Research Article

Lipstick Application And Its Effect On Oral Microflora And Salivary Ph: A Cross-Sectional Study Among Iraqi Women; A Preliminary Analysis

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ABSTRACT

Background: lipstick is cosmetic products that applied directly to the lips, come into continuous contact with the oral cavity and may therefore influence its resident microbial community and local biochemical environment. The microbiological consequences of habitual lipstick use on the oral flora, and its potential effect on salivary pH, remain insufficiently studied, particularly in Iraq.
Aim of the study: the present study aimed to examine the effect of lipstick application on the salivary PH and the oral microflora in a sample of Iraqi women
Methods: A cross-sectional study was conducted on thirty-nine women, randomly recruited from three different Iraqi public institutions and divided into lipstick-applicant (n=30) and non-applicant (n=9) groups. Tongue swabs were cultured on nutrient agar, and bacterial isolates were identified phenotypically and confirmed using the Vitek 2 Compact system. Salivary pH was measured on fasting, undiluted saliva samples using pH indicator paper.
Results: A valid bacterial identification was obtained in 28 of the 39 samples (71.8%); of these, 21 isolates (75.0%) were classified as pathogenic bacteria and 7 (25.0%) as normal oral flora. Sixteen different bacterial genera/species were identified, the most frequent being Stenotrophomonas maltophilia (5/28, 17.9%), Kocuriakristinae and Sphingomonaspaucimobilis (4/28 each, 14.3%). The proportion of pathogenic versus normal-flora isolates was essentially identical between lipstick-applicant and non-applicant women (75.0% vs 75.0% pathogenic; χ^2=0.00, df=1, p=1.00). Salivary pH values across identified samples ranged from approximately 6.6 to 7.6, consistent with a near-neutral oral environment.
Conclusion: Lipstick application was not associated with a difference in the pathogenicity profile of the cultivable oral (tongue) microflora in this sample of Iraqi women, and salivary pH remained within the physiological neutrophilic range in both groups.

Keywords: lipstick; oral microflora; salivary pH; cosmetics; bacterial colonization; Iraqi women.

INTRODUCTION

Cosmetic products are extensively regulated due to their direct and repeated contact with human skin and mucous membranes. According to Article 2 of the European Commission regulation on pharmaceutical and cosmetic products, a cosmetic product is defined as any substance or mixture intended to be placed in contact with the external parts of the human body, or with the teeth and the mucous membranes of the oral cavity, with a view exclusively or mainly to cleaning, perfuming, changing the appearance, protecting, keeping in good condition, or correcting body odors [1]. This regulation lists 1,740 substances in Annex II that are prohibited from intentional addition to cosmetic products, among which reference number 39 corresponds to antibiotics. All cosmetic formulations showed to contain additional additives that function as preservatives for the product integrity and prevent microbial contamination during storage and distribution, but these additives have not been studied comprehensively,and their broader environmental and microbiological impact remains incompletely assessed [2,3]. Some of these additives may affect the microorganisms that present in the surrounding environment in addition to those residing on and within the human body [3].
Antimicrobial resistance (AMR) is recognized as one of the most threats that currently facing health providers globally. AMR-associated and AMR-attributable deaths already number in the millions worldwide each year, and this burden is projected to rise further by 2050 [4]. Reducing antibiotic use across medical and non-medical sectors, including agriculture, has been shown to reduce the emergence of new resistant strains [5,6], suggesting that reducing the use of agents that interfere with microorganisms in and on the human body — whether through medical, veterinary, or cosmetic exposure — may similarly help slow the pace of resistance [7-9].

The resident oral microflora is now well established to play multiple roles in human health, physiological homeostasis, and the body's response to therapeutic agents [10,11]. Bacteria capable of hydrolysing urea in saliva raise the local pH of the oral cavity, which in turn promotes the saturation of calcium phosphate and the formation of dental calculus [12]. Beyond this, the oral microflora participates in immunomodulation and has even been implicated in gallstone formation [13], and imbalances in oral flora composition have been linked to the development of oral cancer [14]. Because the oral microflora is this closely tied to local pH and to overall health outcomes, any factor capable of shifting the oral microenvironment — including cosmetic products applied to and around the mouth — merits investigation.

Lipstick is applied to the lips directly and it can be transferred, even partially, into the oral cavity through licking of the lips, eating, or even by drinking and this possibility makes it closely related to the microenvironment of oral cavity in a comparison with other cosmetics. This intimate relationship has been particularly concerned in places where counterfeit or poorly regulated lipstick products are common. Studies from Bangladesh, for an instance, showed that many of the lipsticks that sold sold in local markets were counterfeit and low-cost brands, and can contain heavy metals such as chromium, lead, and cadmium, in concentrations that may exceed the internationally accepted safety limits [15]. In a similar manner, the concerns about the informal production of cosmetics and the quality control that applied are present in other developing countries, such as in Iraq. One of the drawbacks of these informal cosmetic formulations is their possible effect on the local pH and their possible role in shifting the balance of the oral microflora, either in addition to, or even independently from, any effect of the antimicrobial-related additives discussed above

Given the widespread daily use of lipstick among women, the close physical relationship between lipstick and the oral cavity, and the established sensitivity of the oral microflora to local pH, this study aimed to investigate the effect of lipstick application on oral microflora and salivary pH among Iraqi women.

METHODOLOGY

Study design

The present study is a cross-sectional study that conducted on 39 women, who selected randomlyfrom three different Iraqi public institutions (the Ministry of Science and Technology, the Ministry of Labour and Social Affairs, and a College of Science) and divided into two groups: lipstick-applicant (n=30) and non-applicant (n=9) women. Relevant demographic and clinical information, including age, marital status, institution, current antibiotic use, and contraceptive use, was collected using a predefined data sheet from both groups. Each participant was assigned a serial number that was written on the surface of the sterile swab used for sample collection.

Swabs were collected in the morning under fasting conditions in a well-lit room, with the participant seated comfortably and facing a light source; sampling followed a standardised approach to biological specimen collection, avoiding contact of the swab tip with any surface other than the sampling site [16]. Swabs were obtained from the upper and lower surfaces of the tongue using a gentle rotating motion, then placed immediately into nutrient agar medium to ensure rapid processing after collection, in accordance with standard oral swab culture protocols [17].

Nutrient agar media were sterilised by autoclaving at 121°C for 15-20 minutes. Swabs were cultured directly using a sterilised loop. Isolated colonies were first characterised by Gram staining and colonial/phenotypic (observable) characteristics, and each isolate was then identified to the genus/species level using the Vitek 2 Compact automated identification system (BioMérieux, France), consistent with the phenotypic bacterial identification approach used in previous work by our group [18]. Identifications were reported by the system together with a probability score; among the individual Vitek 2 Compact reports reviewed for this study, identification probability ranged from 88% to 99% (mean ≈ 95.6%), and isolates for which the system could not assign an identification were reported as “Unidentified Organism” and treated as missing at the genus level.

Salivary samples were also collected under fasting conditions. Salivary pH was determined by placing 1 mL of raw, undiluted saliva into a vial and dipping pH indicator paper into the sample; the result was read against a standard colour scale 30 seconds after immersion, following a previously described protocol for assessing pH changes in saliva [19].

Data were analysed using the Statistical Package for Social Sciences (SPSS). Frequencies and percentages were used to describe categorical variables, and cross-tabulation with the Chi-square test was used to examine the association between lipstick application and the pathogenicity classification of the isolated bacteria. A P value of < 0.05 was

considered statistically significant. This statistical approach is consistent with that used in several previous studies by our research group across a range of clinical and laboratory topics, in which SPSS-based descriptive statistics, cross-tabulation, Chi-square testing, and/or analysis of variance (ANOVA) were similarly applied [20,21].

RESULTS

Sample Overview

Of the thirty-nine women enrolled, 30 (76.9%) were lipstick-applicants and 9 (23.1%) were non-applicants (Table 1). A valid bacterial identification was obtained from 28 of the 39 tongue swabs (71.8%); the remaining 11 samples (28.2%) yielded no identifiable growth and were excluded from the genus-level analysis. Of the 28 identified isolates, 21 (75.0%) were classified as pathogenic bacteria and 7 (25.0%) as components of the normal oral flora (Table 2).

Table 1. Distribution of participants by lipstick application

Lipstick application	Frequency	Percent	Valid Percent	Cumulative Percent
Applicant	30	76.9	76.9	76.9
Non-applicant	9	23.1	23.1	100.0
Total	39	100.0	100.0	—

Table 2. Distribution of identified isolates by pathogenicity classification

Pathogenicity	Frequency	Percent (of 39)	Valid Percent (of 28)
Pathogenic	21	53.8	75.0
Normal flora	7	17.9	25.0
Missing / no growth	11	28.2	—
Total	39	100.0	100.0

Bacterial Genus Distribution

Sixteen different bacterial genera/species were isolated across the two groups (Table 3). Stenotrophomonas maltophilia was the most frequently isolated organism (5/28, 17.9%), followed by Kocuriakristinae and Sphingomonaspaucimobilis (4/28 each, 14.3%). Staphylococcus lentus and Serratia ficaria were each isolated twice (2/28, 7.1%), while the remaining eleven genera/species — Pseudomonas luteola, Escherichia coli, Klebsiella pneumoniae, Acinetobacter lwoffii, Chryseobacteriumindologenes, Micrococcus luteus, Kytococcussedentarius, Rhizobium radiobacter, Pantoea spp., Leuconostocmesenteroides spp., and Streptococcus thoraltensis — were each isolated once (1/28, 3.6%). Three of the sixteen taxa (Kocuriakristinae, Staphylococcus lentus, and Micrococcus luteus, together accounting for the 7 normal-flora isolates) were classified as normal oral flora, while the remaining thirteen taxa were classified as pathogenic.

Table 3. Frequency of bacterial genus/species by lipstick application group

Bacterial genus/species	Pathogenicity	Applicant (n)	Non-applicant (n)	Total (n, %)
Stenotrophomonas maltophilia	Pathogenic	5	0	5 (17.9%)
Kocuriakristinae	Normal flora	3	1	4 (14.3%)
Sphingomonaspaucimobilis	Pathogenic	4	0	4 (14.3%)
Staphylococcus lentus	Normal flora	2	0	2 (7.1%)
Serratia ficaria	Pathogenic	1	1	2 (7.1%)
Pseudomonas luteola	Pathogenic	1	0	1 (3.6%)
Escherichia coli	Pathogenic	1	0	1 (3.6%)
Klebsiella pneumoniae	Pathogenic	1	0	1 (3.6%)
Acinetobacter lwoffii	Pathogenic	1	0	1 (3.6%)
Chryseobacteriumindologenes	Pathogenic	0	1	1 (3.6%)
Micrococcus luteus	Normal flora	1	0	1 (3.6%)
Kytococcussedentarius	Pathogenic	1	0	1 (3.6%)
Rhizobium radiobacter	Pathogenic	1	0	1 (3.6%)
Pantoea spp.	Pathogenic	1	0	1 (3.6%)

Leuconostocmesenteroides spp.	Pathogenic	1	0	1 (3.6%)
Streptococcus thoraltensis	Pathogenic	0	1	1 (3.6%)
Total	—	24	4	28 (100%)

As a check on the identification workflow, Table 4 lists a representative subset of the original Vitek 2 Compact chart reports reviewed for this study (Lab IDs 80-90, corresponding to samples 1-14), including the identification probability for each confirmed isolate. Two of these eleven reports (samples 2 and 8) returned "Unidentified Organism", consistent with the overall proportion of samples with no valid identification reported above.

Table 4. Representative Vitek 2 Compact identification reports (Lab IDs 80-90)

Lab ID	Sample No.	Selected organism	Probability
80	1	Sphingomonaspaucimobilis	95%
81	2	Unidentified Organism	—
82	3	Chryseobacteriumindologenes	97%
83	5	Chryseobacteriumindologenes	97%
84	8	Unidentified Organism	—
85	9	Acinetobacter lwoffii	88%
86	10	Stenotrophomonas maltophilia	95%
87	11	Stenotrophomonas maltophilia	95%
88	12	Stenotrophomonas maltophilia	95%
89	13	Stenotrophomonas maltophilia	99%
90	14	Stenotrophomonas maltophilia	99%

It is worth noting that these chart reports record two confirmed isolates of Chryseobacteriumindologenes (samples 3 and 5), one more than the single isolate recorded in the SPSS-derived summary in Table 3; in line with the research team's guidance, the SPSS-derived total was retained as the authoritative figure in Table 3 and throughout this manuscript.

Association Between Lipstick Application and Bacterial Pathogenicity

Among lipstick-applicant women, 18 of the 24 identified isolates (75.0%) were pathogenic and 6 (25.0%) were normal flora. Among non-applicant women, 3 of the 4 identified isolates (75.0%) were pathogenic and 1 (25.0%) was normal flora. The proportion of pathogenic to normal-flora isolates was therefore identical between the two groups, and a Chi-square test of the 2×2 table (pathogenicity × lipstick application) confirmed no statistically significant association (χ^2=0.00, df=1, p=1.00).

Two organisms, Chryseobacteriumindologenes and Streptococcus thoraltensis, were isolated only from non-applicant women, while the remaining fourteen taxa were isolated only, or predominantly, from lipstick-applicant women; given the small number of non-applicant isolates overall (n=4), these single-isolate observations should be interpreted with caution and are not evidence of a genus-specific association.

Salivary pH

Salivary pH values recorded across the identified samples ranged from approximately 6.6 to 7.6, with most readings clustering close to neutrality (≈ 7.0-7.5), consistent with the near-neutral pH range generally preferred by oral bacterial flora.

DISCUSSION

This study found that a high proportion of the cultivable tongue microflora identified in this sample of Iraqi women — three-quarters of all identified isolates — was classified as pathogenic rather than normal flora, irrespective of lipstick use. This proportion was numerically identical between lipstick-applicant and non-applicant women, and the formal Chi-square comparison showed no statistically significant association between lipstick application and the pathogenicity profile of the isolated flora (χ^2=0.00, p=1.00). This null result should be interpreted cautiously given the small and unbalanced group sizes (30 applicants versus only 9 non-applicants, yielding just 4 identified isolates in the non-applicant group); a study with a larger and more evenly distributed non-applicant group would be better powered to detect a true difference, if one exists.

The bacterial genera most frequently isolated in this study — Stenotrophomonas maltophilia, Kocuriakristinae, and Sphingomonaspaucimobilis — are environmental and opportunistic organisms rather than the classical cariogenic

or commensal oral streptococci typically emphasised in oral microbiome literature. Their relative predominance here may reflect the specific institutional and environmental exposures of the participants, sampling and transport conditions, or simply the composition of the cultivable, phenotypically identifiable flora captured by this particular culture-based approach, which cannot detect the full diversity of the oral microbiome the way culture-independent sequencing methods can.

Because the tongue and the labial mucosa are in constant, direct contact with saliva, the composition of the cultivable tongue microflora is expected to reflect, at least in part, the physicochemical conditions of the surrounding oral environment. Most bacteria that colonize the human mouth behave as neutrophiles that prefer a pH range close to neutral for best growing; both strongly acidic and strongly alkaline conditions can disrupt bacterial protein folding and membrane function enough to impair growth. The salivary pH values recorded in this study (approximately 6.6-7.6) fall within this expected neutrophilic range, which is consistent with saliva's normal buffering capacity and does not, on the basis of the descriptive data available, suggest a marked pH shift attributable to lipstick use. This is also broadly consistent with previous reports linking urease-producing oral bacteria to elevated salivary pH and dental calculus formation [12], none of which were identified as predominant isolates in the present sample.

The comparatively high proportion of samples with no identifiable bacterial growth (11/39, 28.2%) is itself a notable finding and may reflect low bacterial load on the tongue surface at the time of sampling, transport or culture conditions, or technical limitations of phenotypic identification for fastidious or slow-growing oral organisms. This should be considered a methodological limitation when interpreting the genus-level findings.

Ultimately, the statistically non-significant association that demonstrated in this study between the use of the lipsticks and oral bacterial pathogenicity or salivary pH does not rule out the possible effect of lipstick composition itself but the pathogenic bacteria were identified in the lipstick-applicant women. Reports from other countries have documented that counterfeit or poorly regulated lipsticks can contain heavy metals at concentrations exceeding accepted safety limits [15]; such contaminants, and other undisclosed cosmetic additives, could plausibly influence the oral microenvironment through mechanisms not captured by bacterial culture alone (for example, direct cytotoxicity or heavy-metal selection pressure on specific bacterial taxa). The present study did not chemically characterise the lipstick products used by participants, which limits its ability to distinguish a true absence of effect from an effect that a culture-based, exposure-undifferentiated design was simply not able to detect.

CONCLUSION

In this cross-sectional study of Iraqi women, valid bacterial identification was obtained in 71.8% of tongue swabs, of which three-quarters were classified as pathogenic and one-quarter as normal oral flora. Lipstick application was not associated with a difference in this pathogenicity profile (χ^2=0.00, p=1.00), and salivary pH remained within the expected near-neutral, neutrophile-favouring range in the samples examined. These findings do not support a strong or easily detectable effect of habitual lipstick use on the cultivable oral microflora or salivary pH in this population, although the small and unbalanced non-applicant group limits the strength of this conclusion.

It is important to note that, despite there was non-significant difference between applicant and non-applicant women, which could be owned to the insufficient sample size, the pathogenic bacteria identified in this study were found in the lipstick-applicant women.

RECOMMENDATIONS

Future studies should recruit a larger and more evenly balanced non-applicant comparison group to improve statistical power.

Chemical or heavy-metal analysis of the specific lipstick products used by participants is recommended to distinguish cosmetic-composition effects from simple application status.

A paired, within-subject design (sampling the same women before and after a defined period of lipstick use) would help control for inter-individual variation in baseline oral flora.

Culture-independent methods (e.g., 16S rRNA sequencing) would capture a broader range of the oral microbiome than phenotypic culture and Vitek 2 identification alone, particularly given the high proportion of samples with no identifiable growth in this study.

STATEMENT OF ETHICS

The study was approved by Ibn Sina University of Medical and Pharmaceutical Sciences, College of Medicine Scientific Committee, with an approval number 1776 in 28/05/2025.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the conception, design, data collection, analysis, and writing of this manuscript.

FUNDING SOURCES

This research received no specific grant or any other form of support from any funding agency in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

The authors would like to thank all the women who volunteered to participate in this study, as well as the staff of the three participating Iraqi public institutions for facilitating sample collection. Thanks is expressed to Dr Muhammed abdul Wahab, Athraa A. Sada, Atyaf Khalid, Shahad Shukri, Noor Nihad, Raafat R. Muhammed for their role and aid in sample handling and analysis.

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