suitable quality and good manufacturing practices should generally lead to the production of preparations with low microbial contamination. Adequate preservation and the use of non-invasive packages (such as tubes, pumps or narrow orifice containers) increase the chances that contamination levels will remain low during storage and use of the product (Brannan and Dille, 1990).

Based on the antibiotic susceptibility testing recorded from the isolates, it was seen that majority of *Staphylococcal* isolates showed resistant to a wide range of antibiotics, this is in agreement with the results of Osungunna et al. (2010) and Gopalkrishna et al. (2010), in which resistance as high as 90% was recorded for *S. aureus*. The characteristic resistance of *S. aureus* can be attributed to a previous encounter of the organisms with various antibiotics in which resistance was conferred (Osungunna et al., 2010). Likewise in the case of the Gram negative isolates, relatively high level of sensitivity was recorded, especially to antibiotics such as Gentamycin and Ciprofloxacin.

Conclusion

The microorganisms isolated from the products in the study were *Escherichia, Bacillus, Micrococcus, Staphylococcus, Pseudomonas, Aspergillus, Mucor* and *Penicillium,* indicating that these products might be unsafe for use. It is undisputable evident that contaminated creams and lotions are dominant in the Nigerian market. Based on the FDA and USP standard of 10^{3}cfu $g^{-1}$for cosmetic products, it was evident that out of the eight products sampled, only three were fit for use as the other five products had counts above this level, as well as fungal contaminants.

RECOMMENDATION

It is therefore recommended that special agencies should be put in place with stringent regulatory standards to check the manufacturing and processing of these widely used applications. Scrupulous attention should be given to all ingredients as well as microbial testing before their usage, so as to make them less sources of microbial

infection. Adherence to good manufacturing practice and aseptic handling of raw materials should be ensured to ensure optimum reduction of the microbial load of the finished products. Individuals responsible for maintenance and sales of the finished products should be aware of the storage conditions of each product.

Conflict of Interests

The authors have not declared any conflict of interests.

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