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ORIGINAL ARTICLE

Molecular Characterization of Bacterial Species Isolated from Beauty Blenders Used by Females from Mosul City, Iraq

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ABSTRACT

Background: *Beauty blenders and other cosmetic sponges are widely used in personal and professional makeup applications. However, these tools are prone to microbial contamination due to frequent handling, inadequate cleaning.* ***Objective:*** *This study aimed to evaluate the microbiological contamination of used cosmetic sponges from female users in Mosul City, Iraq, identify the isolated icroorganisms using phenotypic, biochemical, and molecular methods, and assess their antibiotic resistance profiles.* ***Methodology:*** *A total of 26 used cosmetic sponge samples were collected from volunteer participants. Microbiological analyses were conducted using culture-based techniques, VITEK-2 system identification, and 16S rRNA gene sequencing. Antibiotic susceptibility testing was performed following CLSI guidelines. Additionally, behavioral data were obtained through structured questionnaires to assess hygiene practices among users.* ***Results:*** *Out of 26 samples, 22 (84.6%) showed positive microbial growth. Staphylococcus aureus was the most prevalent isolate (41.3%), followed by S. epidermidis, S. sciuri, S. xylosus, Rothia spp., and Kocuria spp. Antibiotic susceptibility testing revealed multidrug resistance in several strains, notably resistance to Oxacillin and Tetracycline among S. aureus, suggesting the presence of MRSA strains. Phylogenetic analysis demonstrated close genetic relationships among Staphylococcus species, with clear divergence from Rothia and Kocuria. Several isolates were submitted to the NCBI GenBank database as novel entries.* ***Conclusions:*** *The high contamination rate of cosmetic sponges indicates poor hygiene practices among users, including infrequent cleaning, sharing of sponges, and storage in moist environments. Public awareness campaigns on proper cleaning and timely replacement of cosmetic applicators are recommended to reduce infection risks and promote consumer health safety.*

INTRODUCTION

A long time ago, the cultural and psychological importance of cosmetic products was taken advantage of by individuals to beautify themselves and display a sense of identity and self-worth. Nevertheless, there is a health concern with their extensive use due to microbial contamination which jeopardizes skin integrity and other safety issues[1].

Most of the cosmetic applicators have direct contact with the skin where the environment is encouraging microbial growth and multiplication. The risks of contamination can also be increased due to poor hygienic levels, inadequate cleaning, and reuse of production that is past its expiration date[2]. These applicators have gained dominance over the years with porous makeup sponges otherwise referred to as beauty blenders taking center stage with their flexibility to not only apply flawless makeup but also a smooth cosmetic finish. It is estimated that the global beauty blender market size is 7.3 billion USD and in the year 2015 it is projected that there will be a growth of 7.1 percent per year[3].

Although they are functional, when beauty blenders are not properly cleaned, they may end up having cosmetic residues, sebum, as well as dead cells of the skin, which offer an optimal environment to support the growth of microbes. It may cause different dermatological disorders such as acne, irritation, and infections particularly in the immunocompromised individuals[4,5]. Previous research results indicated that cosmetic sponges contain microbial contaminants longer than other types of application instruments despite washing them[6]. Remarkably, some studies at the Aston University indicated 79-90 percent of the beauty products brands that were tested including the lipsticks and sponge had been contaminated with disease-causing microorganisms like *Escherichia coli*[2].

These data show that more studies are required concerning the types of microorganisms that are connected with cosmetic applicators and human behavioral components that lead to contamination.

Thus, the goal of the current study was to describe the morphological, biochemical, and molecular characteristics of the microbial species grown on used beauty blenders that were sampled in female members of the Mosul City, Iraq population and to determine their antibacterial susceptibility patterns in order to determine the existence of any related health risks posed by these microbial species.

METHODOLOGY

Sample Collection

Twenty-six used sponges with beauty blenders belonging to different commercial brands were selected to provide microbiological examination. Structured questionnaire was also issued in order to obtain information about usage patterns and hygiene habits. The female students at Northern Technical University / Medical Technical Institute, Mosul, were used to obtain the samples.

Preparation of Culture Media

Culture media were prepared according to manufacturer instructions and sterilized via autoclaving at 121°C, under 15 psi pressure for 20 minutes.

Isolation of Bacteria

The process of microbial transfer of sponge on culture plates was carried out using the method of applying pressure on sponges with Blood Agar plates gently pushed down[7]. The plates were subsequently incubated aerobically at 37 °C in 24-48 h. Bacterial identification was done by placing the emerging colonies on selective media that included MacConkey Agar and Mannitol Salt Agar to give them the chance to eliminate or clean them ensuring that the bacteria are identified, Figure 1.

Fig. 1: Sample collection and participant information.

Identification of bacteria

1-Phenotypic Identification

Morphological characteristics of the colonies, including shape, size, color, and edge features—were recorded based on growth in the respective media.

2-Microscopic Identification

Smears were prepared from bacterial colonies, air-dried, heat-fixed, and stained using Gram stain. Slides were examined under oil immersion lens to determine cell morphology and gram reaction[8].

3-Biochemical Identification

Biochemical testing was conducted [9,10], including:

- Mannitol Salt Agar (MSA) Growth Test: Assesses bacterial tolerance to high-salt environments and ability to ferment mannitol.
- Blood Hemolysis Test: Evaluates the ability of isolates to lyse red blood cells using Blood Agar medium.

4-VITEK-2 System Identification

The VITEK-2 automated system was employed to identify bacterial species and antibiotic susceptibility profiles. Analyses were performed at Al-Hayat International Hospital in Mosul, following standard operating procedures outlined by the manufacturer.

5-Molecular Identification by 16S rRNA Gene Sequencing

Genomic DNA was extracted from bacterial isolates using a commercial extraction kit from Genoid (Taiwan). PCR amplification of the 16S rRNA gene was carried out using universal primers (F: 5′-AGAGTTTGATCMTGGCTCAG-3′; R: 5′-AAGGAGGTGATCCARCCGCA-3′), yielding amplicons of approximately 1945 bp[11]. Amplified products and primers were submitted to Psomagene Laboratory (USA) for sequencing. Resulting sequences were analyzed using BLAST at the NCBI database to determine species-level identification through alignment with reference sequences.

Antibiotic sensitivity testing

The disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines as recommended by [12,13] was used in the determination of antibiotic susceptibility profiles. Nine antibiotics were assessed involving discs that were produced by the Bioanalyse. 4-5 fresh colonies of bacteria were used to make Bacterial suspensions in Nutrient Broth and allowed to incubate at 37o C in 18-24hours. Turbidity was calibrated to 0.5 McFarland standard (1.5 X 10 8 CFU/mL) and spread 0.1 mL suspension to Muller-Hinton Agar plates using cotton swabs that were sterile. Plates were incubated at 37 C after 30-minutes of absorption and the application of antibiotic discs (24-hour incubation).

RESULTS

Microbial Isolation and Growth Patterns

Out of 26 used beauty blender samples analyzed, microbial growth was detected in 22 samples (84.6%), while 4 samples (15.3%) exhibited no growth under aerobic incubation. Figure 2-A illustrates representative bacterial colony formation on Blood Agar, while Figure 2-B highlights fungal and yeast morphology.

Bacterial contamination was observed in all positive samples (100%), followed by fungal growth in 50% of those samples and yeast presence in 18%. These results reflect a predominance of bacteria as the primary contaminants, likely due to the sponges' porous structure and humid storage environments, which favor bacterial proliferation over fungal colonization.

Bacterial Identification: *Staphylococcus* Dominance

Microscopic and phenotypic characterizations indicated that *Staphylococcus* spp. was the most prevailing bacterial group isolated. The colonies that grew in Mannitol Salt Agar turned yellow, which is the features of mannitol fermentation, whereas Gram-stained cells were observed as cluster of Gram-positive cocci.

S. aureus was the most common species with the isolates making up 41.3 percent. This species exhibited alpha hemolysis on the Blood agar and the presence of mannitol fermentation which confirms its pathogenic mode. Other species grown were *S. epidermidis* (27.5 %), *S. xylosus* (10.3 %), *S. sciuri* (10.3 %) each with colony morphology and biochemical characteristics that are discrete. The results of the Staphylococcus study as a whole are graphically rendered in Figure 3-C.

Identification of *Rothia* spp. and *Kocuria* spp.

Other bacterial genera were found in addition to *Staphylococcal* isolates, even though they were less common. The percentages of the total area isolates attributed to *Rothia* spp. were 6.8 percentages. They had creamy colonies and had tetrads or the cells paired as observed by microscope. *Kocuria* spp. were identified in 3.4 percent of the samples, however, with varying morphological appearance but different antibiotic resistance pattern. Both genera are regarded as opportunistic pathogens and their presence could be indicative of the contamination of the environment or ephemeral colonization. Figure 3-D shows representative colonies of *Rothia* as well as microscopic characteristics.

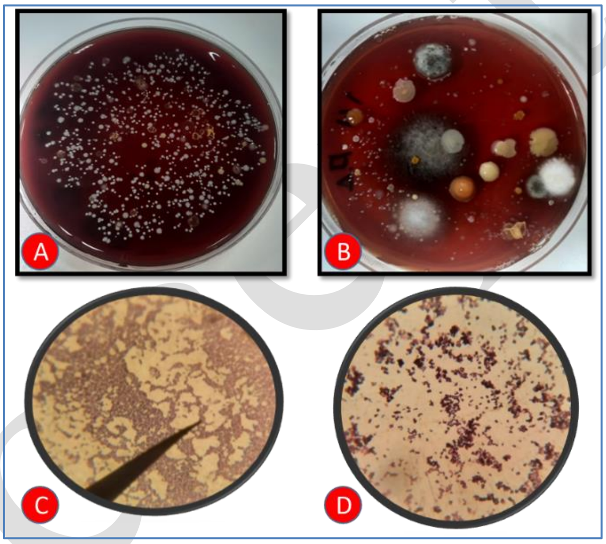

Fig. 2: Microbial growth patterns on selective media. A: Blood Agar plate showing bacterial colonies isolated from beauty blender samples, indicating high bacterial contamination levels with diverse colony morphology. B: Fungal and yeast growth observed on Sabouraud Dextrose Agar, highlighting the presence of opportunistic fungi and yeasts alongside bacterial contaminants. C: Mannitol Salt Agar displaying yellow colonies of *S. aureus* due to mannitol fermentation. Microscopic examination reveals Gram-positive cocci arranged in clusters. D: *Rothia* spp. colonies with creamy appearance and tetrad-shaped Gram-positive cocci observed under oil immersion microscopy, consistent with oral flora contaminants.

Identification using VITEK 2

The identification of bacteria conducted by VITEK 2 automated diagnostic system was as displayed in Table (1). The system makes use of biochemical reasons to classify microorganisms and provides fast and accurate classification in the microbiology field during clinical. By the interpretation of the data presented, the identification probability of *S. aureus* reveals 99 percent, which means there is a highly improved matching between the biochemical profile of the sample and the VITEK 2 reference database. VITEK-2 identified *S. aureus* with 99% probability, confirming the isolate's biochemical consistency with reference data.

The second one that was identified is *S. epidermidis*, and the probability of correct identification is 95 percent. Although *S. epidermidis* is usually less virulent than *S. aureus*, it is a common provider of human intact skin and may turn opportunistic and pathogenic especially when the patient is immunocompromised or has an indwelling medical device. Its reliability in the sample is inspired by its high level of confidence in its detection.

Finally, there was identification of *Rothia* spp. as 88 percent and the probability was slightly lower than the other isolates. The species found in this genus are a normal part of the oral flora though they have been associated with infections in specific clinical conditions. The comparatively smaller probability means a fairly good biochemical fit, yet perhaps with a degree of overlapping with slightly more closely related organisms, which implies that additional molecular data may improve positivity.

Table 1: Identification of bacterial isolates and diagnostic probability via VITEK-2

Bacterial Species	Diagnostic Probability (%)
S. aureus	99
S. epidermidis	95
Rothia spp.	88

Gel -electrophoresis confirmation of 16S rRNA amplification via PCR

The PCR was used to amplify a region of 16S rRNA gene; that is, conserved in bacteria, to confirm bacterial species at a molecular level using universal primers. The code is very informative genetic marker since it is present and varying across bacterial taxa. After PCR, the amplified DNA was separated by gel electrophoresis, when the DNA fragment could be observed and separated by size. Figure 3 indicates the presence of clear bands of different DNA and all the bands tend to align with the anticipated molecular weight of the expected target gene of about 1945bps indicating functional amplification of the target gene. The intensity and clarity of the bands represent purity and quantity of extracted DNA among various isolates of bacteria. This electrophoretic confirmation augers well with the already carried out phenotypic and biochemical identations, and provides the genetic level authentication of isolated microbial species.

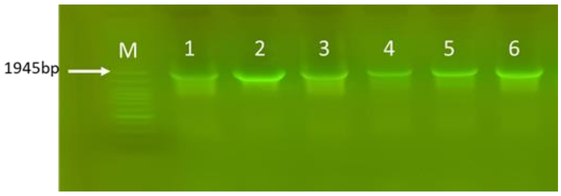

Fig. 3: Gel-electrophoresis of 16S rRNA gene amplification from six bacterial isolates. Lane 1: *S. aureus*, Lane 2: *S. epidermidis*, Lane 3: *Kocuria* sp., Lane 4: *S. xylosus*, Lane 5: *Rothia* sp., Lane 6: *S. sciuri*. Each distinct band corresponds to the amplified 16S rRNA gene product, with an estimated size of ~1945 base pairs, confirming successful molecular detection of the target sequence across all isolates.

Based on the results outlined in Table 2, it is possible to note that a considerable number of individuals (57.6) were likely to share the makeup sponges with others, whereas 42.3 were not. As far as the cleaning procedures are concerned, most (84.6) used water and soap, whereas 15.4 used water - an insufficient mode of cleaning as it is not considered to clean microorganisms, including pathogenic bacteria.

Regarding the frequency of replacement, half of the people surveyed changed their sponges monthly, 26.9 every three months, and 23.07 either every four months or more frequently. On using the sponge, three people used it once a day (73.07 percent), the other 11.5 percent used it two times in a day and 11.5 percent used it three days a week.

The data on the storage practice indicated that the majority of the participants (73.07 percent) kept their sponges, in drawers where they store other cosmetic products, and the rest (26.9 percent), in the handbags which are usually damp and dark environments that are likely to encourage the growth of bacteria and fungi.

Table 2: Participant practices related to makeup sponge sharing, cleaning, replacement, usage, and storage.

Category	Response	Frequency (n)	Percentage (%)
Sharing	Shared with others	15	57.6
	Not shared	11	42.3
Cleaning	Water and soap	22	84.6
	Water only	4	15.4
Replacement Frequency	Monthly	13	50
	Every 3 months	6	23.07
	Every 4 months or more	7	26.9
Usage Frequency	Once daily	19	73.07
	Twice daily	3	11.5
	Three times per week	3	11.5
Storage	Inside drawers	19	73.07
	Inside bags	7	26.9

Sensitivity test results

According to the findings illustrated in Table (3), in keeping with the findings of the antimicrobial susceptibility testing of bacterial isolates by disk diffusion technique most of the isolates were sensitive (S) to most of the antibiotic tested. However, there were pattern of selective resistance in certain strains.

It was found that *S. aureus* was resistant to tetracycline (TE) and oxacillin (OX) which implies that it could qualify to the methicillin-resistant strain (MRSA) since as a strain, the methicillin-resistant strain is broadly resistant to the 12-lactam antibiotic. The only resistance was seen in *S. epidermidis* to erythromycin (E), which is a usual case in this species. *Rothia* spp isolate was highly resistant to erythromycin (E), tetracycline (TE) and gentamicin (CN) whereas other antibiotics were resistant. *Kocuria* spp. exhibited unique resistance to four antibiotics namely gentamicin (CN), tetracycline (TE), erythromycin (E), and oxacillin (OX). It however, remained sensitive to ciprofloxacin (CIP), rifampicin (RA) and vancomycin (VA).

Table 3: Antibiotic susceptibility profiles of bacterial isolates based on disk diffusion method.

Type	TE	CIP	E	VA	CN	RA	OX
S. aureus	R	S	S	S	S	S	R
S. epidermidis	S	S	R	S	S	S	S
S. sciuri	S	S	S	S	S	S	S
S. xylosus	S	S	S	S	S	S	S
Kocuria spp.	R	S	R	S	R	S	R
Rothia spp.	R	S	R	S	R	S	S

Antibiotic Abbreviations:TE – Tetracycline, CIP – Ciprofloxacin, E – Erythromycin, VA – Vancomycin, CN – Gentamicin, RA – Rifampicin, OX – Oxacillin, R – Resistant, S – Sensitive.

Phylogenetic tree of 6 registered strains

Within the identification scope of the molecular identification, there were six different bacterial strains that were successfully uploaded to the NCBI GenBank after the 16S rRNA gene was amplified and DNA sequenced. Such strains are *Staphylococcus* sp. strain A3R1B22 (GenBank: PV476204.1), *S. aureus* strain A3R1B12 (PV617764.1), *S. xylosus* strain A3R1B16 (PV588942.1), *S. epidermidis* strain A3R1B24 (PV617770.1), *Rothia terrae* Comparison of these sequences was carried out to gauge the evolutionary relationship between the isolates.

This led to the phylogenetic tree depicted in Figure (4) where the hierarchy of branching occurs based on the aligned sequences of 16S rRNA genes. Close taxonomic proxity is supported by the grouping in the same lineage of the *S. aureus, S. xylosus, S. epidermidis* and *Staphylococcus* sp. strain A3R1B22. *Rothia terrae* and *Kocuria* sp. on the other hand are located in further more remote branches underpinning their phylogenetic difference to Staphylococci. Phylogenetic analysis validated the molecular identification, highlighting genetic clustering of *Staphylococcus* strains and taxonomic divergence from *Rothia* and *Kocuria*.

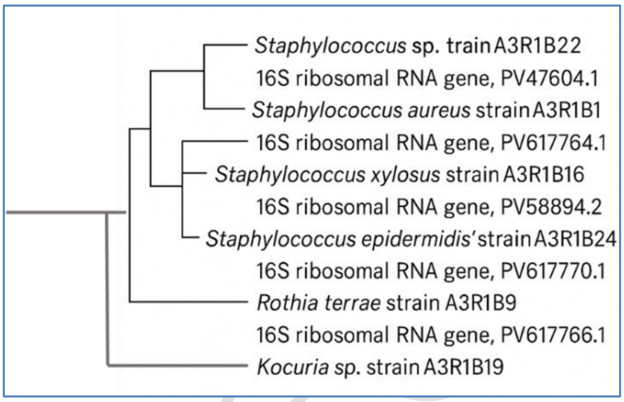

Fig. 4: Phylogenetic relationships of six bacterial isolates based on 16s RNA gene sequences.

DISCUSSION

This study reported the high percentage of microbial contamination of the used beauty blenders taken by female users in Mosul City. The contamination resulted in 84.6 percent suggesting inadequate hygienic behaviour and susceptible nature of these cosmetic accessories to the microbial growth. The result matches with recorded data earlier that microbial growth of 55.14% and 92.41% of Mannitol Salt and Nutrient Agar on similar samples respectively[2].

Behavioral and environmental aspects seem to be contributing factors to contamination as the dropping of sponges on the floor and storage in bathrooms with high moisture levels as well as non-disinfection and replacement of sponges have been implicated [14]. As well, storage in confined areas such as in drawers and handbags leads to further growth of the microbes in these enclosures because of warmth and moisture.

In this study, fungal contamination was also not high which similarly confirms previous results in which bacteria have been found to be prevailing over fungi and yeast in cosmetic settings[15,16]. This might be so because the makeup contains organic materials, minerals and emulsifiers whose nutritional content facilitates the growth of bacteria. Lack of labeled production and expiry dates may compromise preservative efficacy over time, increasing contamination risk.

There were six isolated groups of bacteria. The most common of these isolates included *S. aureus* at 41.3%, which is comparable to other reported isolation rates at approximately 42%[17]. *S. aureus* is a household microbiome inhabitant of the skin that can become a pathogen during skin barrier disruption[18,19], causing such diseases as sepsis or toxic shock syndrome [20].

S. epidermidis was recorded too and this is a commensal organism that can induce infection especially in people with medical implants or people who are in the immunocompromised condition. Although *S. sciuri* is naturally environmental and not pathogenic, it has been linked with rare infections that have severe complications such as endocarditis and septic shock infections[21,22].

Other than *Staphylococcus* species, there were the *Rothia* spp. (6.8%) and *Kocuria* spp. 3.4%. They are normally nonpathogenic bacteria that reside in the mouth cavity and in the skin respectively. Nevertheless, they can cause infections to susceptible patients, like the neonates or people in intensive care units [23, 24]. Both genera strains are now reported to the NCBI GenBank database, widening the list of microorganisms implicated in use of personal hygiene tools.

The survey had alarming hygiene practitioners on the side of the participants. More than 57.6% of them confessed that they shared their beauty blenders, and this substantially made them vulnerable to cross-contamination. Though a majority of them said they cleaned their sponges using soap and water, 15.4% only used water-which is not enough to kill stubborn microbe.

The frequency of changing sponges was different, some users kept them more than four months. They were used daily, but poor storage was a problem too: 73% stored their sponges in drawers along with other cosmetics, whereas 26.9% stored in handbags, a place that has ample chances of microbially colonized.

It is recommended that the sponge be washed and discard after every 30-90 days use[25]. The United States does this with some parts of its countries due to infection risks, including using a sponge with more than one client. However, inconsistent user behavior in terms of keeping using sponges after they have dropped them represents a high danger of infection 64 percent of them were found to be using sponges after dropping them[2].

This aligns with previous studies indicating insufficient hygiene practices among sponge users, especially in the presence of pathogenic bacteria[26]. The potential of transmission of skin infections is also increased by ignorance regarding hand hygiene, and the correct care of cosmetic devices. A good technique used by sponge disinfection was suggested which is microwave treatment: soak the sponge in water and soap and heat up to 60 seconds which can kill those pathogens[27].

Although the given study can provide significant insights into this issue, a number of limitations should be addressed. The small size of the sample (26 cosmetic sponges) limits the extent to which the findings can be used to obtain the wider applicability of the results beyond larger and diverse populations. Besides this, the study had geographical limitation which may not implicate hygiene practices and the microbial profile used in other cities or cities. Fungi and yeast could not be detected very precisely due to detection methods that are mostly based on simple culture methods without large characterization with regard to molecular characterization. Moreover, an assessment of how the microbes survive over time was not conducted, and the effectiveness of various cleaning tools has not been analyzed in detail. These limitations provide possible directions toward future research that may be conducted through more extensive sampling, higher fungal genomics, and comparative studies of disinfection procedures.

Findings underscore the urgency of establishing hygiene guidelines for cosmetic sponge use to prevent microbial infections. The presence of antibiotic-resistant measures, such as those most probably MRSA, is of clinical significance[28, 29]. Such active resistance mechanisms were observed, including β-lactamase production and tetracycline efflux pump gene presence, which are similar trends in other microbial studies[30, 31].

Moreover, the registration of Rothia spp. and Kocuria spp. strain under NCBI database is a new source of microbial surveillance worldwide. This study presents detailed microbiological profiling of isolates from beauty blenders, emphasizing public health risks associated with poor hygiene.

To minimize the infections and safeguard the consumers, education, community intervention, and manufacturer campaigns on specific sponge sanitation, disposal directions, and risk minimizing measures are important.

CONCLUSIONS

The research carried out determined the high microbial contamination of the used cosmetic sponges with *S. aureus* as the predominant isolate. Some of the strains demonstrated antibiotics resistance to oxacillin and tetracycline indicating the existence of antibiotics resistant strains (MRSA). Bacterial identification was correct in terms of molecular and biochemical testing as well as newly isolated strains could be registered in the NCBI database. Its copy questionnaire revealed that participants had low levels of hygiene in their use of them, as there was not enough anti mite cleaning, sharing of sponge, and humid storage. Such actions put more people at risk of transmission of bacteria and skin infections. The findings also point at the possibility of providing people with adequate education about correct sanitation and use of cosmetic tools to protect consumer health.

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Conflict of Interest: The authors do not have financial competing interests.

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