

**ISOLATION AND CHARACTERIZATION OF BACTERIAL ISOLATES  
FROM SCALP HAIR OF MALE UNDERGRADUATE'S STUDENTS IN  
UNIVERSITY OF BENIN**

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF  
MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF  
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REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN  
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**FEBRUARY 2025.**

## CERTIFICATION

This is to certify that this project work was carried out by Osasogie Aganmwonyi in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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Dr. A.S. AZIEGBEMHIN  
(Supervisor)

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Date

## **APPROVAL**

I certify that this work has been accepted in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) in the Department of Microbiology, University of Benin, Benin City.

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**PROF. F. I. AKINNIBOSUN**  
**(Head of Department)**

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**Date**

## **DEDICATION**

This project is dedicated to Almighty God and my supervisor Dr. A.S. AZIEGBEMHIN

## **ACKNOWLEDGEMENT**

I would like to thank God for his continued grace, guidance, and protection throughout my academic journey and during this research project. I am deeply grateful to my parents, whose unwavering love, prayers, and support have been my greatest source of strength. To my siblings, thank you for your encouragement and understanding throughout this process. My heartfelt appreciation goes to my supervisor, Dr. A.S. Aziebeminhin, for his invaluable guidance and support. I also extend special thanks to the Head of Department, Prof. (Mrs.) F.I. Akinnibosun, and all the staff members of the Department of Microbiology for their encouragement and assistance.

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## ABSTRACT

The scalp hair is composed of soft tissue layers that cover the cranium. This study focused on isolation, identification and antibiogram of bacteria isolates from scalp hair. With the aid of a sterile swab sticks, nine scalp hair of undergraduates' students were swabbed and transported to the laboratory for bacteriological analysis. All the samples were analyzed within 24hrs of collection. Collected swabbed stick was submerged in a sterile test tubes label A – I contained Nutrient broth and incubated for 2hrs. 1ml was plated from each test tube. It was then incubated at 37<sup>o</sup> C for 24hours. The result of the bacteria isolated include; *Staphylococcus epidermis* 6 (15%), *Staphylococcus aureus* 6 (15%), *Pseudomonas* sp. 2(5%), *Bacillus* sp. 18(45%), *Streptococcus* spp. 5(12.5%) and *Micrococcus* spp. 3(7.5%). Haemolysin test on the strains of *Staphylococcus aureus* *Streptococcus* sp. *Staphylococcus epidermis*, *Micrococcus* spp. and *Pseudomonas* sp. revealed they had  $\beta$  hemolytic activity and only strains of *Bacillus* spp. showed  $\alpha$  hemolytic activity. While the gelatin test showed that all isolates produce gelatinase enzyme which breakdown gelatin. The antibiogram results revealed that All isolates were highly sensitive to ciprofloxacin, Ofloxacin and clindamycin 40(100%). Ciprofloxacin, Ofloxacin and clindamycin was proved to be the most effective against Gram positive and Gram-negative isolates studied in this work. Contamination of scalp hair from this research, could be from poor hand hygiene and environmental hygiene.

## CHAPTER ONE

### INTRODUCTION

The exploration of microbial diversity within human hair, particularly in male undergraduate students, has drawn increasing attention in recent years. Furthermore, understanding of the bacterial isolates present in hair and their broader implications involve dermatology and microbiology. Kong and Segre, (2011) provide a foundational perspective on the historical context of skin microbiome research, emphasizing the limitations of traditional culture-based methods in isolating fastidious bacteria. The work illustrates the challenges faced when attempting to characterize microbial diversity on the skin, where more prevalent species, such as *Staphylococcus aureus*, can overshadow less common but equally important microbes. They highlighted significant skin colonizers, including various genera of Actinobacteria and coagulase-negative staphylococci, which are particularly abundant in sebaceous regions. Tridico *et al.*, (2014) conducted a research on metagenomic analyses that reveal notable distinctions in microbial communities between male and female hair samples. The research indicated that male scalp hair microbiota show commonalities, the pubic hair microbiome exhibits stability during cohabitation but can shift due to sexual activity. Lo *et al.* (2022) examine the regulation of hair within the context of Hong Kong prisons, highlighting gender-based differences in perceptions of hair and identity. Their research underscores the symbolic nature of hair as a marker of gender, power, and societal norms. The authors note that long hair is often associated with femininity and social conformity, while short hair can symbolize rebellion or independence, especially among male students. This duality illustrates how societal expectations surrounding hair can affect students' choices and self-perceptions, revealing the complex interplay between hair, identity, and social regulation (Wing Lo *et al.*, 2022). This study titled "Isolation and

Characterization of Bacterial Isolates from Scalp Hair of Male Undergraduate Students" focused on identifying and characterizing the bacterial species found on the hair of students, with a particular emphasis on male undergraduates.

## **1.2 Aim and Objectives**

The objective of this research was to;

- isolate and identify bacterial from undergraduate scalp hair
- determine the antibiogram of the bacterial isolates

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.0 Background of The Study**

The scalp is composed of soft tissue layers that cover the cranium. It is an anatomic region bordered anteriorly by the human face, and laterally and posteriorly by the neck. It extends from the superior nuchal lines and occipital turbulences to the supraorbital foramen. Aesthetically, it serves as an area where hair can grow and physically, as a barrier that defends the body from foreign irritation. There are five layers to the scalp: the skin, connective tissue layer, galea aponeurotica, loose areolar connective tissue, and the pericranium. The scalp serves as a physical barrier to protect the cranial vault from physical trauma and potential pathogens that can cause infection (Grimalt,2007). In addition to its physical defenses, the scalp is important aesthetically. Hair grows on the skin of the scalp to not only aid in heat conservation but to also plays a role in an individual's appearance and sexual signaling. The first layer is the skin, which is thick and contains hair follicles and sebaceous glands. The hair follicles can extend into the dense connective tissue layer, where the nerves, lymphatics, and the vascular supply of the scalp reside. The galea aponeurotica, also called the epicranial aponeurosis, is a strong and immobile connective tissue layer continuous with the occipitofrontalis muscle. It is firmly attached to the subcutaneous dense connective tissue layer and serves to prevent stretching of the scalp, especially during surgery, which beneficially prevents complications. The loose connective tissue is important to the mobility of the scalp. It also serves as a flexible plane that separates the top three layers from the pericranium. The pericranium is the deepest layer of the scalp that is composed of dense irregular connective tissue. It tightly adheres to the calvarial bone of the skull. It contains the vascular supply that is vital to supporting the underlying calvarium (Wing, *et al.*,

2022). Scalp hair and its multifaceted implications has gained significant attention across various disciplines, revealing a complex interplay between cultural identity, social norms, and individual experiences. Khumalo, (2007) addresses the secondary scalp disorders linked to hair treatments, highlighting the complications arising from cosmetic practices that often lead to conditions such as traction alopecia and scarring. Furthermore, Antunez, (2013) expands the conversation by situating hair within a broader socio-political framework. The article delves into the historical significance of hair for Black women, illustrating how hair aesthetics are intertwined with concepts of race, gender, and identity (Antunez, 2013).

## **2.2 Blood Supply and Lymphatics of The Scalp**

The vascular supply to the scalp comes from the common carotid artery, posterior intercostal arteries, and the terminal branches of the subclavian artery. These arteries connect through an impressive network of anastomoses, with anastomoses in the temporal region being the most numerous (Chen and Hsieh 2018). The common carotid artery bifurcates into the internal and external carotid arteries at the carotid sinus. Both give off branches that supply different areas of the scalp. The external carotid branches off to form the superficial temporal, posterior auricular, occipital, and the angular artery. The superficial temporal artery, the terminal branch of the external carotid artery, passes over the posterior aspect of the zygomatic and divides into the frontal and parietal branches. The frontal is a terminal branch that runs a tortuous course in an anterosuperior direction across the temple. It supplies the anterior temple, superior to the eyebrows, while the parietal branch supplies the parietal region of the scalp. The posterior auricular artery originates superior to the stylohyoid and digastric muscles and travels to the deep tissues that intersect between the mastoid process and the cartilage of the ear. It supplies scalp posterior and superior to the auricle (Kim, 2017). The posterior auricular artery proximally gives

off the occipital artery. The occipital artery ascends superiorly to penetrate the fascia between the trapezius and sternocleidomastoid. From there it tortuously ascends, and supplies the superficial fascia of the posterior scalp superior to the nuchal line, while also anastomosing with the superficial, posterior auricular arteries and the contralateral occipital artery. The posterior scalp inferior to the nuchal line is supplied by an association of vessels that also supply the trapezius and splenius capitis muscles. These vessels derive from the transverse cervical and posterior intercostal arteries Shevel (*et al.*, 2017)

The internal carotid artery gives rise to the ophthalmic artery that branches into the supratrochlear and supraorbital arteries that supply the anterior portion of the scalp. Both arteries arise from the skull through the supraorbital foramen and anastomose with their contralateral arteries and the superficial temporal artery to dominate the supply to the anterior scalp (Seery, 2002).

The scalp drains into superficial and deep venous systems. The superficial veins follow their respective arteries. The supraorbital and supratrochlear veins drain the superficial scalp anteriorly. While the superficial temporal, occipital, posterior auricular drain the superficial scalp posteriorly. Like the artery, the superficial temporal vein has parietal and frontal branches. The frontal vein communicates with the dural sinuses via a connection with the parietal emissary vein. This vein, found in the loose areolar connective tissue layer, runs superiorly along the lateral side of the head where it penetrates the cranium and communicates with the superior sagittal sinus.

The pterygoid venous plexus is responsible for draining the deep scalp. It is between the temporalis and lateral pterygoid muscles. The plexus is comprised of veins that are named after tributaries of the maxillary artery. These veins include the middle meningeal, sphenopalatine, buccinator, pterygoid, deep temporal, masseteric, infraorbital, and alveolar veins. The pterygoid

plexus also has a communicating vein that travels through the inferior orbital fissure to connect the cavernous sinus to the ophthalmic vein. The plexus eventually drains in the maxillary vein (Germann *et al.*, 2023). The lymphatic vessels of the scalp are located in the subcutaneous connective tissue layer and follow the venous drainage. In general, the anterior portions of the scalp drain through the parotid nodes, which continue to drain through the deep cervical and submandibular lymph nodes. The scalp posterior to the auricle drains to the occipital and posterior auricular (mastoid) lymph nodes. The mastoid lymph nodes specifically drain the area of the scalp located directly posterior to the ear and drain into the occipital lymph nodes. The occipital lymph node drainage covers the rest of the posterior scalp (Germann *et al.*, 2023).

### **2.3 Infections of Scalp Hair**

Although hair and scalp disorders generally are not associated with significant physical morbidity, the psychological impact of visible scalp problems may be very high. In human societies, hair now plays an important role in appearance and sexual signaling to which the original functional roles of protection and heat conservation are secondary, and changes in the appearance of skin and hair affect self-esteem and confidence in social settings. It should also be recognized that scalp changes in some cases may be a sign of a more substantial medical problem, so correct diagnosis is important.

The scalp is unique among skin areas in humans, with high follicular density and a high rate of sebum production. Fingers, combs, hats, styling implements come into contact with the hair and scalp and can introduce microorganisms, increasing the likelihood of infections and infestations. The dark and warm environment of the scalp surface is favorable for the superficial mycotic infections that play a role in dandruff, seborrheic dermatitis, and tinea capitis, and for parasitic infestations such as pediculosis capitis. Scalp changes may also be seen in inflammatory

conditions such as psoriasis. The similarities in clinical signs and symptoms of many scalp conditions can complicate accurate diagnosis. This paper describes the etiology, signs and symptoms, and treatment strategies for these common scalp conditions.

### **2.3.1 Dandruff and Seborrheic Dermatitis**

Dandruff (or pityriasis capitis) and seborrheic dermatitis may be considered to be the same condition, yet on different ends of the disease severity spectrum. Both have been linked to the lipophilic, putative yeast, *Malassezia*, previously known as *Pityrosporum* (Shuster, 1984) . Dandruff and seborrheic dermatitis are extremely common, affecting close to 50% of the world's population (Cardin, 1998). They are overwhelmingly afflictions of adults, occurring most commonly between the ages of 15 and 50 years and very rarely before adolescence, although seborrheic dermatitis can continue to occur in extreme old age. Although dandruff and seborrheic dermatitis usually become apparent during the second and third decades following increased sebum production at the onset of puberty, the severity and duration of the symptoms vary widely. The prevalence is higher in immuno-compromised patients than in healthy adults (Smith *et al.*, 1994). For example, seborrheic dermatitis has been reported to occur in 3–5% of immunocompetent adults, compared with 30–33% of AIDS patients (Farthing and Staughtom, 1985).

### **2.3.2 Etiology of Dandruff and Seborrheic Dermatitis**

Research in recent years has provided an increased understanding of the etiology of dandruff and seborrheic dermatitis, with recognition that microbial activity plays a key role in the development of these conditions. As noted above, dandruff and seborrheic dermatitis have been linked to yeasts of the species *Malassezia* (previously *Pityrosporum*). *Malassezia globosa* and *Malassezia restricta* have been identified as the predominant fungal species present on both

normal and dandruff-affected scalps (Gemmer *et al.*, 2002). The lipophilic yeast is believed to digest sebaceous triglycerides, producing free fatty acids such as oleic acid. The free fatty acids penetrate the stratum corneum and disrupt the skin barrier function (Schwartz *et al.*, 2004), leading to the range of typical symptoms.

### **2..3.3 Treatment strategies**

Before the recognition of the role of *Malassezia* in dandruff etiology, it was hypothesized that seborrheic dermatitis was akin to psoriasis; however, success with antifungal treatments differentiated those suffering seborrheic dermatitis from those with psoriasis (Aron-Brunetiere *et al.*, 1977). Dandruff and seborrheic dermatitis can be treated with products that have both efficacy and cosmetic benefits, and can be conveniently incorporated into a patient's routine hair-care regimen. Treatments to control dandruff and seborrheic dermatitis can be divided into three main classes on the basis of their mechanisms of action; these include keratolytic, antimicrobial, and antiproliferative agents. Simple shampooing and keratolytic treatments (e.g., salicylic acid) will remove a considerable proportion of flakes in patients with milder conditions. The majority of commercially available treatments for dandruff and seborrheic dermatitis contain antifungal agents. These treatments (e.g., pyridione zinc, selenium sulfide, ketoconazole, and ciclopirox) have been shown to improve the visible symptom of flaking and restore the underlying skin condition (Warner *et al.*, 2001). Antiproliferatives (e.g., coal tar) decrease epidermal proliferation and dermal infiltrates (Schwartz *et al.*, 2004). Adjunctive treatment with topical steroids may also be helpful in patients whose condition includes evidence of an inflammatory component. Given that many dandruff and seborrheic dermatitis patients may require regular, long-term use of therapeutic agents, it is important that the treatments be formulated so as to be aesthetically and cosmetically acceptable to the patient.

### **2.3.4 Tinea Capitis**

common mycotic condition of the scalp is tinea capitis, which is also known as ringworm of the scalp because of the characteristic ring-like rash that develops on infected skin. This dermatophytosis is especially common in childhood, unlike dandruff and seborrheic dermatitis, which are more prevalent in adults. Ringworm can be spread by exposure to desquamated cells or through contact with infected people, animals, or soil (Hainer, 2003).

### **2.3.5 Clinical Features**

Symptoms of tinea capitis include well-demarcated or irregular alopecia, scaling, pruritus, and broken hairs. Sporulation inside the hair shaft causes breakage of the hair near the scalp surfaces, leading to “black dot” alopecia (Hainer, 2003). The infection can be inflammatory or noninflammatory. The inflammatory form may produce kerions, boggy, inflammatory scalp masses; these lesions are most often associated with infection by *Trichophyton mentagrophytes* or *Trichophyton verrucosum*. Without early treatment, scarring alopecia may result from kerion formation (Arenas *et al.*, 2006).

### **2.3.6 Etiology of Tinea Capitis**

Dermatophytes are grouped into three genera: *Microsporum*, *Trichophyton*, and *Epidermophyton*. Unlike the dandruff-related *Malassezia*, dermatophytes require keratin for growth and are able to invade the hair shaft. *Microsporum* and *Trichophyton* are the genera most commonly associated with tinea capitis, although the prevalent species will vary by geography and over time. Dermatophytes can be grouped according to their preferred habitat: anthropophilic (infecting humans), zoophilic (infecting animals), or geophilic (infecting soil). Members of all of these groups can infect hair, explaining the vulnerability of humans to infection resulting from

exposure to infected humans, animals, or soil. Wood's lamp examination is useful for detection of *Microsporum*-related tinea capitis cases, as these infections will emit green light upon UV illumination. The majority of tinea capitis cases, however, can be linked to Trichophyten species (Abdel-Rahman and Nahata, 1997), which are nonfluorescent and can be more challenging to diagnose. Recent diagnostic advances use molecular biology techniques, such as PCR analysis of the DNA sequences of nuclear ribosomal components, to identify specific species (Yoshida *et al.* 2006).

### **2.3.7 Treatment Strategies**

Systemic treatments for ringworm are considerably more effective than topical treatments, as the antifungal remedy needs to penetrate into the hair follicle. The most common regimen consists of prolonged dosing with griseofulvin, with addition of topical steroids if needed to control inflammation (Elewski, 2000). Adjunctive topical antifungal therapy, such as shampoos containing pyrithione zinc, selenium sulfide, or ketoconazole, can decrease the number of viable fungi shed from an infected scalp (Higgins *et al.*, 2000) and reduce the risk of transmission of the infection to other people.

### **2.3.8 Psoriasis**

Psoriasis is a chronic, relapsing inflammatory disease that affects at least 2% of the population worldwide, with 50% of those cases involving the scalp (Sinclair *et al.*, 1999). The lack of UV exposure and frequency of friction injury to the scalp may contribute to the scalp's propensity to develop clinically evident psoriatic features (Elewski, 2005). Psoriasis observed on the scalp could be an indication of psoriatic arthritis, as anywhere from 6 to 39% of those with psoriasis develop inflammation of the joints (Myers *et al.*, 2006).

### **2.3.9 Symptoms of Psoriasis**

Psoriasis of the scalp most commonly presents as well-circumscribed, red, scaly plaques, and papules covered by a silver-gray scale. Similar lesions may appear on other body parts, which can aid in diagnosis. Pruritus and burning may accompany the lesions and the severity can fluctuate with time. Hair shafts may appear funneled together, producing what is known as the “tepee sign” (DeVillez, 1994). Hair shafts may also be dry and brittle, and, in some cases, the disease process leads to telogen effluvium, causing extensive hair loss (Comaish, 1969). Videodermoscopy has recently been used clinically to assess features of psoriasis. This technique revealed an extensive array of red dots, believed to relate to tortuous capillaries in the dermal papilla, in all cases of psoriasis. This approach may offer a new diagnostic option for assessment of clinically challenging cases (Ross *et al.*, 2006).

### **2.3.10 Etiology**

The etiology of psoriasis is not clearly understood; however, individual genetic predisposition is generally acknowledged to play a role (Mrowietz *et al.*, 2006). Therefore, a complete family history should be taken and evaluated with regard to previous skin and rheumatologic conditions. Like seborrheic dermatitis, psoriasis involves hyperproliferation, or rapid cell turnover in the epidermis (Sinclair *et al.*, 1999).

### **2.3.11 Treatment Strategies**

Psoriasis is often a life-long condition that warrants long-term treatment strategies and it can be difficult to treat. Shampoos containing keratolytics, such as salicylic acid, can be useful for assisting in the removal of built-up scales. Other common treatments include corticosteroids, vitamin D3 analogs, retinoids, topical coal tar preparations, anthralin, phototherapy, and

immunobiologic agents. Many of these agents are either unpleasant to use (e.g., coal tar shampoos) or can be associated with adverse effects (e.g., drug therapies). Topical treatments are common for milder forms of psoriasis, whereas phototherapy and systemic treatments are used for more severe cases (Gottlieb, 2005). It is commonly thought that tachyphylaxis, the decreasing response to a drug after administration of a few doses, is often seen during treatment with corticosteroids (du Vivier and Stoughton, 1975). However, a recent report suggests that the failure of corticosteroids to clear psoriasis may not be tachyphylaxis but, instead, related to therapeutic efficacy and/or patient compliance (Miller *et al.* 1999).

### **2.3.12 Pediculosis Capitis**

Pediculosis capitis, otherwise known as head lice, is the infestation of the scalp and hair by *Pediculus humanus capitis*; this infestation afflicts millions of people worldwide. It occurs across all socioeconomic groups and may be more common in crowded urban areas (Orion *et al.*, 2006). Girls aged 3–12 are affected most often. In the United States, African Americans have a lower rate of infestation than other races, possibly due to the use of pomades and the curled-shape of the hair (Ko and Elston, 2004). Like tinea capitis, the prevalence is highest among school-aged children, and appears to be on the rise (Chosidow, 2000). Transmission occurs through shared combs and brushes and direct contact with infected hairs, headgear, pillows, and clothing.

### **2.3.13 Clinical Features**

In cases of pediculosis capitis, close visual examination of the scalp will reveal the eggs of the lice as small white nits adhering to the hair shafts, most commonly behind the ears and at the nape of the neck. Microscopic assessment of an infected hair can easily confirm the diagnosis. Movement of adult lice may be visible with the naked eye. However, the search for live adult

lice may take time as the majority of infected scalps may have no more than 10 adult insects (Orion *et al.*, 2006). Pruritus is consistently reported and can lead to excoriations and secondary infections. The infestation may be accompanied by erythema, and papules may also be observed on the back of the patient's neck (Powell *et al.*, 2002)

### **2.3.14 Etiology**

*Pediculus humanus capitis* are blood-sucking insects that live on the head of the host. A sheath 1–2 mm from the scalp envelops the nit and the hair shaft, so the nit is firmly cemented to the hair. Lice hatch within a week and mature to adults within the following week. The spread of the disease is most dependent on the matured adult lice (Ko and Elston, 2004).

### **2.3.15 Treatment Strategies**

Treatment involves a combination of chemical and mechanical approaches. Malathion, natural pyrethrins, permethrin, phenothrin, and lindane, are commonly used insecticides that can be delivered through shampoos or topical treatments. Emerging resistance of the insect to these chemicals is a concern, though, and resistance appears to vary with geography. Therefore, a combination of chemical agents with adjunctive mechanical treatment is common. Hair should be combed with a fine-toothed comb every 3–4 days for 2 weeks to remove lice as they hatch and before they reach maturity (Orion *et al.*, 2006). The environment must also be addressed to avoid reinfestation from contaminated hats, hair brushes, or bedding. People living in close proximity to infested individuals should also be examined and treated as appropriate to prevent the spread of lice.

## **2.4 Scalp Hair Hygiene**

Scalp hair hygiene is a significant aspect of personal grooming that is frequently overlooked. While individuals usually pay attention to the cleanliness of their body and facial skin, scalp hair

hygiene is often disregarded unless there is a noticeable issue. Scalp hair hygiene refers to keeping the scalp and the hair on it clean and well-maintained, preventing the accumulation of dirt, grease, and dead skin cells. It entails various cleansing and care practices for scalp hair, with shampooing being the most common. The scalp, similar to facial skin, is susceptible to sweat, oil, and dirt, and should be cleaned regularly. However, the frequency and methods of hair hygiene vary culturally, with some groups considering hair hygiene less important than other cleanliness practices (Watanabe *et al.*, 2021). Nonetheless, hair hygiene is essential to ensure healthy and well-maintained hair.

Neglecting hair hygiene can result in various issues such as an unkempt appearance, unpleasant odors, and potential scalp infections. One of the main functions of scalp hair hygiene is to clean the scalp, which supports hair growth. A clean scalp is free from sweat, dirt, and excess oil, allowing hair follicles to receive sufficient nutrients and preventing blockages that can lead to hair loss. Thorough hair cleansing also removes residues from hair care products that can accumulate if scalp hair hygiene is neglected. Clogged hair follicles due to product buildup may also cause hair loss. Poor hair hygiene can lead to excessive oiliness, as hair care products tend to accumulate on unwashed hair, making it greasy. Other issues may arise from excessive cleaning. Sharply scented or alcohol-based hair products may cause scalp irritation and dermatitis, while excessive shampooing may damage hair due to the removal of natural oils (Widaty *et al.*, 2023).

## **2.5 Factors Affecting Scalp Health**

scalp is an extension of skin on the head that is covered by hair. The skin comprises different anatomical regions with unique structural, physiological, and developmental features. Scalp skin has several specific attributes, such as a higher number of hair follicles, greater sebum

production, and a unique microbiome composition, such as the predominance of the genus *Malassezia*. Scalp health is essential for maintaining the integrity of hair follicles and hair growth (Saxena *et al.*, 2021). Therefore, scalp disease often leads to abnormal hair loss, such as in androgenetic alopecia and alopecia areata. Scalp skin disease is often misdiagnosed as facial skin disease as it is a distinct anatomical region. Scalp skin disorders can be divided into scaly and non-scaly disorders. Dandruff, seborrheic dermatitis, and psoriasis are common scaly scalp skin diseases, whereas scalp folliculitis, atopic dermatitis, and lichen planopilaris are examples of non-scaly diseases.

The human scalp is naturally colonized by diverse microbial communities, including bacteria, archaea, fungi, and viruses. A certain composition and stability of the microbiome are critical for the health and homeostasis of the scalp. An unbalanced microbiome composition (dysbiosis) has been linked to scalp (and skin) diseases. Some widely used anti-dandruff agents mitigate scalp skin disease by targeting fungal dysbiosis. Recent metagenomic studies have reported significant differences in the microbiome composition of normal and diseased scalps (Pinto *et al.*, 2020). However, almost all studies investigating the scalp microbiome have focused on diseased versus healthy states, and the microbiome composition of “normal” scalp conditions has remained poorly characterized.

## **CHAPTER THREE**

### **MATERIALS AND METHOD**

#### **3.1 Location of the Research**

This study was carried out at the Laboratory Department of Microbiology, Faculty of Life Science, University of Benin.

#### **3.2 Collection of samples**

With the aid of a sterile swab sticks, nine scalp hair of undergraduates' students were swabbed and transported to the laboratory for bacteriological analysis. All the samples were analyzed within 24hrs of collection.

#### **3.3 Dilution Technique**

Collected swabbed stick was submerged in a sterile test tubes label A – I contained Nutrient broth and incubated for 2hrs. 1ml was plated from each test tube. It was then incubated at 37<sup>0</sup> C for 24hours.

#### **3.4 Standardization of the Isolates**

MacFarland standard (0.5) was prepared by mixing 0.05ml of 1% barium chloride (BaCl<sub>2</sub>) with 9.95ml of 1% Sulfuric acid(H<sub>2</sub>SO<sub>4</sub>) to barium sulphate suspension. The turbid solution (McFarland standard) formed was transferred into a test tube for comparison with bacterial inoculums suspension (Cheesbrough, 2006).

#### **3.5 Susceptibility Test**

Kirby-bauer disc diffusion technique was used as to determine the antibacterial activity of isolated bacteria. 20ml Mueller Hinton agar plates were prepared following the manufacturer's instructions. 1ml aliquot of each test organism suspension (standardized) was transferred onto the Mueller Hinton agar plates and was spread evenly following slow rotation of the plates and

excess was decanted. The plates were allowed to dry, with the aid of sterile forcep, antibiotic disc were placed in the well-dried Mueller Hinton agar plates. Zones were interpreted using CLSI standard. (Cheesbrough, 2002).

### **3.6 Hemolysis**

Pure culture of the bacteria isolated were grown on the surface of 5% defibrinated sheep blood agar that is made with nutrient agar and incubated at 37 degree Celsius for 72hrs. Lysing of the red blood cells is indicated by a clear zone around the inoculum spot is indicative of haemolysin production (Ristow and Welch, 2016).

### **3.7 Gelatinase production test**

Pure culture of the bacteria isolated were grown on the surface of Nutrient agar enrich with 2% gelatin and incubated at 37 degree Celsius for 72hrs. A clear zone around the inoculum spot indicated a breakdown of gelatin by the enzyme gelatinase.

## CHAPTER FOUR

### RESULTS

#### 4.1 Result of The Total Bacteria Count of Scalp hair

The result presented in Table 1, shows the Total heterotrophic bacteria count of nine (9) scalp hair samples. The count ranged from  $2.1 \times 10^2$  cfu/ml –  $5.2 \times 10^2$  cfu/ml. Sample A had the least bacterial count while the highest count was observed in Sample D. The reason for this high significant count difference could be trace down to bad hair condition used and personal hygiene.

Table 1: Total bacteria count of undergraduate's scalp hair

Samples	Total bacteria count CFU/ml ( $\times 10^2$ )
A	$5.2 \pm 0.035$
B	$3.0 \pm 0.028$
C	$2.6 \pm 0.014$
D	$2.1 \pm 0.014$
E	$3.9 \pm 0.014$
F	$2.6 \pm 0.014$
G	$2.9 \pm 0.007$
H	$2.6 \pm 0.021$
I	$3.1 \pm 0.014$

KEY:

A – Sample 1    H – Sample 8

B – Sample 2    I – Sample 9

C – Sample 3

D – Sample 4

E – Sample 5

F – Sample 6

G – Sample 7

#### **4.2 Prevalence of Bacteria isolated from scalp hair**

The result of the prevalence of bacteria isolated from scalp hair of undergraduate's students is shown in Table 2. Which include, *Staphylococcus epidermis* 6 (15%), *Staphylococcus aureus* 6 (15%), *Pseudomonas* sp. 2(5%), *Bacillus* sp. 18(45%), *Streptococcus* spp. 5(12.5%) and *Micrococcus* spp. 3(7.5%)

Table 2: Prevalence of bacteria Isolated from undergraduate's scalp hair

Isolates	Percentage occurrence
<i>Staphylococcus epidermis</i>	6 (15)
<i>Staphylococcus aureus</i>	6 (15)
<i>Pseudomonas sp.</i>	2 (5)
<i>Bacillus sp.</i>	18(45)
<i>Streptococcus spp.</i>	5 (12.5)
<i>Micrococcus spp.</i>	3 (7.5)
Total	40 (100)

### **4.3 Virulence factors of the bacteria isolated from scalp hair**

The result of the haemolysin test on the strains of *Staphylococcus aureus* *Streptococcus* sp. *Staphylococcus epidermis*, *Micrococcus* spp. and *Pseudomonas* sp. revealed they had  $\beta$  hemolytic activity and only strains of *Bacillus* spp. showed  $\alpha$  hemolytic activity. While the gelatin test showed that all isolates produce gelatinase enzyme which breakdown gelatin. Haemolysin and gelatinase activity has been regarded as a virulence factor.

**Table 3:** Virulence factors of the bacteria isolates

<b>Isolates</b>	<b>Haemolysin Type</b>	<b>Gelatinase production</b>
<i>Staphylococcus aureus</i>	$\beta$ -haemolysin	+
<i>Bacillus</i> sp.	$\alpha$ -hemolysis	+
<i>Pseudomonas</i> sp.	$\beta$ -haemolysin	+
<i>Streptococcus</i> spp.	$\beta$ -haemolysin	+
<i>Micrococcus</i> sp.	$\beta$ -haemolysin	+
<i>Staphylococcus epidermis</i>	$\beta$ -haemolysin	+

Table 4: Antibiotic resistance patterns of bacterial isolates recovered from undergraduate hair samples.

Isolates	n(%)	Antibacterial Agent							
		CIP	GEN	E	VA	OF	CD	C	TE
<i>Pseudomonas</i> sp.	2(5)	2(100)	2(100)	2(100)	0(0.0)	2(100)	2(100)	2(100)	2(100)
<i>S. Aureus</i>	6(15)	6(100)	0(0.0)	6(100)	3(0.0)	6(100)	6(100)	6(100)	3(50)
<i>S. epidermis</i>	6(15)	6(100)	0(0.0)	6(100)	6(0.0)	6(100)	6(100)	6(100)	3(50)
<i>Streptococcus</i> spp.	5(12.5)	5(100)	0(0.0)	5(100)	0(0.0)	5(100)	5(100)	5(100)	0(0.0)
<i>Micrococcus</i> spp.	3(7.5)	3(100)	0(0.0)	3(100)	0(0.0)	3(100)	3(100)	3(100)	3(100)
<i>Bacillus</i> spp.	18(45)	18(100)	10(55)	10(55)	0(0.0)	18(100)	18(100)	18(100)	12(66)
Total	40(100)								

**KEY:** E-Erythromycin, C-Chloramphenicol, GEN-Gentamycin, TE-Tetracycline, CIP-Ciprofloxacin, VA-Vancomycin, CD-Clindamycin, OF- Ofloxacin

## CHAPTER FIVE

### DISCUSSION

The scalp hair is composed of soft tissue layers that cover the cranium. It is an anatomic region bordered anteriorly by the human face, and laterally and posteriorly by the neck. It extends from the superior nuchal lines and occipital turbulences to the supraorbital foramen. Aesthetically, it serves as an area where hair can grow and physically, as a barrier that defends the body from foreign irritation. Scalp hair hygiene is a significant aspect of personal grooming that is frequently overlooked. While individuals usually pay attention to the cleanliness of their body and facial skin, scalp hair hygiene is often disregarded unless there is a noticeable issue. Scalp hair hygiene refers to keeping the scalp and the hair on it clean and well-maintained, preventing the accumulation of dirt, grease, and dead skin cells. This research focused on isolation and identification as well as antibacterial susceptibility pattern and virulence factors of bacterial isolates from undergraduates scalp hair. The result of this study shows that the total heterotrophic bacterial count from scalp hair samples ranged from  $2.1 \times 10^2$  CFU/ml –  $5.2 \times 10^2$  CFU/ml as shown in Table 1. The high microbial count recorded in this study reflects the high level of contamination of the student's scalp hair and can be attributed to poor personal hygiene as well as lack of hair condition (Mathieu *et al.*, 2013). The mean bacterial counts obtained were lower than those reported by Aram *et al.*, (2010), where mean bacterial counts of plate samples ranged from  $1.20 \times 10^4$  –  $7.2 \times 10^5$  CFU/ml from scalp hair.

Forty (40) species of bacteria were isolated from scalp hair. The bacteria isolated from this study, include; *Staphylococcus aureus* *Streptococcus* sp. *Staphylococcus epidermis*, *Micrococcus* spp. *Bacillus* spp. and *Psuedomonas* sp. Identification of the isolates was confirmed by cultural,

microscopic and biochemical tests, which is in consonance with the findings of Sperling, (2001). The result of the haemolysin test on the strains of *Staphylococcus aureus* *Streptococcus* sp. *Staphylococcus epidermis*, *Micrococcus* spp. and *Pseudomonas* sp. revealed they had  $\beta$  hemolytic activity and only strains of *Bacillus* spp. showed  $\alpha$  hemolytic activity. Haemolysin production has been regarded as a virulence factor (seker, 2010). Pickett *et al.* (1992) reports that haemolysin production is strain dependent, as could be seen from the results of this study, with some strains producing little to no hemolysin. According to the study of Tay *et al.* (1995), the exhibition of haemolytic activity in the majority of campylobacters examined suggests that this cell associated activity could be a potential virulence factor responsible for *Campylobacter* gastroenteritis. The production of haemolysin is a process utilized by microbes to obtain iron for bacterial growth and the expression of virulence factors (Miyamoto *et al.*, 1969). The contact haemolytic activity of *Shigella* spp. was reported to be a contributing factor in the lysis of the phagocytic vacuole for the release of virulent shigellae into the host cytoplasm, where replication occurs (Baudry *et al.*, 1987).

All isolates were highly sensitive to ciprofloxacin, Ofloxacin and clindamycin 40(100%). Ciprofloxacin, Ofloxacin and clindamycin was proved to be the most effective against Gram positive and Gram-negative isolates studied in this work. This agrees with the findings of Geoffrey *et al.* (2013) in which the isolated Gram positive and Gram-negative isolates all showed (100%) sensitivity towards ciprofloxacin. This could be attributed to the broad spectrum of activity of this antibiotic (Arora, 2008). Antibacterial susceptibility testing was done by the Kirby-Bauer disc diffusion method. High resistance to antibiotics like vancomycin, gentamycin and tetracycline was recorded in all isolates. The resistance exhibited by the isolates against some of the conventional antibiotics could be attributed to the ability of these organisms to

acquire mechanisms which might be genetic or acquired features, which allow them to resist the action of the antibiotics. Similar conclusions were drawn by other researchers (Deizell and Laferre, 2000; Bockitwetan *et al.* 2012). Antibiotics resistance has increased worldwide leading to failures in the treatment of human infectious disease (Afroz *et al.*, 2013). Resistance to antibiotics could be as a result of enzymatic inactivation or modification of antimicrobial agents, impermeability of the cell wall or cell membrane, expulsion of the drugs through the efflux pump or alteration of the target receptors (Prescott *et al.* 2002). Contamination of scalp hair from this research, could be from poor hand hygiene and environmental hygiene. I recommend strict management policy in Nigeria against misuse of antibiotics and the general public should be aware of the effects of the misuse of antimicrobials, resistant species persist because of indiscriminate use of antibiotics, hence the development of virulent genes and inability to treat infections caused by bacterial.

## **CONCLUSION**

This study revealed the most common bacterial pathogens found on the scalp hair of undergraduates that could possibly cause infection if proper treatment or hair hygiene is not taken. I recommend better hygiene practices (e.g., regular washing of hair with antimicrobial shampoos) and interventions to prevent scalp infections.

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## APPENDIX ONE

Table 4: Cultural characteristics, Morphological characteristics, Biochemical characteristics of bacterial isolate from cucumber

Cultural characteristics					
Colour	Cream	cream	Cream	Cream	Cream
Shape	Round	Circular	Round	Round	Circular
Elevation	Raise	Raise	Raise	Raise	Raise
Margin	Smooth	Entire	Smooth	Smooth	Entire
Size	medium	Large	medium	medium	Small
Morphological characteristics					
KOH	-ve	-ve	-ve	-ve	+ve
Gram stain	+ve	+ve	+ve	+ve	-ve
Cell morphology	Cocci	Rod	Cocci	Cocci	Rod
Cell arrangement	Cluster	Dispersed	Cluster	Cluster	Singly
Biochemical characteristics					
Catalase	+	+	+	+	+
Indole	-	+	-	-	+
Oxidase	+	-	+	+	-
Voges-proskauer	+	Variable	+	+	
Spore forming	-	-	-	-	+
Glucose	+	+	+	+	+
Lactose	+	-	+	+	+
Sucrose	+	+	+	+	+
Mannitol	+	-	+	+	+
H <sub>2</sub> S production	-	+	-	-	-
Identity	<i>S.aureus</i>	<i>Psuedomonas sp.</i>	<i>S.epidermis</i>	<i>Micrococcus sp.</i>	<i>Bacillus sp.</i>

## **APPENDIX TWO**

### **PREPARATION OF MEDIA**

**Nutrient Agar** 28g of powdered nutrient agar were dissolved in 1000ml of deionized water allowed to soak for 10minutes and then sterilized with an autoclaving for 15minutes at 121<sup>0</sup>C allowed to cool and pour into petri dishes.

### **Eosin Methylene Blue**

37.5g of powdered EMB agar was dissolved in 1000ml of deionized water. Allow to soak for 10minute, swirl to mix and sterilize by autoclaving at 121<sup>0</sup>C for 15minutes. Allow to cool at 47<sup>0</sup>C and pour into petri dishes.

### **Peptone water / Broth**

3.8g of powdered peptone water was dissolved in 280ml of distilled water allow to soak for 10minutes and 5ml was dispensed into 5 labelled sterile test-tubes then sterilized by autoclaving for 15minutes at 121<sup>0</sup>C

### **Thio sulphate citrate Bile Salt (TCBS)**

88g of powdered TCBS cholera medium agar was dissolved in 1000ml of deionized water, allow to soak for 10minutes, swirl to mix the bring to the boil and cool to 45<sup>0</sup>C and pour into petri dishes

### **Sammon citrate Agar**

24g of powdered SCA was dissolved in 1000ml of deionized water, soak for 10minutes, swirl to mix. Dispense into test tubes by adding 5ml and sterilized by autoclaving at 121<sup>0</sup>C for 15minutes. The medium is set as a slope ensuring that the slant is over a butt about 3cm deep.

### **Procedure for sub culturing**

Pure isolates were obtained by selecting discrete colonies and having them subcultured onto petri dishes containing freshly prepared NA media. The bacteria isolates were also transferred by streak method onto free plates respectively.

## **MORPHOLOGICAL IDENTIFICATION**

### **Gram Staining**

1. A thin smear was prepared on clean glass allowed to air dried and then flame it.
2. The smear was stained with crystal violet for 60 seconds.
3. Rapidly wash off the stain with clean water for 5 seconds.
4. Tip off all the water and cover the smear with lugol's iodine for 60 seconds and washed off under slowly running tap.
5. Decolourized using 90% ethanol and washed immediately with clean water.
6. The smear was covered with safranin reagent for 30 seconds then washed off the stain slowly under running tap.
7. The slide was blot dry using paper towel.
8. The strained cell were examined microscopically with oil immersion using only 100 objective lens.
9. Gram positive cells stain purple while gram negative cells stain pink or red.

### **Cultural characteristics**

Cultural characteristics were observed on nutrient agar for the bacterial isolates. The cultural characteristics include size, shape, transparency, elevation, margin, and colour.

### **Gram staining**

The gram staining technique was used for differentiation between gram positive and negative bacterial stains according to Benson (1994). A drop of distilled sterile water was placed on a neat and clean glass slide and a single isolated colony of 24h was picked with a sterile wire loop and used to make a smear on the drop of sterile water. This smear was air dried and rapidly fixed by passing the slide three times over the flame. It was then flooded with crystal violet for min and then washed off with distilled water. Then iodine solution was added to the

smear and left for another 1 min, this serves as a mordant after which it was rinsed off with distilled water. This step was followed by the application of a decolorizing agent (ethanol) for 30sec and washed with distilled water. The smear was counter stained with safranin for one minute. The slide was rinsed with distilled water, air dried and observed under the microscope.

### **Catalase production test**

1ml of hydrogen peroxide solution was discharged into a clean glass slide and a sterile isolating

loop was used to collect the colonies of the test organism which were subsequently immersed in the hydrogen peroxide solution. A positive result was indicated by the production of gas bubbles while its absence was regarded as negative.

### **Citrate utilisation test**

5ml of Simmons citrate broth was inoculated with the test organism. The broth was incubated at 37°C for 48hrs. A positive result was indicated by the change in colour of the medium from green to blue negative tubes remained green.

### **Indole test**

The test organism was inoculated into sterile test tubes containing sterile peptone water, incubated for at 37°C for 48hrs followed by the addition of kovacs reagent. Red ring on the surface layer within 10min indicated positive test for indolence (Cheesebrough,2000).

### **Oxidase test**

Using a sterile wire loop, a colony of the test organism was picked and a smear made on a filter paper. 2 drops of freshly prepared oxidase reagent was placed on it. A positive result was

indicated by the appearance of a deep purple colour within 10sec. (Cheesbrough,2000)

### **Urease production test**

This was carried out by inoculation of the colonies of the test organism into the urea slopes and

incubated at 37°C for 24h. A colour change from yellow to pink indicated a positive response (Cheesbrough, 2000).

### **Coagulase test**

A drop of normal saline was put on the slide and a colony of the test organism was emulsified on the saline. A drop of plasma was added and mixed gently. After 10secs, it was observed for clotting (Cheesbrough, 2000). The presence of agglutination was indicative of a positive result while its absence indicated a negative result

## APPENDIX II

### CULTURE MEDIA

#### SALMONELLA, SHIGELLA AGAR (SSA)

Lab-lemco powder	5.0g/L
Peptone	5.0g/L
Lactose	10.0g/L
Bile salts	8.5g/l
Sodium citrate	10.0g/L
Sodium thiosulphate	8.5g/l
Ferric citrate	1.0g/L
Brilliantgreen	0.00033g/L
Neutral red	0.025g/L
Agar	15.0g/L

#### BILE AESCULIN AGAR

Peptone	14.0g/L
Bile salts	15.0g/L
Ferric citrate	0.5g/L
Aesculin	1.0g/L
Agar	15.0g/L

#### MUELLER-HINTON AGAR

Dehydrated infusion from beef	300.0g/L
Casein	17.5g/L
Starch	1.5g/L
Agar	17.0g/L

#### MAC-CONKEY AGAR

##### *Formula gm/litre*

Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0
pH	7.4 ± 0.2

## **GRAM STAINING AND BIOCHEMICAL REAGENTS**

### **STAIN AND REAGENT**

#### **Gram stain**

The Gram stain was prepared using two stains (crystal violet and safranin or carbol-fuchsin), Gram's iodine, and a decolorizing agent (ethyl alcohol).

#### **A. Gram crystal violet**

##### **Solution A**

Crystal violet - 2.0 g

Dissolved in ethanol (95%) - 20.0 ml

##### **Solution B**

Ammonium oxalate - 0.8 g

Distilled water - 80.0 ml

#### **Gram iodine**

Iodine (crystalline) - 1.0 g

Potassium - 2.0 g

Distilled water - 300.0 ml

3.0g of medium was dissolved in 300.0 ml of distilled water.

It is very important to note that; crystalline iodine, potassium and distilled water were combined to produce iodine solution and that Gram's iodine solution was stored in a dark bottle and protected from light so that it does not degrade.

#### **Decolorizer**

95 % ethyl alcohol was used.

#### **Gram safranin**

Safranin-O (certified) - 0.25 g

Ethyl alcohol (95 %) - 100.0 ml

*Working solution:*

Safranin stock solution – 10.0ml

Distilled water – 90.0 ml

### **Biochemical reagents**

#### **Indole medium**

Peptone – 20.0 g

Sodium chloride – 5.0 g

Distilled water – 1000 ml

pH – 7.4

25.0 g of indole medium was dissolved in 1000 ml of distilled water and autoclaved for 15 min at 121 °C and dispensed aseptically into sterile test tubes.

#### **Oxidase reagent (Kovac's oxidase)**

Amul-alcohol – 15.0 ml

p-dimethyl-aminobenzaldehyde – 0.5 ml

Concentrated HCl – 50ml

Small quantity of Kovac's reagent was prepared by dissolving the aldehyde into alcohol and adding the acid slowly and then kept inside the refrigerator.

#### **Catalase test**

3% Hydrogen peroxide

**APPENDIX III**  
**ANTIBACTERIAL SUSCEPTIBILITY STANDARD**

CLSI		Disk content	Diameter Zone of inhibition			
Antibiotics	Short code	µg	S	I	R	Organisms
1. Meropenem	MEM	10	≥23	20-22	≤19	Enterobacteriaceae
			≥19	16-18	≤15	<i>Pseudomonas aeruginosa</i>
			≥18	14-17	≤13	<i>Acinetobacter spp.</i>
		-	-	-	-	Non-enterobacteriaceae
		10	≥20	-	-	<i>Haemophilus influenzae</i> & <i>H. parainfluenzae</i>
		-	-	-	-	<i>Streptococcus pneumoniae</i>
		-	-	-	-	<i>Streptococcus spp. β – Hemolytic Group</i>
		-	-	-	-	<i>Streptococcus spp. viridans Group</i>
		10	≥30	-	-	<i>Neisseria meningitidis</i>
		-	≥4	8	≥16	Anaerobes
2. Gentamycin	CN	10	≥15	13-14	≤12	Enterobacteriaceae
			≥15	13-14	≤12	<i>Pseudomonas aeruginosa</i>
			≥15	13-14	≤12	<i>Acinetobacter spp.</i>
		-	-	-	-	Non-enterobacteriaceae
			≥15	13-14	≤12	<i>Staphylococcus spp.</i>
3. Vancomycin	VA	-	-	-	-	<i>Staphylococcus spp.</i>
		30	≥17	15-16	≤14	<i>Enterococcus spp.</i>
			≥17	-	-	<i>Streptococcus pneumoniae</i>
			≥17	-	-	<i>Streptococcus spp. β – Hemolytic Group</i>
			≥17	-	-	<i>Streptococcus spp. viridans Group</i>
4. Ciprofloxacin	CIP	5	≥31	21-30	≤20	Enterobacteriaceae
			≥21	16-20	≤15	<i>Pseudomonas aeruginosa</i>
			≥21	16-20	≤15	<i>Acinetobacter spp.</i>
		-	-	-	-	Other Non-Enterobacteriaceae
			≥21	16-20	≤15	<i>Staphylococcus spp.</i>
			≥21	16-20	≤15	<i>Enterococcus spp.</i>
			≥21	-	-	<i>Haemophilus influenzae</i> & <i>H. parainfluenzae</i>
			≥41	28-40	≤27	<i>Neisseria gonorrhoeae</i>
			≥35	33-34	≤32	<i>Neisseria meningitidis</i>

5. Azithromycin	AZM	15	≥13 ≥12 ≥18 ≥18 ≥18 ≥18 ≥20	- - 14-17 14-17 14-17 14-17 -	≤12 - ≤13 ≤13 ≤13 ≤13 -	Enterobacteriaceae <i>Haemophilus influenzae</i> & <i>H. parainfluenzae</i> <i>Streptococcus pneumoniae</i> <i>Staphylococcus spp</i> <i>Streptococcus spp. β – Hemolytic Group</i> <i>Streptococcus spp. viridans Group</i> <i>Neisseria meningitidis</i>
6. Sulfamethoxazole / Trimethoprim	RL	23.75 /1.25  - 23.75 /1.25	≥16 ≥16 ≥16 ≥16 - ≥16 ≥19 ≥16 ≥30	11-15 11-15 11-15 11-15 - 11-15 16-18 11-15 26-29	≤10 ≤10 ≤10 ≤10 - ≤10 ≤15 ≤10 ≤25	enterobacteriaceae <i>Acinetobacter spp</i> <i>Burkholderia cepacia complex</i> <i>Stenotrophomonas maltophilia</i> Other Non-Enterobacteriaceae <i>Staphylococcus spp</i> <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i> & <i>H. parainfluenzae</i> <i>Neisseria meningitidis</i>
7. Ceftriazone	CRO	30  - 30	≥23 ≥21 ≥26 - ≥27 ≥34	20-22 14-20 - - 25-26 -	≤19 ≤13 - - ≤24 -	Enterobacteriaceae <i>Acinetobacter spp</i> <i>Haemophilus influenzae</i> & <i>H. parainfluenzae</i> <i>Streptococcus pneumoniae</i> <i>Streptococcus spp. viridans Group</i> <i>Neisseria meningitidis</i>
8. Amoxicillin/Clavulinic acid	AMC	20/10 - 20/10 -	≥18 - ≥20 ≥4/2	14-17 - - 8/4	≤13 - ≤19 ≤16/8	Enterobacteriaceae <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i> & <i>H. parainfluenzae</i> Anaerobes

9. Erythromycin	E	15	≥23 ≥23 ≥21 ≥21 ≥21	14-22 14-22 16-20 16-20 16-20	≤13 ≤13 ≤15 ≤15 ≤15	<i>Staphylococcus spp</i> <i>Enterococcus spp</i> <i>Streptococcus pneumoniae</i> <i>Streptococcus spp. β – Hemolytic Group</i> <i>Streptococcus spp. viridans Group</i>
10. Ceftazidime	CAZ	30  - - 30	≥21 ≥18 ≥18 ≥21 - - ≥26 ≥31	18-20 15-17 15-17 18-20 - - - -	≤17 ≤14 ≤14 ≤17 - - - -	Enterobacteriaceae <i>Pseudomonas aeruginosa</i> <i>Acinetobacter spp</i> <i>Burkholderia cepacia complex</i> <i>Stenotrophomonas maltophilia</i> Other Non-Enterobacteriaceae <i>Haemophilus influenza &amp; H. parainfluenzae</i> <i>Neisseria gonorrhoeae</i>

Azithromycin (15 µg)

Gentamicin (10 µg)

Vancomycin (30 µg)

Ciprofloxacin (5 µg)

Ceftriazone (30 µg)

Sulfamethoxazole/Trimethoprim (25 µg)

Meropenem (10 µg)

Amoxicillin/Clavulanic acid (30 µg)

Ceftazidime (30 µg)

Erythromycin (15 µg)

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NAME	SEX	LEFT HAND	BREADTH	area	count	cfu	cfu/m <sup>2</sup>	RIGHT HAND	BREADTH	area	count	cfu/m <sup>2</sup>	cfu/m <sup>2</sup>	hand used
Vanessa	F	7	7	49.00	96	960	19.59	7	7	49.00	80	800	16.33	R
Esosa	F	7.5	7	52.50	88	880	16.76	8	8	64.00	150	1500	23.44	R
Ruth	F	7	6.5	45.50	120	1200	26.37	7	7	49.00	113	1130	23.06	R
John	M	8	7	56.00	21	210	3.75	8	7	56.00	49	490	8.75	R
Princess	F	8	7	56.00	11	110	1.96	8	7	56.00	7	70	1.25	R
John	M	8	7	56.00	300	3000	53.57	8	7	56.00	300	3000	53.57	R
Felix	M	8	7	56.00	60	600	10.71	8	7	56.00	8	80	1.43	R
Monday	M	7	7	49.00	90	900	18.37	7	7	49.00	8	80	1.63	R
Osas	M	9	8	72.00	35	350	4.86	9	8	72.00	100	1000	13.89	R
Felicia	F	7	6	42.00	40	400	9.52	7	6	42.00	1	10	0.24	R
Osarentin	F	7.5	7	52.50	2	20	0.38	7.5	7	52.50	4	40	0.76	R
Omon	M	8	7	56.00	2	20	0.36	7.5	7.5	56.25	9	90	1.60	R
Daniel	M	7	6.5	45.50	16	160	3.52	7	6	42.00	13	130	3.10	B
Amaka	F	6.5	6.5	42.25	20	200	4.73	6.5	6.5	42.25	8	80	1.89	R
matilda	F			0.00		0	#DIV/0!			0.00		0	#DIV/0!	R
Joy	F	7	6	42.00	130	1300	30.95	7	6	42.00	105	1050	25.00	R
Martins	M	9	8	72.00	2	20	0.28	9	8	72.00	1	10	0.14	R
Mordecai	M	8	8	64.00	3	30	0.47	8	8	64.00	6	60	0.94	R
Igwe	M	7	6.5	45.50	2	20	0.44	7	6.5	45.50	1	10	0.22	R
Sofia	F	7.5	7	52.50	15	150	2.86	7.5	7	52.50	114	1140	21.71	R
Divine	M	6	6.5	39.00	0	0	0.00	6	6.5	39.00	0	0	0.00	R
Taiye	M	9	8.5	76.50	18	180	2.35	9	8	72.00	300	3000	41.67	B
Jennifer	F	7	7	49.00	8	80	1.63	7	7	49.00	15	150	3.06	R
Ruth	F	9	8	72.00		0	0.00	8	7.5	60.00		0	0.00	B
Joshua	M	8	7.5	60.00	120	1200	20.00	8	7.5	60.00	4	40	0.67	R
King	M	9	8.5	76.50	3	30	0.39	9	8	72.00	3	30	0.42	R
Lordside	F	8.5	8.5	72.25	4	40	0.55	8.5	8.5	72.25	14	140	1.94	R
Joseph	M	8.5	8	68.00	30	300	4.41	8	8	64.00	18	180	2.81	R
Olivia	F	7	7	49.00	27	270	5.51	7	7	49.00	14	140	2.86	R
Priest	M	9	9	81.00	21	210	2.59	9	9	81.00	19	190	2.35	B
Salvation	M	8.5	8.5	72.25	0	0	0.00	8.5	8.5	72.25	0	0	0.00	R
Desmond	M	9	9	81.00		0	0.00	9	8	72.00		0	0.00	R
Nath	M	7.5	7.5	56.25	51	510	9.07	7.5	7.5	56.25	2	20	0.36	R
Peter	M	7	7	49.00		0	0.00	7	7	49.00		0	0.00	R
Comfort	F	7.5	7.5	56.25		0	0.00	7.5	7.5	56.25		0	0.00	R
Matthew	M	7	7	49.00		0	0.00	7	7	49.00		0	0.00	B

MRH	MLH	FRH	FLH
1.728933	0.57	1.212894	1.29
0.154902	1.73	1.369911	1.22
0.212894	0.69	1.362882	1.42
0.490694	0.56	0.118099	0.42
0.857332	0.33	0.277263	0.68
0.028029	0.36	1.39794	1.49
0.658011	0.37	1.336746	0.46
1.619789	1.30	0.485895	0.21
0.176091	0.41	0.28729	0.26
0.380211	0.64	0.455932	0.74
0.449093	0.41		