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Bacteriological Profiling of Commercially Available Eye Cosmetics and their Antibiotic Susceptibility Pattern

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Abstract

Background and Objective: Various types of cosmetics, including the eye cosmetic (EC) one, have been reported worldwide to be contaminated with potential bacterial pathogens causing several infections to humans. The current study determines the bacterial contaminants of commercially available ECs in Malda (West Bengal state, India) and to explore the antibiotic susceptibility patterns of the isolated bacteria.

Methods and Findings: A total of 10 various ECs were purchased from Malda town market, India, and the bacterial growth were enriched in nutrient broth, inoculated with 5-10 mg of each of the test samples. The pure bacteria culture obtained from the samples were identified by phenotypic characterization, as *Bacillus cereus* (n=3), *Bacillus sp.* (n=1), *Chromobacterium violaceum* (n=2), *Pseudomonas aeruginosa* (n=1), and *Listeria monocytogenes* (n=2). The antibiotic susceptibility of the isolated bacteria was determined by disc diffusion method using 10 antibiotics; most of the isolates were resistant to three or more antibiotics, among ampicillin, cefpodoxime, nalidixic acid, trimethoprim and vancomycin. The calculated Antibiotic Resistance Index (ARI) for the test isolates was 0.055, while the multiple antibiotic resistance (MAR) index was 0.3 for *L. monocytogenes*, 0.4 for *C. violaceum*, and 0.5 for *Ps. aeruginosa*; the isolated *Bacillus sp.* had MAR index of zero.

Conclusion: The current data suggest the emergence of antibiotic resistance among bacterial strains in ECs and provide insight into the problems of overuse and/or misuse of antimicrobial agents, and the public awareness on cosmetic safety as well.

Keywords: Eye cosmetics; Bacterial contaminants; Antibiogram; Zone diameter of inhibition; Multiple antibiotic resistance index

Introduction

Microbial contamination of cosmetics is a major public health problem [1,2], and also the cause of concern to the industries, the users as well the clinicians. In the current ages, various cosmetics, including eye-cosmetics (eye liners, eye shadow, mascara, eyelash curlers, kohl) are in use in order to improve self-esteem and appearance, and among those kohl (also called 'kajal' in Bengalee) is a popular eye care product, the use of which has been reported since ancient times. In India, the use of kohl in pediatric age is a common practice to keep the eyes cool, clean and with improved vision [3], while the older infants, children and women apply kohl for beautification and to protect and treat eye diseases. But such agents including the eye-cosmetics may have the capability to serve as the vehicles of bacterial infection into the eyes of the users if contaminated products are used, or can disseminate the infection into others when such agents are shared or misused [4]. Campana et al. [5] studied commercially available cosmetics in order to verify the possible microbial contamination during their use by the consumers. Orus and Leranzo [6] reported the isolation of gram-positive bacteria such as *Staphylococcus aureus*, *S. epidermidis* as well as gram-negative bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* from mascara and eye pencil. Dawson and Reinhardt [7] reported bacterial contamination of eye pencil with the genera Staphylococcus, Micrococcus, Bacillus, Moraxella, Acinetobacter and Pseudomonas. The bacterial strains isolated from different cosmetics including 'kajal' were identified as *E. coli*, *Staphylococcus sp*. and *Bacillus sp.* and the isolates were found resistant to one or more antibiotic tested such as chloramphenicol (CM), tetracycline (TC) and streptomycin (SM) [8].

Abdelaziz et al. [9] reported a large number of potential pathogenic bacteria such as *P. aeruginosa, Citrobacter freundii, K. pneumonia, E. coli, Enterobacter agglomerans, S. epidermidis* and *Micrococcus sp.* from different cosmetics including eye shadow and mascara. Bacteria such as *Bacillus spp., Staphylococcus spp., Pseudomonas spp., P. vulgaris and Serratia marcescens* have been recovered from unused and in-use samples of Al-Kohl [10]. The neonates on application of kohl got infection with microorganisms in their conjunctivae [11]. Akrayi [12] isolated gram-positive (*S. aureus, S.*

epidermidis and *S. capitis*) and gram-negative (*E. coli*) bacteria from the eye lids of eye-cosmetic users and natural eye liner users. Baqer et al. [13] isolated various bacterial strains such as *Proteus, E. coli,* Shigella*,* Citrobacter*,* Klebseilla*, P. aeruginosa, S. aureus* and *S. epidermidis* from used cosmetic samples including mascara. Such bacterial contamination of the cosmetics may cause spoilage of the products [14], or lead to human illness from simple skin infection, conjunctivitis and allergy to keratitis, whole body inflammation and systemic blood infection [5]. However, scientific studies on bacterial contamination of eye-cosmetics and the antibiotic susceptibility of the isolated bacteria are lacking in our part of the globe. Therefore, the current study has been undertaken to isolate and identify the potential bacterial strains from different types of commercially available eye-cosmetics in Malda (West Bengal state, India), and to determine the antibiotic resistance patterns of the bacteria involved.

Methods

Sampling sites and sample collection

A total of 10 randomly selected eye cosmetic (EC) samples: koh-1, kohl-2 and kohl-3; mascara-1 and mascara-2; eye shadow-1 and eye shadow-2; eye liner-1, eye liner-2 and eye liner-3 were collected from Malda town of the West Bengal state, India, and were subjected for bacteriological processing.

Isolation and identification of bacteria

Each of the ECs procured was inoculated into nutrient broth (Hi-Media, India), and following incubation at 37°C for 24 h, a loop-full of the broth cultures (from each sample) were streaked on the surface of blood agar, MacConkey agar, cetrimide agar and nutrient agar (Hi-Media, India), and incubated for 24 h at 37°C. Single and discrete (morphologically different) colonies grown on various agar plates were stored in cystine tryptone agar (Hi-Media, India) stabs. The bacterial strains isolated were identified following gram-staining, biochemical tests (catalase, oxidase, urease, nitrate reduction, gelatine hydrolysis and IMViC) and sugar fermentation [15,16].

Antibiotic susceptibility

The antibiotic susceptibility for the bacterial strains from ECs was determined by disc diffusion method [17], using Mueller-Hinton agar (Hi-Media, India) plates, which were swab-inoculated with overnight grown broth culture of the isolates, and were incubated with ten antibiotic discs (Hi-Media, India): ciprofloxacin (CIP), vancomycin (VA), nalidixic acid (NA), meropenem (MRP), ampicillin (AMP), cefpodoxime (CPD), cefotaxime (CTX), trimethoprim (TR), gentamycin (GEN) and amikacin (AK). The results, in terms of zone diameter of inhibition (ZDI) obtained around each of the antibiotic discs for the isolates, were interpreted following the criteria of the Clinical Laboratory Standards Institute [18], and the isolates were categorized as resistant, sensitive or intermediately susceptible.

Determination of antibiotic resistance and multiple antibiotic resistance indices

The antibiotic resistance index (ARI), and multiple antibiotic resistance (MAR) index for all the isolated bacteria were calculated as follows [19-21]:

$$ARI = \frac{Number\ of\ antibiotic\ resistant\ bacterial\ isolates}{Total\ number\ of\ test\ bacterial\ isolates\ \times\ Number\ of\ antibiotic\ tested}$$

and

$$MAR\ index = \frac{Number\ of\ antibiotics\ to\ which\ the\ isolate\ showed\ resistance}{Number\ of\ total\ antibiotics\ exposed\ to\ the\ isolate}$$

and interpreted according to Krumperman [19]: MAR index ≤ 0.2 was considered low risk, and ≥ 0.2 was indicated as high risk.

Results

Among 10 ECs collected, 8 showed bacterial contamination as per the microbial culture in various media; two (eye liner-1 and eye liner-2) were free from bacterial contamination. Among the isolated bacteria (n=9), 6 were gram-positive [strain code: C3(1)K, C5(A), C5(B), C7(1), C9(A) and C4(A)C, recovered respectively from kohl-1, eye shadaw-1, eye shadow-2, kohl-3, eye liner-3 and mascara-2]. The remaining 3 were gram-negative [strain code: C6(B)D, C8(A) and C2(A)A, which were isolated from kohl-2, liner-3 and mascara-1], respectively. All the bacterial isolates obtained were rod shaped.

In TSI, 6 strains [C5(A), C5(B), C7(1), C4(A)C, C6(B)D and C8(A)] showed acid butt (yellow) and alkali slant (pink), while the C2(A)A strain had red butt and red slant, and the two gram-positive small rod shaped bacteria with strain code C3(1)K and C9(A) had acid butt (yellow) and acid slant (yellow); no strain was found positive for gas (CO_2) and H_2S production. Among the gram-negative bacilli, the C2(A)A strain did not ferment any sugars used in the study, while the other 2 strains [C6(B)D and C8(A)] fermented glucose, but did not ferment lactose, mannitol, sorbitol and xylose. Among the gram-positive bacilli, 3 strains: C5(A), C5(B) and C7(1) fermented sucrose, sorbitol, xylose, glucose but did not ferment mannitol and lactose, while 2 strains [C3(1)K and C9(A)] fermented glucose, sucrose, rhamnose, sorbitol, mannitol and lactose,

but did not ferment xylose. The biochemical test results for the isolated eye-cosmetic bacteria are shown in **Table 1**.

Table 1 Biochemical features of the isolated eye-cosmetic bacteria. CAT: Catalase; CIT: Citrate; GEL: Gelatinase; IND: Indole; MR: Methyl red; NIT: Nitrate; OXI: Oxidase; URE: Urease; VP: Voges-Proskaur.

Strain	CAT	OXI	IND	CIT	URE	MR	VP	NIT	GEL
C3(1)K	+	-	-	-	+	+	+	-	+
C2(A)A	+	+	-	+	-	-	-	-	-
C4(A)C	+	+	-	+	+	-	+	+	+
C5(A)	+	+	-	-	-	-	+	+	+
C5(B)	+	+	-	-	-	-	+	+	+
C6(B)D	+	+	-	+	+	+	-	-	+
C7(1)	+	+	-	-	-	-	+	+	+
C8(A)	+	+	-	+	+	+	-	-	+
C9(A)	+	-	-	-	-	+	+	+	+

Based upon the cultural characteristics (colony morphology, haemolytic activity and pigment production), gram-staining (cell shape), biochemical including TSI test results and sugar fermentation patterns of the eye-cosmetic bacteria, their identities are represented in **Table 2**.

Table 2: Identity of the bacterial isolates from different eye-cosmetics

Strain code	Strain identity
C3(1)K	*Listeria monocytogenes*
C2(A)A	*Pseudomonus aeruginosa*
C4(A)C	*Bacillus sp.*
C5(A)	*Bacillus cereus*
C5(B)	*Bacillus cereus*
C6(B)D	*Chromobacterium violaecium*
C7(1)	*Bacillus cereus*
C8(A)	*Chromobacterium violaecium*
C9(A)	*Listeria monocytogenes*

The antibiotic susceptibility patterns of the isolated bacteria are represented in **Figure 1**.

The *Bacillus sp*. was sensitive to all the test antibiotics having ZDIs 19-40 mm; the three strains of *B. cereus*, such as (C5(A), C5(B) and C7(1), had ZDIs 21-55 mm, 21-54 mm and 20-50 mm, respectively. The isolated *P. aeruginosa* showed resistance to five antibiotics, with 6 mm ZDIs against VA, AMP, CPD and TR, while 10 mm against NA, and thus highest resistance was displayed by *P. aeruginosa* (**Figure 2**).

The *C. violaecium* C6(B)D and C8(A) strains had resistance to AMP, CPD and TR (ZDIs 6 mm, for each) and to VA (ZDI 13 mm), and the *L. monocytogenes* C3(1)K and C9(A) strains had resistance to CPD, VA and NA (ZDIs 6-12 mm). The MAR indices ranged 0.3-0.5, among the antibiotic resistant bacteria (**Figure 3**); the overall antibiotic resistance index was 0.055, for the isolated bacteria.

Discussion

Contamination with pathogenic bacteria of food as well as pharmaceuticals is known [20], and the isolation of potential bacterial pathogens from cosmetics, including eye-cosmetics is not uncommon [2]. Different authors from different parts of the globe isolated bacteria from various types of eye-cosmetics, and identified the contaminants as *S. aureus, S. epidermidis, P. aeruginosa, Citrobacter freundii, K. pneumonia, E. coli, Micrococcus sp., Bacillus spp.,* Shigella and Citrobacter, by phenotypic characterization [6,8,12]. As has been reported by Abdelaziz et al. [9], the eye shadow and mascara samples were heavily contaminated with gram-positive *cocci* as well as gram-negative bacteria including *P. aeruginosa, C. freundii, K. pneumonia, E. coli, Enterobacter agglomerans, S. epidermidis and Micrococcus sp.* Baqer *et al*. [13] reported about the isolation of bacteria, such as *Proteus, E. coli,* Shigella, Citrobacter, Klebseilla, *P. aeruginosa, S. aureus* and *S. epidermidis* from mascara, face sponge and the using brushers. The commonest gram-negative ocular bacterial pathogen included *Ps. aeruginosa* contaminating ophthalmic solutions (eye drops), eye cosmetics and any other substances having a bit of organic carbon [21], as the source of food and energy. In the current study, the bacterial contaminants of different eye-cosmetics included *P. aeruginosa, C. violaceum, L. monocytogenes* and *Bacillus sp*., including *B. cereus*. The isolated bacteria were identified by phenotypic characterization (gram staining, colony morphology, biochemical features and sugar fermentation capacity). The *C. violaceum* isolates in the present study produced light pigment on blood agar plate, and the strains were oxidase positive, however, tested negative for arginine dihydrolase. This current finding was in accordance with the results of Lima-Bittencourt et al. [22], who reported arginine dihydrolase negative *C.*

violaceum isolates along with the arginine dihydrolase positive strains. The *C. violaceum* generally produce pink/violet pigment on agar plates; however, it has been reported that pigmentation is not a vital feature in the characterization of the genus *C. violaceum* [23]. It has also been recorded in some cases that non-pigmented variants develop following subcultures of the pigmented isolates [24]. The eyes might be exposed to various types of eye-cosmetics including liner, shadow, blusher, foundation, as well as kohl and mascara with bacterial contamination. This is due to the fact that the preservatives contained in such cosmetics are insufficient, or are of poor quality so as to prevent colonization of potential bacterial pathogens, which in turn cause damage to the eyes, and spoilage of the cosmetic products too.

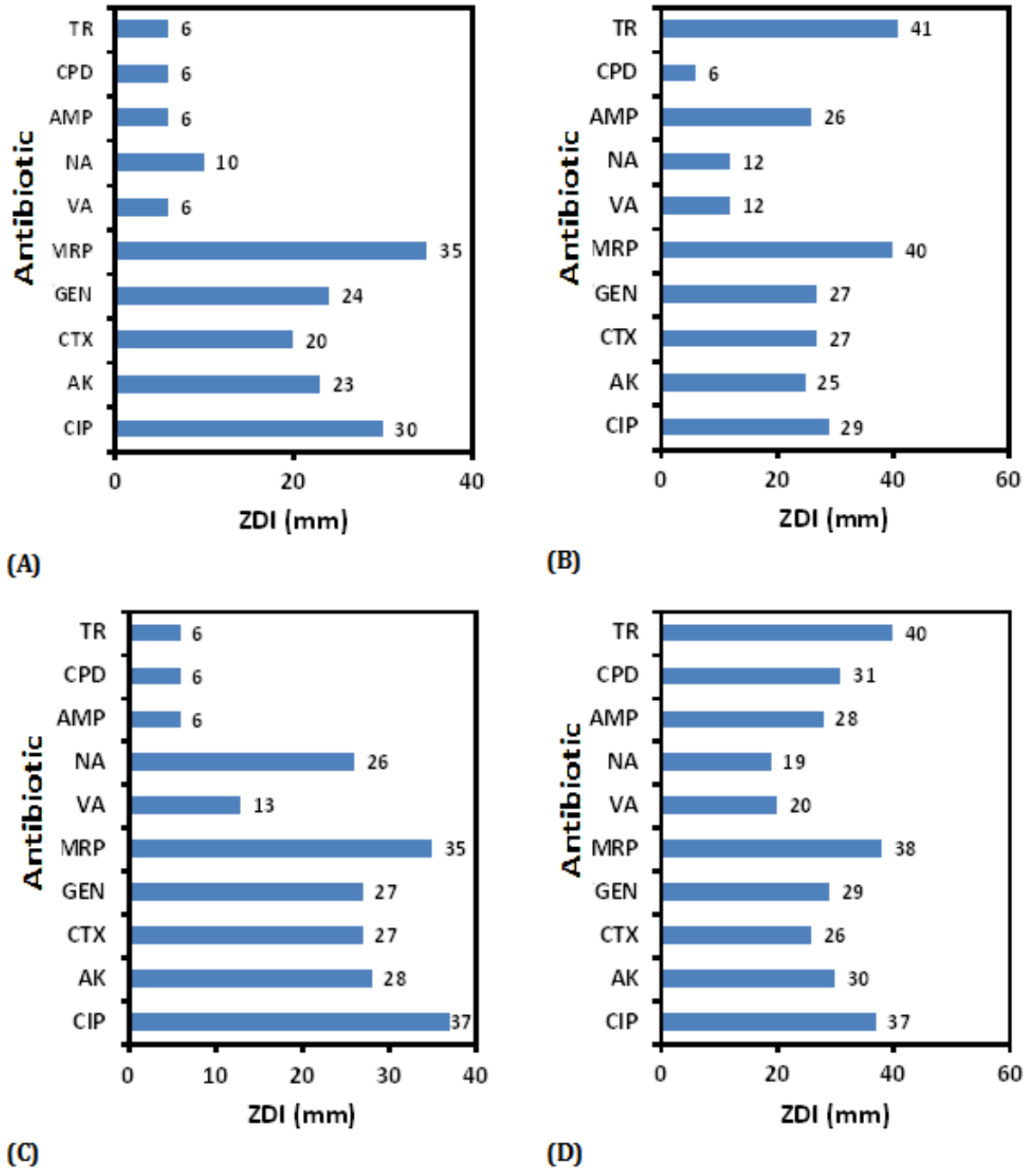

Figure 1 Antibiotic resistance patterns of eye-cosmetic bacteria: (A) P. aeruginosa, (B) *L. monocytogenes*, (C) *C. violaecium*, (D) *Bacillus spp*. AK: amikacin; AMP: ampicillin; CIP: ciprofloxacin; CPD: cefpodoxime; CTX: cefotaxime; GEN: gentamycin; MRP: meropenem; NA: nalidixic acid; TR: trimethoprim; VA: vancomycin; ZDI: zone diameter of inhibition.

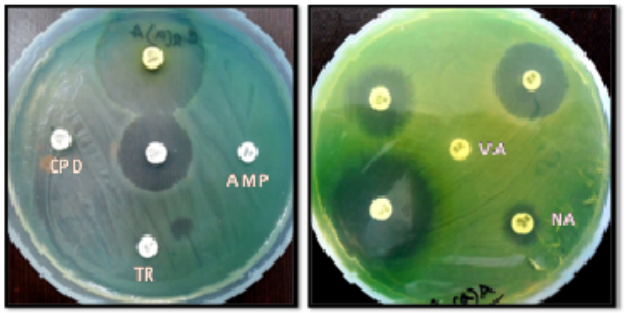

Figure 2 Pseudomonas aeruginosa on nutrient agar plates. The isolate produced characteristic (greenish yellow colour) pigment, and showed resistance to five antibiotics. AMP: ampicillin; CPD: cefpodoxime; NA: nalidixic acid; TR: trimethoprim; VA: vancomycin.

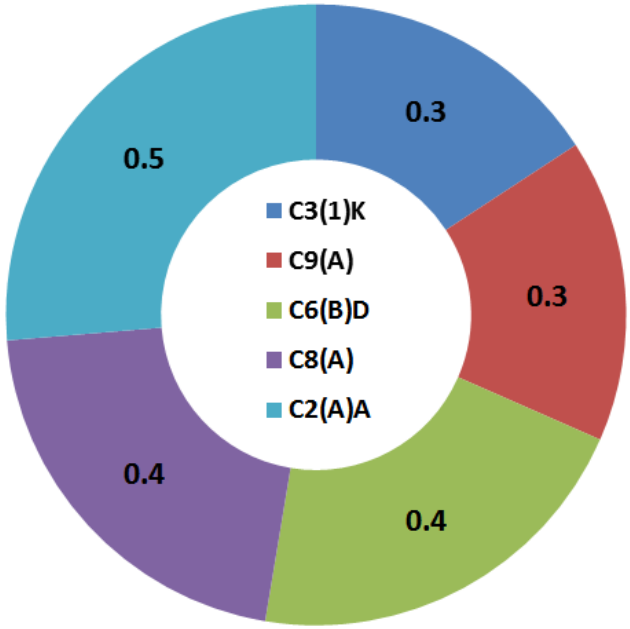

Figure 3 Multiple antibiotic resistance (MAR) indices of various eye-cosmetic bacterial isolates. C3(1)K and C9(A): Listeria monocytogenes; C6(B)D and C8(A): *Chromobacterium violaecium*; C2(A)A: *Pseudomonus aeruginosa*. The digits represented within the figure denote the MAR indices (0.3-0.5).

In the present communication, except *Ps. aeruginosa*, the all isolated bacteria had β-haemolysis capacity *in vitro*, and thus potentially cause pathogenesis on infection through the use of eye-cosmetics in the community (sharing the cosmetics having bacterial contamination). From 58 test positive samples, 32 samples of eye drops were found to be contaminated with *Bacillus spp*. (55.1%), and out of 57 Bacillus isolates, 41 (71.9%) produced different levels of haemolysins [20]. Das et al. [14] isolated *Bacillus sp*., and considered that the bacterium may be responsible for spoilage and unpleasant smell of cosmetic products. Use of cosmetics with microbial contamination has been associated with various diseases: *Clostridium tetani* infections attributed to the use of a talcum powder [25], and clinical eye infection due to the transmission and persistence of microorganisms in eye cosmetics [26] have been demonstrated. Numerous cases of eye infections and the loss of vision were reported to be caused by the contaminated cosmetic products with *P. aeruginosa* [27]. The investigation for microbial contamination of cosmetics has also been reported [28], and death due to the use of such materials contaminated with bacteria has been recorded.

The *C. violaceum* is found in soil and water; reports suggest the bacterium as an emerging pathogen having the capacity to cause fatal infection (skin and localized infection to septicaemia and lesion) in humans in the tropics and sub-tropics [29-31]. In the current study, its (*C. violaceum*) presence in the eye cosmetic might be a potential source of human eye infection. Among the genus Listeria, only *L. monocytogenes* is consistently associated with human illness, called listeriosis. *B. cereus* is ubiquitous in nature, the spores of which are heat-, desiccation-, alcohol- and low-pH (1.5) resistant [32], and hence can be isolated from soil, water, air and dust, and occurs in a range of products used by the customers including foodstuffs. The *B. cereus* strains are associated with human illness like food poisoning (emetic syndrome and the diarrhoeal syndrome) [33,34] as well as some more severe infection including endophthalmitis [35]. *Ps. aeruginosa* is widely distributed in the environment as well as in living hosts, and opportunistically cause severe corneal infection [36,37], and is regarded as the most common pathogen causing bacterial keratitis that progress rapidly and thus results in permanent loss of vision [38-40]. The bacterium *P. aeruginosais* had been used, among others, as an indicator of the official assessment of the effectiveness of cosmetics preservation [41]. *P. aeruginosa* is the etiologic agent of infections to humans, such as that of the cornea and conjunctiva leading to various forms of inflammation, and serious infections of the eye-ball. Beside this, the pathogen causes UTI and lung infections, infection to burn wound, surgical sites, heart muscles and central nervous system infection [41]. In the current study, the all isolated bacteria-beside causing eye and skin infection-can potentially cause bacteraemia, septicaemia, and infection of the central nervous system.

The emergence of drug resistant, including multi-drug resistant (MDR), bacterial isolates from clinical samples, foods, pharmaceuticals, as well as cosmetics, including eye-cosmetics, is a cause of great concern. The bacterial isolates of *S. aureus, Bacillus spp., Klebsiella spp*., and *P. aeruginosa* isolated from cosmetic products (lotion and creams) showed resistance to one or more of the antibiotics: amoxycillin, augmentin, cotrimoxazole (COT), TC, NA, nitrofurantoin, CIP, GEN, ofloxacin and erythromycin [42]. The isolates of *C. violaceum* had sensitivity to GEN, AK, norfloxacin, COT, CM and TC, while resistance to AMP, cefazolin and ceftazidime, as has been reported by Jitmuang [43]. The *B. cereus* isolates from various sources showed resistance to AMP, cephalosporins, penicillin and trimehoprim, and sensitivity to aminoglycosides, CM, CIP, clindamycin, erythromycin, imipenem and VA, [44].

Banerjee et al. [45] reported that the most of the clinical *B. cereus* isolates had resistance to amoxyclav and cephalosporins. The bacterial strains isolated from different cosmetics including 'kajal' were identified as *E. coli*, *Staphylococcus sp*. and *Bacillus sp*., and the isolates were found resistant to one or more antibiotic tested such as CM, TC and SM [8]. Thus, the report on antibiotic resistance of eye-cosmetic bacteria is meagre, and in the current study we have isolated different bacterial strains, of which *Ps. aeruginosa, L. monocytogenes* and *C. violaceum* were resistant to three or more antibiotics tested, while *Bacillus spp*. had sensitivity to all the test antibiotics. Regular surveillance of eye-cosmetic bacteria for antibiotic susceptibility is important and imperative, in order to control the infection caused by such strains in the community.

To the best of the authors' awareness, this research has been the first to be carried out on antimicrobial susceptibility and MAR index determination for eye-cosmetic bacteria in our part of the globe. In this investigation, the two *L. monocytogenes* isolates, one from kohl and another from eye liner, had MAR index of 0.3; the B. cereus isolates had 'zero' MAR index. The MAR index was recorded as 0.4 for *C. violaceum* isolates from eye liner and kohl samples, while MAR index was calculated as 0.5 for mascara isolate of *P. aeruginosa*. Several earlier authors calculated the MAR index for bacteria isolated from different sources in order to evaluate the health risk of the environments, to provide the baseline information about the source of the bacterial contaminants and to identify the origin of resistance; the MAR indices >0.25 pose high risk source of contamination [19,46]. Tambekar et al. [47] reported that bacteria having MAR Index of >0.2 have originated from an environment where several antibiotics are in use. Subramani and Vignesh [48] determined MAR index of >0.2 for the bacterial strains tested, and reported that the isolates were transmitted from an environment of high antibiotic usage. Maloo et al. [49] reported ARI values of 0.03-0.07 for the test bacterial isolates including *P. aeruginosa*, and also recorded that 97% of the isolates were MDR with high MAR index (>0.2), suggesting the origin of the test isolates was of the high antibiotic usage. As has been reported by Oluyege et al. [50], the high level of MAR index (0.81-3.08), as compared to low risk value of 0.2 [19], might be the evidence of public health risk. As per the report of Chandran et al. [51], the MAR indices of 0.33-1 for the bacterial strains tested suggested the probable origin of such contamination from high risk source. Based upon the MAR index calculation, the current eye-cosmetic bacterial isolates (for which overall antibiotic resistance index was 0.055) have been categorized in to three: bacterial group having MAR index "zero" (*Bacillus spp.*), the group having MAR index of less than 0.3 (*L. monocytogenes*), and the group for which MAR index was ≥0.4 (*C. violaceum* and *Ps. aeruginosa*). Thus, findings of the current study suggest that *P. aeruginosa* originated from a very high risk source of contamination with increased number of antibiotic usage, while *L. monocytogenes* and *C. violaceum* from the sources of moderate to high risks. Since the above mentioned bacteria (*L. monocytogenes, C. violaceum* and *P. aeruginosa*) have the capacity to cause nosocomial and community acquired infection, use of such eye-cosmetics with bacterial contaminants might pose a serious threat to humans. Hence, public awareness on cosmetic safety as well as their prudent usage is strongly acclaimed on one side, and on the other maintenance of hygienic setting during production and packing, scientific study-based application of preservatives, phytomedicines and probiotics (since these are excellent antimicrobials) [52-55], as well as judicial use of antibiotics in such products is highly recommended.

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