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Surviving the Ink Matrix: Microbial Persistence and Contamination in In-Use Tattoo Inks

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Short Report

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Abstract

Tattoo inks, due to limited nutrient availability and high biocide concentrations, constitute a unique ecological niche exerting strong selective pressure on microorganisms. This study aimed to characterize the taxonomic biodiversity and adaptive potential of the microflora in in-use tattoo inks (n=152) and soap solutions (n=4) utilized in tattoo parlors. Identification was performed using MALDI-TOF mass spectrometry. Thirty-three bacterial strains were identified, revealing a 34.2% overall contamination rate of the analyzed matrices. The taxonomic profile was dominated by commensal coagulase-negative staphylococci (including *S. hominis*, 28.1% of isolates), followed by environmental spore-forming bacilli (*Bacillus licheniformis*, 25.0%) and enterococci (*Enterococcus faecalis*). Notably, the isolation of *Pseudomonas oleovorans* from the soap solution demonstrates the capacity of this species to survive in a surfactant-rich environment. The presence of spores and opportunistic pathogens in open containers indicates the high adaptive potential of these contaminants. These findings elucidate the population dynamics of microorganisms in tattoo parlors, underscoring the necessity of implementing rigorous aseptic protocols and routine antimicrobial susceptibility profiling to minimize the risk of tattoo-associated infections.

1. Introduction

Despite advancements in infection prevention and increasing public awareness, numerous tattoo-associated complications continue to be reported (Laux et al. 2016). Historically, methodologies for assessing tattoo ink contamination have varied significantly. In studies such as Kluger et al. (2011), direct plating of inks onto solid media resulted in complete microbial growth inhibition due to the high concentration of preservatives and metal salts within the dense ink matrix on the agar surface (Kluger et al. 2011). Conversely, studies by Baumgartner and Gautsch (2011) and Høgsberg et al. (2013) utilized a pre-enrichment step in standard liquid media (tryptic soy broth and serum broth, respectively), followed by aerobic incubation and subsequent subculturing onto agar plates (Baumgartner and Gautsch 2011; Høgsberg et al. 2013). However, this approach without the use of chemical neutralizers also resulted in a high rate of false-negative results; Baumgartner and Gautsch reported a 50% detection failure rate in standard broth due to bacterial exposure to biocides within the bulk volume. Recognizing the limitations of direct culturing inherent in their early work (Nho et al. 2018), researchers from Nho's group adapted their subsequent protocols (Nho et al. 2020; Yoon et al. 2022, 2024) to the methodology outlined in Chap. 23 of the FDA Bacteriological Analytical Manual (BAM), implementing prolonged enrichment in media supplemented with the specific neutralizer Letheen.

Despite the proven efficacy of neutralizing protocols (Jo Huang et al. 2024) in detecting contamination within sealed commercial products, extensive research evaluating the phenotypic tolerance of wild-type microflora in in-use tattoo inks remains scarce. Recent *in vitro* studies (Briancesco et al. 2023) demonstrated that physical dilution of the ink significantly reduces preservative efficacy, enabling the survival of artificially introduced laboratory strains. Prior to undertaking full-scale comparative trials, we conducted this pilot study to isolate wild-type strains surviving within the toxic ink matrix, demonstrating

their capacity to proliferate without the chemical neutralization of preservatives. We propose a micro-volume experimental model (200 µL of ink in 1800 µL of highly nutritious BHI broth), omitting neutralizers. This setup, reflecting the actual volumes utilized during tattooing procedures, facilitates gravitational phase separation, providing adapted strains with the spatial opportunity for migration and metabolic resuscitation. The primary objective was to evaluate the baseline environmental resistance of these microorganisms to matrix preservatives. Consequently, this pilot serves as a proof-of-concept for future comparative methodologies, validating the feasibility of strain isolation without neutralization and providing a detailed phenotypic analysis of the recovered isolates.

2. Materials and methods

Sample Collection

A total of 156 samples were collected from tattoo parlors located across various geographical regions in Poland. The research material comprised 152 in-use tattoo ink samples and 4 samples of working potassium soap solutions ("green soap"), routinely used for cleansing the breached epidermal barrier during tattooing procedures. The soap solution sampling was performed in triplicate. All samples were obtained under strict aseptic conditions and immediately transported to the laboratory.

Cultivation Strategy

To optimize the detection of opportunistic pathogens capable of inducing human skin infections, industrial temperature standards were replaced with clinical incubation conditions (37°C). The collected material was inoculated into enrichment media: liquid brain heart infusion (BHI) broth and solid Columbia blood agar supplemented with 5% sheep blood. Aerobic incubation was maintained at 37°C for 48 hours for solid media and up to 7 days for liquid broth. The methodology intentionally omitted an initial chemical neutralization step for ink preservatives. Uninoculated sterile BHI broth and Columbia blood agar plates were incubated concurrently as negative controls to verify the sterility of the laboratory procedures.

Bacterial Identification

Samples exhibiting macroscopic microbial growth were subcultured to isolate pure strains. Definitive species-level identification of the isolated strains was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) via the MALDI Biotyper system (Bruker Daltonics, Bremen, Germany). Mass spectra analysis was executed automatically using MBT Compass software against the manufacturer's reference database, with identification scores recorded for each individual isolate. According to the manufacturer’s guidelines and established microbiology practices, an identification score of ≥ 2.0 was considered the threshold for high-confidence species-level identification, while a score of 1.80–1.99 was accepted for reliable genus-level identification of spore-forming bacilli, with final species-level assignment cross-verified using macroscopic colony morphology.

3. Results

The study analyzed 156 samples, comprising 152 in-use tattoo inks and 4 working potassium soap water solutions utilized for point-of-care field cleansing. Macroscopic microbial growth was recorded in 52 ink samples, yielding an overall contamination rate of 34.21% (52/152). Growth was also observed in all soap solution replicates. Exhibiting identical growth patterns across all replicates, a single representative soap isolate was selected for species-level identification. All negative controls (uninoculated BHI broth and Columbia blood agar) remained sterile throughout incubation, validating the laboratory procedures.

A representative subset of 39 culture-positive samples (including the soap isolate) was selected for MALDI-TOF MS identification. Mass spectral profiles for 8 isolates were uninterpretable due to total signal absence ("no peaks detected") or sub-threshold reliability scores (< 1.70), rendering taxonomic assignment impossible. Ultimately, successful taxonomic identification was achieved for 33 strains; of these, 26 isolates were classified at the species level (Score ≥ 2.00) while 7 isolates yielded borderline scores (1.80–1.99) and were assigned to the species level based on consistent macroscopic morphology. The identified isolates originated from 31 distinct samples, comprising 30 tattoo inks and 1 soap solution. During the primary subculture phase, polymicrobial contamination was identified in two ink samples, yielding two distinct bacterial strains from each. The detailed taxonomic profile of all identified isolates is summarized in Table 1.

Table 1
Taxonomic distribution of successfully identified bacterial isolates recovered from contaminated in-use tattoo inks and a point-of-care water sample (n = number of identified strains).

Morphotype	Genus	Species	n	Percentage of total identified isolates
Gram-positive cocci	*Staphylococcus*	*epidermidis*	5	15.62
		haemolyticus	2	6.25
		hominis	9	28.13
	Enterococcus	*faecalis*	2	6.25
Gram-positive bacilli	*Bacillus*	*licheniformis* [a]	8	25.00
		pumilus [a]	3	9.38
		subtilis [a]	2	6.25
	Cytobacillus	*oceanisideminis*	1	2
Gram-negative bacilli	*Pseudomonas*	*oleovorans* [b]	1	N/A
Total			33	100

[a] Within these species groups, a specific subset of isolates (4 of 8 B. licheniformis, 1 of 3 B. pumilus, and both B. subtilis strains) yielded MALDI-TOF scores between 1.80 and 1.99. For these particular isolates, species-level assignment was conclusively confirmed based on consistent macroscopic colony morphology and the absence of alternative genus-level identifications.

[b] Strain isolated from a working potassium soap ("green soap") solution sample used in a wash bottle for point-of-care field cleansing. Due to its distinct environmental matrix, this isolate was excluded from the percentage distribution of ink-derived isolates.

4. Discussion

The obtained taxonomic profile is highly consistent with findings from previous studies that utilized chemical neutralizers (Nho et al. 2018, 2020; Yoon et al. 2022, 2024).

The taxonomic profile was dominated by two major groups: Gram-positive bacilli of the *Bacillus* and *Cytobacillus* genera (14 isolates in total) and *Staphylococcus* species (16 isolates). At the species level, the community was characterized by the co-dominance of the commensal coagulase-negative *Staphylococcus hominis* (9 isolates) and the environmental spore-forming *Bacillus licheniformis* (8 isolates). The accumulation of environmental bacilli, which have natural reservoirs in soil and water, indicates continuous exposure of in-use open containers to environmental contaminants within the parlor setting. Interestingly, while controlled experimental models by Briancesco et al. (2023) demonstrated that *Bacillus pumilus* viability in sterile, undiluted ink was strictly limited to a 3-week threshold, our isolation of these strains from active, in-use formulas suggests a far more protracted survival window or a high frequency of recurrent contamination (Briancesco et al. 2023). In real-world parlor settings, the ink matrix is subject to dynamic variables—such as ambient evaporation, micro-fluctuations in preservative efficacy, and the potential introduction of organic micro-debris—which may extend spore persistence beyond baseline values established under standardized laboratory conditions. Notably, unlike environmental bacilli, coagulase-negative staphylococci (CoNS) do not form spores. Their successful cultivation directly from the ink matrix, without prior preservative neutralization, demonstrates that these strains have developed robust adaptive mechanisms to survive chemical stress. Introducing bacteria that constitute the human microbiota into the body poses not only a direct epidemiological threat but also carries the potential for horizontal transfer of antibiotic resistance plasmids (Fišarová et al. 2021). Consequently, we highlight the urgent need for comprehensive studies evaluating the biochemical profiles, antimicrobial resistance, and biofilm-forming capacities of these isolated strains.

Beyond the risks associated with microorganisms isolated directly from inks, we highlight the cultivation of *Pseudomonas oleovorans* from the potassium soap ("green soap") solution. This preparation is routinely applied to the breached epidermal barrier during tattooing and is intended to act as an antiseptic. The detection of viable *P. oleovorans* cells in this product demonstrates that tattoo-associated

infections stem not only from ink contamination but also from critical gaps in parlor sanitary regimens, necessitating further in-depth investigation.

Limitations

This study has certain limitations that must be considered. Primarily, the isolation of *P. oleovorans* from the soap solution represents a single documented contamination incident. Although this finding serves as a critical public health warning, multicenter environmental surveillance encompassing a broader range of point-of-care settings is essential to determine its true prevalence. Furthermore, the in-use study design precludes the use of paired control samples (sealed inks from identical production batches). Consequently, differentiating between primary manufacturing contamination and secondary in-use contamination relies on the behavioral and taxonomic analysis of the isolated microflora rather than direct matrix comparison. Additionally, this is an *in vitro* microbiological study and does not correlate the findings with patient medical records regarding actual *in vivo* skin infections.

Another significant limitation is the reliance on culture-dependent methods and mass spectrometry. Thus, the obtained taxonomic profile may overlook fastidious microorganisms, strict anaerobes, or organisms in a viable but non-culturable (VBNC) state, the detection of which would require next-generation sequencing (NGS) techniques (e.g., 16S rRNA metagenomics). This necessitates cautious extrapolation of the derived epidemiological indicators to the broader industry.

Nevertheless, these findings highlight a dual challenge in tattoo hygiene: the robust adaptive tolerance of the isolated microflora and the compromised efficacy of the preservative systems under in-use conditions. The survival of these strains without prior chemical neutralization suggests potential biocide depletion, which may result from sub-inhibitory initial concentrations, chemical degradation over time, or physical dilution during standard point-of-care procedures. Therefore, this pilot serves as a foundational study for a planned, large-scale research project. Future investigations will focus on the in-depth characterization of the isolated pathogens. Utilizing automated systems for biochemical identification and antimicrobial susceptibility testing (AST) will enable precise epidemiological risk assessment, with a specific focus on their resistance potential.

Conclusion

In conclusion, the high contamination rate of in-use tattoo inks (34.21%), dominated by human skin microbiota and environmental bacilli, indicates significant deficiencies in aseptic standards within tattoo parlors. These findings demonstrate that during repeated use, open ink containers and antiseptic solutions can act as active vehicles for opportunistic pathogens. Implementing rigorous sanitary guidelines and validated hygiene protocols at the point of care is imperative to minimize the risk of cross-contamination and subsequent infections in clients. Although the isolated microorganisms are generally considered harmless environmental flora, their transdermal inoculation during the tattooing process bypasses the epidermal barrier, transforming them into potential opportunistic pathogens. Clinical evidence demonstrates that even these seemingly benign strains can cause significant

complications, ranging from localized cutaneous abscesses to severe systemic infections (Haydushka et al. 2012; Becker et al. 2014; Dieckmann et al. 2016). This highlights the critical public health risk posed by contaminated tattoo inks and the necessity of strict microbiological surveillance.

Declarations

Author statements

Author contributions

J. S.: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Project administration

A.S.: Investigation, Data curation

P.P.: Investigation, Data curation

H. T.: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing

All authors contributed to the article and approved the submitted version.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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Ethical approval

Not applicable. This is an *in vitro* microbiological study on environmental/commercial samples and does not involve human participants or animals.

Consent for publication

Not applicable.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Gemini 3.1 Pro (Google) in order to improve the grammatical accuracy, stylistics, and overall readability of the English text. After using this tool, the authors thoroughly reviewed, checked for factual accuracy, and edited the content as needed. The authors take full responsibility for the integrity, factual accuracy, and originality of the final manuscript

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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