

Determination and Antibigram of Bacterial and Fungal Isolates from *Lawsonia inermis* (Henna Dye) Sold in Port Harcourt Metropolis, Nigeria

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ABSTRACT

Henna is a dye prepared from the plant *Lawsonia inermis*, also known as the henna tree. The dried and grounded leaves of *Lawsonia inermis* is a natural dye for hair, nails and skin for body arts like nail polish, hair dye, treatment of dandruff, hair gloss, makeup, mostly used in Islamic and Hindu cultures. This study was carried out with the aim of determining the bacteria and fungi isolates from henna dye sold in Port-Harcourt metropolis. Natural and synthetic henna samples were bought from Mile 3 market and Rivers State University main gate, using three (3) samples. Isolation and Characterization of bacterial and fungal isolates was done using standard microbiological techniques. The results showed that the Total heterotrophic bacteria (THB) count ranged from 3.0×10^4 Cfu/ml to 6.2×10^5 Cfu/ml. Total heterotrophic fungi (THF) count ranged from 1.2×10^2 Sfu/ml to 2.2×10^2 Sfu/ml. The Total Staphylococcal count (TSC) ranged from 2.2×10^3 to 4.7×10^3 Cfu/ml. Seventeen (17) bacterial isolates belonging to eight (8) genera were identified and include; *Staphylococcus* spp. (17.65%), *Escherichia coli* (11.76%), *Bacillus* spp. (17.65%), *Pseudomonas* sp. (11.76%), *Micrococcus* sp. (11.76%), *Proteus* sp.

(11.76%), *Corynebacterium* sp. (11.76%) and *Streptococcus* sp. (5.90%). A total of six (6) fungal species belonging to six (6) genera were identified and include; *Penicillium* sp. (22.9%), *Mucor* sp. (16.3%), *Aspergillus* sp. (18.9%), *Candida* sp. (22.9%), *Fusarium* sp. (12.2%) and *Microsporum* sp. (6.8%). Antibiotic susceptibility testing was done for the bacteria isolates using the Mueller-Hinton agar. Results showed that *Bacillus* spp., *Staphylococcus* spp. and *Micrococcus* sp. were all susceptible to Ciprofloxacin (100%) and Levofloxacin (100%) respectively, but resistant to Ampicillin (100%) and Gentamycin (100%). *Proteus* sp. and *Pseudomonas* sp. were susceptible to Reflacin (100%) and Ciproflox (100%). However, *Corynebacterium* sp. was resistant to Streptomycin (100%). The study has shown that there is a high rate of bacterial and fungal contamination of henna dye which may result to a risk of breakdown in the integrity of the skin. Therefore, to avoid allergies, complications after use and public health outbreak, it is recommended to consult a dermatologist before use at all.

Keywords: Henna, Dye, Bacteria and Fungi, Antibigram, Public health.

INTRODUCTION

Henna is a natural dye gotten primarily from the dried leaves of *Lawsonia inermis* L., a plant belonging to the family Lythraceae. It has been used for centuries in Africa, the Middle East and South Asia for body decoration, hair colouring, cultural ceremonies and traditional medicinal purposes. *L. inermis* contains several phenolic compounds, flavonoids, terpenoids and other phytochemicals that have been associated with antioxidant and antimicrobial activities [16].

Despite its natural antimicrobial properties, henna should not be assumed to be sterile. As a plant-derived material, henna may become contaminated at different stages, beginning from cultivation and harvesting through drying, grinding, packaging, transportation, storage and eventual preparation for use. Soil, dust, contaminated water, processing equipment, packaging materials and handling by sellers or end users can introduce

microorganisms into plant-based products. A study by [4] investigated herbal products sold in Ota, Ogun State, and reported both bacterial and fungal contamination, with microbial counts varying considerably among the products. The authors also observed that microbial counts increased with storage time, demonstrating that storage conditions can influence the microbiological quality of plant-derived preparations.

The possibility of microbial contamination is particularly important when henna is used as a cosmetic product because it is normally applied directly to the skin or hair. Although many microorganisms present in environmental materials may be harmless, some bacteria and fungi can act as opportunistic pathogens, particularly when applied to damaged or irritated skin.

Microbial contamination of cosmetic products may also cause product deterioration, unpleasant odours, changes in appearance and reduced product quality. In a local study, [6] examined 45 commercial cosmetic products and found that 84% were contaminated, yielding 70 microbial isolates comprising 11 bacterial genera and nine fungal genera. The organisms isolated included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter gergoviae*, *Aspergillus flavus*, *Penicillium* spp. and *Candida albicans*. Of particular concern, a substantial proportion of the bacterial isolates demonstrated multidrug resistance. The study concluded that contaminated cosmetic products could potentially serve as vehicles for transmission of microorganisms to consumers. Furthermore, henna products sold in Port Harcourt may be gotten through different commercial channels, including cosmetic shops, markets, beauty outlets and informal vendors. Differences in product handling, storage conditions, packaging and duration of storage could influence their microbiological quality. End users may also prepare powdered henna into a paste before application, which might introduce additional opportunities for contamination through water, containers and repeated handling. Consequently, determining the microorganisms associated with commercially available henna is important for establishing the microbiological quality of these products and identifying potential health risks to the users. This study is designed to determine the bacterial and fungal isolates associated with henna dye sold in Port Harcourt metropolis and to determine the antibiotic susceptibility patterns of the bacterial isolates.

Materials and Methods

Study Area

The study area include, Mile 3 market and Rivers States University (RSU) main gate, in Port Harcourt City Local Government Area of Rivers State, Nigeria. Its geographical coordinates are: 04°48.2234N 006°59.4060E, 04°48.2234N 006°59.4060E and 04°48.2565N 006°59.2116E respectively.

Sample Collection

The three (3) henna sample (Natural henna leaves, Synthetic black henna and Synthetic red henna), were purchased from different shops in Mile 3 Market and Rivers State University (RSU) main gate and immediately taken to the Microbiology laboratory in Rivers State University, for further analysis. The dried leaves of henna were ground to a powder (using a thoroughly washed mortar), weighed and kept in dry sterile container to avoid contamination. The three (3) different samples were coded NHL, SBH and SRH respectively.

Microbiological Analysis of Henna Samples

Serial Dilution

Dilution of henna samples adopted the two-fold serial dilution techniques. One millilitre (1ml) of the samples was aseptically introduced into 2ml of sterile normal saline (diluent), this was shaken for even distribution of the samples. The first test tube (10^{-1}) dilution was further diluted to obtain a dilution of 10^{-5} .

Isolation and Enumeration of Bacterial and Fungal Counts

Aliquot (0.1ml) from 10^{-4} and 10^{-5} dilution were aseptically seeded (inoculated) unto already prepared sterile Mannitol Salt Agar and Nutrient Agar plates in duplicates using the spread plate technique.

The inoculated sterile agar plates were spread evenly using bent L shape glass rod and it was incubated at 37°C for 24 hours, for the enumeration of total heterotrophic bacteria (THB). After the incubation period, bacterial colonies that grow in the agar plates were counted and recorded as colony forming unit per millilitre (Cfu/ml) of the samples and the formula below were used to calculate the CFU of the bacterial population.

Cfu = Number of colonies

Dilution × Volume Plated

The counts for viable fungi count in the samples was determined by spread plate technique on Sabouraud dextrose agar (SDA) medium. 0.1ml from 10^{-2} and 10^{-3} dilutions of the samples were inoculated in duplicates on dried, sterile SDA plates and evenly spread with flamed glass rod. The plates were incubated inverted at 28°C to 30°C for 3 to 5 days. After the incubation period the emerging colonies were counted and recorded.

Identification of Bacterial and Fungal Isolates

Identification of bacterial isolates (discrete), followed Bergey's manual of determination bacteriology based on observations on appropriate cultured medium for their colour, shapes, elevation size, edge, opacity, texture and margin, Gram's staining reaction and biochemical test were also conducted for the identification purposes.

The identification of the isolated fungi was done macroscopically and microscopically. The gross morphology of the fungal growth on plates were studied including their colours. Small portions of the fungal pure culture were teased and mounted on a clean gross-free glass slide and covered with a cover slip and observed under the microscope. The results were later compared by referencing fungal characteristics in the book of fungi identification manual [14]

Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was carried out using the Kirby-Bauer Method (disc diffusion method) according to Clinical laboratory standard institute. Twenty-four (24) hours old culture were standardized using the 0.5 McFarland standard [8]. The identified bacterial isolates were tested with their respective concentration (in brackets) for the following antibiotics-Tarivid (10µg), Reflacine (10µg), Ciproflax (10µg), Augmentin (30µg) Gentamycin (10µg), Streptomycin (30µg), Ceporex (10µg), Nalidixic acid (30µg), Septrin (30µg), Norfloxacin (10µg), Chloramphenicol (30µg), Ampicillin (30µg), Levofloxacin (20µg), Erythromycin (30µg), Amoxil (20µg), Ampicillin (20µg) and Rifampicin (20µg).

Test bacterial isolates were taken from 24 hour old culture inoculated and grown on sterile nutrient broth (NB) in test tubes. The nutrient broth containing the isolates were incubated at 37°C for 24 hours. Each bacterial suspension turbidity was then compared to that of a 0.5 McFarland standard solution containing about 1.5×10^8 Cfu/ml. The bacterial suspension in nutrient broths were aseptically streaked onto already prepared Mueller-Hinton Agar (MHA) plates using sterile Swab stick and allowed to dry a little then, the antibiotic disc was aseptically placed using sterile forceps onto Miller-Hinton Agar Plate and incubated at 37°C for 16-24 hours. The plates were examined, and the diameters of the zones of inhibition were measured in millimeter (mm) using a meter rule.

The zone diameter for each antimicrobial agents was then translated to Susceptible(S), Intermediate (1) and Resistant (R) categories according to the interpretation table of the CLSI [8].

Results

At the end of the study, the Total heterotrophic bacterial count (THBC) of all samples ranged from 3.0x10⁴Cfu/ml to 6.2x10⁴Cfu/ml, Total *Staphylococcal* count ranged from 2.2x10³ to 4.7x10³, while Total fungal count (TFC) of the three samples ranged 1.2x10² to 2.0x10²Sfu/ml.

Table 1: Microbial Population Count of Bacterial and Fungal Isolates

SC	THB (Cfu/ml)	TSC (Cfu/ml)	TFC (SFU/ml)
NHL	6.2X10 ⁵	2.7X10 ³	2.0X10 ²
SBH	4.1X10 ⁴	4.7X10 ³	1.8X10 ²
SRH	3.0X10 ⁴	2.2X10 ³	1.2X10 ²

Key: SC – Sample Code, NHL – Natural henna leaves, SBH – Synthetic black henna, SRH - Synthetic red henna, THB - Total heterotrophic bacteria, TSC -Total Staphylococci count, CFU - colony forming unit, SFU-Spore forming unit, ml-millilitre

Table 2: Biochemical Identification and characterization of bacterial isolates

Isolate code	Grams reaction	CAT	OXI	ST	MOT	COAG	CIT	URS	IND	SH	MR	VP	MAN	MAL	GLU	LAC	Identified organism
NHL 1A	GPC	+	-	+	-	+	+	+	-	+	+	+	A	A	AG	A	<i>Staphylococcus</i> sp.
SRH 3A	GNR	+	-	-	+	-	-	-	+	-	+	-	A	N	A	AG	<i>Escherichia coli</i>
NHL 1B	GNR	+	-	-	+	-	+	+	-	-	+	-	N	N	A	N	<i>Proteus</i> sp.
SBH 2A	GNR	+	+	-	+	-	+	-	-	-	-	+	AG	AG	A	A	<i>Bacillus</i> sp.
NHL 1C	GNR	+	+	+	+	-	+	-	-	+	-	-	A	N	N	N	<i>Pseudomonas</i> sp.
NHL 1D	GNR	+	-	-	-	-	-	-	-	+	+	-	N	AG	AG	N	<i>Corynebacterium</i> sp.
SBH 2B	GPC	+	+	+	+	-	+	+	-	-	-	-	AG	A	A	N	<i>Micrococcus</i> sp.
SRH 3B	GPR	+	+	-	+	-	+	-	-	-	-	+	AG	AG	A	A	<i>Bacillus cereus</i>
SBH 2C	GNR	+	-	+	+	-	-	-	+	-	+	-	A	N	A	AG	<i>Escherichia coli</i>
SRH 3C	GPC	-	-	+	-	+	+	+	-	+	+	+	A	A	A	A	<i>Staphylococcus</i> sp.
NHL 1E	GPC	+	-	-	-	-	+	-	-	-	+	-	N	A	A	AG	<i>Streptococcus</i> sp.
SRH 3E	GPC	+	+	+	+	-	+	+	-	-	-	-	AG	A	A	N	<i>Micrococcus</i> sp.
SRH 3D	GNR	+	+	+	+	-	+	-	-	+	-	-	A	N	N	N	<i>Pseudomonas</i> sp.
SBH 2E	GPR	+	-	-	-	-	-	-	-	+	+	-	N	AG	AG	N	<i>Corynebacterium</i> sp.
SBH 2D	GPC	+	-	+	-	+	+	+	-	+	+	+	A	A	A	A	<i>Staphylococcus</i> sp.
SBH 2F	GNR	+	-	-	+	-	+	+	-	-	+	-	N	N	A	N	<i>Proteus mirabilis</i>
NHL 1F	GPR	+	+	-	+	-	+	+	-	-	-	+	AG	A	A	A	<i>Bacillus subtilis</i>

Key:
NHL – Natural henna leaves
SBH – Synthetic black henna
SRH – Synthetic red henna
GPR – Gram position rod, GNR – Gram negative rod, GPC – Gram positive cocci, CAT – Catalase, MOT – Motility, OXI – Oxidase, ST – Salt tolerance, URS – Urease, COAG – Coagulase, VP – Vogues proskauer, CIT – citrate, IND – Indole, MR – Methyl Red, GLU – Glucose, LAC – Lactose, MAN – Mannitol, MAL – Maltose, AG – Acid/Gas, A – Acid, N – Negative, += Positive, -= negative

Table 3: Macroscopic and Microscopic Description of Fungi

Fungal Isolates	Macroscopic	Microscopic
<i>Aspergillus</i> sp.	Green colonies which change to black	Septate hyphae bearing conidia on conidiophores
<i>Fusarium</i> sp.	White cottony lawn like growth with reverse yellow colour	Septate hyphae, with presence of banana shaped septate conidia
<i>Microsporium</i> sp.	Radially furrowed white wooly growth with a yellowish white centre and white periphery	Septate hyphae with solitary conidia
<i>Candida</i> sp.	Cream coloured, growth round glistening colonies	Oval shaped colls
<i>Mucor</i> sp.	White fluffy growth, with reverse white colour	Non septate hyphae, with non-septate sporangioophores
<i>Penicillium</i> sp.	Radially furrowed blue-green velvety growth with white periphery; with reverse white colour	Septate hyphae with branched Conidiophores bearing Phialides. Conidia are arranged chains on the Phialides.

Table 4: Percentage of Bacterial Isolates from all Samples

Organisms	Number	Percentage (%)
<i>Bacillus</i> spp.	3	17.65
<i>Staphylococcus</i> spp.	3	17.65
<i>Proteus</i> sp.	2	11.76
<i>Micrococcus</i> sp.	2	11.76
<i>Streptococcus</i> sp.	1	5.90
<i>Pseudomonas</i> sp.	2	11.76
<i>Corynebacterium</i> sp.	2	11.76
<i>Escherichia coli</i>	2	11.76

Table 5: Susceptibility pattern of Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus* sp.) Isolated from NHL

Bacillus (3) Staphylococcus (3)						
Antibiotics (conc. µg)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Chloramphenicol (30µg)	1(33.3)	0(0.0)	2(66.7)	1(33.3)	0(0.00)	2(66.7)
Ampicillin (20µg)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Levofloxacin (20µg)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	3(100)
Erythromycin (30µg)	0(0.00)	3(100)	0(0.00)	1(33.3)	2(66.7)	0(0.00)
Gentamycin (10µg)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Ciprofloxacin (10µg)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	3(100)
Streptomycin (30µg)	3(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	3(100)
Amoxil (20µg)	3(100)	0(0.00)	0(0.00)	2(66.7)	1(33.3)	0(0.00)
Norfloxacin (10µg)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Rifampicin (20µg)	0(0.00)	2(66.7)	1(33.3)	3(100)	0(0.00)	0(0.00)

KEY: R; Resistance, I; Intermediate, S; Susceptible
NHL: Natural henna leaves, SBH: Synthetic black henna, SRH: Synthetic red henna.

Table 6: Susceptibility pattern of Gram-positive bacteria (*Corynebacterium* and *Streptococcus* sp.) Isolated from NHL

Corynebacterium(2)			Streptococcus (1)			
Antibiotics (conc. µg)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Chloramphenicol (30µg)	1(33.3)	0(0.00)	2(66.7)	0(0.00)	0(0.00)	1(100)
Ampicillin (20µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)
Levofloxacin (20µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)
Erythromycin (30µg)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)
Gentamycin (10µg)	2(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
Ciprofloxacin (10µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)
Streptomycin (30µg)	2(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
Amoxil (20µg)	1(50)	1(50)	0(0.00)	0(0.00)	1(100)	0(0.00)
Norfloxacin (10µg)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)	0(0.00)
Rifampicin (20µg)	0(0.00)	1(50)	1(50)	1(100)	0(0.00)	0(0.00)

KEY: R; Resistance, I; Intermediate, S; Susceptible
NHL: Natural henna leaves, SBH: Synthetic black henna, SRH: Synthetic red henna.

Table 7: Susceptibility pattern of Gram-negative bacteria (*Proteus* sp. and *Pseudomonas aeruginosa*) Isolated from NHL

Proteus(2) Pseudomonas (2)						
Antibiotics (conc. µg)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Tarivid (10µg)	0(0.00)	1(50)	1(50)	0(0.00)	2(100)	0(0.00)
Reflacin (10µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)
Ciproflo (10µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)
Augmentin (30µg)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)
Gentamycin (10µg)	2(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)
Ceporex (10µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)
Streptomycin (30µg)	2(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)
Nalidixic acid (30µg)	1(50)	1(50)	0(0.00)	0(0.00)	2(100)	0(0.00)
Septtrin (30µg)	0(0.00)	2(100)	0(0.00)	2(100)	0(0.00)	0(0.00)
Amplicin (30µg)	0(0.00)	1(50)	1(50)	0(0.00)	0(0.00)	2(100)

KEY: R; Resistance, I; Intermediate, S; Susceptible
NHL: Natural henna leaves, SBH: Synthetic black henna, SRH: Synthetic red henna.

Table 8: Susceptibility pattern of Gram-positive bacteria (*Bacillus* sp. and *Corynebacterium* sp.) Isolated from SBH

Micrococcus (2) Staphylococcus (3)						
Antibiotics (conc. µg)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Chloramphenicol (30µg)	0(0.00)	0(0.00)	2(100)	1(33.3)	0(0.00)	2(66.7)
Ampicillin (20µg)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Levofloxacin (20µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	3(100)
Erythromycin (30µg)	0(0.00)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)
Gentamycin (10µg)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Ciprofloxacin (10µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	3(100)
Streptomycin (30µg)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Amoxil (20µg)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Norfloxacin (10µg)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
Rifampicin (20µg)	1(50)	0(0.00)	1(50)	0(0.00)	0(0.00)	3(100)

KEY: R; Resistance, I; Intermediate, S; Susceptible
NHL: Natural henna leaves, SBH: Synthetic black henna, SRH: Synthetic red henna.

Table 9: Susceptibility pattern of Gram-positive bacteria (*Micrococcus* sp. and *Staphylococcus* sp.) Isolated from SBH

Antibiotics (conc. µg)	<i>Bacillus</i> (3)			<i>Corynebacterium</i> (2)		
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Chloramphenicol (30µg)	1(33.3)	0(0.0)	2(66.7)	1(50)	0(0.00)	1(50)
Ampicillin (20µg)	3(100)	0(0.00)	0(0.00)	0(0.00)	2(100)	0(0.00)
Levofloxacin (20µg)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)
Erythromycin (30µg)	0(0.00)	3(100)	0(0.00)	0(0.00)	1(50)	1(50)
Gentamycin (10µg)	3(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)
Ciprofloxacin (10µg)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)
Streptomycin (30µg)	3(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)
Amoxil (20µg)	3(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Norfloxacine (10µg)	1(33.3)	2(66.7)	0(0.00)	0(0.00)	0(0.00)	2(100)
Rifampicin (20µg)	0(0.00)	2(66.7)	1(33.3)	0(0.00)	0(0.00)	2(100)

Table 10: Susceptibility pattern of Gram-negative bacteria (*Proteus* sp. and *Escherichia coli*) Isolated from SBH

Antibiotics (conc. µg)	<i>Proteus</i> sp.(2) <i>Escherichia coli</i> (2)					
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Tarivid (10µg)	0(0.00)	1(50)	1(50)	0(0.00)	2(100)	0(0.00)
Reflacin (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Ciproflo (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Augmentin (30µg)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)
Gentamycin (10µg)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)
Ceporex (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Streptomycin (30µg)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)
Nalidixic acid (30µg)	1(50)	1(50)	0(0.00)	1(50)	1(50)	0(0.00)
Septrin (30µg)	0(0.00)	2(100)	0(0.00)	2(100)	0(0.00)	0(0.00)
Amplicin (30µg)	0(0.00)	1(50)	1(50)	0(0.00)	0(0.00)	2(100)

Table 11: Susceptibility Pattern of Gram positive bacteria (*Bacillus* sp., *Staphylococcus aureus* and *Micrococcus* sp.) Isolated from SRH

Antibiotics (conc. µg)	<i>Bacillus</i> (3)			<i>Staphylococcus</i> (3)			<i>Micrococcus</i> (2)		
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Chloramphenicol (30µg)	1(33.3)	0(0.00)	2(66.7)	1(33.3)	0(0.00)	2(66.7)	0(0.00)	0(0.00)	2(100)
Ampicillin (20µg)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)
Levofloxacin (20µg)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)
Erythromycin (30µg)	0(0.00)	3(100)	0(0.00)	1(33.3)	2(66.7)	0(0.00)	0(0.00)	2(100)	0(0.00)
Gentamycin (10µg)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)
Ciprofloxacin (10µg)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)
Streptomycin (30µg)	3(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	3(100)	2(100)	0(0.00)	0(0.00)
Amoxil (20µg)	3(100)	0(0.00)	0(0.00)	2(66.7)	1(33.3)	0(0.00)	2(100)	0(0.00)	0(0.00)
Norfloxacine (10µg)	3(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)
Rifampicin (20µg)	0(0.00)	2(66.7)	1(33.3)	3(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)

Table 12: Susceptibility pattern of Gram negative bacteria (*Pseudomonas* sp. and *Escherichia coli*) Isolated from SRH

Antibiotics (conc. µg)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Tarivid (10µg)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)
Reflacin (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Ciproflo (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Augmentin (30µg)	0(00.0)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)
Gentamycin (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Ceporex (10µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Streptomycin (30µg)	0(0.00)	0(0.00)	2(100)	2(100)	0(0.00)	0(0.00)
Nalidixic acid (30µg)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	2(100)
Septrin (30µg)	0(0.00)	2(100)	0(0.00)	2(100)	0(0.00)	0(0.00)
Amplicin (30µg)	0(0.00)	1(50)	1(50)	0(0.00)	0(0.00)	2(100)

Table 13: Comparison and percentage occurrence of fungal isolates from three (3) different henna dyes sold in Port Harcourt metropolis

Fungal isolates	SBH	NHL	SRH	Total	Percentage%
<i>Fusarium</i> sp.	3	4	2	9	12.2
<i>Mucor</i> sp.	4	3	5	12	16.3
<i>Aspergillus</i> sp.	7	2	5	14	18.9
<i>Microsporium</i> sp.	3	2	0	5	6.8
<i>Penicillium</i> sp.	6	3	8	17	22.9
<i>Candida</i> sp.	7	5	5	17	22.9
Total	30	19	25	74	100
%	40.5	25.7	33.8	100	

DISCUSSION

The study showed that henna products in this study contained bacterial and fungal populations, although the microbial loads differed among the samples. The total heterotrophic bacterial (THB) count ranged from 3.0 × 10⁴ CFU/mL to 6.2 × 10⁵ CFU/mL. The considerably higher bacterial load in NHL may be related to its plant origin. Although henna contains compounds with antimicrobial activity, this does not necessarily make commercial henna products sterile.

In fact, [11] reported that commercial henna powder contained non-pathogenic *Bacilli* and *Aspergillus niger*, suggesting that microorganisms can survive in henna products despite the plant's antimicrobial properties.

The THB count recorded for the natural henna in the present study (6.2×10^5 CFU/mL) was also higher than that reported by [7], who reported bacterial contamination of approximately 10^2 – 10^3 CFU/mL in many used cosmetic products, although some beauty blenders had loads exceeding 10^6 CFU/mL.

The total staphylococcal count (TSC) ranged from 2.2×10^3 CFU/mL to 4.7×10^3 CFU/mL. The highest TSC in SBH is notable because staphylococci are common members of the human skin microbiota and can be introduced into cosmetic products through handling, water, packaging, or poor manufacturing standard. [10] similarly reported the presence of *Staphylococcus* spp. among the predominant bacterial contaminants of shared cosmetic products. A similar study of commonly used cosmetics also identified *Staphylococcus aureus* as the most frequently isolated organism [17].

The total fungal count (TFC) ranged from 1.2×10^2 CFU/mL to 2.0×10^2 CFU/mL. The lower fungal counts compared to THB suggest that bacteria were the predominant microbial group in the samples. Nevertheless, the isolation of fungi remains important because moulds and yeasts can survive in cosmetic and plant-derived materials and may contribute to product deterioration or opportunistic infections in susceptible individuals. Microbial contamination studies of cosmetics have reported organisms belonging to genera such as *Candida*, *Aspergillus*, *Penicillium*, and *Fusarium* [10].

The identified bacteria included *Staphylococcus* spp., *Escherichia coli*, *Proteus* spp., *Bacillus* spp., *Pseudomonas* spp., *Corynebacterium* spp., *Micrococcus* spp. and *Streptococcus* spp. (Table 2).

The occurrence of these organisms suggests possible contamination from human handling (*Staphylococcus*, *Micrococcus*, and *Streptococcus*), the surrounding environment and contamination from fecal sources (*Bacillus* spp., *E. coli*, *Proteus* sp., *Pseudomonas* spp., and *Corynebacterium* spp.). These organisms may be transferred to cosmetic products during processing, packaging, retail handling, or direct application by consumers. The isolation of these organisms agrees with the studies of [2], who isolated *Pseudomonas* sp., *E. coli*, *Proteus* sp., *Bacillus* spp. and *S. aureus* from commercially available cosmetic products in Akure. Also, [9] identified *S. aureus* and *Pseudomonas aeruginosa* among the predominant bacterial contaminants from cosmetics sold in Aba, Nigeria.

The findings important because *Lawsonia inermis* (henna) itself has demonstrated antimicrobial activity against several bacteria, including *S. aureus*, *E. coli*, and *P. aeruginosa*. [12] [5].

Six fungal genera were identified, namely *Aspergillus*, *Fusarium*, *Microsporum*, *Candida*, *Mucor*, and *Penicillium* (Table 3). The isolation of these organisms from henna is important because they are either associated with plant materials and powdered herbal products or are dermatophytes capable of infecting keratinized tissues such as skin, hair, and nails. In a study of powdered herbal medicinal preparations, *Aspergillus* spp. were the most frequently isolated fungi [1] [3] [17].

The diversity of fungi reported in the present study is consistent with previous studies showing that cosmetic products can become contaminated with fungi during production, packaging, storage, and repeated handling [10] [17].

Table 4 shows the distribution and percentage occurrence of bacterial isolates from the natural henna leaves (NHL), synthetic black henna (SBH), and synthetic red henna (SRH) samples. A total of 17 bacterial isolates were reported, representing eight bacterial genera. *Bacillus* spp. and *Staphylococcus* spp. were the most frequently isolated organisms, with 3 isolates each (17.65%), followed by *Proteus* spp., *Micrococcus* spp., *Pseudomonas* spp., *Corynebacterium* spp., and *Escherichia coli*, each accounting for 2 isolates (11.76%), while *Streptococcus* spp. was the least isolated, with 1 isolate (5.90%).

The pattern indicates that the henna samples contained a mixture of environmental, skin-associated, and potentially pathogenic bacteria. And agrees with the reports of [10] [17]. The presence of *E. coli*, *Pseudomonas*, and *Staphylococcus* is particularly important from a public-health perspective because these organisms are recognized indicators or potential causes of contamination and infection.

Table 5 presents the antibiotic susceptibility pattern of *Bacillus* spp. and *Staphylococcus* spp. isolated from natural henna leaves (NHL). Among the *Bacillus* isolates, 100% susceptibility was reported for levofloxacin and ciprofloxacin. In contrast, all three *Bacillus* isolates (100%) were resistant to ampicillin, gentamicin, streptomycin, amoxicillin, and norfloxacin.

Resistance in environmental *Bacillus* isolates is of public-health interest because environmental bacteria can serve as reservoirs of antimicrobial-resistance determinants that may potentially be transferred to other bacteria. Studies of antibiotic resistance among bacteria isolated from cosmetics and personal-care products have similarly reported resistance to multiple antimicrobial agents [7].

The *Staphylococcus* isolates also demonstrated that all three isolates (100%) were susceptible to levofloxacin, ciprofloxacin, and streptomycin, whereas complete resistance was recorded against ampicillin, gentamicin, norfloxacin, and rifampicin. The resistance observed in the present study is comparable with reports [17] who found different levels of antibiotic resistance among *Staphylococcus* sp. isolated from commonly used cosmetic products in Kaduna State, Nigeria.

Table 6 presents the antibiotic susceptibility pattern of *Corynebacterium* sp. and *Streptococcus* sp. isolated from natural henna leaves (NHL). Complete susceptibility (100%) was recorded against ampicillin, levofloxacin, and ciprofloxacin. In contrast, both *Corynebacterium* isolates (100%) were resistant to gentamicin and streptomycin.

The susceptibility of *Corynebacterium* to levofloxacin and ciprofloxacin is noteworthy because fluoroquinolones can have activity against several non-diphtherial *Corynebacterium* species, although susceptibility varies considerably between species. The observed resistance to gentamicin and streptomycin, however, indicates that the isolates may have limited susceptibility to some aminoglycosides. Resistance among environmental *Corynebacterium* isolates is important because some members of the genus can act as opportunistic pathogens and may carry antimicrobial-resistance determinants. Therefore, their presence in a product applied directly to the skin deserves microbiological attention.

The *Streptococcus* isolate showed susceptibility (100%) to chloramphenicol, levofloxacin, gentamicin, ciprofloxacin, and streptomycin. Complete resistance (100%) was observed to norfloxacin and rifampicin.

The resistance and intermediate responses observed in this table are consistent with the broader concern regarding antimicrobial resistance among bacteria associated with cosmetic and personal-care products [6] [17].

Table 7 presents the antibiotic susceptibility pattern of *Proteus* spp. and *Pseudomonas* sp. isolated from natural henna leaves (NHL). The two *Proteus* isolates were 100% susceptible to Reflacin, ciprofloxacin, and Ceporex. In contrast, both isolates (100%) were resistant to gentamicin and streptomycin.

The *Pseudomonas* sp. isolates showed that both isolates (100%) were susceptible to Reflacin, ciprofloxacin, gentamicin, Ceporex, streptomycin, and ampicillin, while both isolates (100%) were resistant to Septrin.

The differences observed between *Proteus* and *P. aeruginosa* may be related to differences in their intrinsic resistance mechanisms and antimicrobial susceptibility profiles. *P. aeruginosa* possesses several mechanisms that can contribute to antimicrobial resistance, including restricted outer-membrane permeability, efflux pumps, antimicrobial-inactivating enzymes, and alterations in antibiotic targets [15].

Table 8 presents the antibiotic susceptibility pattern of *Bacillus* spp. and *Corynebacterium* spp. isolated from synthetic black henna (SBH).

The *Bacillus* isolates (100%) were susceptible to levofloxacin and ciprofloxacin. In contrast, all three isolates (100%) were resistant to ampicillin, gentamicin, streptomycin, and amoxicillin. This pattern indicates that the *Bacillus* isolates were resistant to several commonly used antimicrobial agents but retained good susceptibility to the fluoroquinolones tested. Similar resistance among bacterial isolates from commercial cosmetic products has been reported [6] [17].

The *Corynebacterium* isolates showed 100% susceptibility to levofloxacin, gentamicin, ciprofloxacin, norfloxacin, and rifampicin. Chloramphenicol produced an equal distribution of resistance and susceptibility (50% each), while erythromycin resulted in 50% intermediate susceptibility and 50% susceptibility. Both isolates were completely resistant to streptomycin (100%). Notably, neither isolate showed resistance to levofloxacin, gentamicin, ciprofloxacin, norfloxacin, or rifampicin.

The complete susceptibility of the *Corynebacterium* isolates to several antibiotics contrasts with the marked resistance observed in the *Bacillus* isolates. Such differences may be influenced by intrinsic resistance mechanisms and acquired resistance genes.

The resistance to streptomycin observed in both *Bacillus* and *Corynebacterium* isolates is noteworthy. Streptomycin resistance may result from mechanisms such as modification of the antibiotic, reduced uptake, or alteration of its ribosomal target. The complete resistance of the *Bacillus* isolates to ampicillin, gentamicin, and amoxicillin also indicates a broader resistance pattern that deserves attention. Similar multidrug-resistant patterns have been reported among bacteria recovered from cosmetic products, suggesting that personal-care products may occasionally harbour bacteria with reduced susceptibility to commonly used antibiotics [6].

The findings are particularly relevant because the organisms were isolated from synthetic black henna, a product intended for direct application to the skin.

The antibiotic susceptibility pattern of *Micrococcus* spp. and *Staphylococcus* spp. isolated from synthetic black henna (SBH) is presented in Table 9.

The *Staphylococcus* isolates showed complete susceptibility to levofloxacin, ciprofloxacin, and rifampicin, but complete resistance to ampicillin, gentamicin, streptomycin, amoxicillin, and norfloxacin.

The two *Micrococcus* isolates were 100% susceptible to chloramphenicol, levofloxacin, and ciprofloxacin. However, both isolates (100%) were resistant to ampicillin, gentamicin, streptomycin, amoxicillin, and norfloxacin. Resistance to gentamicin and streptomycin suggests reduced susceptibility to aminoglycosides. This agrees with the study of [6].

Table 10 shows the antibiotic susceptibility pattern of Gram-negative bacteria isolated from SBH henna dye. The *Proteus* sp. isolates showed 100% susceptibility to Reflacin, Ciproflox and Ceporex. Resistance was highest against Gentamycin and Streptomycin, with both isolates (100%) resistant to each antibiotic.

The *E. coli* isolates (100%) were resistant to Reflacin, Ciproflox, Gentamycin, Ceporex, Streptomycin and Septrin. In contrast, Ampicillin was the only agent that demonstrated complete susceptibility (100%) against the *E. coli* isolates.

The extensive resistance observed among the *E. coli* isolates is important from a public health perspective. Similar results have been reported by [13], where multidrug-resistant Gram-negative bacteria, including *E. coli* and *Proteus* species were recorded

Table 11 presents the antibiotic susceptibility pattern of *Bacillus* spp., *Staphylococcus* sp. and *Micrococcus* spp. isolated from synthetic red henna (SRH).

Among the three *Bacillus* isolates, 100% susceptibility was reported against levofloxacin and ciprofloxacin. All three isolates (100%) were resistant to ampicillin, gentamicin, streptomycin, amoxicillin, and norfloxacin.

The three *Staphylococcus aureus* isolates also showed 100% susceptibility to levofloxacin, Streptomycin and ciprofloxacin, while complete resistance (100%) was observed against ampicillin, gentamicin, norfloxacin, and rifampicin.

The two *Micrococcus* isolates showed that (100%) were susceptible to chloramphenicol, levofloxacin, and ciprofloxacin, but were completely resistant to ampicillin, gentamicin, streptomycin, amoxicillin, and norfloxacin.

The repeated susceptibility to levofloxacin and ciprofloxacin across the three bacterial groups is of importance. Also, the high level of resistance to ampicillin, amoxicillin, gentamicin, streptomycin, and norfloxacin is of public-health interest. The presence of such organisms in henna is particularly relevant because the product is applied directly to the skin. These results agree with the study of [6].

Table 12 presents the antibiotic susceptibility pattern of *Pseudomonas* spp. and *Escherichia coli* isolated from synthetic red henna (SRH). The two *Pseudomonas* isolates showed 100% susceptibility to Reflacin, ciprofloxacin, gentamicin, Ceporex, and streptomycin. No complete resistance was recorded among the *Pseudomonas* isolates to any of the antibiotics tested.

The *E. coli* isolates showed that (100%) were susceptible to nalidixic acid and ampicillin, but they were completely resistant to Reflacin, ciprofloxacin, gentamicin, Ceporex, streptomycin, and Septrin. Tarivid. The contrast between *Pseudomonas* and *E. coli* is particularly striking for ciprofloxacin. While both *Pseudomonas* isolates were susceptible, both *E. coli* isolates were resistant. The observed resistance may be due to previous exposure of bacterial populations to antimicrobial agents and the acquisition of resistance genes. The findings are consistent with concerns raised in studies of cosmetic products [6].

Table 13 shows the distribution of fungal isolates from natural henna leaves (NHL), synthetic black henna (SBH), and synthetic red henna (SRH) sold in Port Harcourt metropolis. A total of 74 fungal isolates were reported, with SBH accounting for the highest proportion (40.5%), followed by SRH (33.8%) and NHL (25.7%). The higher fungal isolation from the synthetic products suggests that the formulation, processing, handling, packaging, or storage conditions of these products may provide opportunities for fungal contamination. Similar contamination of cosmetic products with fungi has been reported, including *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Candida* species [6] [18].

Among the isolates, *Candida* spp. and *Penicillium* spp. were the most frequently isolated, with 17 isolates each, representing 22.9% of the total fungal isolates. *Candida* was isolated from all three henna types, occurring 7 times in SBH, 5 times in NHL, and 5 times in SRH. Similarly, *Penicillium* was reported in all three products, with the highest occurrence in SRH (8 isolates), followed by SBH (6) and NHL (3). The widespread occurrence of these fungi is consistent with previous studies of topical cosmetic products. [18] reported *Fusarium*, *Penicillium*, *Candida* and *Aspergillus* among fungi associated with body lotions used in Port Harcourt. The occurrence of multiple fungal genera in all three henna categories is important from a public-health and product-quality perspective. Cosmetics and products applied directly to the skin should be manufactured and stored under conditions that minimize microbial contamination.

Conclusion

The findings of this study show that henna dyes sold in Port Harcourt metropolis can harbor a variety of bacterial and fungal contaminants, indicating potential public-health concerns associated with their use. Natural henna leaves (NHL), synthetic black henna (SBH), and synthetic red henna (SRH) all had bacterial and fungal isolates, although the level and diversity of contamination varied among the products. Synthetic black henna recorded the highest fungal occurrence (40.5%), followed by synthetic red henna (33.8%) and natural henna leaves (25.7%).

The bacterial isolates included potentially significant organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* spp., *Bacillus* spp., *Corynebacterium* spp., *Streptococcus* spp., and *Micrococcus* spp. The isolation of organisms such as *S. aureus* and *E. coli* is particularly important because their presence may indicate contamination arising from raw materials, handling, processing, packaging, or storage. The fungal isolates included *Candida*, *Penicillium*, *Aspergillus*, *Mucor*, *Fusarium*, and *Microsporum* spp., with *Candida* and *Penicillium* being the most frequently reported.

The antimicrobial susceptibility results further showed that several bacterial isolates exhibited resistance to multiple antibiotics, although susceptibility to some agents, particularly fluoroquinolones such as ciprofloxacin and levofloxacin, was observed in several isolates. The occurrence of resistant organisms in a product intended for direct application to the skin is significant because contaminated products may serve as potential reservoirs for antimicrobial-resistant bacteria.

The present study established that commercially available henna dyes examined in Port Harcourt were not sterile and contained a mixture of bacterial and fungal organisms of potential public-health significance. The findings therefore emphasize the importance of microbiological quality control and high hygiene standards throughout the production, packaging, distribution, and storage of henna products.

Recommendations

1. Manufacturers and regulators particularly the National Agency for Food and Drug Administration and Control (NAFDAC), should routinely examine commercially available henna products for bacterial and fungal contamination before they are released for sale. Also, microbial isolates from cosmetic products should be periodically subjected to antimicrobial susceptibility testing to monitor the emergence and distribution of resistant organisms.
2. End users should be educated about the importance of buying properly labelled and registered products from reliable sources and avoiding products with damaged, leaking or unsealed packaging.
3. Future studies should include a larger number of henna brands and samples from different locations in Rivers State. Molecular identification techniques could also be used to confirm the species identity of the bacterial and fungal isolates and to investigate antimicrobial-resistance genes.

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