Original Article

The effect of long-pulsed 1064 nm Nd:YAG laser-assisted hair removal on some skin flora and pathogens: an *in vivo* study

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Abstract

Background: The effect of NDYag on normal skin flora and pathogenic microbes has not been studied.

Objectives: Evaluation of immediate (before versus after each session) and delayed (pre-first session *versus* pre-fourth session) antimicrobial effect of Nd:YAG laser-assisted hair removal.

Methods: Thirty females scheduled for axillary Nd:YAG laser hair removal were included. Skin swabs were collected from the vault of the dominant axilla before and after each of the four sessions. Bacteriological cultures were performed to record the counts of total aerobes, total anaerobes, lipophilic bacteria, total staphylococci, *Staphylococcus epidermidis* (*S. epidermidis*), *S. saprophyticus*, *S. hominis,* and *S. aureus*. Reported changes in sweat odour and folliculitis (if present) were recorded.

Results: *S.hominis* was the predominant species in all subjects before and after all sessions. Counts of total aerobes, total anaerobes, lipophilic bacteria, total staphylococci, and *S.hominis* significantly decreased after all 4 sessions. A significant reduction was noted in the median colony counts before the fourth session as compared to the baseline count before the first session in total aerobes (278.9 *versus* 126.3 $\times$ 10^5 CFU/cm^2, $p = 0.003$), total anaerobes (338.7 *versus* 103.7 $\times$ 10^5 CFU/cm^2, $p = 0.002$) and total staphylococci (248.5 *versus* 105.0 $\times$ 10^5 CFU/cm^2, $p = 0.004$). Most subjects reported worsened or unchanged axillary sweat odour. There was a statistically significant positive correlation between sweat odour and the counts of total aerobes ($r = 0.433$, $p = 0.017$), total anaerobes ($r = 0.377$, $p = 0.040$), total staphylococci ($r = 0.383$, $p = 0.036$) and *S.hominis* ($r = 0.497$, $p = 0.005$) ; lower counts were associated with a worsened odour.

Limitations: Small sample size; few laser sessions; short follow-up; subjective assessment of sweat odor and quantity.

Conclusions: Laser caused an immediate and delayed reduction in axillary aerobes, anaerobes, lipophilic bacteria, and staphylococci. This form of dysbiosis might lead to sweat odour changes.

Keywords: Nd:YAG laser, skin flora, staphylococci, folliculitis, total aerobic count

Plain Language Summary

A possible antimicrobial effect of laser has been recently described but this has not adequately studied. This study aimed to evaluate the immediate and delayed antimicrobial effects of four Nd:YAG laser-assisted hair removal sessions for the underarms using bacteriological cultures for different bacterial species. Thirty female subjects were studied. Results revealed that post-session bacterial counts were significantly decreased with all sessions. This included various bacterial subtypes (total aerobes, total anaerobes, lipophilic bacteria, total staphylococci, and *S.hominis*). Most subjects reported either a worsened or unchanged underarm sweat odour; lower bacterial counts were associated with a worsened odour. We conclude that laser hair removal could cause immediate and delayed reductions in underarm bacteria, and this might lead to sweat odour changes.

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Introduction

Laser hair removal has become the treatment of choice for removing unwanted hair.[1] The most commonly used lasers for this purpose include diode (800 nm), alexandrite (795 nm), and neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers (1064 nm).[2]

Absorption by melanin at 1064 nm is lower than at shorter wavelengths but is still sufficient to permit selective photothermolysis of the pigmented hair follicle with permanent destruction.[3,4] This reduces the thermal damage to the surrounding epidermis, making Nd:YAG laser the safest option in dark-skinned patients.[5]

A distinctive axillary odour emanates when a large and permanent population of microorganisms thrives on secretions from eccrine, apocrine, and sebaceous glands. The resident axillary microbiota consists mainly of bacteria of the genera Staphylococcus, Micrococcus, Corynebacterium, and Propionibacterium. In females, Staphylococci predominate over Corynebacteria.[6] The use of laser for hair removal can alter the microbial flora, and an alteration of the smell of sweat can accompany this. The duration of this antimicrobial effect is undetermined, and so are the accompanying skin changes such as sweat odour and amount.[7]

Studies on the effect of Nd:YAG laser on skin flora are scarce. The present study aimed to evaluate the effect of Nd:YAG laser hair removal on the density of some skin flora and pathogens in the axilla (both immediate and delayed effects), and the relation between changes in bacterial counts and changes in axillary sweat odour and amount.

Subjects and Methods

This quasi-experimental interrupted time series study (ClinicalTrials.gov ID NCT05034237) was conducted on 30 females recruited at the dermatology outpatient clinic of Alexandria Main University Hospital, Egypt over 10 months from January to November 2021. Approval of the Medical Ethics Committee of Alexandria Faculty of Medicine was obtained (IRB NO 00012098). Informed written consent was taken from every participant.

Participants aged 20–40 years were selected, who had Fitzpatrick skin types III–V, and had had no previous laser hair removal sessions involving the axillae. Those with inflammatory skin conditions such as atopic dermatitis or psoriasis, or fungal or herpetic infections were excluded, as well as those with diabetes, those receiving immunosuppressive therapy, and pregnant or lactating females. A pre-designed data sheet was used to collect patients' data as their age, the number of laser session, their skin type, history of folliculitis as well as their laboratory results. Results of changes in sweat odour and amount were also recorded in the last session as compared to the first session.

Laser sessions

All participants were subjected to four sessions of laser-assisted hair removal at 4 week intervals using long-pulsed 1064 nm Nd:YAG laser (Cynosure Elite+™, Westford, USA). Parameters were set (15–18 mm spot size, fluence 24–35 J/cm^2, pulse duration 30–40 ms) according to hair colour, thickness, and density, and the skin phototype. All participants were instructed to avoid using oral or topical antibiotics for 72 hours before sampling, avoid washing the axilla with soap for 24 hours before the session, avoid using deodorants, antifungal or antibacterial cleansing products in the axilla throughout the study, and not to use a topical anaesthetic before sessions.

Sample collection

Swab were taken immediately before and after the session, as any time lapse would have affected the bacterial count (bacteria multiply with time). Each time (pre- or post-laser), two sterile swabs were used simultaneously for skin swabbing; these were then placed in 2 ml sterile thioglycolate broth (an enrichment broth that supports the growth of anaerobes, aerobes, and microaerophilic microorganisms). A modified standardized swabbing method described by Selwyn and Ellis (1972) was used.[8] The moistened with sterile saline swabs were vigorously rubbed on a 2 × 2 cm^2 area of the right axilla, with moderate pressure for 30 seconds. The tube into which the two swabs were placed was labeled, placed in an ice box and transported within 2 hours to the Microbiology Laboratory at High Institute of Public Health, Alexandria University for immediate processing.

Laboratory methods

The tube containing the two swabs and thioglycolate broth was vortexed for 30 seconds to disperse any bacteria adhering to the swabs and to distribute bacteria evenly in the liquid broth. A volume of 1 mL of the broth was transferred to each of six dilution tubes to obtain dilutions from 10^{-1} to 10^{-6}. These dilutions were used to culture the required plates. An automated pipette took a volume of 100 µl from each dilution tube to culture each of the following plates: two blood agar plates (for the total bacterial count; one aerobic and the other one for anaerobic incubation), mannitol salt agar plate (for enumeration of total Staphylococci and detection of *S.aureus*), blood agar supplemented with 0.5% Tween 80 (for enumeration of lipophilic bacteria) and Staphylococcus Chromogenic Agar plates (Condalab, Madrid,Spain, # Cat. 2076) for staphylococcal species identification and enumeration. All plates were incubated at 37°C for 24–48 hours aerobically, except for the blood agar plate reserved for the enumeration of anaerobes which was incubated using gas packs at 37°C for 5 days.

Bacterial colony counting and identification procedures

Plates from dilutions showing the best countable colonies (30–300 colony forming unit (CFU)/plate) were chosen to be

counted. Identification of *S. aureus* on mannitol salt agar was done according to standard microbiological methods[8] Isolates growing on Chromogenic (CHROM) agar were identified based on their colour according to the manufacturer's instructions,[9] and biochemical tests were used to confirm the identity of the isolates. *S.aureus* was tested for cefoxitin susceptibility to identify methicillin-resistant *S. aureus* (MRSA).[8] *S.hominis* were identified by being coagulase-negative, followed by automated identification by Vitek 2 Compact (bioMérieux, France).

The final colony count was calculated in relation to the swabbed surface area and dilution used, and the colony count was expressed as colony forming units (CFU)/cm^2. Automated bacterial identification using Vitek 2 Compact (bioMérieux, France) was done when needed to confirm isolates.

Assessment of sweat odour and quantity and presence or absence of folliculitis

Participants were asked about changes in sweat amount and odour by the end of the study (fourth laser session). Change in the amount of sweating was classified as decreased, increased, or unchanged, while change in odour was expressed as a score: improved (+1), worsened (–1), or unchanged (0). Any history of recurrent axillary folliculitis was re-evaluated by the end of the study.

Statistical analysis

Data was fed to the computer and analysed using IBM SPSS software package version 25.0. Qualitative data were described using numbers and percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution, data were not normally distributed. Quantitative data were described using the range (minimum and maximum), mean, standard deviation, and median. Significance was judged at the 5% level. The tests used were: Wilcoxon signed-rank test as a non-parametric test used for quantitative variables to compare between two related groups (paired comparison), Friedman's test as a non-parametric test used for quantitative variables to compare between more than two related groups (paired comparison), Spearman's rho correlation (for abnormally distributed quantitative variables) and Mann-Whitney test for quantitative variables to compare between two independent groups.

Results

The mean age of our participants was 26 years, with skin phototypes III-V. Five subjects gave a history of recurrent folliculitis.

The immediate antimicrobial effect of Nd:YAG laser

This was evaluated by comparing bacterial counts before and after each laser session. With the first session, there were significant reductions ($p < 0.001$) of colony forming units (CFU/cm^2) of total aerobes (278.9 versus 111.3 × 10^5), total anaerobes (338.7 versus 66.3 × 10^5), lipophilic bacteria (205.0 versus 78.7 × 10^5), total staphylococci (248.5 versus 85.0 × 10^5) and *S. hominis* (120.0 versus 25.0 × 10^5) [Table 1] [Figure 1]. This pattern of reduction was also found in the second, third and fourth laser sessions.

Table 1: Axillary bacterial colony counts of before and after the first Nd:YAG laser session in 30 female subjects

Type of bacteria	Colony count (×10^5 CFU/cm^2)		p-value
	Before first session	After first session	
Total aerobes			
Mean ± SD	778.3 ± 1429.7	511.4 ± 1318.7	Wilcoxon Signed Rank test = 30.0 P < 0.001*
Median	278.9	111.3	
Min-Max	2.5–6250.0	0.0–6012.0	
Total anaerobes			
Mean ± SD	787.3 ± 1422.9	305.1 ± 693.4	Wilcoxon Signed Rank test = 30.0 P < 0.001*
Median	338.7	66.3	
Min-Max	2.5–6662.5	0.0–3737.0	
Total staphylococci			
Mean ± SD	408.6 ± 536.1	455.9 ± 1703.6	Wilcoxon Signed Rank test = 37.0 P < 0.001*
Median	248.5	85.0	
Min-Max	12.5–2720.0	0.0–9425.0	
Lipophilic bacteria			
Mean ± SD	936.5 ± 2268.7	244.7 ± 646.2	Wilcoxon Signed Rank test = 19.0 P < 0.001*
Median	205.0	78.7	
Min-Max	0.0–9262.0	0.0–3532.5	
S.epidermidis			
Mean ± SD	1.4 ± 7.3	4.8 ± 22.0	Wilcoxon Signed Rank test = 4.0 P = 0.593
Median	0.0	0.0	
Min-Max	0.0–40.0	0.0–120.0	
S.saprophyticus			
Mean ± SD	9.4 ± 27.6	11.4 ± 53.3	Wilcoxon Signed Rank test = 8.0 P = 0.161
Median	0.0	0.0	
Min-Max	0.0–125.0	0.0–292.0	
S.hominis			
Mean ± SD	246.3 ± 300.6	108.4 ± 157.6	Wilcoxon Signed Rank test = 25.0 P < 0.001*
Median	120.0	25.0	
Min-Max	3.4–1283.7	0.0–578.5	

P*: p-value significant at level < 0.05

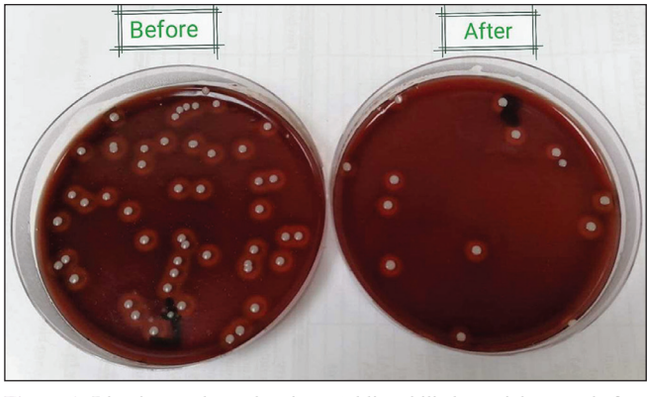

Figure 1: Blood agar plates showing total lipophilic bacterial counts before (left) and after (right) the third session of one participant.

The delayed antimicrobial effect of Nd:YAG laser

This was evaluated by comparing bacterial counts before the first and fourth laser sessions. There was a significant reduction in the median colony counts (CFU/cm^2) for total aerobes (278.9 versus 126.3 × 10^5, p = 0.003), total anaerobes (338.7 versus 103.7 × 10^5, p = 0.002) and total staphylococci (248.5 versus 105.0 × 10^5, p = 0.004). There were also observed reductions in the median lipophilic bacterial and *S.hominis* counts, but these did not reach statistical significance [Table 2].

With regard to staphylococcal species, *S.hominis* represented 96.6% and 83.3% of species before the first and fourth sessions, respectively. *S.saprophyticus* comprised 3.3% and 10% before first and fourth sessions respectively, while the corresponding figures for *S.epidermidis* were 0% and 3.3% respectively (data not shown). Four participants yielded *S.aureus* transiently in their axillary swabs; 3 yielded *S.aureus* prior to their third laser sessions with a median colony count of 10 × 10^5 CFU/cm^2) and one participant had positive *S.aureus* both before and after her third laser session with colony counts of 307.5 × 10^5 and 5 × 10^5 CFU/cm^2 respectively. None of the *S.aureus* isolates were found to be MRSA, as they were all sensitive to cefoxitin.

Table 2: Axillary bacterial colony counts of before the first session and before the fourth session of Nd:YAG laser in 30 female subjects

Type of bacteria	Colony count (×10^5 CFU/cm^2)		p-value
	Before first session	Before fourth session	
Total aerobes			
Mean ± SD	778.3 ± 1429.7	172.6 ± 176.6	Wilcoxon Signed Rank test = 89.0 p = 0.003*
Median	278.9	126.3	
Min-Max	2.5–6250.0	1.0–762.5	
Total anaerobes			
Mean ± SD	787.3 ± 1422.9	400.6 ± 1069.7	Wilcoxon Signed Rank test = 84.0 p = 0.002*
Median	338.7	103.7	
Min-Max	2.5–6662.5	2.5–5037.5	
Total staphylococci			
Mean ± SD	408.6 ± 536.1	510.1 ± 1659.0	Wilcoxon Signed Rank test = 91.0 p = 0.004*
Median	248.5	105.0	
Min-Max	12.5–2720.0	1.5–8612.5	
Lipophilic bacteria			
Mean ± SD	936.5 ± 2268.7	189.3 ± 295.5	Wilcoxon Signed Rank test = 144.5 p = 0.070
Median	205.0	110.0	
Min-Max	0.0–9262.0	0.0–1250.0	
S. epidermedis			
Mean ± SD	1.4 ±7.3	4.0 ±21.9	Wilcoxon Signed Rank test = 3.0 p = 1.000
Median	0.0	0.0	
Min-Max	0.0–40.0	0.0–120.0	
S.saprophyticus			
Mean ± SD	9.4 ± 27.6	18.3 ± 47.7	Wilcoxon Signed Rank test = 35.0 p = 0.445
Median	0.0	0.0	
Min-Max	0.0–125.0	0.0–237.5	
S.hominis			
Mean ± SD	246.3 ± 300.6	145.8 ± 198.8	Wilcoxon Signed Rank test = 150.0 p = 0.090
Median	120.0	70.0	
Min-Max	3.4–1283.7	0.0–825.0	

P*: p-value significant at level <0.05*

On comparing the median bacterial counts before each session, there was a significant difference between the four sessions in total aerobe counts (post hoc test, p = 0.010), total anaerobe counts (post hoc test, p = 0.008), total staphylococci (post hoc test, p = 0.001), and *S.hominis* (post hoc test, p = 0.036). Comparing the median bacterial counts after each session revealed a significant difference between the four sessions in total aerobes (post hoc test, p = 0.002), total anaerobes (post hoc test, p < 0.001), total staphylococci (post hoc test, p = 0.031), and lipophilic bacteria (post hoc test, p = 0.022). [Figures 2 and 3].

Subjective changes in sweat odour and amount

By the end of the study, twelve participants (40%) reported worsened sweat odour, two (6.7%) reported improved odour, and sixteen (53.3%) reported no change in odour [Table 3]. The predominant species among those with worsened and improved sweat odour was *S.hominis*. One participant who yielded *S.aureus* transiently in her axillary swabs reported a worsened axillary odour. As regards sweat amount, sixteen females (53.3%) reported no change, ten (33.3%) reported an increased amount and four (13.4%) reported decreased sweating.

The baseline bacterial counts before the first laser session were not significantly different in subjects who reported worsened sweat odour later than in those who reported no change in odour (data not shown). However, counts prior to the fourth laser session showed marked differences, where statistically significant lower counts of total aerobes (52.5 versus 142.5 × 10^5 CFU/cm^2) (p = 0.029), total anaerobes (23.7 versus 128.7 × 10^5 CFU/cm^2) (p = 0.047) and *S. hominis* (17.5 versus 117.5 × 10^5 CFU/cm^2) (p = 0.033) were found among those with worsened odour, indicating that lower counts of these organisms were associated with a perceived malodour of sweat [Table 4]. Since only two participants reported improved sweat odour, they were excluded from the statistical analysis correlating sweat odour and bacterial counts [Table 4]. These two participants had median bacterial counts (CFU/cm^2) before their first and fourth laser sessions respectively, as follows: total aerobes (386.4 versus 391.3), total anaerobes (244.7 versus 1718.7 ×10^5) and *S.hominis* (301.2 versus 300 ×10^5 CFU/cm^2).

The twelve females who reported worsened sweat odour showed significant reductions in the following median bacterial counts (CFU/cm^2) from their first to fourth sessions respectively (pre-session samples): total aerobes (248.7 versus 52.5 × 10^5, p = 0.005), total anaerobes (365 versus

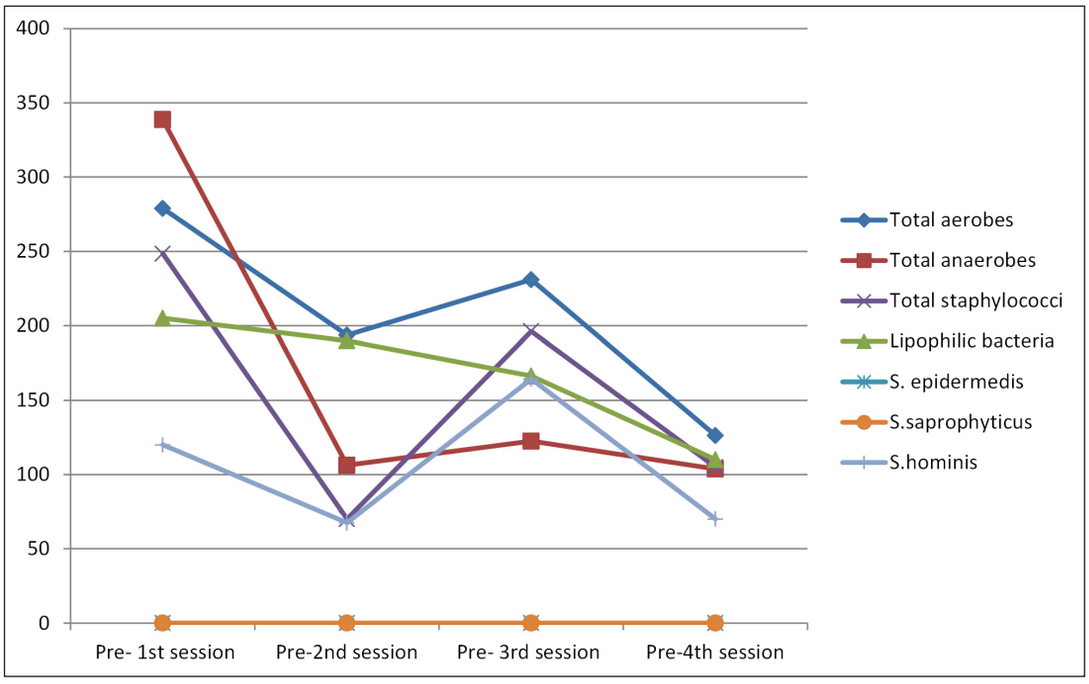

Figure 2: Distribution of the studied participants (n = 30) according to the median bacterial colony count before each session (S1, S2, S3 and S4)

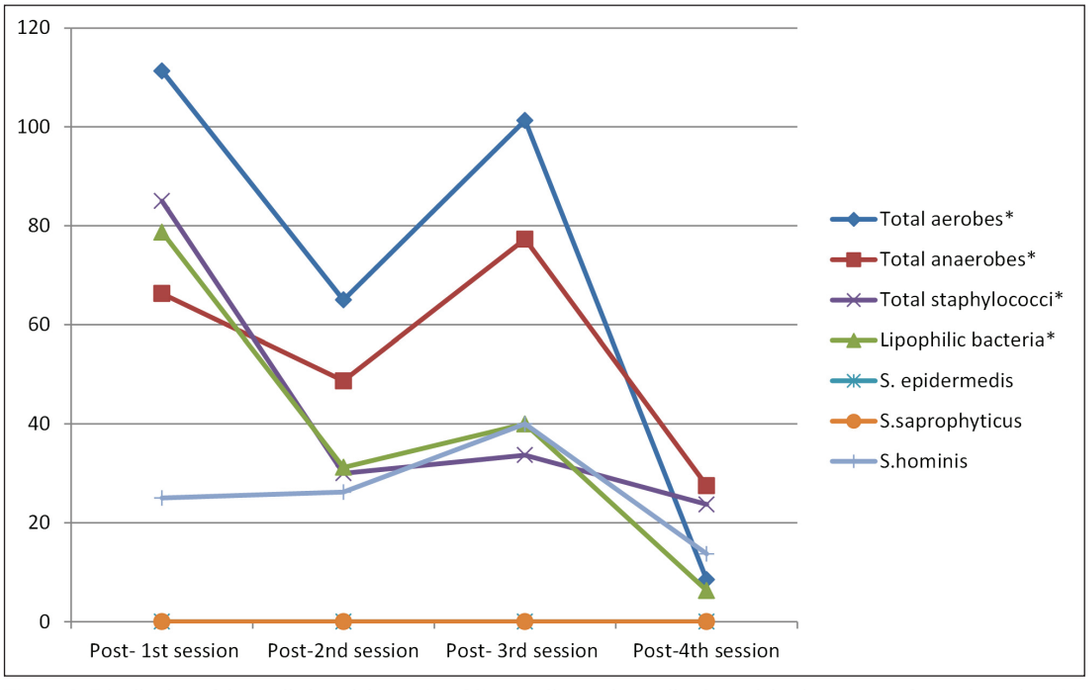

Figure 3: Distribution of the studied participants (n = 30) according to the median bacterial colony count after each session (S1, S2, S3 and S4)

23.7 × 10^5, p = 0.010), total staphylococci (232.5 versus 18.5 × 10^5, p = 0.008) and *S.hominis* (45 versus 17.5 × 10^5, p = 0.019). Further, there was an observed reduction, although statistically insignificant, in the median lipophilic bacterial count (307.5 versus 56.2 × 10^5). On the other hand, all the sixteen females who reported unchanged sweat odour had no significant difference in their bacterial counts [Table 5].

There was a statistically significant positive correlation between sweat odour and total aerobic count (r = 0.433, p = 0.017), total anaerobic count (r = 0.377, p = 0.040), total staphylococci (r = 0.383, p = 0.036) and *S.hominis* (p = 0.497, p = 0.005) i.e., higher the bacterial counts, higher the likelihood of normal sweat odour, and vice versa. Lipophilic bacteria and *S.saprophyticus* were not correlated with sweat odour [Table 6].

Table 3: Distribution of subjective changes in sweat odour and amount by the end of four Nd: YAG laser sessions

	n	%
Odour of sweat		
Worsened	12	40.0
Improved	2	6.7
Unchanged	16	53.3
Amount of sweat		
Decreased	4	13.4
Increased	10	33.3
Unchanged	16	53.3

Table 4: Comparison between axillary bacterial counts before the fourthe Nd:YAG laser session among participants who reported worsened or unchanged sweat odour

Type of bacteria	Colony count (×10^5 CFU/cm^2)		p-value
	Worsened sweat odour	Unchanged sweat odour	
Total aerobes			
Mean ± SD	102.5 ± 136.8	197.9 ± 158.3	p = 0.029* U = 142.5
Median	52.5	142.5	
Min-Max	1.0–485.0	7.5–647.5	
Total anaerobes			
Mean ± SD	97.4 ± 160.2	463.5 ± 1225.2	p = 0.047* U = 138.5
Median	23.7	128.7	
Min-Max	52.0–572.5	2.5–5037.5	
Total staphylococci			
Mean ± SD	72.1 ± 99.8	872.5 ± 2238.0	p = 0.082 U = 133.5
Median	18.5	111.3	
Min-Max	1.5–310.0	2.5–8612.5	
Lipophilic bacteria			
Mean ± SD	103.2 ± 110.1	205.3 ± 306.3	p = 0.108.5 U = 1.980
Median	56.2	153.7	
Min-Max	0.0–342.5	0.0–1250.0	
S.saprophyticus			
Mean ± SD	4.1 ± 11.5	24.2 ± 62.4	p = 0.837 U = 101.0
Median	0.0	0.0	
Min-Max	0.0–37.5	0.0–237.5	
S.hominis			
Mean ± SD	53.6 ± 73.1	198.8 ± 242.2	p = 0.033* U = 148.0
Median	17.5	117.5	
Min-Max	0.0–242.5	2.5–825.0	

P*: p value significant at level <0.05

Five participants gave a history of repeated folliculitis in their axillae before having their laser sessions; all five of them reported an improvement in this problem by the end of the study. On assessing the bacterial counts in these five participants, all median bacterial counts were found to be reduced. However, a significance test could not be performed due to the small number.

Discussion

There have been no previous studies evaluating the effect of Nd:YAG laser on skin flora and pathogens *in vivo*. Studying the antibacterial effect of Nd:YAG laser has important implications, firstly due to its widespread use for hair removal. Secondly, the antibacterial effect of Nd:YAG laser could be used for skin decolonization; this is a potential use not hitherto suggested, to our knowledge. Skin decolonization aims to reduce the bacterial load on the skin preoperatively, preventing surgical site infections that occur when high bacterial loads are present. Important pathogens implicated in such infections are *S. aureus*, especially MRSA. The results of our study might help develop new decolonization strategies, especially in the era of multidrug resistance. The degree of reduction of bacterial load either in the pre- versus post session counts or in the pre-S versus pre-S4 counts was found to be variable among participants (50%–70%). This variation among participants might be attributed to personal factors such as the composition and quantity of sebaceous secretions as well as the bacterial virulence factors which are strain-specific and may contribute to re-growth of bacteria. The extent to which bacterial reduction might be beneficial in alleviating sweat odour requires further studies, as the beneficial role of skin flora requires adequate balance of their counts.

Studies have reported the dominance of Staphylococci over other aerobic axillary flora (aerobic coryneforms and micrococci), especially among females.[10,11] Staphylococci were thus selected for our study as an important axillary microbial flora since our participants were all females.

In the present study, the baseline median total aerobic bacterial count was 778.3 ± 1429.7 × 10^5 CFU/cm^2, which is higher than that reported by Fazel *et al* (179.7 × 10^5 CFU/cm^2).[12] In the present study, the most predominant staphylococcal species was *S. hominis* followed by *S. Saprophyticus* and *S. epidermidis*. In line with our findings, Kloos & Musselwhite[13] reported that the most abundant species on axillary skin was *S. hominis*. However, Fazel *et al*.[12] reported that the predominant baseline bacteria were *S. aureus* followed by *S. epidermidis* and then *S. saprophyticus*. These differences might be due to differences in study populations including age, personal habits and precautions taken before sample collection. Variations in laboratory media and techniques used might also have contributed to these differences.

In the present study, when comparing median bacterial counts of pre-session versus post-session bacterial counts, there was a significant reduction of colony counts of total aerobes, total anaerobes, lipophilic bacteria, total staphylococci, and *S.hominis*. This finding was consistently seen in the four laser sessions, where post-session colony counts were significantly lower than pre-session ones. No similar significant reduction was seen in counts of *S. epidermidis*, *S. saprophyticus,* and *S. aureus*. This is probably owing to the lower colony counts

Table 5: Distribution of a subgroup of participants (n = 28) concerning sweat odour according to their axillary bacterial colony counts before first and fourth Nd:YAG laser sessions

Type of bacteria	Colony count (×10^5 CFU/cm^2)					
	Unchanged sweat odour (n = 16)			Worsened sweat odour (n = 12)		
	Pre S1	Pre S4	p-value	Pre S1	Pre S4	p-value
Total aerobes						
Mean ± SD	893.3 ± 1759.9	197.9 ± 158.3	=0.163 Wilcoxon =41.0	690.2 ± 1052.6	102.5 ± 136.8	= 0.005* Wilcoxon = 3.0
Median	253.4	142.5		248.7	52.5	
Min-Max	40.0–6250.0	7.5–647.5		2.5–3817.5	1.0-485.0	
Total anaerobes						
Mean ± SD	343.0 ± 918.5	463.5 ± 1225.2	= 0.196 Wilcoxon = 43.0	1121.6 ± 297.9	97.4 ± 160.2	0.010* Wilcoxon = 6.0
Median	311.2	128.7		365.0	23.7	
Min-Max	3.9–1121.2	2.5–5037.5		2.5–6662.5	52.0–572.5	
Total Staphylococci						
Mean ± SD	344.3 ± 368.0	872.5 ± 2238.0	= 0.214 Wilcoxon = 44.0	460.0 ± 746.1	72.1 ± 99.8	0.008* Wilcoxon = 5.0
Median	228.5	111.3		232.5	18.5	
Min-Max	12.5–1397.5	2.5–8612.5		25.0–2720.0	1.5–310.0	
Lipophilic bacteria						
Mean ± SD	735.0 ± 2277.8	205.3 ± 306.3	= 0.266 Wilcoxon = 46.5	1314.1 ± 2606.1	103.2 ± 110.1	= 0.099 Wilcoxon = 15
Median	142.5	153.7		307.5	56.2	
Min-Max	2.5–9262.3	0.0–1250.0		0.0–8287.0	0.0–342.5	
S.saprophyticus						
Mean ± SD	9.5 ± 22.7	24.2 ± 62.4	0.600 Wilcoxon = 13.0	0.42 ± 1.5	4.1 ± 11.5	= 0.317 Wilcoxon = 1.000
Median	0.0	0.0		0.0	0.0	
Min-Max	0.0–79.7	0.0–237.5		0.0–5.0	0.0–37.5	
S.hominis						
Mean ± SD	238.6 ± 325.5	198.8 ± 242.2	0.642 Wilcoxon = 59.0	232.1 ± 308.9	53.6 ± 73.1	= 0.019* Wilcoxon = 9.0
Median	107.5	117.5		45.0	17.5	
Min-Max	7.5–1283.7	2.5–825.0		3.4–952.5	0.0–242.5	

p value significant at level <0.05, S1: first session of Nd: YAG laser, S4: fourth session of Nd: YAG laser

Table 6: Correlation between the odour of sweat and bacterial counts

Bacterial parameter	rs	p value
Total aerobes	0.433	0.017*
Total anaerobes	0.377	0.040*
Total staphylococci	0.383	0.036*
Lipophilic bacteria	0.163	0.390
S.saprophyticus	0.301	0.105
S.hominis	0.497	0.005*

rs: Spearman rho correlation co efficient
*: p value significant at level <0.05

of these species isolated in our study. The immediate antibacterial effect of Nd:YAG laser has also been reported in an *in vitro* study on *S. aureus* and *Pseudomonas aeruginosa* (after 24 hours).[14]

A delayed antibacterial effect of Nd:YAG laser was documented in our study, with reductions in total aerobic, anaerobic, staphylococcal, and *S.hominis* counts on comparing samples obtained before the first and fourth sessions. There was a significant immediate, but not delayed, inhibitory effect of the Nd:YAG laser on the lipophilic bacterial count as well. This might be due to variations in bacterial susceptibility to laser and the ability of some species to recover better than others. It was noted that there was no significant difference between counts of the second and third sessions while the difference was significant between the first and fourth sessions, indicating that the bacterial delayed inhibitory effect of Nd:YAG laser requires at least four sessions to become significant. Fazel *et al*.[12] reported a significant reduction in the mean bacterial colony in the axillary region of laser participants after the sixth session of hair removal.

In more than half the participants (50%–96.3%), and in all laser sessions, the percentage of reduction of bacterial counts between each pre- and post-session reached ≥50% (except for the anaerobic count of the 2nd session). The most frequent rates of bacterial reduction was noted in counts of lipophilic bacteria, where 55.2%–96.3% of participants had ≥50% reduction in their pre-*versus* post session counts, in all four laser sessions, p = 0.003) (Table S1).

Concerning the delayed inhibitory effect of laser (pre-S *versus* pre-S4), ≥50% reduction of counts was seen in 50%–70% of participants in different bacterial parameters.

The antibacterial effect of Nd:YAG laser can explain the clearance of repeated folliculitis reported by five participants in the present study. Staphylococci, especially *S. aureus*, are the most frequent cause of bacterial folliculitis, while less commonly implicated genera include Streptococcus, Pseudomonas, and coliform bacteria.[15] On assessing the median bacterial count in these five participants, all bacterial counts done were reduced, but a test of significance could not be performed due to the small number. Reduction of Staphylococci (including *S. aureus*) might explain the improvement of folliculitis following Nd:YAG laser sessions.

Possible explanations for the antibacterial effect of laser include (a) suppression of DNA metabolism and cell division, leading to degenerative changes up to cell pyknosis. There are three-phase responses of bacteria to laser irradiation: phase 1, bacteria inhibited but viable; phase 2, bacteria non-viable but intact; and phase 3, physical dissolution of bacteria,[16] (b) irradiated endogenous bacterial photosensitiser resulting in induction of reactive oxygen species,[17] (c) modulation of transmembrane convection directly by laser,[18] (d) For pigmented bacteria, absorption of laser light by the pigments inside the bacteria leads to vaporization of water and cell lysis,[19] (e) stimulatory effect on human lymphocytes.[20]

Side-effects of laser hair removal include pain, erythema, edema, dyschromia, blistering, erosions, or purpura.[1] Changes in sweating have been reported but not thoroughly investigated. In the present study, most females reported either no change in sweat odour (53.3%) or worsened odour (40%). Only two females (6.7%) reported improvement in odour. In the study by Fazel *et al*.[12] most (63.3%) participants who underwent six sessions of alexandrite laser treatment reported improved sweat odour; an unchanged odour was reported in 20%, and the odour was exacerbated in 16.7%. The differences in this respect between the studies may be attributed to several factors including differences in laser machines, different laser parameters used, number of sessions, and ethnic and individual variations.

Laser-induced changes in sweat odour maybe due to personal factors (such as the amount and composition of sweat, personal hygiene habits, and genetic factors) as well as the interplay of bacterial species by antagonism or synergism. In the present study, a significant correlation was noted between worsened sweat odour and lower counts of total aerobes (Figures already in results section), total anaerobes, total staphylococci and *S.hominis*. Our findings contradict those of Troccaz *et al*.,[21] who reported that sweat odour improved with decreased axillary bacterial counts of *S. hominis* and higher counts of *S. epidermidis*. In contrast, Rennie *et al*.[22] reported a lack of association between the total bacterial count of staphylococci and axillary odour, whereas coryneform bacteria had a statistically significant association with odour intensity.[22] Fazel *et al*.[12] reported that *S.epidermidis* was predominant in those with improved odour, *Micrococcus luteus* in those with worsened odour and *S. aureus* in those with unchanged odour. In contrast, in our study, *S.hominis* was predominant in all participants regardless of their sweat odour. Troccaz *et al*.[21] also found that offensive body odour was linked to higher proportions of Corynebacterium, Anaerococcus, Peptoniphilus, and *S. hominis*, possibly due to their production of malodorous volatiles from apocrine sweat. In the present study, although higher numbers of aerobes, anaerobes and staphylococci were associated with better sweat odour in contrast to most other studies, it is possible that other untested bacterial species might have decreased simultaneously contributing to an improved sweat odour in our subjects. The association of certain species with improved odour has been investigated in some studies as a therapeutic option for malodour, with axillary bacterial transplantation where the malodor-causing microbiome is replaced with a healthy non-odorous axillary microbiome.[23] Transplantation of axillary microbiome has been performed in only a few studies as clinical trials to improve axillary odour. To the best of our knowledge, these studies did not report side effects following transplantation of microbiome, whether these bacteria were transplanted from the same person but from a different body site (homologous), or from the axillary skin of a different person. Further studies in the future might give us more evidence of the safety of this transplantation, and the occurrence of any possible side effects which might include hypersensitivity or, possibly, gradual disappearance of the transplanted microbiome and replacement by the primary undesired one.

Females who reported unchanged sweat odour revealed no significant differences in the median bacterial counts before and after laser. However, laser might have a species-dependent effect leading to dysbiosis, with some species increasing relative to others, as reported by Troccaz *et al*.[21] and Fazel *et al*.[12] In our study however, dysbiosis could not be proved in relation to other species, since only staphylococci were studied.

In our study, 53.3% of females reported no change in sweat amount, 33.3% reported increased amount while 13.4% reported decreased sweating. Increased axillary sweating induced by 1064 nm Nd:YAG laser was studied by Aydin *et al*.[24] after 6–10 monthly sessions of laser hair removal (10-mm spot size, 30–60 J/cm fluence and 55–65 ms pulse width). This agrees with some reports on induction of sympathetic skin response after laser stimulation[25,26] On the other hand, reduction of axillary sweating by 1064 nm Nd:YAG for treatment of bromidrosis acting subdermally rather than transdermally. The laser reaches its target directly resulting in histopathologic alterations in eccrine glands with effective reduction of hyperhidrotic activity. They also suggest that heating affects the surrounding nerve fibres contributing to the clinical effect.[27] Along with our results, similar findings were reported with diode and alexandrite laser for axillary hair removal and even inguinal hyperhidrosis following inguinal laser hair removal.[28–30] From previous data, one can

speculate that 1064 nm Nd:YAG laser may have both stimulatory effect on axillary sweating when applied transdermally and inhibitory effect when applied subdermally. According to Helou *et al*, the mean number of laser sessions needed for onset of hyperhidrosis was 6, with a minimum of 3 sessions and a maximum of 11 sessions.[29]

The underlying mechanism for excessive sweating after the laser is still not fully understood. The laser may induce direct thermal injury or indirect stimulation via nerve fibres to sweat glands close to the hair follicles. Devices operating in the near-infrared wavelength region penetrate deeply into the dermis and even the subcutaneous tissue targeting the heavily pigmented matrix of the hair. Therefore, the deep-going energy could activate the sweat glands lying near the deep terminal hair bulbs of the axilla whenthe laser is applied through the skin surface. This hyperactive state is transient in 76.7%.[29] This is possible through the influence of periglandular temperature on glandular metabolism, neurotransmitter release and metabolism, membrane transport, and receptor affinity.[30]

On the contrary, Letada *et al*.[31] reported a significant reduction in axillary sweating after five sessions of 1064 nm Nd:YAG laser. Histologic analysis of axillary tissue did not demonstrate any notable change between pre- and post-treatment. This led Letada *et al*.[31] to conclude that the mechanism of action might involve cellular or subcellular alterations through the heating of melanin or water chromophores, thus impacting the sympathetic cholinergic transmissions in the axilla.[32]

Limitations of the present study included a small sample size, few laser sessions, a short follow-up period, and subjective assessment of sweat odour and quantity; only limited bacterial species were included in the study. Lack of control group, only woman study subjects and inclusion of only axillary site are other limitations.

Conclusion

Nd:YAG laser (1064 nm) may have an antibacterial effect against certain bacterial species. Both immediate and delayed antibacterial effects were observed, but longer follow-up is required to evaluate the duration of these effects. Nd:YAG laser may also affect sweat odour by altering the local skin microbiome.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflict of interest

There are no Conflict of Interest.

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