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(RESEARCH ARTICLE)

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Microbial contamination and antimicrobial resistance of lipstick testers from local cosmetic brands

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Abstract

Multiple people commonly use cosmetic testers, especially lipsticks, which raises the possibility of microbiological contamination. The purpose of this study was to determine the isolated bacteria using the VITEK method, assess their antimicrobial susceptibility, and examine the microbial presence in lipstick testers. Microbial analysis was performed on lipstick samples that were gathered from different retail locations. Upon identification of the isolated microbes, it was discovered that *Staphylococcus gallinarium* and *Micrococcus luteus* were among the possible pathogens. Common antibiotics were employed to assess these isolate's antimicrobial susceptibility. The results raised questions regarding the safety of shared cosmetic testers since they showed a notable prevalence of antibiotic resistant bacteria. These findings are consistent with other research emphasizing the microbial hazards connected to cosmetics. The study emphasizes the necessity of strict hygienic procedures in retail settings for cosmetics as well as heightened customer knowledge of the dangers of sharing testers. Microbial contamination might be reduced by using single use applicators and routinely cleaning cosmetic testers. Alternative antimicrobial agents that might improve the safety of cosmetic goods should be investigated further.

Keywords: Microbial Contamination; Lipstick Testers; VITEK Identification; Antimicrobial Resistance; Cosmetic Hygiene

1. Introduction

Cosmetic testers are widely used in retail settings to allow customers to try products before purchase. However, their frequent exposure to multiple users raises significant concerns regarding microbial contamination [1, 5]. Improper handling and repeated contact can turn these products into potential reservoirs of pathogenic microorganisms, posing health risks ranging from minor skin infections to severe systemic illnesses, particularly in individuals with compromised immunity [1, 6].

Lipsticks, one of the most commonly used cosmetic items, are especially prone to microbial contamination when shared [3, 8]. Studies have identified a variety of bacterial and fungal contaminants in shared lipstick testers, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis* [2, 7, 12]. Fungal organisms such as *Candida albicans, Aspergillus Niger*, and *Penicillium species* have also been isolated from these products, further emphasizing the hygienic concerns [4, 10].

The hypothesis of this study is that lipstick testers in retail environments harbor significant microbial contamination, presenting a tangible health risk to consumers. This hypothesis is driven by prior research, such as Lundov et al. [1] and Di Maita et al. [3], demonstrating the presence of pathogenic bacteria and fungi in shared cosmetic products, coupled with observations of inconsistent hygiene protocols in retail settings [9, 13]. The importance of this research lies in its

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potential to address a critical public health concern: the unchecked spread of pathogens through cosmetic testers, which millions of consumers use daily to enhance their appearance and self-care routines [5, 14]. Ensuring the safety of these products is vital not only for individual health but also for informing regulatory standards that govern the beauty industry [9, 15].

The current study aims to evaluate the microbiological contamination of lipstick testers available in retail stores, identify the isolated bacterial and fungal species using the VITEK identification system, and assess their antibiotic susceptibility profiles. The purpose is to raise awareness about the microbial hazards of shared cosmetics and highlight the urgent need for improved hygienic practices and routine microbiological monitoring in the cosmetic industry [11, 13].

2. Materials and methodology

2.1. Materials Used

The present study was conducted to analyse the microbial contamination of lipstick testers available in local cosmetic shops. Two different types of lipstick testers were selected: one bullet lipstick and one liquid lipstick. These samples were chosen based on the assumption that shared cosmetic testers are frequently exposed to unhygienic conditions, increasing the risk of microbial contamination.

The research was conducted over a period of six months at a microbiology laboratory equipped with necessary resources for microbial isolation, identification, and antibiogram profiling.

2.2. The primary materials used in this study included

- Lipstick Testers: Bullet and liquid lipstick samples collected from various local shops.
- Culture Media: Sterile Nutrient Agar, Sterile Sabourauds Agar, Sterile MacConkey Agar, and Sterile Nutrient Broth.
- Sterile Equipment: Cotton swabs, inoculation loops, Petri dishes, and test tubes.
- Biochemical Test Reagents: Used for Sugar Fermentation, Urease, Motility, Coagulase, Catalase, Salt Tolerance, and Voges-Proskauer tests.
- VITEK System: For advanced microbial identification using MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) technology.
- Antibiotic Discs: Trimethoprim, Nitrofurantoin, Penicillin, Gentamicin, Tetracycline, Oxacillin, Sulphathiazole, Methicillin, Norfloxacin, Chloramphenicol, Vancomycin, Clindamycin, and Erythromycin.

2.3. Collection and Preparation of Samples

Samples of lipstick testers were gathered from many nearby cosmetic stores. Direct viable count enumeration was considered unnecessary since a low microbial load was anticipated. Rather, an enrichment method was used. The enrichment media was Sterile Nutrient Broth. A sterile cotton swab was used to swab each lipstick tester, and the swab was then instantly injected into the enrichment broth. In order to promote microbial development, the broth was vortexed to guarantee complete mixing and incubated for 24 hours at 37°C.

Isolation of Microbes: To get well-isolated colonies of microorganisms, a loopful of the enriched broth was streaked onto Nutrient Agar and Sabourauds Agar plates following incubation. The inoculation plates were incubated for 24 to 48 hours at 37°C. Three different colony types from both agar plates were chosen based on their morphological characteristics after incubation, and they were purified by repeated subculturing.

Identification of microorganisms: Gram staining was used to classify the isolates as either Gram-positive or Gram-negative. Lactose fermenters and non-fermenters were distinguished by further subculturing on MacConkey Agar. Sugar fermentation, urease, motility, coagulase, catalase, salt tolerance, and Voges-Proskauer assays were among the biochemical tests used for initial characterization.

The purified bacterial cultures underwent VITEK identification using the MALDI-TOF technique for confirmation identification, enabling accurate microbiological characterization at the species level.

Antibiogram Profiling: Following isolate identification, antibiogram profiling was done to determine the isolated bacteria's pattern of antibiotic susceptibility. For this, the Kirby-Bauer Disc Diffusion Method was used. Antibiotic discs

were positioned at appropriate intervals from the bacterial isolates, which were equally distributed on sterile nutrient agar plates. Trimethoprim, Nitrofurantoin, Penicillin, Gentamicin, Tetracycline, Oxacillin, Sulphathiazole, Methicillin, Norfloxacin, Chloramphenicol, Vancomycin, Clindamycin, and Erythromycin were among the many antibiotics that were studied. To determine whether the isolates were susceptible or resistant, the zone of inhibition surrounding each antibiotic disc was measured after the plates had been incubated for 24 hours at 37°C. To ascertain the possible dangers connected to the usage of shared lipstick testers, the outcomes of microbiological identification and antibiotic susceptibility tests were examined and contrasted with the results of existing research.

3. Results and discussion

On Nutrient Agar, the separated enriched sample displayed a varied population of bacterial growth with several colony forms. Below Table I gives the colony characteristics of the bacterial isolates from lipstick testers After incubation, Saburou's agar plate showed no signs of growth. Mac Conkey's Agar was used to isolate the chosen isolates. After a day of incubation, no growth was observed on the plates. These findings verified that the isolates were Gram-positive cocci, as did the Gram's Staining.

Table 1 Colony Characteristics of Bacterial Isolates from Lipstick Testers

Colony Characteristics	Isolate 1	Isolate 2	Isolate 3
Size	1mm	2mm	2mm
Shape	Circular	Irregular	Circular
Colour	Colourless	Colourless	Yellow
Margin	Entire	Irregular	Entire
Elevation	Flat	Flat	Slightly elevated
Consistency	Dry	Bayrous	Dry
Opacity	Opaque	Opaques	Opaques
Gram's Nature	Gram positive	Gram positive	Gram positive
Morphology	Cocci	Cocci	Cocci

Biochemical assays were then performed on the isolates. When isolated on blood agar, isolates 1, 2 and 3 were non-hemolytic; they tested positive for urease and catalase, grew in 6.5% NaCl, and tested negative for coagulase and the Vogues Pauker test. Each of the three isolates had a different result for the sugar utilization test. Below Table II show biochemical Characteristics of bacterial isolates from the lipstick testers.

Table 2 Biochemical Characteristics of Bacterial Isolates from Lipstick Testers

Biochemical tests	Isolate 1	Isolate 2	Isolate 3
Haemolysis on blood	Non-haemolytic	Non-haemolytic	Non-haemolytic
Urease Test	Positive	Positive	Positive
Catalase test	Positive	Positive	Positive
Growth in 6.5% NaCl	Positive	Positive	Positive
Coagulase Test	Positive	Positive	Positive
Voges-Proskauer Test	Negative	Negative	Negative
Glucose Fermentation (0.5%)	Aerobic and Anaerobic	Aerobic and Anaerobic	Aerobic and Anaerobic
Mannitol Fermentation (0.5%)	Aerobic and Anaerobic	Negative	Negative

Figure 1 Growth in 6.5% NaCl (Isolate 2)

Figure 2 Growth in 6.5% NaCl (Isolate 3)

Figure 3 Urease

Figure 4 Glucose Fermentation and Mannitol Fermentation

Figure 5 Glucose Fermentation and Mannitol Fermentation (Isolate 2)

Figure 6 Glucose Fermentation and Mannitol Fermentation (Isolate 3)

By Vitek Analysis using MALDI-TOF method, isolate 1 and Isolate 2 were identified to be Staphylococcus glirarium and Isolate 3 was identified as Micrococcus luteus.

Table 3 Biochemical Test details for Vitech Analysis

TEST	ISOLATE 1	ISOLATE 2	ISOLATE 3
AMY (Amylase)	-	-	-
APPA (Aminopeptidase A)	-	-	-
Lea (Leucine Biosynthesis Protein A)	-	-	+
AlaA (Alanine Transaminase)	-	-	+
dRIB (Deoxyribose)	-	-	-
NOVO (Novobiocin (an antibiotic))	+	+	-
dRAF (Deoxyribose Raffinose)	+	+	-
OPTO (Optochin)	+	+	-

O129R (O/129 Resistance)	-	-	-
NC6.5 (Neutral Casein at pH 6.5)	+	+	-
1LATk (1-Lactate)	-	-	-
TyrA (Tyrosine Biosynthesis Protein A)	-	-	+
ProA (Proline Biosynthesis Protein A)	-	-	+
CDEX (Cyclodextrin)	-	-	-
PIPLC (Phosphatidylinositol-Specific Phospholipase C)	-	-	-
dXYL (Deoxyribose Xylose)	+	+	-
AspA (Aspartate Aminotransferase)	-	-	-
BGURr(Beta-Glucuronidase)	-	-	-
dSOR (Deoxy sorbitol)	-	-	-
LAC (Lactose)	-	-	-
dMAN (Deoxymannose)	+	+	-
SAL (Salicin)	+	+	-
ADH1 (Alcohol Dehydrogenase 1)	+	+	-
BGAR (Beta-Galactosidase (Arabinoside substrate))	-	-	-
AGAL (Alpha-Galactosidase)	-	-	-
URE (Urease)	+	-	+
NAG (N-Acetylglucosamine)	-	-	-
dMNE (Deoxy mannitol)	+	+	-
SAC (Sucrose)	+	+	-
BGAL (Beta-Galactosidase)	-	-	-
AMAN (Alpha-Mannosidase)	-	-	-
PyrA (Pyrimidine Biosynthesis Protein A)	-	-	+
POLYB	-	-	-
dMAL (Deoxy maltose)	+	+	-
MBdG (Methyl umbelliferyl-Beta-D-Glucuronide (substrate for beta-glucuronidase))	+	+	-
dTRE (Deoxy trehalose)	+	+	-
AGLU (Alpha-Glucosidase)	-	-	-
PHOS (Phosphatase)	-	-	-
BGUR (Beta-Glucuronidase)	+	+	-
dGAL (Deoxy galactose)	-	-	-
BACI (Bacitracin)	-	-	-
PUL (Pullulan's)	-	-	-
ADH2s (Alcohol Dehydrogenase 2)	-	-	-

Antibiotic susceptibility tests of all three isolates gave varied results.

3.1.1. Isolate 1*: Staphylococcus gallinarium*

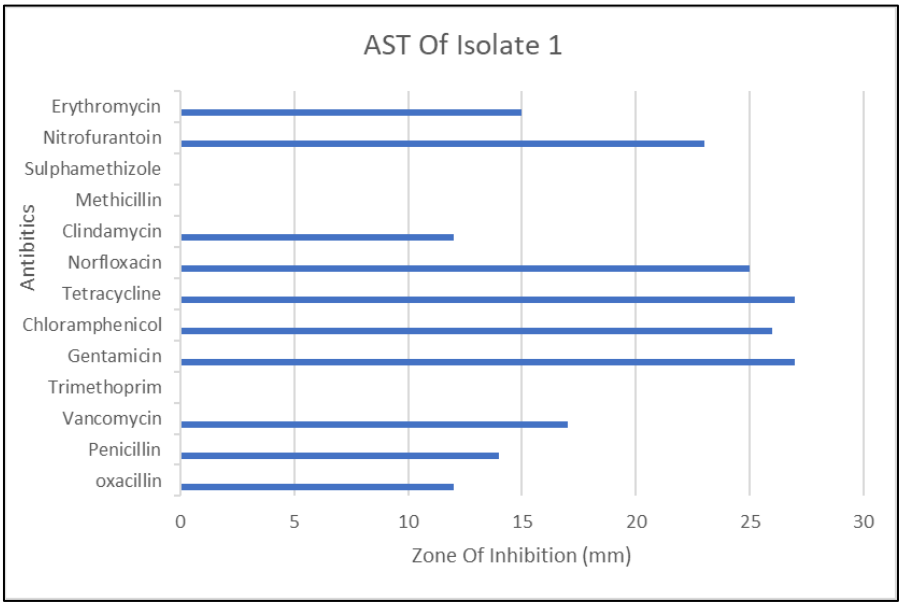

Figure 7 Graphical Representation of AST of Isolate 1

Figure 7 shows graphical representation of AST of isolate 1 (Staphylococcus gallinarium) , Figure 8 shows AST of Isolate 1. According to the AST, isolate 1 is sensitive to Nitrofurantoin, Clindamycin, Norfloxacin, Gentamicin, Vancomycin resistant to Sulphathiazole, Methicillin, Trimethoprim, Penicillin and showed intermediate results with Chloramphenicol, Erythromycin and Oxacillin.

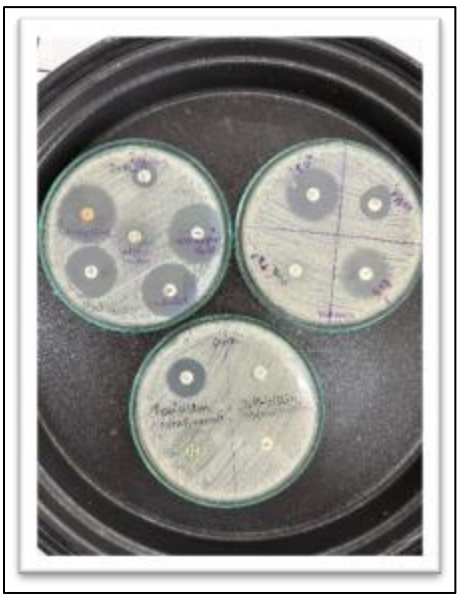

Figure 8 AST of Isolate 1

3.1.2. Isolate 2: *Staphylococcus gallinarium*

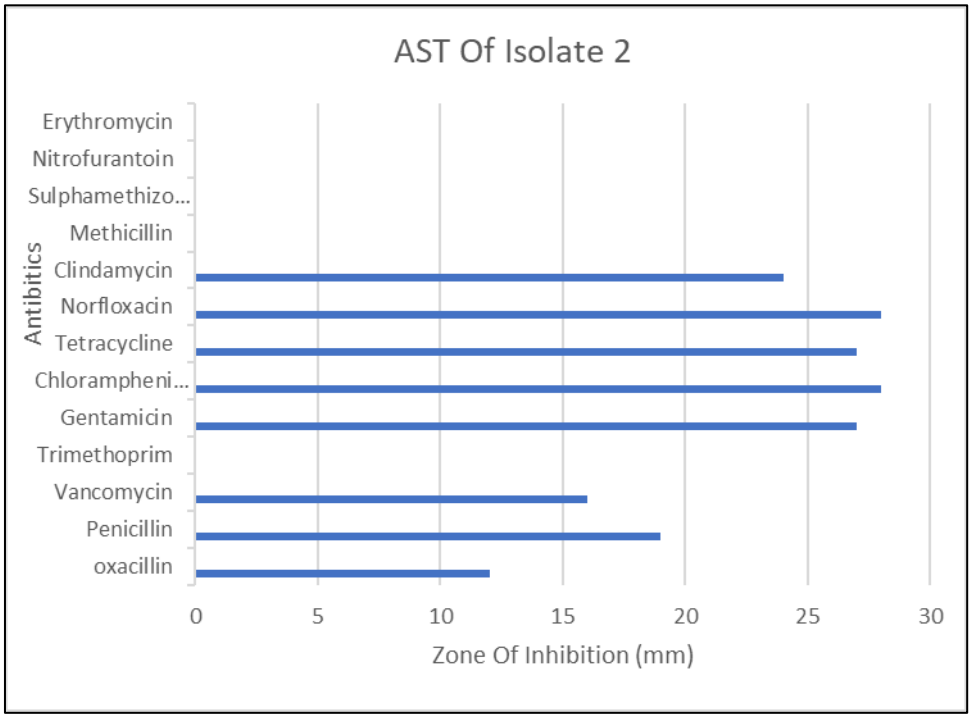

Figure 9 Graphical Representation of AST of Isolate 2

Figure 9 shows graphical representation of AST of isolate 2 (Staphylococcus glirarium), Figure 10 shows AST of Isolate 2. According to the AST, isolate 2 is sensitive to Tetracycline, Chloramphenicol, Clindamycin, Norfloxacin, Gentamicin resistant to Trimethoprim, Methicillin, Erythromycin, Nitrofurantoin, Sulphathiazole and Penicillin. and showed intermediate results with oxacillin and Vancomycin.

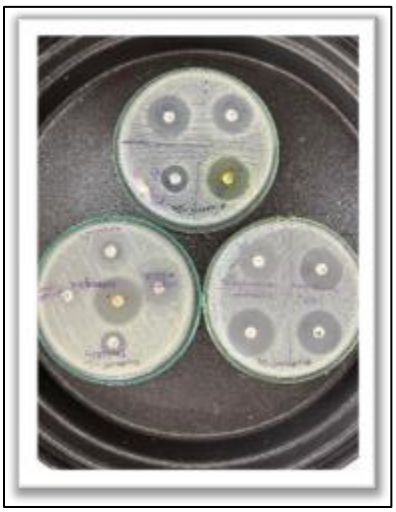

Figure 10 AST Isolate 2

3.1.3. Isolate 3*: Micrococcus luteus*

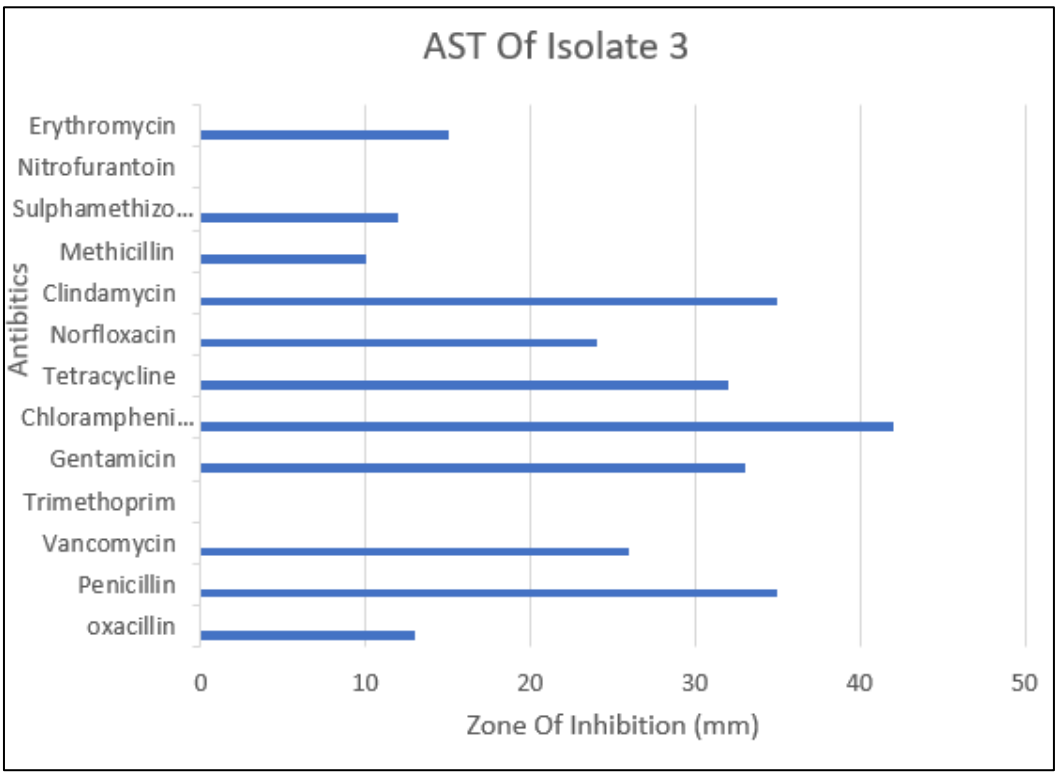

Figure 11 Graphical Representation of AST of Isolate 3

Figure 11 shows graphical representation of AST of isolate 3 (Micrococcus luteus), Figure 12 shows AST of Isolate 3. According to the AST, isolate 3 is sensitive to Sulphathiazole, Vancomycin, Penicillin, Oxacillin, Tetracycline, Chloramphenicol, Clindamycin, Norfloxacin, Gentamicin and resistant to Trimethoprim, Nitrofurantoin. and showed intermediate results with Methicillin and Erythromycin.

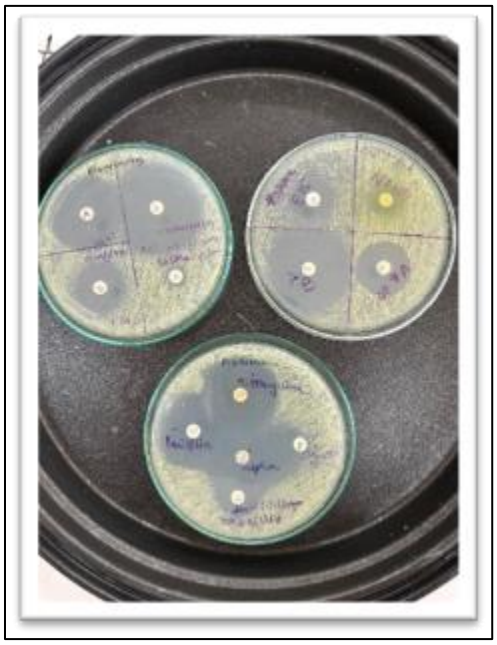

Figure 12 AST Isolate 3

This study sheds light on an issue that many of us never think about, lipstick testers in stores might be a breeding ground for bacteria, putting customers at risk of infections. The presence of *Staphylococcus gallinarium* and *Micrococcus luteus* in the samples proves that these testers aren't as safe as they seem. What's even more surprising is that, although both *Staphylococcus gallinarium* isolates came from the same species, they behaved differently in biochemical tests and had unique antibiotic resistance patterns. This suggests they might be different strains with distinct characteristics.

The findings of this study highlight a significant public health concern regarding microbial contamination in lipstick testers, particularly the presence of antibiotic-resistant bacteria. Several bacterial isolates exhibited resistance to commonly used antibiotics, including Trimethoprim, Methicillin, and Penicillin. This resistance is concerning, as infections caused by these bacteria could be challenging to treat, potentially leading to prolonged illness or complications, especially in immunocompromised individuals. The high frequency of customer interaction with lipstick testers in retail settings amplifies the risk of pathogen transmission, underscoring the need for improved hygiene practices and stricter regulatory oversight in the beauty industry.

To mitigate these risks, one potential strategy is the incorporation of antimicrobial agents into lipstick formulations to inhibit bacterial growth. However, the use of traditional antibiotics in cosmetics raises concerns about exacerbating antibiotic resistance, a growing global health crisis. A more sustainable alternative could involve the use of plant-based antimicrobial agents, such as essential oils (e.g., tea tree oil) or flavonoids, which have demonstrated effective antibacterial properties in prior studies without contributing to resistance. These natural compounds could provide a safer means of reducing microbial contamination in cosmetic products.

At the consumer level, adopting safer practices can significantly reduce the risk of infection. For instance, avoiding direct application of testers to the lips or other mucous membranes, where bacteria can easily enter the body, is a practical precaution. Retailers could further minimize risks by providing disposable applicators or single-use tester sachets, which would limit cross-contamination between users. Implementing such measures could enhance consumer safety while maintaining the interactive experience of product testing.

4. Conclusion

The presence of antibiotic-resistant bacteria in lipstick testers highlights the urgent need for enhanced hygiene protocols and regulatory measures in the beauty industry. While plant-based antimicrobial agents and single-use applicators offer promising solutions, further research is needed to evaluate their effectiveness and feasibility. By addressing these challenges, the industry can better protect consumers and reduce the public health risks associated with shared cosmetic testers.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors of this manuscript declare the following regarding any potential conflicts of interest or competing interests related to the publication of this study on microbial contamination in lipstick testers

All authors have no financial or non-financial conflicts of interest to disclose. has no affiliations with or involvement in any organization or entity with a financial interest in the cosmetic industry or products mentioned in this manuscript, nor does she have any competing interests that could influence the outcome of this study.

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