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Virulence Potentials and Pattern of Bacterial Contamination of Palm-Oil Vended Within Rivers State, Nigeria

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ABSTRACT

Bacterial contamination of palm-oil is mediated by diverse groups of bacteria which do not only affect the quality of the oil but causes foodborne ailments. The study assessed Virulence potentials and pattern of bacterial contamination of palm-oil vended within Rivers State, Nigeria**.** Palm-oil samples were randomly collected from major markets (Eleme, Creek Road and Mbiama) in the three senatorial districts of Rivers State and subjected to standard bacteriological analysis, including, cultural and molecular techniques. Data obtained showed the mean total heterotrophic bacterial count (THBC) varied from $0.61 \pm 1.19 \times 10^6$ CFU/ml (Creek Road Market) to $1.76 \pm 3.48 \times 10^6$ CFU/ml (Mbiama Market). Total coliform count (TCC) varied from $5.25 \pm 4.57 \times 10^3$ CFU/ml (Mbiama Market) to $6.25 \pm 4.78 \times 10^3$ CFU/ml (Eleme and Creek Road Markets). Faecal coliform count (FCC), varied from $0.80 \pm 0.54 \times 10^2$ CFU/ml (Creek Road Market) to $9.1 \pm 11 \times 10^2$ CFU/ml (Mbiama Market). *Salmonella* and *Shigella* count (SSC) varied from $0.40+0.81 \times 10^3$CFU/ml (Creek Road Market) to $7.9 \pm 13.96 \times 10^3$ CFU/ml (Mbiama Market). *Staphylococcus* count (SC) varied from $2.2+0.37 \times 10^1$ CFU /ml (Mbiama Market) to $7.0 \pm 3.91 \times 10^1$ CFU /ml (Creek Road Market). Analysis of variance (Anova) of the various samples showed there was no significant difference ($P>0.05$) in the bacterial counts obtained. Amongst the Sixteen bacteria genera were identified, the prevalence of the bacterial species showed that *Bacillus cereus* and *Klebsiella* spp. had the highest prevalence (100%) in Eleme samples, *Bacillus cereus* was also the highest in Creek Road sample (100%). *Bacillus* spp., *Enterobacter* spp. and *Shigella* spp., had the highest prevalence in Mbiama samples. The presence of these potential pathogens in palm oil signals a critical public health hazard driven by poor hygiene standards and inadequate regulatory oversight, necessitating stricter quality control measures.

Keywords: Palm-Oil, Shigell, Market, Poor Hygiene, Contamination, Bacteria, Virulence, Pathogens, Public Health Hazard.

Introduction

The global need for palm oil is fast increasing because of an increase in population (Nesaretnam, 2017). Palm oil is widely consumed edible oil globally, with Nigeria being one of the largest producers (Ohimain and Izah, 2016). The world's leading and second largest producers of palm oil, Indonesia and Malaysia, produces more than 85% of the global palm oil supply (Absalone *et al.,* 2020). However, as the demand of palm oil continues to appreciate, the production is predicted to reach 240 million tonnes by 2050 (Sehgal and Sharma, 2021). The oil is extracted from fruit of the oil palm tree (*Elaeis guineensis*), which is one of the species of palm and is a significant source of revenue for many tropical countries.

Furthermore, it is a vital raw material for food – producing companies (Nesaretnam, 2017). It has been widely grown for its oil-rich nuts and fruit in recent decades, however, it is considered as the world's oldest and fastest growing fruit and oil crop (Di Genova *et al*., 2018). In Nigeria, palm oil represents the most cost - effective source of cooking oil and a significant agricultural crop, distinguished by its rich carotene profile. Nevertheless, palm oil serves as the primary raw material used for producing detergents, margarine, confectionery, lubricants, pharmaceuticals, bakery products and shoe polish. Additionally, it is used as an additive in animal feeds (Ohimaim and Izah, 2016).

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The quality and safety of palm oil are affected due to microbial contaminations, posing serious health risks to consumers (Osei – Amponsah *et al*., 2018). Microbial contamination of palm oil can occur during processing, storage and transportation, and these microbes are capable of producing toxins that can cause food borne illness (Madhusudhan *et al*., 2015). The palm oil industry is an important contributor to Nigeria economy, with a population capacity of over 1 million metric tons per annum (Ohimain and Izah, 2016). However, the palm oil industryies faces challenges related to quality control processing and storage which can lead to microbial contamination that is of public health interest (Madhusudhan *et al*., 2015). In Nigeria, palm oil is a staple food, and its contamination with microbes poses a significant risk to public health (Liao *et al*., 2023).

Bacteria such as *Staphylococcus*, *Pseudomonas* and *Bacillus* have been reported to be associated with palm oil contamination (Okogbenin *et al*., 2014). These microorganisms can cause deterioration of palm oil leading to change in its characteristics. For instance, *Pseudomonas* species are known to cause spoilage of dairy products and food that contain high amount of lipids (Okogbenin *et al*., 2014). *Pseudonomas* species can adapt itself favourably in different kind of environmental conditions and can survive and replicate in minimum nutritional conditions such as drinking water, containing traces of organic compounds (Okogbenin *et al*., 2014).

Nevertheless, the presence of other microorganisms in palm oil, including *Enterobacter* and *Aspergillus* spp., is likely due to environmental contamination from water and soil (Izah *et al*., 2018). The presence of *Enterobacter* species could be attributed to unhygiene conditions that smallholder processors maintained during palm oil processing from palm oil fruit (Ngangjoh *et al*., 2020). The ingestion of most of the microorganisms found in palm oil could be deleterious to the body system especially with people with impaired immune system (Ohimain and Izah, 2015).

Some of these microorganisms, particularly *Proteus* species, can cause health problems when they enter the urinary tract, potentially infecting the urethra, bladder, and kidney (Ohimain and Izah, 2015). Most species of *Micrococcus* present in palm oil are non–pathogenic but could become a health issue with persons with suppressed immune system (Enyoh *et al*., 2018).

Some species of *Micrococcus luteus* cause a rare form of infective endocarditis and septic shock in immune suppressed individuals (Izah *et al*., 2018). *Candida* species are important opportunistic pathogens in immunocompromised patients, commonly causing skin and mucosal infections. They can colonize and invade the host's skin, evade immune responses, as well as degrade critical host defence proteins like immunoglobulins. *Candida albicans*, in particular, possesses a wide array of virulence that enhances its ability to colonize and establish infection (Kumari *et al*., 2018).

Based on the high content of vitamins and other essential minerals like magnesium, potassium and iron in palm oil, it is considered widely consumed edible oil. However, the quality and safety of palm oil sold in the markets are questionable due to the potential presence of microbes. These microbes can cause spoilage (Faluso & Oluwafemi, 2020), as well as the nutritional value and produce toxins that are harmful to human health when consumed.

The aim of this study therefore is to assess the virulence potentials and pattern of bacterial contamination of palm-oil vended within Rivers State, Nigeria as to ascertain the public health risk of the consumption of contaminated palm oil.

Materials and Methods

Study Area

The study was conducted in Rivers State, Nigeria. Rivers State is a State in the Niger Delta region of Southern Nigeria, which was formed on the 27 May, 1967, from the former Eastern Region of Nigeria, the State lost territory to the formation of Bayelsa State in 1996 (Britannica, 2021). The State is named after numerous rivers and is divided into three senatorial districts namely Rivers East, Rivers South East and Rivers West (Britannica, 2022).

Rivers State borders include Anambra and Imo to the north, Abia and Akwa Ibom to the east and Bayelsa and Delta to the west, the state capital Port Harcourt is a metropolis that is considered to be commercial center of the Nigerian oil and gas industry (Britannica, 2021). However, fishing, farming and Palm oil production are the principal occupations of the region.

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Palm oil (*Elaeis guineensis*), samples were obtained from the three different Markets, and each market represents the three senatorial districts of Rivers State. The markets are Creek Road market, Port Harcourt, in Port Harcourt city local government area with the Latitude 4.759047^{o} and longitude 7.024923^{o}, which represents Rivers East senatorial district, Alesa- Eleme Market, in Eleme local government area with the Latitude 4.792233^{o} and Longitude 7.120172^{o} , which represents Rivers South East senatorial district and Mbiama Market, in Ahoada West local government area with the Latitude 5.063917 and Longitude 6.448571^{o}, represent Rivers West senatorial district all in Rivers State (Britannica, 2022). These areas and markets were selected based on the population density and easy accessibility to the Palm oil.

The Map of the study area is shown in Figure 1.

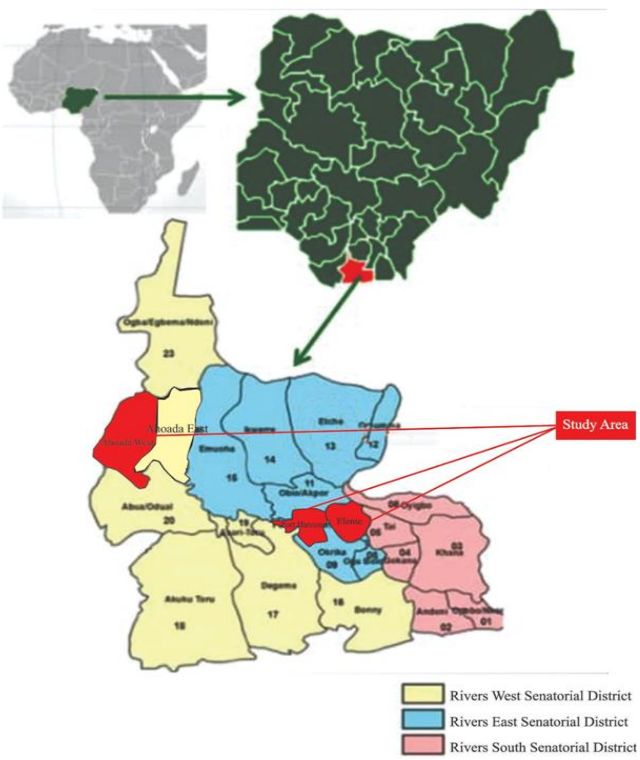

Figure 1: Map of Rivers State showing the study areas

Sample Collection

Palm oil samples were purchased randomly from four (4) different local sellers in three (3) different markets in three Senatorial districts, namely Rivers East senatorial district (Creek Road Market), Rivers South East senatorial district (Alesa, Eleme Market) and Rivers West senatorial district (Mbiama Market). Samples were collected monthly for three months and total number of 36 samples was collected. The samples were collected using sterile containers and transported to the laboratory for microbiological analysis.

Enumeration of Bacterial Species

The 10-fold serial dilution and pour plating procedures were used to isolate bacteria from the palm oil samples. Media were all obtained from the Titan Biotech Limited, Rajasthan, India. All the media were sterilized and autoclaved at 121^{o} C at 15 psi for 15 minutes. An aliquot of the diluted sample was inoculated on sterile Petri dishes containing Nutrient Agar, MacConkey agar, Eosine methylene blue agar, *Salmonella* and *Shigella* agar, Mannitol salt agar and inoculated using the spread plate method and incubated at 37^{o} C for 24 hours, faecal coliforms were incubated at 44.5^{o} C for 48 hours, while fungal plates were incubated at 37^{o} C for 72 - 96 hours.

Statistical Analysis

Statistical package for social sciences (SPSS) version 22 was used to analyze the data obtained from the plate counts as well as measurement of the zone of inhibition. Descriptive statistics (mean and standard deviation) were used to summarize all data obtained. One-way Analysis of variance (ANOVA) was carried out to test for significant differences in the microbial counts between the sampled locations. Since the ANOVA results indicated no significant difference ($P > 0.05$) among the sampled locations for these parameters, Post-hoc tests such as Duncan's Multiple Range Test (DMRT) were not performed. The data are as mean ± standard deviation.

Results

The bacterial population obtained from the different locations of the study is presented in Table 1. The total heterotrophic bacterial count (THBC) varied from 0.61$\pm$ 1.19 × 10^{6} CFU/ml (Creek Road Market) to 1.76 $\pm$ 3.48 × 10^{6} CFU/ml (Mbiama Market) (Table 1). For Total coliform count (TCC), the value ranged between 5.25 $\pm$ 4.57× 10^{3} CFU/ml (Mbiama Market) to 6.25 $\pm$ 4.78 × 10^{3} CFU/ml (Eleme and Creek Road Markets). The faecal coliform count (FCC), Mbiama Market had the highest value of9.1 $\pm$ 11.00 × 10^{2} CFU/ml.

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Table 1: Mean Bacterial Population in the Palm Oil from the various Markets sampled

Location (Market)	Bacterial Population in the Palm Oil Samples				
	Total Heterotrophic Bacteria ($\times10^6$)	Total Coliform ($\times10^3$)	Faecal Coliform ($\times10^2$)	*Salmonella/ Shigella* ($\times10^3$)	*Staphylococcus* ($\times10^1$)
Eleme	1.60±1.204	6.25±4.78	7.62±12.79	6.05±10.71	6.50±1.91
Creek Road	0.61±1.19	6.25±4.78	0.80±0.54	0.40±0.81	7.0±3.91
Mbiama	1.76±3.48	5.25±4.57	9.1±11.00	7.9±13.96	2.2±0.37
***p*-value**	0.739	0.942	0.47	0.490	0.574

Eleme Market was next with the value of 7.62 $\pm$ 12.79 $\times$ 10^2 CFU/ml, while Creek Road Market had the least value of 0.80 $\pm$ 054 $\times$ 10^2 CFU/ml. For *Salmonella Shigella* count (SSC), the value ranged between 0.40 $\pm$ 0.81 $\times$ 10^3 CFU/ml (Creek Road Market) to 7.9 $\pm$ 13.96 $\times$ 10^3 CFU/ml (Mbiama Market). For *Staphylococcus* count (S C), the value ranged between 2.2 $\pm$ 0.37 $\times$ 10^1 CFU/ml (Mbiama Market) to 7.0 $\pm$ 3.91 $\times$ 10^1 CFU/ml (Creek Road Market). There was however, no significant difference between the values obtained from the locations (P > 0.05), as shown in Table 1.

Data obtained on the prevalence of the bacterial species as presented in Table 2 showed that for Eleme samples, *Bacillus cereus* and *Klebsiella* spp. had the highest prevalence (100%). For Creek Road samples, *Bacillus cereus* had the highest prevalence (100%), while for Mbiama samples, *Bacillus* spp., *Enterobacter* spp and *Shigella dysenteriae* had the highest prevalence (100%) In the overall, *Bacillus cereus* was the most isolated bacterial species in the study (91.7%). Data obtained showed that for Eleme samples, *Candida* spp. and *Fusarium* spp. had the highest prevalence (50%).

Data obtained from virulence phenotypes of the isolates for motility study, revealed that *Citrobacter* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella* spp., had the highest isolates that were motile (100%). For Hemolysis, data obtained showed that all the isolates of *Rhodococcus* spp., 66.7% of Coagulase negative *Staphylococcus* spp. were alpha hemolytic. For beta hemolysis, all (100%) of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus* and *Salmonella* spp were beta hemolytic. For gamma hemolysis, all the isolates (100%).

Table 2 Prevalence of the Bacterial Species in the study locations

Organism	Markets			Prevalence n (%)
	Eleme n(%)	Creek Road n (%)	Mbiama n (%)	
Bacillus cereus	4(100)	4 (100)	3 (75)	11 (91.7)
Bacillus spp.	1 (25)	2 (50)	4 (100)	4 (58.3)
Citrobacter fleundii	-	-	2 (50)	2 (16.7)
Enterobacter spp.	1 (25)	-	4 (100)	5 (41.7)
Escherichia coli	-	-	2 (50)	1 (16.7)
Klebsiella spp.	4(100)	-	2 (50)	6 (50)
Pseudomonas spp.	1 (25)	1 (25)	1 (50)	4 (33.3)
Proteus spp.	-	-	3 (75)	3 (25)
Rhodococcus	1 (25)	2 (50)	1 (25)	4 (33.3)
Staphylococcus aureus	-	-	3 (75)	3 (25)
Staphylococcus epidermis	-	-	2 (50)	2 (16.7)
S. saprophyticus	1 (25)	-	-	1 (8.3)
Shigella. dysenteriae	-	2 (50)	4 (100)	6 (50)
Shigella sonnei	1 (25)	-	-	1 (8.3)
Salmonella spp	1 (25)	-	2 (50)	3 (25)
Serratia marcescens	-	-	1 (25)	1 (8.3)
Acinetibacter baumanii	-	1 (25)	-	1 (8.3)
Clostridium spp	-	1 (25)	-	1. (8.3)
Micrococcus leteus	-	1 (25)	-	1 (8.3)
Enterococcus	-	-	2 (50)	2 (16.7)

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Citrobacter spp., *Enterobacter* spp., *Klebsiella* spp., and *Shigella* spp. were gamma hemolytic.

For Biofilm formation, *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Rhodococcus* spp., *Staphylococcus* spp. and *Salmonella* spp., were all biofilm formers (100%). For DNase, all the isolates (100%) of *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*, were DNase positive (100%). For Capsule formation, *Citrobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Rhodococcus* spp., *Staphylococcus aureus* and *Salmonella* spp. were all (100%) capsule formers as shown in Table 3.

Table 3: Virulence Characteristics of the Isolates

Organism	**Number (%)**						
	Motility	**α**	**β Hemolysis**	**γ**	**Biofilm formation**	**DNase**	**Capsules**
Bacillus spp	22 (88)	2(8)	20(80)	3(12)	23(92)	17(68)	18(72)
Citrobacter spp	2 (100)	0	0	2(100)	2(100)	0	2(100)
Enterobacter spp	5(100)	0	0	7(100)	7(100)	0	6(85.7)
Escherichia coli	2(100)	0	2(100)	0	2(100)	2(100)	2(100)
Klebsiella spp	0	0	0	7(100)	6(85.7)	7(100)	7(100)
Pseudomonas aeruginosa	4(100)	0	4(100)	0	4(100)	4(100)	4(100)
Proteus vulgaris	3(100)	0	3(100)	0	3(100)	3(100)	3(100)
Rhodococcus spp	0	4(100)	0	0	4(100)	0	4(100)
Staphylococcus aureus	0	0	3(100)	0	3(100)	3(100)	3(100)
Coagulase Negative *Staphylococcus* species	0	2(66.7)	1(33.3)	0	3(100)	0	0
Shigella spp	0	0	0	7(100)	0	0	0
Salmonella spp	3(100)	0	3(100)	0	3(100)	0	3(100)
Serratia marcescens	+	-	+	-	+	+	+
Acinetibacter baumanii	-	-	-	+	+	-	+
Clostridium spp	+	+	-	-	+	-	-
Micrococcus leteus	-	-	-	+	-	-	-
Enterococcus spp	-	-	-	+	+	-	+

Discussion

The global need for palm oil is fast increasing due to the increase in human population (Nesaretnam, 2017). Nigeria is one of the largest producers of Palm oil, and it has been a vital sources in the majority of Nigerian's diet, as a widely consumed edible oil globally (Izah and Ohimain 2016). The bacterial load obtained from the study revealed that the total heterotrophic bacterial count (THBC) was above the Nigeria Agency for Food and Drugs Administration (NAFDAC, 2019) maximum acceptable microbiological level, which stipulates that the maximum allowable number of organisms in a sample unit of Palm oil should not exceed 2 with a microbiological level of 10^4 CFU/ml. The results from this research however, showed that the microbial load obtained from Total coliform count (TCC) x10^3 CFU/ml exceeded the maximum threshold of 10^2 CFU/ml which is of a great concern. The faecal coliform count contamination in the oil samples 10^2 CFU/ml is generally considered high and not acceptable in food or water meant for consumption, it is however, a cause for concern. Ideally, faecal coliform should be minimal or absent in food products like palm oil. For *Salmonella* and *Shigella* count (SSA), data obtained revealed that high bacterial load was above the threshold stipulated by NAFDAC which stipulates that *Salmonella* and *Shigella* should be absent in 25g of sample. This means that if *Salmonella* or *Shigella* are detected on food products like palm oil, it is considered major safety concern to consumers.

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The *Staphylococcus* load obtained from this research $x10^1$ CFU/ml is below the NAFDAC threshold which is relatively low and acceptable. Although, food can have a low microbial load but contain a particular microorganism whose presence could be harmful to the consumer or that could contribute to the deterioration of that food (Ngono *et al*., 2016).

Consuming pathogenic organisms, particularly *Staphylococcus aureus,* can trigger adverse physiological responses in human. *S. aureus* produces enterotoxins that cause food poisoning and gastroenteritis, especially when contaminated oil is consumed raw or without adequate heat treatment (Tesfaye *et al*., 2015). A significant public health concern arises because these enterotoxins are heat - stable and can persist in the oil for prolonged periods maintaining their toxicity even after initial processing. There was however, no significant difference between the values obtained from the locations ($P > 0.05$).

The prevalence of the bacterial isolates identified in this study showed revealed that *Bacillus cereus* had the highest prevalent rate of 91.7%. However, fifteen bacterial genera were isolated in this study. The bacterial species identified from the oil samples included Bacillus *cereus*, *Bacillus spp*., *Citrobacter fleundii*, *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., Proteus spp., *Rhodococcus* spp., *Staphylococcus aureus*, *Staphylococcus epidermis*, *Staphylococcus saprophyticus*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella* spp., *Serratia marcescens*, *Acinetobacter baumanii*, *Clostridium* spp., *Micrococcus leteus* and *Enterococcus* spp.. *Enterobacter* spp. are found in water and environment.

Their presence in palm oil samples is an indication of contamination from water and the environment. Some strains of this bacterium are opportunistic pathogens that occasionally cause meningitis, septicemia and also associated with respiratory tract infections Okogbenin *et al*., (2014). The presence of *E. coli* and *Klebsiella* are associated with faecal matters, these organisms are indicators of faecal contaminations Okogbenin *et al*., (2014) Ngangjoh *et al* (2020).

This contamination is however, attributed to unhygienic practices that food handlers and processors maintained during and after processing Madhusadhan *et al*., (2015).

While six strains of *E.coli* can cause diarrhea, the bacterium is also responsible for other serious infections, including sepsis, septic shock, urinary tract infections, and meningitis.

P. aeruginosa is opportunistic pathogen which is associated with nosocomial infections that are likely severe on patients with immuno suppressed conditions Izah and Ohimain (2016). This organism can reproduce and survive in different kind of environmental condition Okogbenin *et al*., (2014). *Bacillus cereus* which was isolated in this study is well known cause of food borne illness due to its ability to produce toxins. This bacterium is commonly found in the dust, soil and vegetation in rare cases can also cause non-gastrointestinal infections, especially in immunocompromised individuals.

Their presence in palm oil typically indicates post-processing contamination, since palm oil undergoes high-temperature during processing greater than 100^o C, vegative *B. cereus* cells would be killed. However, spores can survive if present before or introduced after heat treatment because of their dormancy, much resistant to lethal effect of heat and ultraviolet radiation (Enumuor *et al*., 2021). *Proteus* species can turn pathogenic upon entering the urinary tract (Ohimain and Izah 2016).

Conclusion

By systematically enumerating total heterotrophic bacteria, coliforms, *Salmonella, Shigella*, *Staphylococcus*, the research establishes a clear baseline of microbial contamination levels, highlighting significant hygiene deficits in post-harvest handling and marketing practices. The detection of these indicator organisms confirms that locally sold palm oil serves as a potential vehicle for foodborne pathogens, posing direct health risks to consumers.

Furthermore, the investigation into virulence potentials reveals that many of the isolated microbes possess traits associated with pathogenicity, such as toxin production and adhesion capabilities.

This finding underscores that the threat extends beyond mere presence; the microbes are equipped to cause severe clinical infections.

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