CHAPTER ONE

INTRODUCTION

The human hand represents one of the chief organs in the body that always interacts with the environment and as such a principal agent in the dissemination of pathogenic microflora from the environment to humans and *vice versa* (Ekrami *et al*., 2011). Microorganisms associated with hands can be grouped into transient or resident and usually form part of the normal flora (Burton *et al*., 2011). Transient micro-organisms are usually picked by the hands from surfaces such as gate pass, ATM machines, mobile phones, currency notes as well as door handles (Pittet *et al*., 2006). Non-living surfaces serve as environmental reservoirs for pathogen transmission, occurring either through direct contact with mucous membranes and broken skin or indirectly via the contamination of hands, which then transfer microbes to the eyes, nose, or mouth (Kraay *et al*., 2021). Coming into contact with these contaminated surfaces can result in illness within vulnerable individuals (Burton *et al*., 2011). These objects, known as fomites, facilitate the spread of infectious agents—including bacteria, viruses, and fungi—to new hosts (Abrar et al., 2016). Research into the prevalence and dispersal of these pathogens has highlighted the significant role fomites play in infection acquisition (Medrano-Félix *et al*., 2011). The risk of microbial transfer from surfaces to hands is especially critical in high-density environments where frequent human interaction occurs (Ahmed *et al*., 2019).

A gate pass serves as a vital plastic card designed to track vehicle entries and exits within an organization's premises. Issuing these passes helps maintain accurate records of all vehicles entering and leaving designated areas. As noted by Aliyu *et al.* (2018), this card plays a key

role in deterring vehicle theft and protecting organizational assets, making it an indispensable tool for employees who drive to work.

Researchers have highlighted the gate pass's involvement in infection spread (Hamza *et al*., 2018; Adeleye *et al.,* 2020). Evidence shows that pathogens transfer from surfaces to handlers, persisting despite standard cleaning protocols. Gate passes often harbor microbes from both indoor and outdoor settings (Al-Harbi *et al.,* 2017). Items like food, pets, fabrics, rugs, wooden surfaces, and upholstery can release fungal spores into the air, leading to surface contamination on these cards (Badger *et al.,* 2015). Users of gate passes encounter diverse environmental factors that pose health risks (Aliyu *et al*., 2018). Common microbes detected on gate pass surfaces include;*Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* as well as *Candida albicans* (Hamza *et al.*, 2018; Adeleye *et al*., 2020). Pathogenic bacteria and fungi on such surfaces present significant public health threats (Shiaka and Yakubu, 2013).

The study of microorganisms on gate pass is important to understanding the dissemination of microbes particularly the pathogenic ones (Uaboi-Egbenni *et al.,* 2010). And the number and type of surface microorganisms on gate pass can be used to determine the degree of cleanliness of a system (Ekhaise *et al.,* 2010).

Currently, information concerning microbial contamination of gate passes particularly in Nigeria, is still scanty (Lagier *et al*., 2015). The increased availability of multiple gate pass users would indicate that on a daily basis, these passes are subjected to physical handling by a large variety of human users. As these gate passes are not subjected to routine

disinfection procedures, there is an increased likelihood of these used gate passes playing a role or functioning as a fomite in the cross-transmission of pathogenic microflora of potential public health importance amongst users (Omoigberale *et al*., 2014).

1.1 Aim and Study Objectives

The aim of this study was to ascertain the likely presence of potentially pathogenic bacterial and fungal isolates associated with swabbed gate pass / tags used at a tertiary educational institution in Benin City, Nigeria.

Specific objectives were as follows:

1) Isolate and enumerate bacteria and fungi associated with gate pass/tag surfaces.
2) Identify the bacterial and fungal isolates using microbiological, biochemical and molecular biology techniques.
3) Determine the antibiotic susceptibility profiles of exposedpurified bacterial cultures to selected commercially available antibiotic discs.

CHAPTER TWO

LITERATURE REVIEW

2.1. BRIEF DESCRIPTION AND IMPORTANCE OF GATE PASS

Non-living surfaces serve as environmental reservoirs for pathogen transmission, occurring either through direct contact with mucous membranes and broken skin or indirectly *via* the contamination of hands, which then transfer microbes to the eyes, nose, or mouth (Kraay *et al*., 2021). Coming into contact with these contaminated surfaces can result in illness within vulnerable individuals (Burton *et al*., 2011). These objects, known as fomites, facilitate the spread of infectious agents including bacteria, viruses, and fungi to new hosts (Abrar *et al.*, 2016). Research focused on the prevalence and dispersal of these pathogens has highlighted the notable role fomites play in infection acquisition (Medrano-Félix *et al.*, 2011). The risk of microbial transfer from surfaces to hands is especially critical in high-density environments where frequent human interaction occurs (Ahmed *et al.*, 2019).

2.2. MICROBIOLOGICAL ASSESSMENT OF GATE PASS AND SURFACES

Gate pass surfaces can be contaminated by fungal molds, bacteria, viruses and any activity that can introduce organiccontaminants onto the surface (Ahmed *et al*., 2019). The concern for proper sanitation is very relevant particularly in commercial/industrial settings that accommodate a large number of people and visitors because infections can trigger both mild and seriously irritating health outcomes (Tambeker *et al*., 2007).Gate pass can act as a microbial "harbor" Including *Escherichia coli* (Blom *et al*., 2000). Contaminated gate pass have unique microbial problems and their control is a complex task. The microbiological integrity of gate pass has been a focus of several studies varying from bacterial

contamination of gate pass to the assessment of the barrier attributes of gate pass micro-environments (Fekety *et al*., 2001).

Fungal growth on the surfaces of plastic gate passes can occur primarily not because fungi directly feed on plastic, but due to the fact that fungi can grow on organic matter such as dust, dirt as well as residues that accumulate on plastic surfaces (Okal *et al*., 2023). Pure plastic itself is an inorganic substrate and generally does not provide food for mold or fungi. However, additives in plastics (like cellulose, plasticizers, lubricants, stabilizers, and colorants) or dirt on the surface can support fungal growth. Warm, humid environments further encourage fungal growth on these surfaces (Srikanth *et al*., 2022).

Some fungal strains have been documented to be capable degrading certain types of plastics by secreting enzymes that break down the polymer structures, but this biodegradation process is different from simple fungal growth on plastic surfaces (Okal *et al*., 2023). Fungi capable of plastic degradation often belong to common genera such as *Aspergillus* (e.g., *A. flavus, A. fumigatus,A. niger*) as well as *Penicillium* (e.g., *P. griseofulvum, P. oxalicum* and *P. chrysogenum*), *Cladosporium* (e.g., *C. cladosporioides* and *C. ramotenellum*) and *Fusarium* (e.g., *F. solani*) which are commonly found in indoor and outdoor environments and are capable of producing spores (Srikanth *et al*., 2022).

2.3. FACTORS AFFECTING BACTERIAL ADHESION TO GATE PASS

The surface physicochemical characteristics of bacterial cells and materials—such as hydrophobicity and roughness play a critical role in the initial adhesion stage (Zhao *et al*., 2023). Factors like nutrient levels in the surrounding environment, bacterial growth phase, medium pH and temperature, cellular features (including extracellular polymeric substances

[EPS], flagella), and ionic strength also influence bacterial attachment (Wala'a *et al.,* 2013). Previous research has examined the adhesion potential of pathogenic bacteria to various contact surfaces (Zahra *et al.,* 2016), with researchers proposing two primary strategies: (i) altering material surface properties to reduce bacterial colonization; and (ii) creating more effective disinfectants and protocols targeting attached bacteria (Al-Harbi *et al.,* 2017).

2.4.1. Adhesion of Selected Bacterial Isolates to Different Contact Surfaces

Research has indicated that *S. aureus* is capable of adhering to and developing biofilms on various inert materials, including vehicle gate passes (Hamadi *et al.,* 2014). The propensity of this pathogen to colonize surfaces such as plastic, rubber, stainless steel, and glass is well-established in the literature (Lund, 2014; Hamza *et al.,* 2019; Adeleye *et al.,* 2022). Nevertheless, the speed and scale of this adsorption vary significantly based on the specific material (Rattanasena and Somboonwatthanakul, 2010). The exact mechanisms driving S. aureus adhesion remain complex and poorly defined, as researchers struggle to correlate specific surface characteristics with the degree of bacterial attachment (Lund, 2014).

To better understand these dynamics, the adhesive capabilities of ten (10)*S. aureus* strains were tested across diverse substrates, including marble, granite, glass, and two types of polypropylene. The study also included "silestone," a quartz-based material embedded with biocides. This antimicrobial protection, such as Microban, works by infiltrating the microbial cell wall and neutralizing vital internal functions, thereby halting the growth and reproduction of prokaryotes and molds (Uaboi-Egbenni *et al*., 2010).

It is important to note that no direct relationship was observed between the quantity of attached cells and the hydrophobicity of the material surfaces. Significant adhesion occurred

on highly hydrophobic surfaces, yet these levels were comparable to those found on moderately hydrophobic marble and hydrophilic granite (Allerberger and Wagner, 2010). Similarly, surface topography did not appear to correlate with cell attachment; although rougher materials showed high levels of adhesion, the results were nearly identical for the smoothest surfaces (Allerberger and Wagner, 2010).

Furthermore, the origin of the *S. aureus* isolates did not seem to influence their adhesive capacity or their ability to form biofilms (Allerberger and Wagner, 2010). Interestingly, while the lowest adhesion levels were found on the two polymers, the two silestone varieties showed slightly higher counts, suggesting that the integration of Microban may not be fully effective in preventing the attachment of these pathogens (Allerberger and Wagner, 2010). Other species, such as *Proteus vulgaris*, also demonstrate the ability to colonize various inert surfaces, though the degree of attachment varies (Yuan *et al*., 2021). For instance, *Providencia rettgeri* has been shown to form biofilms on plastic, cement, and stainless steel, with the highest density occurring on plastic (Huang *et al*., 2022). Investigations into *P. vulgaris* on polyethylene, polypropylene, and granite further confirm that surface roughness and hydrophobicity are insufficient to explain differences in adhesion (Boyer *et al*., 2012), a finding supported by additional studies on stainless steel 304 (Lund, 2014).

The actual progression rate of adhesion to stainless steel varied from 2.0×10^4 cells/mm^2 for *P. vulgaris*cultured from chicken breast to 4.67×10^3 cells/mm^2 for *Proteus vulgaris*cultured from the water of packaged chicken (Boyer *et al*., 2012). As such, the absence of variation in hydrophobicity or in roughness can cause changes as the adhesion substratum was similar for all strains examined (Marzano and Balzaretti, 2011).

Numerous investigations have demonstrated a direct, nearly linear relationship between the hydrophobicity of a solid surface and the degree of bacterial attachment. This phenomenon was particularly evident when evaluating high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), and polymethylmethacrylate (PMMA) as potential carriers for *Proteus vulgari*s (Protocarrero *et al*., 2002; Ekrami *et al.*, 2011). Similarly, a linear link for surface hydrophobicity as well as cell adhesion was observed for *Staphylococcus epidermidis* (strain ATCC 35984) across various medical-grade polymers, including silicone and cellulose diacetate (Pep *et al.*, 2016). This correlation also extends to porous materials like clay and foam glass when used as biomass supports in anaerobic reactors (Protocarrero *et al.,* 2012).

In addition to hydrophobicity, surface texture is often cited as a major influence on microbial colonization. For example, research into *Yersinia ruckeri*—a pathogen affecting fish—showed that its ability to stick to wood, concrete, and fiberglass was strongly tied to the roughness of those materials (Ranjit-Singh *et al.,* 2011). Protocarrero *et al.* (2012) found comparable results when comparing S. aureus adhesion on polished versus unpolished stainless steel. However, despite these findings, establishing a consistent correlation between surface roughness and the attachment of foodborne pathogens remains elusive.

2.5. SOURCES AND CONTROL OF BIOLOGICAL CONTAMINATION OF THE OUTER SURFACES OF INANIMATE OBJECTS

The external surfaces of inanimate objects, such as gate passes, are susceptible to biological contamination from several sources, including human contact, organic dust, stored materials, and the circulation of both indoor and outdoor air (Misgana *et al*., 2015). With respect to

their specific functional roles, these environments create a distinct micro-ecological niche where the risk of microbial development and subsequent air contamination is elevated. Under conducive microclimatic conditions, these microorganisms can colonize materials and trigger the process of biodeterioration (Kalwasińska *et al.*, 2012).

The degradation of materials like wood, masonry, cabinetry, and paper is largely attributed to microfungi specifically those within the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Alternaria*, *Mucor*, and *Rhizopus* while heterotrophic bacteria play a smaller role (Kang and Frank, 2000). Generally, bacterial populations on surfaces like metal and wood remain low; however, their numbers surge if these materials become damp, flooded, or fail to dry quickly (Kalwasińska *et al*., 2012). These high-moisture environments specifically encourage the growth of cellulolytic bacteria, such as *Cellulomonas, Cellfalciculata*, *Cellvibrio,* and *Cytophaga* (Rahkio and Korkeala, 2007; Kotula and Emswiler, 2008).

As microorganisms are ubiquitously distributed in built environments, a way of controlling their ability to contaminate outer surfaces of inanimate objects such as gate pass would require the implementation of strict adherence to hand hygiene protocols (Muhammad *et al*., 2013). Effective disinfection of inanimate objects such as gate pass and the environment must take place. Some strains of bacteria can be more virulent and resistant to normal cleaning methods. Cleaning products containing chlorine appear to be most effective in disinfecting inanimate surfaces such as gate pass/tags (Gould and Freeman, 2003).

2.6. PUBLIC HEALTH SIGNIFICANCE OF BACTERIA AND FUNGI ASSOCIATED WITH GATE PASS

The microbial flora present on plastic gate passes is of public health relevance as these plastic surfaces can serve as reservoirs and vectors for pathogenic microflora (Adeleye *et al.*, 2022). Plastic gate passes, frequently handled by many people, can accumulate diverse prokaryotes exemplified by *E. coli*, *S. aureus* and *K. pneumoniae* respectively (Adeleye *et al*., 2022).These bacteria, often pathogenic, have the potential to cause infections and spread diseases among the user population. It has been demonstrated that bacterial contamination on plastic surfaces like gate passes poses a risk for epidemic outbreaks due to their ability to harbor and transmit these microorganisms (Adeleye *et al*., 2022).

In addition to bacteria, fungi also colonize plastic surfaces, including plastic wastes and materials similar to plastic gate passes (Gkoutselis *et al*., 2021). Certain fungi are capable of degrading plastics, but importantly, plastic debris acts as selective microhabitats—or plastisphere that accumulate fungal pathogens, including opportunistic human pathogens and plant pathogens (Munir *et al.*, 2024). For example, species such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium solan*i, and others are found thriving on plastics (Srikanth *et al.*, 2022). These fungi can cause infections and contribute to public health risks by potentially serving as sources of fungal disease transmission, particularly in environments with poor waste management and sanitation (Srikanth *et al*., 2022).

Plastic surfaces, due to their durability and persistence in the environment, can harbor both bacterial and fungal pathogens for extended periods (Gkoutselis *et al*., 2021; Adeleye *et al*.,

2022). This trend can increases the likelihood of spreading antibiotic-resistant bacteria and fungal pathogens in both community and healthcare settings. The role of plastic gate passes as fomites (objects that transmit infection) is important to consider in public health strategies, emphasizing the need for hygiene practices such as regular disinfection of these plastic items and education on hand hygiene to reduce pathogen transmission risks (Adeleye *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This research was conducted at University of Benin (UNIBEN) and University of Benin Teaching Hospital (UBTH)both located in Benin City, Edo State, Nigeria.

Verbal consent was obtained from the Chief Security Officers of the University of Benin (UNIBEN) and the University of Benin Teaching Hospital (UBTH) prior to sample collection. Permission was granted to aseptically swab gate pass/tags at the main entrance points of both institutions.

3.2 Collection of Swabbed Samples

A grand total of 100 gate pass/tags were respectively swabbed between August and November, 2023. The size of the swabbed surface area of the respective gate pass was $10cm^2$ (5cm (length) by 2cm (breadth). Swabbing was done aseptically using sterile swab sticks moistened with sterile peptone water under aseptic conditions. Ten (10) swab samples from each of the sample sites were collected every two weeks. Appropriate labeling was ensured and swabs were immediately taken to the laboratory for microbiological examination.

3.3 Enumeration and Isolation of Bacteria and Fungi

The pour plate method as described by Holt *et al.*(2000) for the determination of bacterial and fungal agar counts was utilized to ascertain the total mean viable counts of the respective swabbed surfaces. Stock homogenates (solution) were prepared from the labeled swab sticks by dipping the swab in 9 ml of the peptone water diluent and these homogenates

were respectively diluted up to 10^{-5} under aseptic conditions. One (1) millilitre was taken from the stock solution and serially diluted up to 10^{-5} with 1 ml of the 10^{-5} diluent subsequently plated under aseptic conditions. Plating for each diluent was done in triplicates and the nutrient agar and (heterotrophic bacterial count) McConkey agar (coliform count) were incubated at 35 ^{o}C for 24 and 48 hours respectively. Diluted samples were also used to inoculate potato dextrose agar(PDA) plates and the agar plates were incubated at ambient room temperature for 5 days to isolate and enumerate fungi. At the end of the incubation time line, the observed discrete fungal colonies were enumerated and documented.

3.4. Identification of Bacterial isolates

In the course of enumerating the bacterial colonies, uniquely occurring colonies were noted and sub-cultured on freshly prepared nutrient agar (NA) plates under aseptic conditions and the plates were incubated at 35 ^{o}C for 24 hrs. The cultural characteristics of the purified cultures were observed and documented. The examined pure bacterial cultures were transferred to freshly prepared NA slants. Further physiological, morphological and biochemical attributes of the selected isolates were used along with their cultural characteristics for purpose of identification (sections 3.4.1 below)

3.4.1. Physiological Tests

3.4.1.1. Motility test

This procedure effectively differentiates between motile and non-motile bacteria based on their ability to disperse within the medium during growth. This test was conducted using stab inoculation method as described by Sharma (2009). The sterilized half strength nutrient agar medium in the tubes were left to cool to45 ^{o}C. In each tube, the molten agar was allowed to

set in an vertical alignment and then stab-inoculated with 24 hr broth culture of each bacterial isolate, with the aid of a sterile inoculating needle to about half the depth of the slanted agar medium. The test tubes were incubated at room temperature for 2-6 days and at each day, the tubes were observed (Sharma, 2009). Non-motile bacterial isolates exhibited growth patterns, restricted to the stab-line, and these patterns showed well defined margins and leaving the surrounding medium clearly transparent (Sharma, 2009). Motile bacterial isolates showed diffused growth which extended as a turbid zone from the stab line to the surrounding medium (Sharma, 2009).

3.4.2. Morphological Assay

3.4.2.1 Gram Staining

The working principle behind Gram staining of bacterial cultures involves the differentiation of prokaryotes (bacteria) into two main groups—Gram-positive and Gram-negative with respect to their respective cell wall composition. Twenty-four (24) hour bacterial cultures on nutrient agar were Gram stained in accordance with procedure described by Cappuccino and Welsh (2020). Smears of the bacterial cultures were prepared and heat fixed on labeled clean glass slides. The smears were then stained with one (1) drop of the primary stain, crystal violet for one (1) minute. The stain was washed off with distilled water and flooded with dilute Gram iodine solution for one (1) minute. This was washed off with distilled water and the smears were decolourized with 95% alcohol for 30 seconds and rinsed off with distilled water. The decolourized smears were then counter stained with the secondary stain; safranin solution for one (1) minute. The counter stain was washed off with distilled water, left to air dry and viewed under the microscope using the immersion oil objective. Gram positive isolates were recorded as those that retained the purple coloration associated with

the primary stain whilst the Gram negative cultures were those that retained the red coloration of the secondary stain (Cappuccino and Welsh, 2020).

3.4.3. Biochemical Tests

3.4.3.1. Catalase test

A fresh smear of 24 hr culture of the bacterial culture was prepared on a clean slide and drops of 3% hydrogen peroxide were added. Effervescence (production of gas bubbles) was indicative of a positive reaction (Sharma, 2009).

3.4.3.2. Coagulase test

This procedure involves the detection of bound coagulase enzyme, on the surface of bacteria like *S. aureus*. This test helps to differentiate between *S. aureus* (coagulase-positive) and other staphylococci that are generally negative for this enzyme. The slide test procedure for bound coagulase as described by Cheesebrough (2006) was utilised to ascertain if any of the purified bacterial cultures could exhibit the coagulase enzyme. A wet smear of the bacterial culture was prepared on clean glass slide using a sterile inoculating loop. A drop of plasma was placed on the wet smear and mixed gently. Observed immediate clumping within the smear was indicated as a positive result (Cheesebrough, 2006).

3.4.3.3. Oxidase test

This procedure aids in differentiating between various bacterial groups with reference to their ability to synthesize cytochrome C oxidase. A few drop of oxidase reagent (Tetramethyl- p- phenyl-diamine-dihydrochloride) was placed on a piece of filter paper. A

smear of the test bacterial culture was placed on the soaked filter paper. It was observed that oxidase producing bacterial culture oxidizes the phenyl-diamine in the reagent to generate a purple colour. This alteration in colour to purple within 10 secs was documented as a positive result; otherwise, the observation was documented as a negative result

3.4.3.4. Indole test

This procedure helps to differentiate bacteria within families like Enterobacteriaceae (e.g., distinguishing *Escherichia coli* from *Enterobacter* and *Klebsiella*) as part of broader biochemical identification protocols. Prepared and sterilized peptone water was inoculated with 24 hour culture of the respective bacterial isolates and incubated at 35 ºC for 48 hours (Sharma, 2009). One milliliter (1 mL) of Kovac's reagent was then added to each of the incubated tubes. The development of a red precipitate at the top of the broth was documented as a positive result; otherwise, the observation was documented as negative (Sharma, 2009).

3.4.3.5. Citrate test

The test bacterial culture was inoculated on prepared Simmons citrate agar slants and the slants were incubated for 48 hours at 35ºC (Harley and Prescott, 2002). An observed colour change in the incubated agar slant from green to blue was documented as a positive result (Harley and Prescott, 2002).

3.4.3.6.Sugar utilization test

Several bacterial groups and genera can be differentiated on the basis of the sugars they utilize and ferment. The fermentation medium was prepared by the composition of 0.1 g of

peptone, 0.1 g of sodium chloride and 0.1 g of fermentable sugar (glucose, mannitol, lactose, sucrose or mannose) in 10ml of distilled water. About 4ml of the fermentation medium was dispensed into Bijou bottles containing Durham tubes. About 1ml of phenol red indicator was also added to each of the tubes. The tubes were then autoclaved at 121^{o}C for 15 mins. One per cent (1%) solution of the sugar was prepared and separately sterilized at 115^{o}C for 10 mins. Five millilitre (5 mL) of the sterilized sugar solution was dispensed into the tubes that contained peptone water and the pH indicator under aseptic conditions (Aneja *et al*., 2003). The tubes were inoculated with the respective isolates with the aid of a sterile inoculating wire loop and incubated at 37^{o}C for 24 hrs (Aneja *et al.*, 2003). Acid production was indicated by the colour change in the medium from red to yellow while gas production was indicated by the presence of gas bubbles in the Durham tubes (Aneja *et al*., 2003).

3.4.3.7. Urease test

The ability of the bacterial isolate to produce urease enzyme was determined according to procedure described by Sharma (2009). Prior to inoculation, the urea medium was autoclaved at 121^{o}C for 15 mins. The urea was filter sterilized seperately using a membrane filter. The isolates were cultured on the sterilized culture medium with urea serving as a nitrogen source and phenol red as indicator. The culture was incubated at 37^{o}C for 24 hrs and the production of red coloured slant was indicative of urease production (Sharma, 2009).

3. 5 Macroscopic and Microscopic Identification of Fungal isolates

The cultured fungal isolates were tentatively characterized after being subjected to wet mount staining which entailed the staining of a prepared wet fungal smear with lactophenol cotton in blue stain and viewing of the stained smear under the microscope. The wet smear

was prepared by placing a drop of the stain on the grease free slide followed by the placement of the excised part of the fungal mycelium on the stain drop. This mixture was covered with a coverslip and viewed under the ×40 objective lens of a compound light microscope (Sharma *et al*., 2009). The documented results obtained were compared with standard reference illustrations as documented by Barnett and Hunter (1998).

The macroscopic colonial and morphological characteristics of each isolate was determined by observing several attributes of the fungal colonies and these features included the surface appearance of the colony as well as the colour of the reverse side of the colony (Sharma *et al.,* 2009).

3.6. Molecular Identification of Selected Isolates

3.6.1. Extraction of Fungal and bacterial DNA

DNA extraction was achieved with the aid of a ZR fungal/bacterial DNA mini prep extraction kit obtained from Inqaba Biotech, South Africa. A heavy growth of the respective pure cultures of themicrobial isolates was sprepared and suspended in 200 microliters of isotonic buffer placed into a ZR Bashing Bead Lysis tubes; 750 microliters of lysis solution was then added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximal speed for 5 mins (Whitman *et al*., 2012). The ZR bashing bead lysis tube was then centrifuged at 10,000 ×g for 1 minute.

Four hundred microlitre (400 µl) of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 × g for 1 min. One thousand two hundred microlitre (1200 µl) of fungal/bacterial DNA binding buffer was then added to the filtrate in the collection tubes bringing the final volume to 1600 µl; 800 µl was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000 × g for

1 min, the flow through was then discarded from the collection tube (Whitman *et al.*, 2012). The remaining volume was transferred to the same Zymo-spin and spun. Two hundred microlitre (200 µl) of the DNA Pre-Wash buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000 × g for 1 min., followed by the addition of 500 µl of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000 × g for 1 min.

The Zymo-spin IIC column was transferred to a clean 1.5 µl centrifuge tube; 100 µl of DNA elution buffer was added to the column matrix and centrifuged at 10,000 × g for 30 secs to elute the DNA. The ultra-pure DNA was then preserved at -20 ^{o}C for subsequent downstream reactions.

3.6.2. DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE 19810 U.S.A) . The equipment software was launched*via* double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked with the aid of normal saline. Two microlitre of the extracted DNA was placed onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was quantified *via* clicking on the "measure" button.

3.6.3. 16S rRNA Amplification

The 16s rRNA genes harbored by the purified bacterial cultureswere amplified with the aid of the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler operatedat a final volume of 50 µl for 35 cycles. The PCR mix included: the ×2 Dream Taq Master mix provided by Inqaba Biotech, South Africa (taq polymerase, DNTPs,

MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were: initial denaturation, 95ºC for 5 mins; denaturation, 95ºC for 30 secs; annealing, 52ºC for 30 secs; extension, 72ºC for 30 secs for 35 cycles and final extension, 72ºC for 5 mins. The product was resolved on a 1% agarose gel at 120V for 15 mins and then visualized with the aid of a UV transilluminator.

3.6.4. Internal Transcribed Spacer (ITS) Amplification

The ITS sequence of the rRNA genes harbored by the purified fungal isolates were amplified with the aid of the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3,primers on a ABI 9700 Applied Biosystems thermal cycler operatedat a terminal quantity of 50µl for 35 cycles. The PCR mix encompassed: the ×2 Dream Taq Master mix acquired from Inqaba Biotech, South Africa (Taq polymerase, DNTPs, MgCl), the primer concentrates (0.4M) and the extracted DNA as template. Operating conditions of the PCR include: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 53ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles as well as the final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator (Tagg *et al*., 2020).

3.6.5. Sequencing of 16S rRNA and ITS Genes

Sequencing activity was performed with the aid of the BigDye Terminator kit on a 3510 ABI sequencer manufactured by Inqaba Biotech., Pretoria, South Africa. Sequencing was achieved at a terminal quantity of 10 µL, and some of the components were; 0.25 µl BigDye® terminator v1.1/v3.1, 2.25 µl of 5 x BigDye sequencing buffer, 10 µM PCR

primer, and 2-10ng PCR template per 100bp. Sequencing conditions were; 32 cycles of 96°C for 10 sec., 55°C for 5sec, as well as 60°C for 4minutes .

3.6.6. Phylogenetic Analysis

The collected gene sequences were edited with the aid `of the bioinformatics algorithm Trace edit; and identical gene sequences were collected from the NCBI website with the aid of BLASTN. These gene sequences were aligned with the aid of ClustalX. The evolutionary history was inferred with the aid of the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates was interpreted to represent the evolutionary archive of the examined taxa. The respective evolutionary distances were then derived with the aid of the Jukes-Cantor procedure (Oleghe *et al.,* 2025).

3.7. Antibiotic Susceptibility Pattern of the Isolates

The antibiotic sensitivity/resistance profiles of the selected bacterial cultures was evaluated with the aid of the modified Kirby-Bauer disc diffusion procedure as detailed by Vandepitte *et al*. (2003) and the resultant growth inhibitory zones were interpreted as described by the Clinical and Laboratory Standards Institute (CLSI, 2020). All the test bacterial cultures were cultured in sterilized nutrient broth and the turbidity of the cultured broth were adjusted to 0.5 McFarland turbidity standard prior to determination of the antibiogram profile by matching the turbidity of the overnight broth to that of prepared Barium sulphate opacity standard (0.36 Normal) (Vandepitte *et al*., 2003). Mueller Hinton agar (MHA) plates were subsequently prepared and labeled. The MHA plates were seeded with the standardized bacterial isolates with the aid of spread plate method with the aid of swab sticks

(Cappuccino and Welsh, 2020). The seeded plates were left to dry for 5 mins (Vandepitte *et a*l., 2003).

Commercially available antibiotic discs utilised included; Ofloxacin (5 µg OFX), levofloxacin (30 µg LBC) Ciprofloxacin (10 µg CIP), Gentamycin (10 µg CN), Chloramphenicol (10 µg CH), cefixime (30 µg ZEM), cefoxitin (30 µg CFX), cefuroxime (30 µg CXM), ampicillin (30 µg AZM), Augmentin (30 µg AUG), Imipenem (IMP), Erythromycin (30 µg E), Naladixic acid (10 µg NA), Ceftriaxone (30 µg CRO), Nitrofurantoin (10 µg NF) and ampicillin (10 µg ACX). The observed inhibitory growth zones were measured in millimetre across the disc with the aid of a rulerandthe respective growth zones were interpreted as sensitive (S), intermediate (I) or resistant (R) as described by CLSI (CLSI, 2020).

3.7.1.Evaluation of the multiple antibiotic resistance index

The multiple antibiotic resistance (MAR) index for the respective exposed bacterial cultures was ascertained with the aid of a formula described by Mir *et al.* (2021). The formula is given below:

MAR= A/B

wherein;

A: represent thenumber of drugs to which the exposed bacterial culture was resistant

B: total number of drugs to which the bacterial culture was exposed to

MAR was categorized as resistance to more than two classes of antibioticsamong all tested antibiotics (Mir *et al*., 2021).

3.8. Statistical Analysis

The mean bacterial counts were subjected to parametric unpaired T test using Microsoft excel version 2010. This was done to ascertain if the observed differences between the mean counts obtained from the respective swabbed gate passes were significantly different (α=0.05)

CHAPTER FOUR

RESULTS

4.1. Microbial Counts

The mean bacterial and coliform counts of swabbed gate pass/tags from the respective locations are shown in Table 4.1. The mean heterotrophic bacterial count (THBC) for swabbed UNIBEN and UBTH Gate Tags ranged from $2.60 \times 10^5 \pm 0.71$ (week 12) to $10.4 \times 10^5 \pm 0.90$ cfu/cm^2 (week 5), and $2.17 \times 10^5 \pm 0.31$ (week 10) to $8.17 \times 10^5 \pm 1.09$ cfu/cm^2 (week 2) respectively. Mean total coliform counts (TCC) for swabbed UNIBEN gate tags ranged from $1.33 \times 10^5 \pm 0.06$ (week 12) to $8.20 \times 10^5 \pm 1.45$ cfu/cm^2 (week 2). Mean total coliform counts (TCC) for swabbed UBTH gate tags varied from $1.33 \times 10^5 \pm 0.03$ (week 12) to $7.10 \times 10^5 \pm 0.57$ cfu/cm^2 (week 9).The observed differences between the weekly heterotrophic bacterial and coliform counts for the swabbed gate passes from both institutions was statistically insignificant ($p>0.05$) (Appendix 1).

The mean total fungal counts (TFC) observed for the swabbed gate passes is shown on Table 4.2. The mean TFC for swabbed UNIBEN and UBTH Tags ranged from $2.10 \times 10^5 \pm 0.80$ (week 9) to $6.00 \times 10^5 \pm 0.11$sfu/cm^2 (week 8) and $2.01 \times 10^5 \pm 0.66$sfu/cm^2(week 12) to $11.3 \times 10^5 \pm 0.34$ sfu/cm^2 (week 1). The observed differences between the weekly heterotrophic fungal counts for the swabbed gate passes from both institutions was statistically insignificant ($p>0.05$) (Appendix 1).

Table 4.1: Mean total heterotrophic bacterial and coliform counts for the swabbed gate passes

Sampling Weeks	THBC ($\times 10^5$ cfu/cm^2)		TCC ($\times 10^5$ cfu/cm^2)	
	UNIBEN	UBTH	UNIBEN	UBTH
1	[+]6.35±1.05	5.30±10.4	7.30±1.57	3.94±0.29
2	3.33±0.19	8.17±1.09	8.20±1.45	4.17±0.31
3	3.01±0.08	2.70±0.73	4.00±0.60[a]	5.27±0.41
4	5.33±0.20	3.23±0.68	2.20±0.34	2.70±0.86
5	10.4±0.90	5.45±3.74	4.43±0.65	5.17±0.20
6	7.20±0.63	4.12±0.42	2.55±0.41	2.70±0.86
7	9.20±1.46	5.12±0.91	2.05±0.38	4.29±0.40[a]
8	6.50±1.17	2.22±0.33	2.46±0.66	2.10±0.46
9	4.75±0.17	5.17±0.20	3.33±0.33	7.10±0.57
10	4.20±0.38	2.17±0.31	7.00±2.88	3.33±0.33
11	6.66±0.28	6.17±0.81	5.00±0.30	3.00±0.00
12	2.60±0.71	7.13±1.76	1.33±0.06	1.33±0.03

Key: +; Overall mean ± Std. Deviation of three swabs, THBC; Total heterotrophic bacterial count, TCC; Total coliform count

Table 4.2: Mean total fungal counts for the swabbed gate pass/tags from different locations

Period (Weeks)	UNIBEN ($\times 10^5$ sfu/cm^2)	UBTH ($\times 10^5$ sfu/cm^2)
1	[+]3.15±0.72	11.3±0.34
2	3.76±0.76	5.33±0.28
3	5.72±1.20	3.14±0.52
4	2.58±0.55	4.41±0.52
5	2.51±0.51	3.54±0.23
6	4.53±1.52	4.27±0.33
7	3.20±0.99	2.55±0.05
8	6.00±0.11	2.90±0.01
9	2.10±0.80	2.20±0.00
10	3.23±0.73	4.05±1.05
11	4.51±0.65	3.01±0.88
12	3.09±0.00	2.01±0.66

Key:+; Overall mean ± Std. Deviation of three swabs,

4.2. Identities of Bacterial and fungal Isolates based on Cultural, Morphological and Biochemical Attributes

The cultural, morphological as well as biochemical attributes of the respective bacterial cultures is shown in Table 4.3. The identified bacterial isolates were *Staphylococcus aureus, Providencia rettgeri, Streptococcus pyogenes, Proteus vulgaris, Serratia marcescens, Escherichia coli, Bacillus altitudinis* and *Pseudomonas aeruginosa.*

Result for the cultural and microscopic characteristics of fungal isolates is shown on Table 4.4. Presumptive fungal isolates identified were *Aspergillus niger*, *Trichoderma harzianum,Microsporum* sp., *Cladosporium* sp. and *Penicillium* sp.

Table 4.3: Cultural, morphological and biochemical attributes of the bacterial cultures

	Isolate Code							
Characteristics	A	B	C	D	E	F	G	H
Cultural								
Elevation	Convex	Convex	convex	Flat	Convex	convex	Convex	convex
Margins	Smooth	Entire	Smooth	Serrated	Smooth	smooth	Smooth	Entire
Colour	Yellow	Milky-white	White	Pink	Red	Pink	White	Grey
Shape	Circular	Circular	Circular chains	Circular	circular	circular	circular	Circular
Morphology								
Gram stain	+	-	+	-	-	-	+	-
Cell type	Cocci	Rod	Cocci	Rod	Rod	rod	Rod	Rod
Cell Arrangement	Cluster	Single	Chain	Single	Single	single	Single	Single
Biochemical								
Catalase	+	+	-	-	+	+	+	+
Indole	-	+	-	+	-	+	-	-
Urease	+	+	-	+	-	-	-	+
Oxidase	+	-	-	-	-	-	-	+
Coagulase	+	-	-	-	-	-	-	+
Citrate	-	+	+	+	+	-	+	+
Glucose	+	+	+	+	+	+	+	+
Lactose	+	-	-	-	-	+	-	-
Mannitol	+	-	-	-	-	+	-	-

KEY:A= *Staphylococcus aureus* B =*Providencia rettgeri*, C = *Streptococcus pyogenes,* D= *Proteus vulgaris*, E = *Serratia marcescens*, F = *Escherichia coli*, G = *Bacillus altitudinis*, H = *Pseudomonas aeuruginosa*

Table 4.4: Cultural and morphological characteristics of the fungal cultures

	Fungal Culture Code				
Characteristic attributes	**F1**	**F2**	**F3**	**F4**	**F5**
Appearance of colony on PDA	Black	Dark grey	Dark grey	Grey-green	green
Morphological attributes	flask-shaped	concentric ring	spindle-like shape	shield-shaped	brush-like appearance
Nature of hyphae	Septate	Non septate	Septate	Non septate	Septate
Observed spore	Conidiophores	Conidiophore	conidia	Conidiophore	Conidiophores
Colour of spore	Black	Dark blue	black	Brownish	Green

KEY:F1 = *Aspergillus niger*, F2 = *Trichoderma harzianum*, F3 = *Microsporum sp.* F4 = *Cladosporium* sp, F5 = *Penicillium* sp.

4.3. Molecular Identities of Bacterial and Fungal Cultures

The documented 16S rRNA sequence from the selected bacterial culture generated a precise match in the course of doing the megablast search for veryidentical molecular sequences present in the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the bacterial isolates revealed a percentage similarity (100%) to other bacterial species. The evolutionary distances which were computed with the aid of the Jukes-Cantor procedure were very similar with the phylogenetic positioning of the respective bacterial isolate 16S rRNA within *Serratia, Bacillus, Providencia* spp and also indicated a very close relatedness to *Serratia* sp., *Bacillus altitudinis* and *Providencia rettgeri* (Figure 4.1 and Plate 4.1).

The documented ITS sequence from the selected fungal culture generated a precise match in the megablast search for very identical sequences present in the NCBI non-redundant nucleotide (nr/nt) database. The ITS profiles of the respective fungal isolates revealed a percentage (100%) exactness to the relevant species. The computed evolutionary distances done with the aid of the Jukes-Cantor technique were identical to the phylogenetic positioning of the isolate's ITS within the *Trichoderma* and *Cladosporium* spp. and indicated a very near relatedness to *Trichoderma harzianum* as well as*Cladosporium* sp. isolate IPBCC_22_1543 (Figure 4.2 and Plate 4.2)

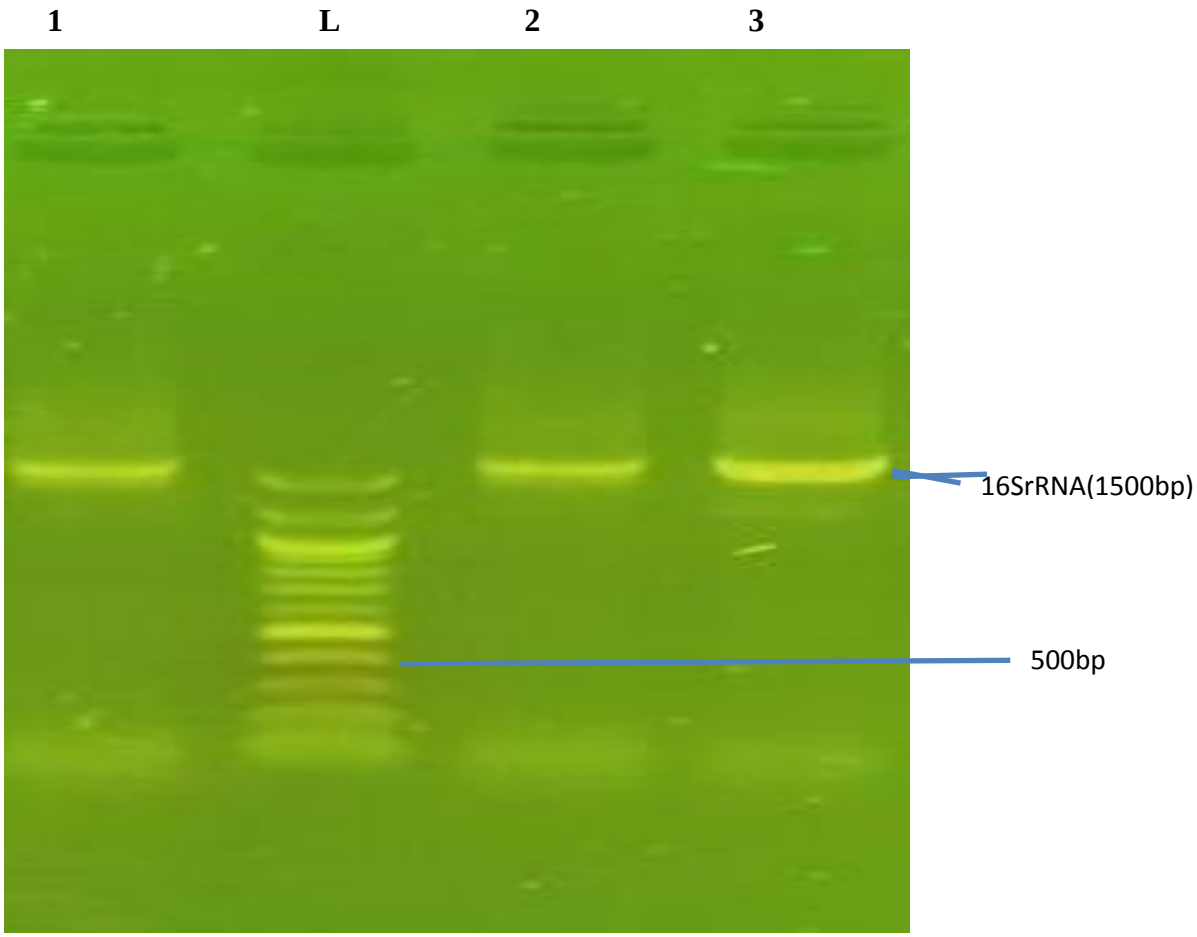


Plate 4.1: Agarose Gel Electrophoresis of plasmid profile of bacterial isolates. Lanes 1, 2 and 3 represent the amplified 16srRNA gene at 1500bp

L: 100bp DNA ladder, 1 =*Serratia* sp. , 2= *Bacillus altitudinis*3= *Providencia rettgeri*

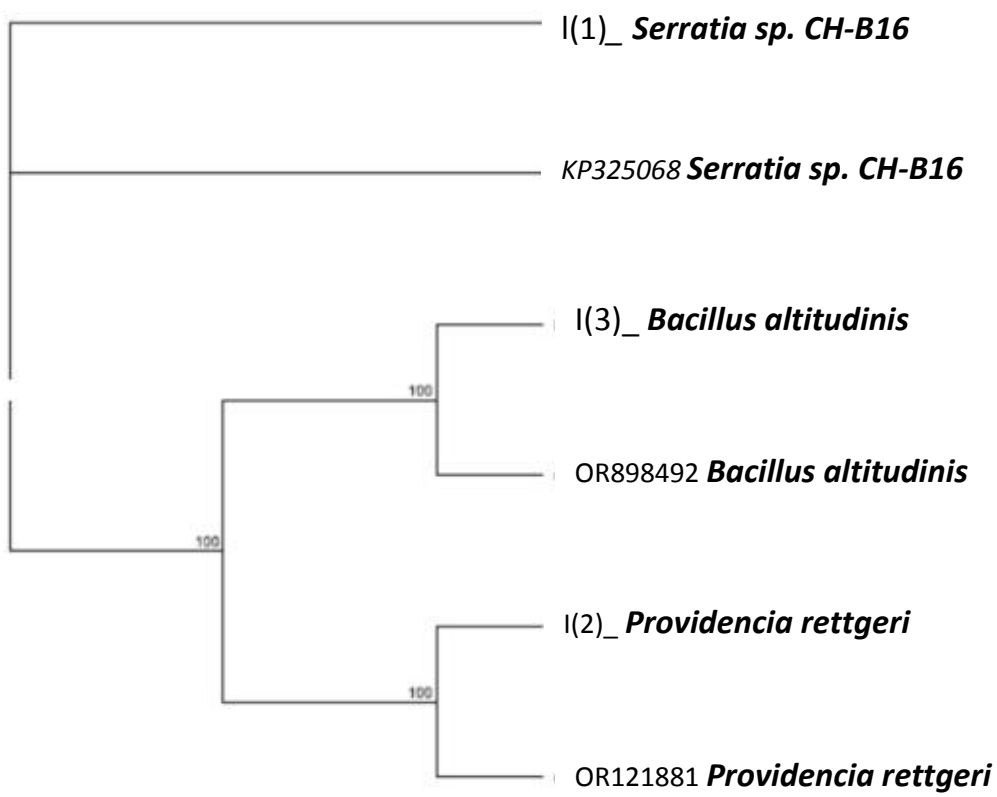


Fig 4.1: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

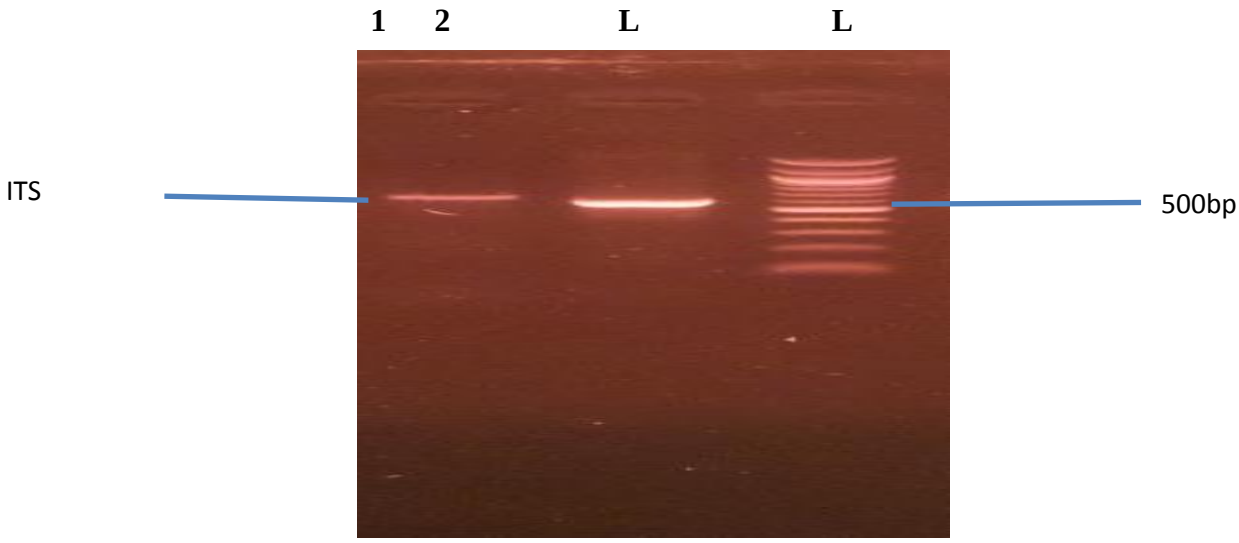


Plate 4.2: Agarose gel electrophoresis showing the amplified ITS. Lanes 1-2 represent the amplified ITS at 500bp while lane L represents the 100bp DNA ladder.

L: 100bp DNA ladder, 1 = *Trichoderma harzianum*, 2= *Cladosporium*sp

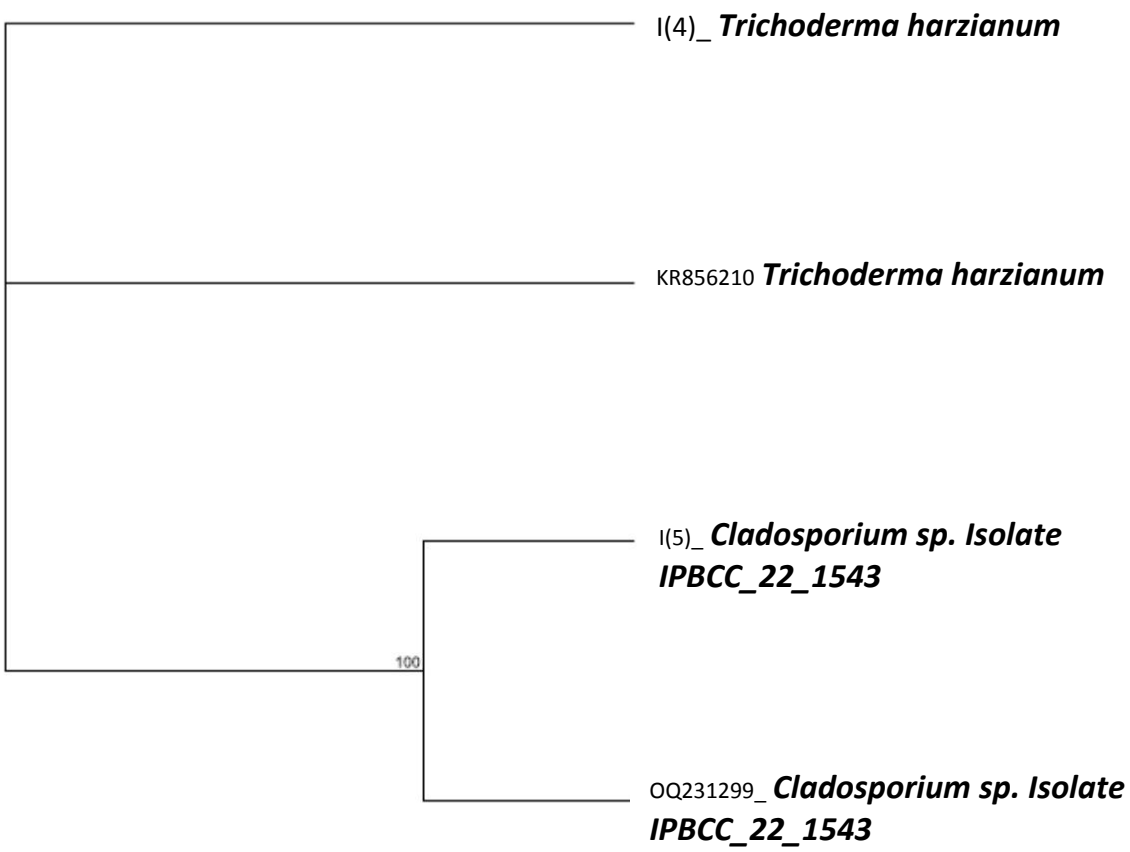


Figure 4.2: Phylogenetic tree showing the evolutionary distance between the fungal isolates

4.4. Percentage Occurrence of Bacterial and Fungal Isolates

Table 4.5 shows the percentage occurrence of the bacterial isolates of gate pass from all the locations. A total of Fifty six (56) Bacteria species were isolated from the swabbed UNIBEN and UBTH Tags respectively. *Escherichia coli*(20.7%) was the most frequently occurring bacterial isolate from the swabbed UNIBEN gate pass/tag while *Serratia marcescens*(6.90%) was the least occurring bacterial isolatesfrom the swabbed UNIBEN gate/pass. *Providencia rettgeri*and *Streptococcus* sp. were the most frequently occurring bacterial isolates from the swabbed UBTH gate pass/tags (18.55%) while *Staphylococcus aureus* (3.70%) was the least occurred bacterial isolates.

The frequency of occurrence of fungal isolates in gate pass from all the location is shown in Table 4.6. Twelve (12) fungal strains were isolated from the swabbed UNIBEN gate/pass with *Aspergillus niger* (38.5%) having the highest percentage frequency of occurrence. For the fungal isolates cultured from the swabbed UBTH gate pass/tags (9), *Cladosporium* sp. was the most predominant (44.4%) .

Table 4.5: Percentage occurrence of bacteria isolates of gate pass from all the locations

Bacterial	LOCATION		Total	Frequency of Occurrence (%)
	UNIBEN	UBTH		
Bacillus altitudinis	3(10.3%)	4(14.8%)	7	12.5
Escherichia coli	6(20.7%)	3(11.1%)	9	16.1
Staphylococcus aureus	5(17.2%)	1(3.70%)	6	10.7
Providencia rettgeri	2(6.90%)	5(18.5%)	7	12.5
Streptococcus pyogenes	3(10.3%)	5(18.5%)	8	14.3
Serratia marcescens	2(6.90%)	4(14.8%)	6	10.7
Proteus vulgaris	3(10.3%)	2(7.41%)	5	8.93
Pseudomonas aeruginosa	5(17.2%)	3(11.1%)	8	14.3
Total	**29**	**27**	**56**	100

Table 4.6: Occurrence of fungal isolates of gate pass from all the location

Fungi	UNIBEN	UBTH	Total	Occurrence (%)
Trichoderma harzianum	2(15.4 %)	0(0.00 %)	2	9.09
Aspergillus niger	5(38.5 %)	1(11.1%)	6	27.3
Penicillium sp.	3(23.1 %)	2(22.2 %)	5	22.7
Microsporum sp.	1(15.4 %)	2(22.2 %)	3	13.6
Cladosporium sp.	1(7.69 %)	4(44.4 %)	5	22.7
Total	12	9	21	100

4.5. Antibiotic Susceptibility/Resistance Pattern of Bacterial Isolates

The antibiotic resistance/susceptibility patterns of the Gram positive bacterial isolates is presented in Table 4.7. For the tested bacterial isolates; *S. aureus* (83%,), *B. altitudinis* (71%) and *S. pyogenes* (88%) were respectively resistant to ciprofloxacin while 86% and 50% of *B. altitudinis* and *S. pyogenes* strains exhibited resistance to levofloxacin. Eighty six per cent (83%) and 75% of *B. altitudinis* and *S. pyogenes* strainswere susceptible to cefxime while 83% of the *S. aureus* strains were resistant to cefxime. *S. aureus* (83%,), *B. altitudinis* (86%) and *S. pyogenes* (88%) were suspectible to cefoxitin while 83% of the exposed *S. aureus* strains were resistant to ampicillin, imipenem and cefuroxime respectively. Eighty six per cent (86%) of exposed *B. altitudinis* cultures were susceptible to ofloxacin, imipenem and erythromycin while 88% of the exposed *S. pyogenes* cultures were sensitive to ofloxacin, gentamycin, ampicillin, cefuroxime and cefoxitin respectively. The MAR index for the Gram positive bacterial isolates were; *S. aureus* (0.58), *B. altitudinis* (0.42) and *S. pyogenes* (0.33).

The antibiotic resistance/susceptibility patterns of the Gram negative bacterial isolates is presented in Table 4.8. Eighty eight per cent (88%) of the exposed *E. coli* isolates were suspectible to levofloxacin, ofloxacin,cefoxitin, ceftriaxone and nitrofurantoin while 88% of the *E. coli* cultures were resistant to ampicillin and imipenem respectively. Eighty six per cent (86%) of the exposed *P. rettgeri* cultures were resistant to naladixic acid, gentamycin and cefuroxime while 86% of the *P. rettgeri* strains were suspectible to augmentin, imipenem, ampicillin, cefoxitin and cefxime respectively. Seventy five per cent (75%) of the exposed *P. aeruginosa* cultures were suspectible to ofloxacin and nitrofurantoin while 100%

of the *P. aeruginosa* cultures were resistant to levofloxacin and naladixic acid respectively. Eighty per cent (80%) of the exposed *P. vulgaris* strains were resistant to gentamycin, cefoxitin, ceftriaxone and augmentin while 80% of the *P. vulgaris* strains were sensitive to naladixic acid, ofloxacin and nitrofurantoin respectively. Eighty three per cent (83%) of the exposed *S. marcescens* cultures were resistant to gentamycin and imipenem while 100% of *S. marcescens* cultures were suspectible to cefuroxime and nitrofurantoin respectively. Sixty seven per cent (67%) of exposed *S. marcescens* strains were resistant to levofloxacin, cefxime and ceftriaxone while 83% of *S. marcescens* cultures exhibited susceptibility to naladixic acid and augmentin respectively. The MAR index for the Gram negative bacterial isolates were; *E. coli* (0.25), *P. rettgeri* (0.42), *P. aeruginosa* (0.83), *P. vulgaris* (0.33) and *S. marcescens* (0.42) respectively.

Table 4.7 Antibiotic susceptibility profile of selected Gram-positive isolates

S/N	Antibiotics	Antibiogram of Isolates								
		S. aureus (6 strains)			*B. altitudinis* (7 strains)			*S. pyogenes* (8 strains)		
		S (%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)
1	OFX	50	50	0	86	14	0	88	12	0
2	LBC	100	0	0	0	14	86	0	50	50
3	CIP	0	17	83	0	29	71	0	12	88
4	CN	10	67	33	0	14	86	88	12	0
5	CH	0	33	67	0	43	57	75	25	0
6	ZEM	0	17	83	86	14	0	75	25	0
7	CFX	83	17	0	86	14	0	88	12	0
8	CXM	0	17	83	43	57	0	88	12	0
9	AZM	0	17	83	0	14	86	88	12	0
10	AUG	0	33	67	29	71	0	0	25	75
11	IMP	0	17	83	86	14	0	0	38	62
12	ERY	67	33	0	86	14	0	75	25	0
	MARI	**0.58**			**0.42**			**0.33**		

KEY: OFX = Ofloxacin, LBC = levofloxacin, CIP= Ciprofloxacin, CN = Gentamycin, CH = Chloramphenicol, ZEM = cefxime, CFX = cefoxitin, CXM = cefuroxime, AZM= ampicillin, AUG = Augmentin, IMP = Imipenem, ERY= Erythromycin, S = Sensitive, I = Intermediate, R = Resistance, MARI = Multiple Antibiotic Resistance index (MAR index ≥ 0.2 (public health significance)

Table 4.8: Antibiotic susceptibility profile of selected Gram-negative isolates

S/N	Antibiotics	Antibiogram of Isolates														
		E. coli (9 strains)			*P. rettgeri* (7 strains)			*P. aeruginosa* (8 strains)			*P. vulgaris* (5 strains)			*S. marcescens* (6 strains)		
		S (%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S (%)	I(%)	R(%)	S (%)	I(%)	R(%)
1	LBC	88	12	0	0	29	71	0	0	100	100	0	0	0	33	67
2	NA	0	67	33	0	14	86	0	0	100	80	20	0	83	17	0
3	CN	0	67	33	0	14	86	0	25	75	0	20	80	0	17	83
4	OFX	88	12	0	71	29	0	75	25	0	80	20	0	67	33	0
5	ZEM	67	33	0	86	14	0	0	12	88	100	0	0	0	33	67
6	CFX	88	12	0	86	14	0	0	37	63	0	20	80	60	40	0
7	CRO	88	12	0	71	29	0	0	37	63	0	20	80	0	33	67
8	CXM	67	33	0	0	14	86	0	37	63	60	40	0	100	0	0
9	NF	88	12	0	0	29	71	75	25	0	80	20	0	100	0	0
10	AUG	0	33	67	86	14	0	0	12	88	0	20	80	83	17	0
11	ACX	0	12	88	86	14	0	0	25	75	60	40	0	67	33	0
12	IMP	0	12	88	86	14	0	0	12	88	60	40	0	0	17	83
	MARI	**0.25**			**0.42**			**0.83**			**0.33**			**0.42**		

KEY: OFX = Ofloxacin, LBC = levofloxacin, CN = Gentamycin, ZEM = cefxime, CFX = cefoxitin CXM = cefuroxime, AUG = Augmentin, IMP = Imipenem, NA = Naladixic acid, CRO = Ceftriaxone, NF = Nitrofurantoin, ACX = ampicillin, S = Sensitive, I = Intermediate, R = Resistance, MARI = Multiple Antibiotic Resistance index (MAR index ≥ 0.2 (public health significance)

CHAPTER FIVE

DISCUSSION

The range of bacterial counts cultured from the respective gate passes/tags in this study was at variance with data reported by Adeleye *et al*. (2022) which revealed lower bacterial bio-load (1.68×10^2 cfu/ml) with respect to swabbed plastic gate passes given to individual visitors by a Federal University located in Dutse, Jigawa State, Nigeria. There were variations in the viable counts obtained from the swabbed gate passes being used in UNIBEN and UBTH. A likely reason for this trend could be the extent and magnitude of usage of these gate passes which could impact directly the resultant microbial load present on the surface of these fomites. The human hand is known to harbor several bacterial commensals and the contact between the hand and these gate passes would invariably lead to the introduction of bacteria onto the surfaces of the tags. These reasons were previously posited by Adeleye *et al*. (2022) and the authors further stated that the volume of human traffic visiting a particular area within a gated premise can also have a direct effect on the microbial bio-load present on the surface of these gate passes.

The likely direct effect of human usage on the surface microbial bio-load of gate pass has also been reported by Ulger *et al*. (2009) and the authors further opined that the surfaces of these objects could function as a reservoirs of microbiota that could subsequently serve as a infection source when touched by humans.

The swabbed gate passes were observed to harbor a diverse species of bacteria as well as fungi and this trend was similar to a study conducted by Aliyu *et al*. (2018) who isolated seven bacterial species including *Proteus vulgaris*, *Pseudomonas*

aeruginosa, *Staphylococcus aureus*, and *Escherichia coli* (amongst other bacteria spp.) and *Trichoderma* sp., *Penicillium* sp., and *Aspergillus flavus* (amongst other fungi) from swabbed surfaces of gate passes issued at the Umaru Musa Yar'adua University main gate. Badger-Emeka (2015) suggested that bacteria present on gate passes could serve as potential source of infection which can easily be transmitted through contact with the hands. Al-Harbi *et al*. (2017) observed that fomites can act as microbial reservoirs which could subsequently aid in the dissemination of infection when in contact with different skin surfaces of the users.

Escherichia coli (16.1%) was observed to be the most dominant prokaryote from the swabbed surfaces of the respective gate passes. This trend was similar with a study by Hamza *et al.* (2019) which revealed the predominance of *E. coli* in gate passes used in Aminu Kano Teaching Hospital, Kano and attributed their isolation from these fomite surfaces to the fact that the bacterium was part of the normal body flora

S. aureus has been identified as a etiologic agent responsible for nosocomial infections attributed to multiple antibiotic resistant strains, staphylococcal food poisoning and skin infections exemplified by boils, styles as well as as furuncules (Eggimann *et al*., 2003). This Gram positive prokaryote has also been identified as a causative agent of several health outcomes which include; pneumonia, urinary tract infections (UTI), bacteremia as well as endocarditis (Medrano *et al.*, 2011).

The isolation of *P. aeruginosa* from the respective swabbed gate passes was similar to an earlier study executed by Ivan *et al*. (2018) who revealed a percentage

isolation rate;19.7% with respect to the detection of this prokaryote from swabbed gate passes. Misgana *et al*. (2015) described the role(s)attributed to contaminated fomites in the spread and dissemination of microbial pathogens. Frequent transfer of inanimate objects to different units could aid microbial proliferation in these fomites (Ekrami *et al*., 2011).

The detection and culturing of *Bacillus* spp. from some of the swabbed gate pass samples was in contrast with an earlier study executed by Adeleye *et al*. (2022) which documented the absence of this group of endospore forming bacterial genera. However, the detection of this bacterium in the swabbed gate pass could be attributed to the fact that this endospore producing prokaryote can generally tolerate unsuitable environmental conditions exemplified by desiccation and ultraviolet irradiation due to its endospore forming abilities and the dormancy of these endospores for years (Nicholson *et al.*, 2000).

*Proteusvulgaris*is known to thrive in a broad variety of environments which include; water, dust, surfaces as well as soil. The isolation of *Proteus* spp. from swabbed gate pass surfaces in this study was similar to an earlier research conducted by Samy *et al*. (2012) who detected the presence of the bacterium from gate pass surfaces. Greenblat *et al*. (2004) opined that *Proteus* sp. strains are known opportunistic pathogens especially in immuno-compromised individuals. *Proteus* sp. can cause infections such as recurrent bacteraemia, septic shock and septic arthritis (Palusiak, 2022). *Proteus* sp. can thrive in conditions where there is little water or a lot of salt (Greenblat *et al*., 2004).

Research by Zahra *et al*. (2016) indicates that *Aspergillus* sp. is ubiquitous, appearing commonly on human skin and across diverse environments. This is supported by Eggimann *et al.* (2003), who noted that inanimate objects serve as vectors for spreading *Aspergillus* spp. in contaminated areas—a finding consistent with the observations in this study. Similarly, the detection of *Penicillium* sp. on gate passes aligns with Santos *et al*. (2010), who identified this fungus as a primary microorganism found on fomites. As a globally distributed mould, *Penicillium* is a frequent contaminant and a known cause of food spoilage. Beyond its role in degradation, its spores are potent allergens (Oliveira *et al*., 2023). While generally low in virulence, certain species can infect immunocompromised patients (Bloomfield, 2011) or lead to ulcerative dermal lesions if the skin barrier is compromised (Ge and Wang, 2022).

The prevalent distribution of fungal species on gate passes can likely be attributed to frequent human contact and the cosmopolitan nature of these microfungi. Furthermore, their high rate of occurrence is supported by their inherent resilience; as noted by Nada and Faiza (2017), these organisms possess a documented capacity to endure fluctuating moisture levels and ambient temperatures, allowing them to thrive in varied environmental conditions.

The antibiotic susceptibility profiles of the identified bacterial cultures from the swabbed gate passes indicated that Gram negative bacterial isolates which include; *E. coli, P. rettgeri, P. aeruginosa, P. vulgaris* and *S. marcescens* were resistant to Gentamycin and susceptible to Ofloxacin and Nitrofurantoin while Gram positive bacterial isolates namely *S. aureus*, *B. altitudinis* and *S. pyogenes* were resistant to

Ciprofloxacin and susceptible to cefoxitin and erythromycin respectively. Current observations indicate that several commercial antibiotic discs failed to inhibit the growth of the bacterial isolates. These findings contrast with the work of Mohammed *et al.* (2019), who found that *P. aeruginosa* and *E. coli* were susceptible to ciprofloxacin. Conversely, the results align with Sahile *et al*. (2016), who reported that isolates such as *E. coli* and *P. aeruginosa* exhibited resistance to multiple antibiotics, including ciprofloxacin, gentamicin, ofloxacin, ampicillin, and erythromycin. Such resistance patterns may be attributed to the unregulated or indiscriminate use of these drugs by humans (Manisha *et al*., 2017).

In the present study, several isolates exhibited multi-drug resistance (MDR), with P. aeruginosa and S. aureus recording the highest Multiple Antibiotic Resistance (MAR) indices at 0.7 and 0.6, respectively. These results echo the findings of Abebaw et al. (2018), who noted that *P. aeruginosa*, *E. coli*, and *S. aureus* demonstrated resistance to ciprofloxacin, gentamicin, and augmentin. Furthermore, species including *B. altitudinis, S. pyogenes, P. rettgeri, P. vulgaris* as well as *S. marcescens* were resistant to at least two antibiotic classes. This aligns with Tigist *et al*. (2018), who identified *S. pyogenes, P. vulgaris* and *Klebsiella* spp. as primary multidrug-resistant pathogens. The expansion of a robust resistance gene pool would likely accelerates this trend, highlighting a concerning spread of resistant strains within the study area. Consequently, antibiotics that were once effective may quickly become obsolete. This would pose a notable challenge in resource-limited regions like Nigeria, where the high cost of medical treatment is often secondary to basic survival needs. The variations in resistance patterns across different sampling

sites may be attributed to the specific multi-drug resistant strains present in those locations (Yah e*t al*., 2007).

5.1 Contribution to Knowledge

This study contributed to knowledge in the following ways;

1. The study demonstrated that the swabbed gate pass/tags was a reservoir for multiple drug resistant bacteria of public health relevance.
2. Potentially pathogenic fungi were also isolated from the examined swabbed gate passes/tags.
3. The feedback from this study to the affected community shall help to increase awareness of the likely health hazards directly or indirectly linked with gate pass usage with the expectation that management and other stakeholders will take appropriate measures in the interest of public health.

5.2 Conclusion

Several potentially pathogenic bacterial (*B. altitudinis, E. coli, S. aureus, P. rettgeri,S. pyogenes, S. marcescens, P. vulgari*s, *P. aeruginosa*)and fungal isolates (*T. harzianum,*

A. niger, Penicillium sp., *Microsporum* sp. and *Cladosporium* sp.) were cultured from swabbed gate passes being utilized in two public institutions; UNIBEN and UBTH respectively. All the Gram positive bacterial isolates demonstrated drug resistance to ciprofloxacin while the Gram negative isolates were resistant to gentamycin. The MAR index of the selected isolates varied from 0.83 for *P. aeruginosa* to 0.25 for *E. coli* indicting that the bacterial isolates were of public health importance. With reference to this

study's findings , it is essential to promote hand hygiene immediately before and after using gate passes via the use of hand sanitizers or practice of hand washing with soap (when convenient). It is also necessary to conducttraining of personnel with respect to good contact control and good work habits as it relates to the use of gate passes/tags.

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APPENDIX I

CULTURE MEDIA

Nutrient Agar

Beef extract	3.0g
Agar No.2	12.0g
Peptone	5.0g
Sodium chloride	8.0g
Distilled water	1000ml

Nutrient broth

Gelatin Peptone	5.00
Beef Extract	3.00

Preparation

Nutrient Broth (NB) was used for serial dilution and Nutrient Agar (NA) was used for culturing. In preparing the media, 13 g of nutrient broth was dissolved in 1000 ml of distilled water and 28 g of nutrient agar was dissolved in 1000ml of distilled water. The media were autoclaved at 121 ^{o} C for 15 minutes.

APPENDIX II

MacConkey Agar Test

MacConkey agar (MAC) is a selective and differential bacterial culture media. MacConkey medium is selective for gram-negative bacteria and differentiates the gram-negative bacteria based on lactose metabolism.

Composition (g/l)

Peptone	17 g
Polypeptone	3 g
Lactose	10 g
Bile salts	1.5 g
Sodium chloride	5 g
Agar	13.5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Distilled water	1L
Final pH	7.1

Preparations

Weigh and suspend 50 grams of MacConkey agar powder in 1 Litre of purified water and mix thoroughly. Read and follow the instruction of the manufacturer if you have purchased dehydrated agar media from a commercial supplier.Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.Autoclave at 121°C for 15 minutes. Cool to 45-50°C, mix well, pour about 20- 25ml into sterile Petri plates and allow solidifying before use.

APPENDIX III

GRAM STAINING AND BIOCHEMICAL REAGENTS

GRAM STAIN

Gram's crystal violet

Solution A

Crystal violet	2.0g
Ethanol (95ml)	20.0ml

Solution B

Ammonium oxalate	0.8g
Distilled water	8.0ml
Iodine	1.0g
Potassium iodide	2.0g
Water	300ml

3.0g of medium dissolved in 300ml distilled water

Gram's safranin

Safranin	0.25g
Ethanol	10.0ml
Distilled water	100ml

BIOCHEMICAL REAGENTS

Indole medium

Peptone	20.0g
Sodium chloride	5.0g
Distilled water	1000ml
PH	7.4

Preparation

15g of peptone was dissolved in 1000ml of distilled water and autoclaved at 121^{o}C for 15 minutes, allow to cool for a temperature of about 47 ^{o}C and dispense aseptically into sterile test bijou bottles.

Simmon citrate agar

Composition

Sodium ammonium phosphate	1.50g
Potassium dihydrogen phosphate	1.0g
Magnesium sulphate	0.2g
Sodium citrate	2.5g
Bromothymol blue	0.016g
Distilled water	1000ml
PH	7.0

Preparation

24.0g of Simmon's citrate medium was dissolved in 1000ml of distilled water and autoclaved at 121^{o}C for 15 mins, allowed to cool for a temperature of about 47 ^{o}C and dispense aseptically into sterile test tubes

Urea base agar

Composition

Peptone	1g
Sodium chloride	5g
Potassium dihydrogensulphate	2g
Glucose	5g
Agar powder	20g
Distilled water	1000ml

Preparation

The powdered urea agar was used and prepared according to manufacturer's instruction. Urea agar was prepared by suspending 2.1g of the powdered medium in 95ml of distilled water and dissolve by boiling. The medium was sterilized by autoclaving for 20mins at 115^{o}C. The medium was cooled to about 50^{o}C and 5ml of 40% v/v sterile urea solution was added. The medium was dispensed into culture tubes in 15ml aliquot and were allowed to solidify in slant positions.

Kovac's reagent

This reagent is used for indole test, which is used to determine which organism has the ability to split indole from tryptophan.

Composition

Amul Alcohol 15.0ml

P-dimethylaminobenzaldehydre 0.5ml

Concentrated hydrochloric acid 50 ml

Preparation

The aldehyde was dissolved in the alcohol and slowly the prepared acid was added in small quantities and stored in refrigeration. Shake before use. 0.5 Kovac's reagent is added on the 48hrs peptone food containing the isolates for organism

Sugar utilization medium

Glucose, fructose, lactose, mannitol, maltose and sucrose fermentation tests were carried out using Phenol red carbohydrate broth (Merck, 2007). This test is done to evaluate the ability of a particular bacterial isolate in using a particular sugar for energy. The broth was sterilized separately from the carbohydrate solution to avoid hydrolysis of carbohydrates caused by excessive heating. These ingredients were dissolved in 800ml distilled water to prepare phenol red base broth and 4.5ml of this transferred to capped test tubes. Durham tubes were then inserted inside the test tubes to detect gas

production during fermentation. The broth was sterilized using an autoclave at 121°C for 15 minutes.

Composition

Peptone	2.0g
Sodium chloride	5.0g
Water	1000ml
Phenol red	7.0

Preparation

10% v/v of each of the following sugar is dissolved in 1000ml of distilled water and autoclaved at 121^{o}C for 15 mins. This was allowed to cool and dispense aseptically into sterile test tubes containing Durham's tubes. Observations were then made on color changes with yellow color indicating fermentation of the sugar hence positive results while pink-red/red color implied no fermentation hence negative Reaction. Gas production was indicated by presence of bubbles trapped within the durham tube.

APPENDIX IV

Statistical Analysis — Unpaired T-Test Results for Microbial Counts

Table 1: Unpaired T-test results for Total Heterotrophic Bacterial Counts (THBC) — UNIBEN vs UBTH

Week	UNIBEN THBC ($\times 10^5$ cfu/cm^2)	UBTH THBC ($\times 10^5$ cfu/cm^2)	p-value	Significance
1	6.35±1.05	5.30±10.4	0.88	Not significant
2	3.33±0.19	8.17±1.09	0.08	Not significant
3	3.01±0.08	2.70±0.73	0.65	Not significant
4	5.33±0.20	3.23±0.68	0.12	Not significant
5	10.4±0.90	5.45±3.74	0.21	Not significant
6	7.20±0.63	4.12±0.42	0.07	Not significant
7	9.20±1.46	5.12±0.91	0.10	Not significant
8	6.50±1.17	2.22±0.33	0.09	Not significant
9	4.75±0.17	5.17±0.20	0.24	Not significant
10	4.20±0.38	2.17±0.31	0.07	Not significant
11	6.66±0.28	6.17±0.81	0.58	Not significant
12	2.60±0.71	7.13±1.76	0.10	Not significant

Key: THBC = Total Heterotrophic Bacterial Count; cfu/cm^2 = colony forming units per cm^2. All weekly comparisons yielded $p > 0.05$, indicating no statistically significant difference between UNIBEN and UBTH bacterial counts.

Table 2: Unpaired T-test results for Total Coliform Counts (TCC) — UNIBEN vs UBTH

Week	UNIBEN TCC ($\times 10^5$ cfu/cm^2)	UBTH TCC ($\times 10^5$ cfu/cm^2)	p-value	Significance
1	7.30±1.57	3.94±0.29	0.11	Not significant
2	8.20±1.45	4.17±0.31	0.10	Not significant
3	4.00±0.60	5.27±0.41	0.24	Not significant
4	2.20±0.34	2.70±0.86	0.56	Not significant
5	4.43±0.65	5.17±0.20	0.31	Not significant
6	2.55±0.41	2.70±0.86	0.84	Not significant
7	2.05±0.38	4.29±0.40	0.07	Not significant
8	2.46±0.66	2.10±0.46	0.68	Not significant
9	3.33±0.33	7.10±0.57	0.07	Not significant
10	7.00±2.88	3.33±0.33	0.31	Not significant
11	5.00±0.30	3.00±0.00	0.07	Not significant
12	1.33±0.06	1.33±0.03	1.00	Not significant

Key: TCC = Total Coliform Count; cfu/cm^2 = colony forming units per cm^2.

Table .3: Unpaired T-test results for Total Fungal Counts (TFC) — UNIBEN vs UBTH

Week	UNIBEN TFC ($\times 10^5$ sfu/cm^2)	UBTH TFC ($\times 10^5$ sfu/cm^2)	p-value	Significance
1	3.15±0.72	11.3±0.34	0.07	Not significant
2	3.76±0.76	5.33±0.28	0.15	Not significant
3	5.72±1.20	3.14±0.52	0.19	Not significant
4	2.58±0.55	4.41±0.52	0.11	Not significant
5	2.51±0.51	3.54±0.23	0.17	Not significant
6	4.53±1.52	4.27±0.33	0.89	Not significant
7	3.20±0.99	2.55±0.05	0.53	Not significant
8	6.00±0.11	2.90±0.01	0.07	Not significant
9	2.10±0.80	2.20±0.00	0.87	Not significant
10	3.23±0.73	4.05±1.05	0.56	Not significant
11	4.51±0.65	3.01±0.88	0.31	Not significant
12	3.09±0.00	2.01±0.66	0.25	Not significant

Key: TFC = Total Fungal Count; sfu/cm^2 = spore forming units per cm^2.

APPENDIX V

Antibiotic Susceptibility Profiles of Bacterial Isolates (Kirby-Bauer Disc Diffusion)

Table 4: Antibiotic susceptibility and resistance profiles of all bacterial isolates recovered from gate pass / tags (UNIBEN and UBTH)

Antibiotic	*S.aureus*	*P.rettgeri*	*S.pyogenes*	*P.vulgaris*	*S.marcescens*	*E.coli*	*B.altitudinis*	*P.aeruginosa*
Ofloxacin (OFX)	S	R	S	S	S	S	R	S
Levofloxacin (LBC)	R	R	S	S	R	S	R	R
Ciprofloxacin (CIP)	R	R	R	S	S	R	R	R
Gentamycin (CN)	S	R	S	R	R	S	R	S
Chloramphenicol (CH)	S	S	S	S	S	S	S	S
Cefixime (ZEM)	R	S	S	S	R	S	S	R
Cefoxitin (CFX)	S	S	S	R	S	S	S	S
Cefuroxime (CXM)	R	R	S	S	S	S	S	S
Ampicillin (AZM)	R	S	S	S	S	R	R	R
Augmentin (AUG)	S	S	R	R	S	R	S	R
Imipenem (IMP)	R	S	R	S	R	R	S	R
Erythromycin (E)	S	S	S	S	S	S	S	S
Nalidixic acid (NA)	S	R	S	S	S	S	S	R
Ceftriaxone (CRO)	S	S	S	R	R	S	S	S
Nitrofurantoin (NF)	S	S	S	S	S	S	S	S
Ampicillin (ACX)	R	S	S	S	R	R	R	R

Key: S = Sensitive I = Intermediate R = Resistant | Colour code: Green = Sensitive Yellow = Intermediate Red = Resistant

Organisms: SA = Staphylococcus aureus, PR = Providencia rettgeri, SP = Streptococcus pyogenes, PV = Proteus vulgaris, SM = Serratia marcescens, EC = Escherichia coli, BA = Bacillus altitudinis, PA = Pseudomonas aeruginosa

APPENDIX VI

Multiple Antibiotic Resistance (MAR) Index of Bacterial Isolates

Table 5: Multiple Antibiotic Resistance (MAR) index values for all bacterial isolates recovered from swabbed gate pass / tags

Isolate	Gram Reaction	Antibiotics Resisted (A)	Total Antibiotics Tested (B)	MAR Index (A/B)
Staphylococcus aureus	+ve	7	12	**0.58**
Providencia rettgeri	−ve	5	12	**0.42**
Streptococcus pyogenes	+ve	4	12	**0.33**
Proteus vulgaris	−ve	4	12	**0.33**
Serratia marcescens	−ve	5	12	**0.42**
Escherichia coli	−ve	3	12	**0.25**
Bacillus altitudinis	+ve	5	12	**0.42**
Pseudomonas aeruginosa	−ve	10	12	**0.83**

Key: A = Number of antibiotics resisted by isolate; B = Total antibiotics tested (n = 12); MAR Index = A/B. MAR index ≥ 0.2 indicates isolates from high antibiotic-use environments and is of public health significance (Mir et al., 2021).

APPENDIX VII

Ethical Consideration and Consent Documentation

This study involved non-invasive environmental sampling (surface swabbing) of gate pass / tags at the entry points of University of Benin (UNIBEN) and University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. No human subjects, patient records, or clinical specimens were involved in this research.

Prior to the commencement of sample collection, verbal consent was obtained from the Chief Security Officers of both institutions. Permission was expressly granted to the researcher to aseptically swab gate pass / tags at the main entrance points of UNIBEN and UBTH over the 12-week sampling period (August – November 2023). Both institutions were informed of the purpose of the research and the non-invasive nature of the sampling procedure.

Institution	Form of Consent Obtained
University of Benin (UNIBEN)	Verbal consent — Chief Security Officer
University of Benin Teaching Hospital (UBTH)	Verbal consent — Chief Security Officer

The research was conducted in full compliance with standard ethical principles governing environmental microbiological research. No identifiable personal data were collected from any individual during the course of the study.