Volume : 3 | Issue : 2 | February 2014 • ISSN No 2277 - 8179

Research Paper

Isolation And Identification of Bacteria From Different Cosmetic Samples And to Check Antimicrobial Activity of Antibiotics on Bacteria Isolated

Microbiology

KEYWORDS : Streptomycin, Cosmetics,*E.coli*, Chloramphenicol, Microscopy, Nutrient Agar

Aakriti Guleria

Abhilashi Institute of Life Sciences, Department of Microbiology, Mandi - 175008, Himachal Pradesh, India.

ABSTRACT

In present study,investigation has been performed for Isolation and Identification of different types of bacteria from Cosmetic Samples. Samples were taken as Powder ,Kajal, Moisturizer, Lipstick and Eyeliner. Microorganisms like E.coli ,Staphylococcus and Bacillus were isolated from cosmetic samples. E.coli were isolated from Kajal ,Staphylococcus from Powder and Bacillus from Moisturizer.No microorganisms were isolated from Lipstick and Eyeliner.Isolation of three bacteria from three different samples of cosmetics showed Gram positive,rod shaped cocci and Gram negative bacilli.These microorganisms are identified on the basis of colony morphology on Nutrient Agar,by Microscopy,growth on selective media and final identification by biochemical analysis.Then antimicrobial activity of the antibiotics was checked.Three antibiotics were used namely Chloramphenicol,Streptomycin and Tetracyclin. Chloramphenicol showed greater or maximum zone of inhibition for E.coli i.e 11mm and minimum zone of inhibition for Staphylococcus i.e 8mm.Tetracyclin showed maximum zone of inhibition for Bacillus i.e 14mm and minimum zone of inhibition for Staphylococcus i.e 8mm. Streptomycin showed maximum zone of inhibition for Staphylococcus i.e 9mm. E.coli was the most prevalent bacteria among most of the cosmetic samples while Staphylococcus and Bacillus were found in least quantity.Results declared that Chloramphenicol is effective against E.coli. Tetracyclin is effective against Bacillus and Streptomycin has intermediate effect on Bacillus.

INTRODUCTION:

Microbial contamination of cosmetic products is a matter of great importance to the industry and it can become a major cause of both product and economic loss. The water and nutrients present in cosmetics make them susceptible to microbial growth,although only a few cases of human injury due to contaminated cosmetics have been reported.More often, microorganisms are the cause of organoleptic alterations such as offensive odours and changes in viscosity and colour (Orus,P.1993).

Methods to det ect microbial contamination in cosmetics and their raw materials are usually based on traditional plate counts.However,little is known about the metabolic state of microorganisms residing in cosmetic products or in specific areas of a manufacturing plant.Viable microorganism are often metabolically injured as a result of adverse physical or chemical conditions(high processing temperature,cleaning,sanitizati on agents and preservatives).As a result,these microorganisms are in a viable but non-culturable state and thus cannot multiply in a nutritive agar medium.The recovery of stressed microorganisms is a challenge for cosmetic microbiologists,since appropriate diluents,preservatives ,neutralizing agents,culture media,etc. are needed.The validation of microbiological detection methods is therefore an indispensable prerequisite for the detection of microorganisms.

A variety of new methods,such as Bioluminescence,impedance and cytometry,which are based on the metabolic state of microorganisms,are the most reliable for detecting stressed cells.These "fast"methods allow the detection of microbial contamination ,both in the finished products and in raw materials with in 24 hours.Fast methods are of great industrial importance,since they facilitate the rapid release of products into the market.However,despite the advantages offered by fast methods,they are not yet able to detect specific microorganisms,including pathogens.Thus,classical microbiological approaches remain indispensable for the isolation and identification of microorganisms (Leranoz,S.1994).

Historically,cosmetic term includes the products whose purpose was to enhance the appearance or modified the odour of the human body.However this concept has evolved due to the pressure to continually adapt cosmetics to changing market demands and to fulfil the consumers need and expectations.This rapid evolution forced the authorities of different countries,in the middle of the 20th century to regulate cosmetic products in order to ensure consumer safety.

However, with the continuos increased in the variety of raw materials and cosmetic products,it is frequenty necessary to rely on pharmaceutical, food regulations and on pharmacopoeias due to the lack of official cosmetic guidelines.While regulation regarding microbiological content in cosmetic products do not exist,foods are classified according to their nature and health regulations including microbiological limits are defined.Although some recommendations have been published by governments and cosmetics associations,the only requirement of cosmetics is that they "must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use".

Differences between cosmetics and food/pharmaceuticals are often a matter of disagreement.Health authorities would like cosmetic companies to achieve the "gold standards"imposed by pharmaceutical regulations and recommendations on areas such as manufacturing,filling and testing.While the general intention is correct,cosmetics are not meant to be ingested or injected in the human body nor are they to be used for therapeutic purpose.

Microbiologists working in the fields of cosmetics are frequently required to design preservative systems that provide good protection of cosmetic products against microbial contamination.However,scientific information on this issue is scarce,since most biocide studies deal with antibiotics for human treatment. Microbiologists must therefore work within a narrow range of preservative concentrations in order to achieve effectiveness against microorganisms while avoiding toxicity for consumers.For this reason,regulations in the EU and in other countries have specified preservatives allowed,their maximum concentrations and other directions specifically related to the kind of cosmetic products.

Over 150 mascaras representing eight popular brands were examined for their susceptibility to microbial contamination during their use by study group members.Early in the studies,two brands without preservatives supported reproducing populations of microorganisms,including potential eye pathogens. Microbes associated with the facial skin and the fingers of the study group users were typically isolated from mascaras after use .Four patients with Staphylococcal blepharitis and cosmetic heavily laden with *Staphyloccus epidermidis* showed marked clinical improvement when they stopped using the contaminated cosmetics .The application of used eye area makeup prior to and following ocular surgery should be avoided (Ergun,H.1987).

Antimicrobial of antibiotics was checked on bacteria isolated.Antibiotics are among the most frequently prescribed medication in modern medicine.Some antibiotics are bactericidal,means that they work by killing bacteria.Other

Volume : 3 | Issue : 2 | February 2014 • ISSN No 2277 - 8179

antibiotics are bacteriostatic,means that they work by stopping bacteria multiplication.The selective action of antibiotics upon bacteria and other microorganism is known as the Antibiotic Spectrum.Som e are active against certain bacteria and not upon others whereas some are active against fungi and viruses (Davies,1994).

Mechanism of action is inhibiting protein synthesis on the ribosomal level (Smadel,2003).Antibiotics used here are Streptomycin,Tetracycline and Chloramphenicol.

MATERIAL AND METHODS:

A) MATERIAL USED

a) For Sampling Sterile containers were used for sampling .
b) For Culturing Different types of selective as well as non se lective media were used for culturing as given below:

i) Nutrient agar medium
ii) MacConkey agar medium
iii) Eosin Methylene Blue (EMB) agar medium
iv) Mannitol Salt Agar (MSA) medium
v) Deoxycholate Agar (DCA) medium
vi) Bacillus Medium (BM)

c) For Microscopy Gram staining kit(Crystal violet,Gram's iodine,saffranin,decolorizer) was used for most of the microscopic examination.

d) For Biochemical analysis

Different types of reagents and chemicals were used for the purpose of biochemical analysis which are given below

i) Hydrogen Peroxide(3%)
ii) Triple sugar iron agar medium
iii) Simmons Citrate agar
iv) Christens medium (Urea,Phenol red,agar)
v) 24hours old culture
e) For Antimicrobial activity
i) Muller Hinton agar medium
ii) Standard Antibiotics
B) APPARATUS AND GLASSWARE USED

Bacteriological incubater,oven,refrigerator ,testtubes ,graduated pippets of 10ml. capacity, inoculation loop, conical flask of 100ml.and 250ml. capacity,measuring cylinder of 50ml. & 100ml. capacity ,cotton aluminium foil,beaker of 100ml. &200ml. capacity ,tissue paper and marker,autoclave,microscope ,petriplates,laminar air flow cabin,burner and matchbox.

C) METHODS USED

Various methods were employed for isolation and identification of bacteria from five different cosmetic samples and testing antimicrobial activity of antibiotics on them.

i) Sample collection

Various cosmetic samples like powder, moisturizer, kajal-lipstick and eye liner were collected from various branded companies those were used in homes.

ii) Isolation

It was done in three step

a) Serial Dilution

One gram of powder sample was mixed in 10ml of distilled water.Dilutions were made upto 10^{-9} by adding 1ml from previous dilution to next dilution.Serialy diluted samples were than inoculated on the nutrient agar plates by plating out from test tubes having dilutions 10^{-5} and10^{-6}.

b) Spread plate methods

Using sterile swab,each dilution was spreaded on the Nutrient agar patriplates and then observed the characteristics of colonies after incubating them for 24hrs at 37°C.We have studied the colony morphology with naked eyes and with the help of magnifying glass.Following features were studied:

* Form of Colonies (circular or irregular)
* Colour of the colonies
* Size of the colonies

Eleva tion of the colonies (raised,flat or wavy)

* Surface of the colonies (wrinkled, smooth or irregular)
* Opacity of the colonies (Opaque and translucent)
* Odour of the colonies

c) Microscopy:

After observing colony morphology of bacterial isolates, the morphology (shape,size,appearance and motility) of microorganisms were observed with the help of light microscope and Gram staining techniques were adopted to differentiate between Gram positive and Gram negative microorganisms.

Procedure for Gram Staining:

Taken a clean slide, put a drop of saline on it and then transferred an isolated colony on it with the help of Sterile loop near burner flame.Prepared a thin smear of microorganism and dry it .Added to this Grams reagents,i.e. crystal violet for 1min.,Grams's iodine for 2 min.,decolorizer for 3-4sec. and at last counter stain i.e.safranine is added .Dried the slide and observed under microscope .

TEST	RESULT
Gram Staining	Positive/Negative
Shape	Rods/Coccus
Endospore	Positive/Negative
Motility	Positive/Negative

iii) Secondary identification on Selective media:-

Selective media were used for Secondary identification and morphology of isolates were studied on the Selective media. Different media for different isolates were used as given below:

MEDIA	MICROORGANISMS
MacConkey	E.coli and Staphylococcus
EMB	E.coli
MSA	Staphylococcus
BM	Bacillus

iv) Final identification of isolates: It was done on the basis of biochemical analysis.

Different biochemical tests were performed for each isolates as given below:

* Catalase Test
* Indole production Test
* Citrate Utilization Test
* TSI (Triple Sugar Iron)agar Test
* Methyl Red and Vogues-Proskauer Test

For all these tests, 24 hrs old broth or culture was taken from different microorganisms isolated:

1.CATALASE TEST

Taken 24hrs. old culture and put it on the slide with the help of loop.Poured 3% of hydrogen peroxide on it.Effervescence indicated positive results.During aerobic respiration certain microorganisms produce H_2O_2 which is lethal to cell. The enzyme catalase present in these microorganism produce H_2O and O_2 which help them in their survival

$$2H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2$$

2.Indol Production test

Tryptophan is an essential amino acid which is oxidized by some bacteria with the help of enzyme Tryptophanase and result in the formation of Indole,Pyruvic acid and Ammonia .Bacteria taken from broth were inoculated into tryptone broth. Then kovac's reagent is added to it. Cherry red reagent layer indicates positive results.

3. Citrate utilization test
It is used to differentiate among enteric bacteria on the basis of their ability to utilize Citrate as the carbon source by Citrate producing microorganisms.

Citric acid---------->Oxaloacetic acid + acetic acid---------->Pyruvic acid

The Citrate test is performed by inoculating the microorganism into an organic synthetic medium i.e Simmon Citrate agar ,where Sodium Citrate is the only carbon source and Bromothymol blue is used as an indicator when the Citric acid is metabolized.The carbondioxide generated combined with sodium and water to form carbonate which changes the colour of the medium.

CO_2+2Na+H_2O---------->Na_2CO_3 (Alkaline Ph, blue colour)

4. Triple sugar iron agar (TSIA) Test
TSIA medium is composed of three sugar (lactose ,sucrose and little amount of glucose),iron and 1% phenol indicator.The medium was made with little slant and with deep butt and organisms were inoculated by spreading and swabbing.TSIA slant is a multi test medium. It is mainly useful to the microorganisms of Enterobacteriaceae group.

* Gas production was detected by blackening of slant and butt .If gas production was there then butt got splitted forming an empty area in the medium.
* If medium became yellow ,there is production of acid
* In aerobic conditions,there is redness in slants and yellow in butt.

5.Methyl Red Test
This test detects the production of sufficient acid by fermentation of glucose so that pH of the medium falls and maintained below 4.4.Inoculated the microorganisms into the medium. Added 5ml of glucose phosphate into it .The medium was incubated at 37°c for 2-5days.Added 5drops of 0.04 methyl red. The medium turns to bright red colour which indicates the positive results While yellow colour indicates the negative results.

6.Vogues-proskauer Test(VP Test)
This test was done to determine the capacity of some microorganisms to ferment carbohydrate with production of non acidic or natural end product such as Acetyl-Methyl Carbinole or its reduction products i.e. 2,3-Butylene glycol.These products are produced from Organic acids that results from glucose metabolism.Pink colour appear in just 2-5min. that ultimately get deepened either to magenta or crimson red in about 30min. duration. Negative tests shows no colour change for about 30min.

v) Testing of Anti-Microbial activity
24hrs. old broth culture of test isolated were spread over the Muller Hinton agar with the help of Sterile glass rod and wells were made on the agar Petriplates with the help of stainless steel borer of diameter 6 to 8mm.Different antibiotics in different amount were added to the wells in each plates.Allowed the antimicrobial agent to diffuse for 30 minutes.Finally petriplates were incubated at 37°c for 24hrs and zone of inhibition was observed and measured with the help of scale.

RESULT AND DISCUSSION
RESULT
A) Identification of isolates on Nutrient agar and Selective media.
B) Biochemical analysis of isolates
C) Antimicrobial studies of antibiotics

A) Identification of isolates on Nutrient agar and Selective media.

i) Results on Nutrient Agar:- When incubated at 37°c for 24-48 hrs.

Sapl Name	Form	Colour	Size	Elevation	Surface	Opacity	Microorganisms isolated
Powder	Circular	Golden Yellow	Small	Raised	Smooth	Opaque	Staphylococcus
Kajal	Circular	Creamy White	Large	Raised	Smooth	Opaque	E.coli
Moisturizer	Circular	White	Small	Raised	Smooth	Opaque	Bacillus

ii) Results of Microscopy
Microscopic examination of recovered isolates on Nutrient Agar

Sample Name	Arrangement	Shape	Colour	Gram's Staining	Microorganism identified
Powder	Cluster	Cocci	Purple	Gram positive	Staphylococcus
Kajal	Single	Rod	Pink	Gram Negative	E.coli
Moisturizer	Single	Rod	Purple	Gram positive	Bacillus

iii) Results on Selective media:-
Morphological study of Cosmetic isolates on selective media

Sample Name	Media used	Size	Colour	Surface	Microorganism identified
Powder	EMB	Small	Metallic	Smooth	E.coli
Kajal	MSA	Small	Yellow	Smooth	Staphylococcus
Moisturizer	BM	Small	White	Rough	Bacillus

B) Biochemical analysis of isolates
Biochemical tests of cosmetic isolates

Sr. No.	Catalase Test	Indole Test	MR	VP	TSI	Citrate	Gas Production	Microorganisms confirmed
1	+ve	+ve	+ve	-ve	+ve	-ve	+ve	E.coli
2	+ve	-ve	+ve	+ve	+ve	-ve	+ve	Staphylococcus
3	+ve	-ve	-ve	+ve	+ve	-ve	-ve	Bacillus

Different Bacterial isolates from various cosmetic samples

Sample	Microorganism
Powder	E.coli
Kajal	Staphylococcus
Moisturizer	Bacillus

No microorganisms were isolated from Lipstick and Eyeliner.

C) Antimicrobial studies of antibiotics.
Diameter of zone of inhibition shown by various isolates against different antibiotics.

	Chloramphenicol	Tetracyclin	Streptomycin
	Zone of Inhibition (mm)		
Staphylococcus	6mm	8mm	9mm
E.coli	11mm	9mm	7mm
Bacillus	8mm	14mm	6mm

DISCUSSION

Results from this study showed that bacteria isolated from various cosmetic sources were identified on the basis of growth on nutrient agar(primary identification),microscopy,growth on selective media(secondary identification) and biochemical analysis .Finally the antimicrobial activity of antibiotics were checked against bacterial isolates of cosmetics.We have isolated three different bacteria from three cosmetics (Powder , Kajal, Moisturizer) and no microorganisms were isolated from Lipstick and Eyeliner.The most frequently identified microorganism was Staphylococcus aureus,which is the most common bacterial skin pathogen.Cosmetics application is largely restricted to the skin. Staphylococcus aureus is a common skin microorganisms that can cause boils, impetigo, conjunctivitis, folliculitis and food poisoning (Brannan,1987).Pseudomonas aeruginosa, the most frequently found contaminant in cosmetics infects wounds and burns and can also cause pneumonia in immunosuppressive patients.It was also detected in cosmetic products (Tenenbaum,1967).One death due to use of shampoo by immunosuppressive patients was reported by Hopfer et al. This shampoo was contaminated with Pseudomonas aeruginosa (Geis,2006).

Several cases of eye infections and even loss of vision were also caused by contaminated cosmetic products contaminated with Pseudomonas aeruginosa (Reid and Wood,1979).A survey on recalls of microbiologically contaminated cosmetics in Europe between 2005-2008 reported 24 different cosmetic products which were contaminated with Pseudomonas aeruginosa ,Bacillus cepacia, Staphylococcus aureus , Enterococcus sp. etc.(Lundov,2008).Similar results showed 10 commerically available cosmetic creams and lotions which were purchased and their microbiological contents was evaluated. Investigators identified Staphylococcus aureus ,Streptococcus sp. and Bacillus sp. similar to previous studies (Hugbo et al,2003).

There were also outbreak investigation in the identification of these opportunistic pathogens in contaminated cosmetic products (Alvarez-Lerma et al,2003)Additional to well known cosmetic preservatives , product also includes other antimicrobial components, such as alcohols, chelating agents, phenolic antioxidants, plant – derived essential oils, extracts and fragrance ingredients(Varvaresou et al.,2009).

Although parabens have ideal preservative properties, some recent in vitro and in vivo studies indicated that they adversely affect endocrine and reproductive system(Oishi,2002). It was also reported that using parabens in cosmetics , particularly in under arm deodorants and anti perspirants, can be associated with breast cancer (Darbre et al.,2002) .Our results showed that cosmetic products produced in our country,can be contaminated during the production process.Therefore it is important to take precautions during production process in order to prevent infections due to microbial contamination.It is necessary to comply with GMP standards strictly during the production. Preservatives should be added to products as determined by regulation and in accordance with toxic dose limits,for consumers health.

REFERENCE

Alvarez L.,Arvidson,C.J.(2003).Cultivation media for Bacteria. 23,375-382. | Brannan,D.K.,Dille,J.C., Kauffman,D.J. (1987).Correlation of in-vitro challenge testing with consumer.Applied Environmental Microbiology,53,1827-1832. | Brannan,D.K., Dille,J.C. (1990).Type of closures prevents microbial contamination of cosmetics during consumer use.Applied Environmental Microbiology,56(5),1476-1479. | Darbre H.,Crooks,H.M.(2002).Chloramphenicol Synthesis. American Journal of Chemical Sciences,71,2463-2466 | Davies,J.(1994).Inactivation of antibiotics and the dissemination of resistance genes.In Science,23,375-382. | Ergun,H. (1987).Microbiological analysis of some cosmetics.21(4),301-307 | Geis,P.A.(2006).Biology of Microbes.Cosmetic Microbiology,Taylor and Francis group,New York,2,19-69 | Hugbo,P.G.,Onyekweli,A.O.,Igwe,I.(2003).Microbial contamination and preservative capacity of some brands of Cosmetics.Tropical Journal of Pharmaceutical Research,2(2),229-234 | Leranoz,S.(1994).Current Trends in Cosmetic Microbiology.8(2),2152-2154 | Lundov,D.,Garrod,M.D.(2008).Streptomycin British Medical Journal,15,212-218 | Orus,P.,Elane,B. (1993).The Hazards of Cosmetics,New York.1-5 | Oishi,P.,Lawrence,P.(2002).Streptomycin.British Medical Journal,15,212-218 | Reid,F.R.,Wood,T.O.(1979).Pseudomonas Corneal Ulcer.The causative role of contaminated eye cosmetics.Arc Opthalmol,97,1640-1641 | Smadel,J.E.(2003).Chloramphenicol Journal of antimicrobial chemotherapy,52,856-859 | Tenenbaum,K.,Zhang,D.(1967).History of antibiotics.Journal of antibiotics,61,40-42 | Varvaresou .V.,Wallhausser,K.H.(2009).Microbiological quality control of skin care preperations.Cosmetics and Toiletries,93,42-48 |