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## RESEARCH ARTICLE

**BIO-AEROSOLS AND THEIR VIRULENCE FACTORS IN EDONWHII BEACH ATMOSPHERE, EASTERN OBOLO LGA, NIGERIA**

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## ARTICLE DETAILS

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## ABSTRACT

Standard microbiological techniques were used to evaluate bio-aerosols and associated virulence factors in the Edonwhii Beach Atmosphere in Eastern Obolo LGA, Nigeria. The microbial components of the beach atmosphere were evaluated using Settle Plate and Metagenomics methods. All the culturable bacteria but *Escherichia coli*, elaborated virulence factor(s), indicating potential to cause disease. At the molecular level, pathogenic genes *fliC* and *hlyA* that code for flagellin C and hemolysin A virulence factors were detected. Also detected were strains of *Fusarium verticillioides*, *Candida krusei*, *Candida albicans*, *Trichophyton sporon* sp and *Cladosporium* sp and are capable of causing keratitis or other dermal infections in human. High concentrations of microbial pollutants in Edonwhii beach atmosphere are a clear evidence of air pollution which can adversely impact on human health. As a result, continual monitoring of air quality, as well as devising and developing instruments to identify contaminants, is advised. Open defecation near the beach should be avoided, and overall environmental cleanliness should be maintained.

## KEYWORDS

Bio-aerosols, Virulence Factors, Atmospheric Aerosols, Settle Plate, Open defecation, Metagenomics

## 1. INTRODUCTION

Aerosols in the atmosphere can be found in both natural and manmade settings. Bio-aerosols make up a significant portion of atmospheric aerosols. Bio-aerosols are very small airborne particles of biological origin that range in size from 0.001 to 100 m and, due to their small size and low weight, may be readily transferred from one environment to another (Lindsley et al., 2017). A group researchers expect that bio-aerosols will account for 5-50% of atmospheric particles greater than 0.2m in diameter (Smith et al., 2018). Fundamental environmental parameters such as temperature and moisture content, on the other hand, can greatly influence the extent of bio-aerosol formation and dispersion due to their regulatory impact on the development of microorganisms (Dedesko et al., 2015). According to pathogenic bacteria in bio-aerosols can spread in a variety of situations because to their small diameter and lightweight (Hsu et al., 2022). Exposure to bio-aerosols in aquatic ecosystems has gained a lot of attention in recent years due to public health concerns.

Water bodies, open defecation, marine waste, dead and decaying aquatic creatures, aquatic food processing activities, and so on are all potential sources of bio-aerosol exposure in the aquatic environment (Spring et al., 2018; Zhang et al., 2019). Some researchers discovered soil to be one of the key sources of microorganisms released into the atmosphere (Xie et al., 2021). Rainfall can also speed up the discharge of bio-aerosols from the soil into the atmosphere. Coughing, washing, toilet flushing, talking, strolling, sneezing, and cleaning floors are all capable of producing bio-aerosols (Nazaroff, 2016). Airborne or droplet transmission has been recognized as the primary pathways for the spread of human and animal

germs such as *E. coli*, *Staphylococcus aureus*, *anthrax*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Zheng et al., 2018). According to a study, exhaled breath may account for around 17% of total bio-aerosol emissions from humans (Xu et al., 2017). Another study developed an aerosol droplet sampling technique to extract pulmonary surfactant from alveolar lining fluid (ALF), which might be utilized to assess lung functions (Mizev et al., 2018).

Bio-aerosols, which are tiny airborne particles containing biological agents, have the potential to cause respiratory infections or give rise to symptoms like allergic asthma, rhinitis, and airway inflammation. Researchers have reported these health risks, particularly among people living near riversides who are exposed to bio-aerosols (Kim et al., 2017). Over the last few decades, the field of bio-aerosol research has seen significant developments, as pointed out by (Mainelis, 2019). This progress encompasses various aspects, including the investigation of adverse health effects caused by bio-aerosols, the role of bio-aerosols in environmental processes like cloud formation and ice nucleation, their presence at high altitudes, their interactions with ecosystems, and their role in the transmission of plant diseases.

According to bio-aerosols can have a significant influence on public health through a variety of mechanisms, including inhalation, skin contact, and ingestion (Jiayu et al., 2019). These agents have the ability to cause infectious as well as non-infectious illnesses. Q fever, Legionnaire's disease, and Pontiac fever are some of the infectious diseases associated with bio-aerosols, while non-infectious diseases such as asthma, allergic rhinitis, bronchitis, atypical conjunctivitis, and organic dust toxic

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syndrome can also be triggered by bio-aerosol exposure. The composition of bio-aerosols can contain various viruses, such as enteric viruses (e.g., Noro- and rotaviruses) and respiratory viruses (e.g., influenza and coronaviruses), as well as bacteria (like staphylococci, legionellae, tuberculous and non-tuberculous bacteria), bacterial spore formers (*Clostridium difficile* and *Bacillus anthracis*), and fungi (including *Aspergillus*, *Penicillium*, *Cladosporium* spp, and *Stachybotrys chartarum*).

The standard procedures and culture-dependent methods used for analyzing microorganisms in air samples have limitations, as they cannot capture the full diversity of microorganisms present, including those that are non-culturable. To overcome this, molecular methods have become crucial tools for studying microbial communities. These techniques involve directly isolating genetic material from environmental samples, enabling the analysis of all microorganisms, including the non-culturable ones (Otlewska et al., 2014). To live within their hosts, microorganisms have developed a variety of virulence factors, leading to the development of severe diseases. Recent advances in genomics data and bioinformatics techniques for common human infections have yielded important insights into the molecular understanding of virulence features. This understanding is likely to pave the door for the development of novel compounds and techniques for combating infectious illnesses (Leitoa, 2020). Toxins, enzymes, exopolysaccharides, capsules, lipopolysaccharides, glyco- and lipoproteins, and other compounds in pathogenic microbes boost their capacity to elude human defenses and cause illness. Furthermore, specific microbial products can infiltrate host cells and modify their activities, aiding the infection's progress (Amaretti et al., 2020). Notably, capsules, siderophores, lipopolysaccharide (LPS), and fimbriae all have a key role in hypervirulence (Zhu et al., 2021).

Bio-aerosols aid in the transmission of pathogenic bacteria containing virulence genes. These genes code for virulence factors, which allow microorganisms to assault the host's immune system and cause a variety of illnesses. Biofilm production, hypermucoviscosity, capsule synthesis, adhesions, iron absorption, and lipopolysaccharide creation are examples of virulence factors (Aljanaby and Alhasani, 2016). This study evaluated the virulence factors producing potential of the dominant microbial contaminants; and revealed microbial bio-aerosols and their virulence factors in Edonwhii Beach Atmosphere, Eastern Obolo LGA, Nigeria.

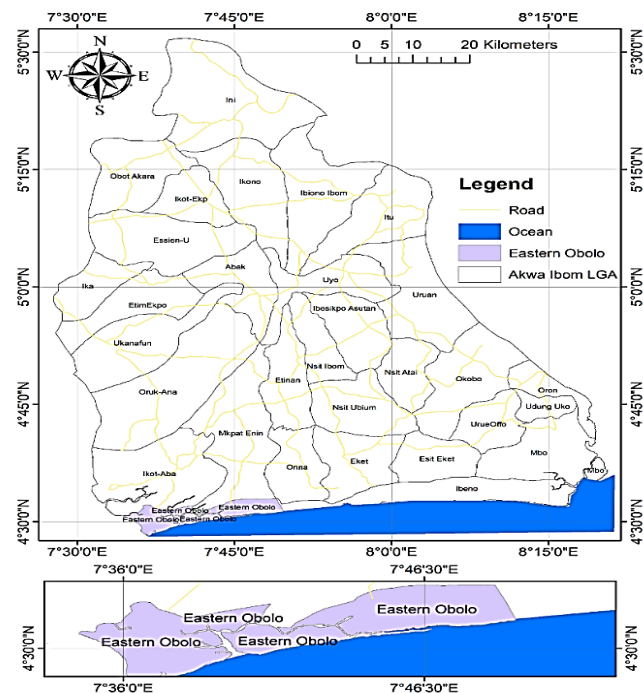
## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Area and Sampling Locations

Edonwhii Fishing Settlement is located in Akwa Ibom State's Eastern Obolo Local Government Area (LGA), on the outskirts of the Niger Delta. The Eastern Obolo LGA is located between the Imo and Qua Iboe estuaries, with latitudes ranging from 4° 28 to 4° 53 and longitudes ranging from 7° 50 to 7° 55 East. The LGA is bounded to the north by Mkpato Enin Local Government Area, to the northeast by Onna, to the west by Ikot Abasi, to the southeast by Ibeno Local Government Area, and to the south by the Atlantic Ocean. The area's overall landmass is 117,008 square kilometers,

with an estimated coastline length of 184 kilometers (Ekpe et al., 1995). Eastern Obolo is one of Nigeria's most densely inhabited coastal districts, with several villages involved in diverse socioeconomic activities such as artisanal fishing, timbering, and boat transport.

The waterways of Eastern Obolo are rich in vegetation, including *Rhizophora* mangle, *Avicennia africana*, *Lanuncularia*, *Raphia hookeri*, and *Nypa fruticans*. *Sargassum* sp. is common during the wet season. Furthermore, oil palm (*Elaeis guineensis*) and coconut palm (*Coccoloba nucifera*) are found in abundance throughout the settlements. The area is also notable for oil production, since it is home to multiple oil exploration wells. *Nypa* palm and red mangrove are the major plant species. Numerous creeks define the terrain, and there is a huge mudflat at Iko creek and other spots around the area. A big sand bar may be seen near Etizar and Edonwhii at neap low tide. As a tropical location, the area has two distinct seasons: dry (October to May) and rainy (April to October), with an average annual rainfall of roughly 2500 mm (AKUTEK Report, 2006; Effiong et al., 2018).



**Figure 1:** Coastal Region of Akwa Ibom State Showing the Study Area

Ten (10) sampling stations (Figure 2) were established and marked (geo-referenced) with a Global Positioning System (GPS) device.



**Figure 2:** Satellite Imagery of Edonwhii Beach showing the sample stations.

### 2.2 Preparation of Media

Two distinct types of medium were used to extract microorganisms from the beach environment. Nutrient Agar (NA) [Oxoid], Saboraud Chloramphenicol Agar (SCA) [Scharlau], and MacConkey Agar NO. 1 (MA) [Fluka] were among them. The NA was employed for bacterial isolation, whereas the SCA and MA were used for enteric bacteria isolation. The makers' guidelines were followed in the manufacturing of these media. In a water bath, the mixture of each medium was heated and brought to a boil

until entirely dissolved. After that, the mixture was autoclaved at 121°C for 15 minutes to assure sterility. The Agar was autoclaved and allowed to cool to 45°C before being placed into sterilized plates to harden.

### 2.3 Enumeration of Microbial Loads of the Beach Atmosphere

In this work, the sedimentation (or settle plate) method described by Downes and Ito was applied (Downes and Ito, 2001). Allowing bacteria-carrying particles to settle onto a solid medium for a defined amount of

time, followed by incubating the medium at the desired temperature, is the basis for this procedure. The number of colonies produced on the medium reflects the quantity of bacteria-containing particles that have settled. It is a method that does not require any equipment and focuses on viable particles (bio-aerosols) that deposit on the surface of a solid medium during a certain exposure duration (APHA, 1992). Nutrient Agar (NA) and Sabouraud dextrose agar (SDA) were employed to count aerobic bacteria, following the procedures described by APHA and Downes and Ito respectively (APHA, 1992; Downes and Ito, 2001). For fungi, chloramphenicol (-µg/ml) was added to the medium, while bacteria were treated with a mixture of 100 g/ml Cycloheximide and 50 g/ml Benomyl. In addition, employing MacConkey Agar as the analytical medium, coliform densities were measured.

Allowing the bio-aerosol to settle on uncovered Petri plates holding the proper culture medium. The exposure took place in an open space with no walls or obstructions. Pre-prepared and sanitized 9 cm diameter Petri plates with 20ml of the corresponding culture media were put on a 4ft high wooden platform and exposed for 20 minutes at each sample point. The experiment was performed three times for each microbiological characteristic, yielding a total of ten samples from various sampling locations. Following exposure, the Petri dishes were closed, transferred to the laboratory, and incubated according to the type of bacteria being studied. The NA and MA plates were specifically incubated at 37°C for 2 days to measure the numbers of heterotrophic bacteria and coliforms.

## 2.4 Calculation

After incubation, the colonies on the Petri plates were counted (Pasquarella et al., 2000). The Polish standard PN89/2-04088/08 gave Equation 1 to estimate the number of microorganisms in CFU/m<sup>3</sup> using the Settle Plate Technique and Koch's sedimentation technique.

$$\text{CFU/m}^3 = a \times 1000 / P \times t \times 0.2 \quad (1)$$

Where:

- "a" represents the number of colonies counted on the Petri plate.
- "P" denotes the surface measurement of the Petri plate used.
- "t" signifies the exposure time of the Petri plate (Friberg et al., 1999).

In summary, the following method is used to compute microbe concentration, given as colony-forming units per cubic meter (CFU/m<sup>3</sup>), based on the number of colonies detected on the Petri plate, the surface area of the plate, and the exposure duration.

## 2.5 Determination of Virulence Factors Producing Potential of the Isolates

### 2.5.1 Coagulase Test

The approach provided by Turkyilmaz and Kaya was utilized to determine coagulase activity (Turkyilmaz and Kaya, 2006). The Tube Coagulase (TC) test required combining several colonies of each organism in a sterile test tube with 0.5 ml of citrated rabbit plasma. The tube was then incubated at 37°C for 4 and 24 hours before being examined. Clot formation was reported as a positive result for coagulase activity if it was seen at either of these time periods.

### 2.5.2 DNase Test

The experiment was carried out using Difco's commercially available DNase agar. The DNase agar was inoculated with spots, and the plates were incubated at 37°C for four days. Following incubation, 1 N HCl was poured onto the agar. The appearance of a clearing zone around the bacterial growth suggested that DNase activity was present (Turkyilmaz and Kaya, 2006).

### 2.5.3 Thermonuclease (TNase) Test

The approach provided by Turkyilmaz and Kaya was utilized to assess TNase activity (Turkyilmaz and Kaya, 2006). They produced toluidine blue-deoxy nucleic acid agar and placed it into disposable Petri dishes to detect TNase activity. A pink halo (width 1 to 3 mm) surrounded each test well, indicating the presence of thermostable nuclease.

### 2.5.4 Capsule Test

Turkyilmaz and Kaya devised a technique for doing the capsule test (Turkyilmaz and Kaya, 2006). Using a loop, a tiny number of microorganisms were mixed with Indian ink on a microscope slide. The slide was then topped with a cover slide. Against the brown-black backdrop, the capsule looked as a distinct zone enclosing the bacterium.

### 2.5.6 Slime Formation

The Congo Red Agar (CRA) technique established by Freeman et al., 1989). The medium included Brain Heart Infusion Broth (Oxoid) at a concentration of 37 g/l, sucrose at a concentration of 50 g/l, agar at a concentration of 10 g/l, and Congo red at a concentration of 0.8 g/l. The Congo red stain was made as a concentrated aqueous solution and autoclaved separately for 15 minutes at 121°C. When it had cooled to 55°C, it was added to the agar. The test samples were injected into the plates, which were then incubated aerobically at 37°C for 24 hours. Slime-positive isolates developed black colonies with a dry crystalline quality, while slime-negative isolates generated pink colonies.

### 2.5.7 Bio-film Formation

The Micro-plate technique (MP) developed by Pfaller et al., 1988). This was accomplished using tissue culture plates with 96 flat-bottomed wells. Each well was filled with 0.2 ml of a bacterial solution in Tryptic Soy Broth (TSB) containing 105 CFU/ml. The contents were aspirated after 48 hours of incubation in aerobic conditions at 37°C, and the plates were washed twice with phosphate-buffered saline (PBS) at pH 7.2. Following that, the wells were stained for 30 seconds with 0.25% safranin. The plates were then scanned at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader (BioTek, ELx808). There was also a negative control with sterile TSB. The experiment was done at least twice, with the optical density (OD) measurements averaged. The ability of the strains to generate slime was graded on a three-point scale: no biofilm producer (-) with OD 0.500, weak biofilm producer (+) with OD between 0.500 and 1.500, and strong biofilm producer (++) with OD > 1.500.

### 2.5.8 Hemolysis

Tryptic Soy Agar (TSA) mixed with 5% washed rabbit erythrocytes was used to test alpha hemolysin. Positive samples showed a large zone of full hemolysis with fuzzy borders after 24 hours of incubation at 37°C. Strains were tested for beta-hemolysin on TSA containing 5% sheep blood. The plates were incubated at 37°C for 24 hours before being moved to 4°C overnight. Positive strains had a broad zone of partial hemolysis with sharp edges. Gamma hemolysis was defined as non-hemolysis on TSA with 5% sheep blood (Turkyilmaz and Kaya, 2006).

### 2.5.9 Hemagglutination

Turkyilmaz and Kaya presented a comparable approach for performing the hemagglutination test (Turkyilmaz and Kaya, 2006). The test was performed using U-shaped 96-well microtiter plates. McFarland standard 1 was used to adjust bacterial suspensions in phosphate-buffered saline (PBS). In the microtiter plates, two successive dilutions of the bacterial culture were made, resulting in a total volume of 50 l per well. Following that, each well received 50 l of a 1% human erythrocyte suspension in PBS. After shaking the plates, they were incubated at room temperature for 2 hours. The hemagglutination was then classified as positive or negative.

### 2.5.10 Determination of Virulence Potential of the Fungal Isolates

The purpose of this test was to identify fungi commonly responsible for skin diseases in both humans and animals, particularly fungi belonging to the genera *Microsporum*, *Epidermophyton*, and *Trichophyton*. Suspected isolates were streaked onto plates of Dermatophyte Test Medium (DTM) that had been sterilized at 121°C for 15 minutes and then cooled down to 45°C. After reaching room temperature, the agar surface dried before inoculation. The specimen was directly incubated onto the medium by gently pressing it onto the agar's surface. The plate was then incubated at room temperature (20-30°C) for up to 10-14 days. Daily examination of the plate was conducted to check for any color changes. A positive reaction was indicated by the development of a red color change in the medium, along with white aerial hyphae. Most pathogenic dermatophytes would produce a color change within 3 to 6 days. Negative results would show no color change to red (Campbell and Stewart, 1980).

## 2.6 Molecular Analysis of Air Samples

### 2.6.1 Collection of Samples

Phosphate Buffered Saline (PBS Buffer) was used in this investigation to catch microorganisms from the atmosphere. 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na<sub>2</sub>HPO<sub>4</sub>, and 0.24 g of KH<sub>2</sub>PO<sub>4</sub> were dissolved in 800 ml of distilled water to make the PBS Buffer. HCl was used to alter the pH of the solution to 7.4. The capacity was then increased to 1L by adding more distilled water. The mixture was autoclaved to sanitize it. Following that, metagenomic DNA samples were extracted and purified for further examination.

A workflow scheme for metagenomic (molecular) studies applied to air samples (Figure 3).

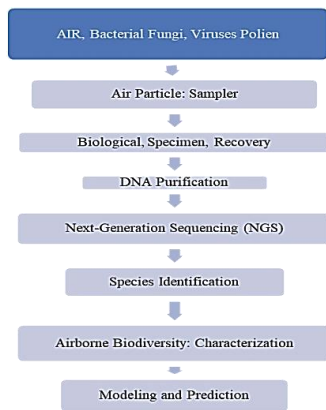


Figure 3: A workflow scheme for metagenomic studies applied to air samples (Tringe and Rubin, 2005)

### 3. RESULTS AND DISCUSSION

#### 3.1 Results

##### 3.1.1 Virulence of Microbes Isolated from the Edonwhii Beach atmosphere

Almost all isolated bacterial genera such as *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus* sp., *Staphylococcus aureus*, *Proteus* sp., *Klebsiella* sp., et cetera, had shown virulence factor except *Escherichia coli*, meaning that they have potential to cause disease (Table 1). Virulence factor had also been observed in fungal isolate *Fusarium verticillioides* capable of causing keratitis in man (Table 1). At the molecular level, pathogenic genes *fliC* and *hlyA* that codes for flagellin and hemolysin A virulence factors were detected (Table 2; Figure 3).

Table 1: Virulence potential of microorganism isolate from the beach atmosphere

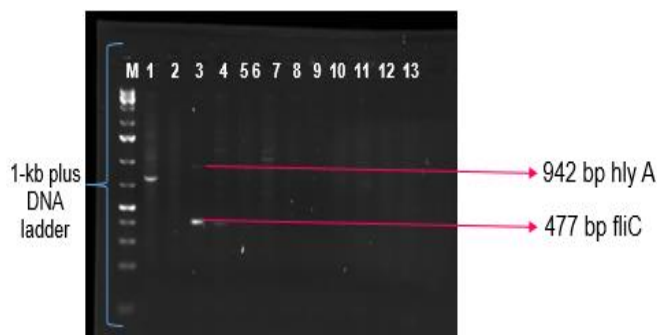
Isolate	Coagulase	Dnase	Tnase	Capsule	Slime Form.	Biofilm Form.	Hemolysis	Haemagglutination	Growth on DTM
<i>Micrococcus</i> sp	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	-	-	-	-	+	+	-	+	-
<i>Serratia</i> sp	-	+	-	-	+	-	+	+	-
<i>Bacillus</i> sp	-	+	-	+	-	+	-	-	-
<i>Staph. aureus</i>	+	-	+	-	-	+	β	-	-
<i>Shigella</i> sp	-	-	-	-	+	+	-	-	-
<i>Proteus</i> sp	-	+	-	-	-	-	-	-	-
<i>Salmonella</i> sp	-	-	-	-	-	-	-	-	-
<i>Pediococcus</i> sp	-	-	-	-	-	-	-	-	-
<i>Staphylococcus</i> sp	+	-	+	-	-	+	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-	+	+	+	-
<i>Aspergillus fumigatus</i>	-	-	-	-	-	+	+	-	-
<i>Aspergillus</i> sp.	-	-	-	-	-	+	-	+	-
<i>C. tropicalis</i>	-	-	-	-	-	-	-	-	-
<i>C. krusei</i>	-	-	-	-	+	+	-	-	+
<i>C. albicans</i>	-	+	-	-	+	+	-	-	+
<i>C. utilis</i>	-	-	-	-	-	+	-	-	-
<i>Pichia</i> sp	-	-	-	-	-	-	-	-	-
<i>Fusarium</i> sp.	-	-	-	-	-	+	-	-	+
<i>Trichosporon</i> sp.	-	-	-	-	-	+	-	-	-
<i>Sacchromyces</i> sp.	-	-	-	-	-	-	-	-	-
<i>Eurotium</i> sp	-	-	-	-	-	-	-	-	-
<i>Monascus</i> sp	-	-	-	-	-	-	-	+	-
<i>Cladosporium</i> sp	-	-	-	-	-	+	-	-	+
<i>Trichophyton</i> sp	-	-	-	-	-	-	-	-	+
<i>Fusarium verticillioides</i>	-	-	-	-	-	+	-	-	+

+ = Present - = Absent

Source: Field data

Table 2: Detection of Virulence Genes in Air Sample UB<sub>1</sub>

Genes	Detection		Virulence Factor
	n	%	
<i>fliC</i>	2	15.4	Flagellin
<i>stx1</i>	0	0	Shiga toxin 1
<i>stx2</i>	0	0	Shiga toxin 1
<i>Eae</i>	0	0	<i>E. coli</i> attachment effacement
<i>rfbE</i>	0	0	O-antigen transporter
<i>hlyA</i>	2	15.4	Hemolysin A



**Figure 4:** Virulence Genes.

### 3.2 Discussion

A group of researchers did a study that gave insight on the possible spread of infectious illnesses in sandy beach habitats (Matallah-Boutiba et al., 2016). They identified two key mechanisms for microbe transmission: direct exposure to microorganisms in the sand and migration of germs from water to sand inside the swash or intertidal zone. This highlights the necessity of studying the microbial dynamics at beaches in order to properly estimate the risk of human and animal illnesses. Building upon this notion, a comprehensive examination of beach sand microorganisms and their significant presence (Velonakis et al., 2014). The beach sand served as a reservoir for various genera and species of microorganisms, some of which possess pathogenic properties with the potential to cause diseases in humans and animals. The identification of potential pathogens in beach sands raises concerns about the safety of beachgoers and the need for improved sanitary conditions.

The current study focused on Edonwhii sandy beach and revealed a notable abundance of proteobacteria. Proteobacteria represent a diverse phylum known for their capability to engage in complex metabolic processes, such as the oxidation of methane and hydrogen sulfide or the reduction of sulfur-containing compounds. The prevalence of proteobacteria in this coastal environment warrants further investigation into their specific roles and impacts on the beach ecosystem. Moreover, marine beaches have been found to harbor aerobic Gram-positive bacteria commonly found in the human gut. The presence of these bacteria in beach environments may have implications for the health of beach visitors, especially those with compromised immune systems. Additionally, the discovery of Planctomycetes, a phylum of bacteria that may have beneficial relationships with marine algae, opens up possibilities for exploring the ecological interactions between microorganisms and the beach ecosystem.

However, it is essential to note that the research findings also bring attention to the poor sanitary conditions of Edonwhii beach, which contribute to the proliferation of microorganisms in the atmosphere. This unsanitary environment provides a favorable breeding ground for various pathogens, potentially exposing beachgoers to harmful microbes. Such findings emphasize the urgent need for improved beach management and sanitation practices to protect public health. A group of researchers conducted investigations that support the existence of harmful bacteria in beach sands (Yamahara et al., 2012; Matallah-Boutiba et al., 2016). The discovery of *Vibrio vulnificus*, *Salmonella*, *Campylobacter*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in beach sands emphasizes the significance of monitoring and managing possible health concerns associated with sandy beach settings.

In the context of this study, the focus on culturable bacteria provided valuable insights into their virulence factors, indicating their potential to cause diseases. The presence of pathogenic genes *fliC* and *hlyA*, responsible for encoding flagellin and hemolysin A virulence factors, respectively, highlights the capacity of these bacteria to pose health threats to beachgoers. Furthermore, the detection of strains such as *Fusarium verticillioides*, *Candida krusei*, *Candida albicans*, *Trichophyton* sp, and *Cladosporium* sp, capable of growing on Dermatophytic Test Medium (DTM), raises concerns about the possibility of keratitis and other dermal infections in humans. These findings align with the research and reinforce the importance of addressing the potential health risks associated with sandy beach environments (Matallah-Boutiba et al., 2016).

The research presented here underscores the significance of understanding the microbial dynamics in sandy beach environments. It provides valuable insights into the prevalence of various microorganisms, including potential pathogens, and their interactions within the beach ecosystem. The implications of these findings call for improved beach management practices, enhanced sanitation measures, and increased

awareness among beach visitors and communities to safeguard public health and preserve the pristine beauty of sandy beaches. Further research in this area will contribute to our understanding of beach microbial ecology and enhance our ability to protect human and environmental health in these unique coastal settings.

### 4. CONCLUSION

The current study focused on Edonwhii sandy beach and discovered an abundance of proteobacteria, a diverse phylum capable of complex metabolic processes. This finding calls for further investigation into the specific roles and impacts of proteobacteria on the beach ecosystem. Furthermore, aerobic Gram-positive bacteria present in the human gut and Planctomycetes, which may have favorable associations with marine algae, were discovered on maritime beaches. These findings pave the way for further research into the ecological interactions between microorganisms and the beach environment.

The research findings also draw attention to the poor sanitary conditions of Edonwhii beach, contributing to the proliferation of microorganisms in the atmosphere. This unsanitary environment provides a favorable breeding ground for various pathogens, posing potential health risks to beachgoers. Improved beach management and sanitation practices are urgently needed to protect public health. The study's focus on culturable bacteria provided valuable insights into their virulence factors, indicating their potential to cause diseases. The presence of pathogenic genes *fliC* and *hlyA* highlights the capacity of these bacteria to pose health threats to beachgoers. Furthermore, the detection of strains capable of growing on Dermatophytic Test Medium raises concerns about the possibility of dermal infections in humans. Addressing these potential health risks in sandy beach environments is essential.

Overall, this research underscores the significance of understanding microbial dynamics in sandy beach environments, providing insights into various microorganisms, including potential pathogens, and their interactions within the beach ecosystem. Enhanced beach management, sanitation measures, and increased awareness are crucial to safeguard public health and preserve the beauty of sandy beaches. Further research in this area will contribute to our understanding of beach microbial ecology and enhance our ability to protect human and environmental health in these unique coastal settings.

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