

The Diversity and Antibigram of Bacterial Isolates of *Olea europaea* (Olive) Oil Brands Sold in Port Harcourt Metropolis

Ogbonna, S. I¹., Isomah, C. J^{2*}., Amad-Wali, O². and Elisha, P. H¹.

¹Department of Microbiology, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria. ²Department of Medical Microbiology, Faculty of Medical Laboratory Science, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria.

*Corresponding Author: chiladi.isomah@ust.edu.ng.

ABSTRACT

A significant percentage of the population in Port Harcourt metropolis consumes olive oil for mostly cooking and religious reasons. The production of good quality olive oil free from pathogenic microorganism is essential to prevent diseases or illnesses associated with contamination of olive oil. This study was aimed at determining the microbial composition of different olive oil brands sold in Port Harcourt metropolis. Five (5) samples of different olive oil brands were used for the study. Isolation and enumeration of microorganisms was done on appropriate Agar medium using standard microbiological techniques. The results showed that the Total Heterotrophic Bacteria Count (THBC) ranged from 1.0×10^5 to 7.6×10^5 CFU/ml and Total Heterotrophic Fungi Count (THFC) was zero. Twenty five (25) bacteria isolates belonging to 5 genera with their percentage of occurrence were; *Pseudomonas* sp. (16%), *Bacillus* sp. (16%), *Micrococcus* sp. (8%), *Staphylococcus* spp. (28%), *Escherichia coli* (28%) and *Klebsiella* sp. (4%). Percentage occurrence from bacterial isolates showed that *Staphylococcus* spp. and *Escherichia coli* were the most dominant bacterial species while *Klebsiella* sp. was the least occurring bacterial species. Antibiotic sensitivity results showed that *Staphylococcus* sp. were more susceptible to Ciprofloxacin, Erythromycin, and Levofloxacin but showed resistance to Gentamicin, Cefuroxime and Ceftazidime. *Escherichia coli* was more susceptible to Ofloxacin, Augmentin, Peflacin, Ciprofloxacin, and Ceporex, but resistant to Ceftriaxone and Gentamicin. *Pseudomonas* sp. was more susceptible to Ofloxacin, and Ciprofloxacin but resistant to Ceftriaxone. *Bacillus* sp. was susceptible (100%), to all antibiotics tested, except Levofloxacin (75%) and Cefuroxime (50%). *Klebsiella* sp was resistant to Ceftriaxone but susceptible to all other antibiotics tested. The presence of potential pathogens such as *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* spp., *Escherichia coli*, and *Klebsiella* sp. which exhibited antibiotic resistance as shown in this study buttress the urgent need for actions to safeguard public health. Microbiological testing of olive oil brands for microbial contaminants is important, to ensure compliance with standards and prevention of public health outbreak.

Keywords: Olive Oil, Bacteria, Antibigram, Potential Pathogens, Antibiotic Resistance, Public Health.

Introduction

Olive oil is one of the oldest and most valuable edible oils consumed by humans and has been used for thousands of years for nutritional, medicinal, cosmetic, and religious purposes. It is extracted from the fruits of *Olea europaea* and is widely appreciated for its high content of monounsaturated fatty acids, phenolic compounds, tocopherols, and other bioactive substances that promote cardiovascular health, possess antioxidant and anti-inflammatory properties, and contribute to disease prevention.

Consequently, olive oil has become an increasingly popular dietary product worldwide, including in Nigeria, where imported brands are readily available

in supermarkets, pharmacies, and retail outlets (Ghanbari et al., 2012; Servili et al., 2014).

Beyond its nutritional value, olive oil occupies a unique place in the religious traditions of many communities. In Christianity, olive oil symbolizes holiness, healing, peace, divine blessing, and consecration, and is frequently used during baptisms, ordinations, anointing of the sick, and other sacred rites. These religious beliefs have contributed to sustained and growing use of olive oil among Christian populations, thereby increasing the need to ensure that products available to consumers are microbiologically safe and of high quality (Catechism of the Catholic Church, 1997; Vatican Press Office, 1983).

Although olive oil has a chemical environment that can inhibit many microorganisms, it should not be regarded as completely sterile. Microorganisms may be introduced into olive oil during harvesting, processing and handling, particularly through contaminated olives, processing water and equipment. Research has demonstrated that the microbiota associated with olives can influence the microbiological and chemical characteristics of virgin olive oil during extraction, while viable microorganisms, particularly yeasts, may persist in the oil after production (Vichi *et al.*, 2011; Ciafardini & Zullo, 2018). In addition, experimental evidence has shown that the survival of bacteria in virgin olive oil is influenced by the concentration of phenolic compounds: *Escherichia coli* survived longer in oils with relatively low concentrations of polar phenols, whereas higher phenolic concentrations produced stronger inhibitory effects (Zullo *et al.*, 2018). Therefore, although the low availability of water and the presence of phenolic compounds contribute to the antimicrobial characteristics of olive oil, these properties do not necessarily eliminate all microorganisms that may be introduced during production or handling (Medina *et al.*, 2019; Zullo *et al.*, 2020).

Studies on the microbiology of olive oil have shown that the product may contain a diverse microbial flora comprising bacteria, yeasts, and moulds. Yeasts appear to be particularly important because some species can survive during storage and influence the physicochemical and sensory characteristics of the oil. While some oil-associated yeasts may contribute positively to sensory development, others can adversely affect quality through activities such as triacylglycerol hydrolysis and the production of undesirable compounds (Ciafardini & Zullo, 2018; Zullo & Ciafardini, 2020). Bacterial diversity has also been demonstrated in extra virgin olive oils. Fancello *et al.* (2020), isolated several bacterial genera, including *Bacillus*, *Brevibacillus*, *Micrococcus*, *Staphylococcus*, *Pantoea*, *Kocuria*, *Lysinibacillus*, and *Lactobacillus*, from extra virgin olive oils. Their findings demonstrate that olive oil can harbour viable bacterial populations despite its relatively inhibitory physicochemical environment.

Although olive oil use has increased considerably in Nigeria due to its nutritional, medicinal, and religious significance, there is limited information regarding the microbial quality and antibiotic susceptibility profiles of microorganisms associated with commercially available olive oil brands sold within

Port Harcourt Metropolis. Most available studies have focused on the chemical composition, antioxidant activity, authenticity, or adulteration of olive oil, while comparatively few have investigated its microbiological safety. This scarcity of local data limits effective food safety assessment and public health risk evaluation.

This study aims to determine the microbial flora associated with selected olive oil brands sold in Port Harcourt Metropolis and to evaluate the antibiotic susceptibility patterns of the bacterial isolates.

Materials and Methods

Study Area

The study was undertaken in Port Harcourt Local Government Area, Rivers State, Nigeria. The samples were coded RS, G, RC, BF, and SH, respectively. Samples RS, G, RC, BF, and SH were bought collected from Mile 3 Market in Port Harcourt, at the coordinates 4.804550 N, 6.992040 E.

Sample Collection and preparation

Five (5) olive oil samples of different brands were bought from Mile 3 market in Port Harcourt City Local Government Area. Samples were labelled/coded according to the manufacturers and then put in a sterile container and taken to the Microbiology laboratory in Rivers State University for Microbiological analysis.

Each sample was first dissolved in dimethyl sulfoxide (DMSO) to obtain a uniform solution suitable for microbiological analysis. A solution was prepared by accurately weighing 0.5 ml of olive oil into a sterile container and adding 0.5 mL of dimethyl sulfoxide (DMSO). The mixture was vortexed vigorously until homogenous (Mothana, 2019).

Microbiology Analysis of the Samples

Serial Dilution

Using sterile pipette, 1ml of olive oil sample was transferred into first test tube (10^{-1}) containing normal saline (diluent). This was properly mixed and from 10^{-1} dilution it was serially diluted tube by tube till 10^{-3} dilution. This process was repeated for the number of samples (5).

Cultivation and Enumeration of Total Heterotrophic Bacteria (THB)

An aliquot of 0.1ml of olive oil samples each was transferred into 9ml of normal saline to obtain a dilution of 10^{-1} . The whole serial dilution process was conducted to a maximum dilution of 10^{-3} . 0.1ml aliquot was inoculated separately on the nutrient agar plates at dilution of 10^{-2} and 10^{-3} respectively. After incubation period, counts of the colonies that grew on the nutrient agar were recorded as colony forming unit per millilitre (CFU/ml) of the samples, and were used to calculate the total heterotrophic bacterial population.

$$\text{THB (CFU/ml)} = \frac{\text{No of colony}}{\text{Dilution} \times \text{volume plated}}$$

Cultivation and Enumeration and of Total Fungi Count (TFC)

The total count for viable fungi in the samples was determined by spread plate technique on Sabouraud Dextrose Agar (SDA) medium. About 0.1 aliquot was inoculated on sterile SDA plate and spread evenly with a sterilized bent L shaped glass rod. The plates were incubated inverted at 28°C to 30°C for 5 days. It was observed that, no fungal colony developed on the SDA plates at the end of the incubation period.

Isolation of Pure Culture (sub culture) of Bacteria

After counting of isolated bacteria colonies, discrete colonies were sub cultured from the primary plate to a fresh prepared nutrient agar plate to obtain a pure culture. This is done by sterilizing a wire-loop and inoculating needle to pick a viable colony and streak into prepared sterile plates and incubated at 37°C for 24 hours.

Identification of Bacteria and Fungi Isolates

The Cowan and Steel's (1993) identification manual was used for the identification of the various bacteria isolates. Discrete colonies were identified based on characteristics such as colour, shape, size, margin, elevation, texture, surface, etc. the morphological and biochemical characteristics which include; catalase, oxidase, coagulase, indole, motility, Methyl-red, Voges Proskauer and Sugar Fermentation tests of the prospective bacteria isolates were compared with the recommendation in Bergey's manual of determination bacteriology (Barrow & Feltham, 1993; Holt *et al.*, 1994).

Antibiotics Sensitivity Test

The antibiotic susceptibility testing for all bacterial isolated from the study was carried out on Mueller- Hinton agar, according to National Committee for Clinical Laboratory Standards (NCCLS, 2003). A sterile swab stick was dipped into the tube containing the bacterial suspension, and the swab was used to swab the surface the plates to ensure even distribution of the organism. The agar was allowed to dry for 3 – 5 minutes. Using sterile forceps, the impregnated antimicrobial discs were placed evenly on the surface of the inoculated plate. The head of the forceps was used to press down each disc slightly to make contact with the agar. After applying the discs, the plates were incubated in an inverted position at 37°C for 24 hours. After incubation, the test plates were examined to ensure confluence growth or near confluence.

The diameter of each zone of inhibition was measured in millimetre using a ruler on the underside of the plate and each zone observed interpreted (narrow or broad effect) and recorded (CLSI, 2024).

Results

The microbial load from the various olive oil brands had a Total Heterotrophic Bacteria Count (THBC) which ranged from 1.0×10^5 to 7.6×10^5 CFU/ml, with RC showing the highest count of 7.6×10^5 while SH had the lowest count of 1.0×10^5 . While the Total Heterotrophic Fungi Count (THFC) was zero (Table 1).

Table 1: Microbial Population (Count in CFU/ml) of Bacterial and Fungal Isolates

Sample Code	Microbial Count (CFU/ml)	
	Total Heterotrophic Bacteria	Total Heterotrophic Fungi
G	1.4×10^5	0
RC	7.6×10^5	0
BF	1.6×10^5	0
SH	1.0×10^5	0
RS	2.9×10^5	0

Key: CFU= Colony Forming Unit, ml = Milliliter

The results of the morphological and physiological (Biochemical) Characterization showed the presence of *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Micrococcus* sp., *Pseudomonas* sp., and *Staphylococcus* spp. in the various brands olive oil samples. The percentage occurrence (%) of bacteria in the various brands of olive oil samples are presented in Figure 1. While the percentage occurrence of bacteria isolates from all the olive oil samples are presented in Figure 2.

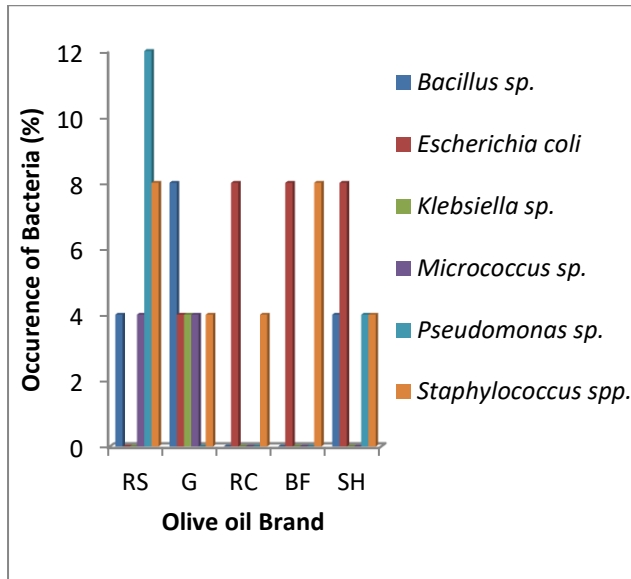


Fig. 1: Occurrence (N %) of Bacteria in the Various Brands of Olive Oil Samples

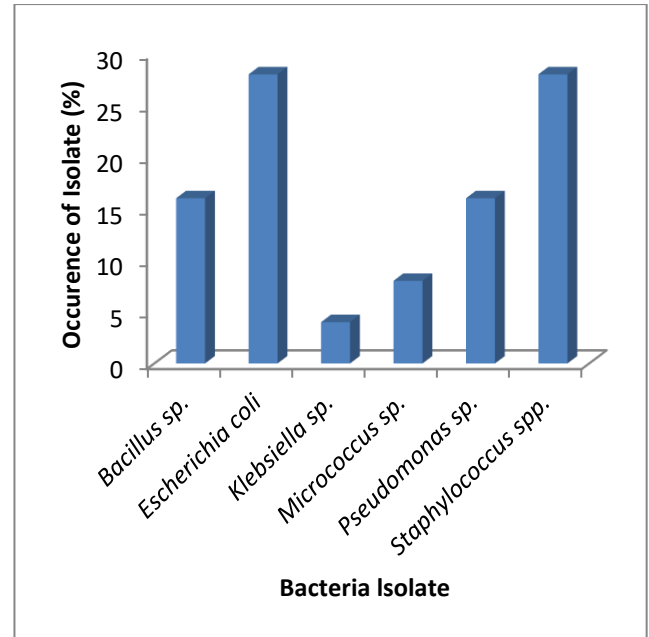


Fig. 2: Percentage Occurrence of Bacteria Isolates from all Samples

Table 2 show the susceptibility pattern of *Escherichia coli* and *Pseudomonas* sp. from olive oil samples. The results show that *Escherichia coli* was more susceptible to Ofloxacin, Augmentin, Peflacin, Ciprofloxacin, and Ceporex, but resistant to Ceftriaxone and Gentamicin. *Pseudomonas* sp. was more susceptible to Ofloxacin, and Ciprofloxacin but resistant to Ceftriaxone.

Table 2: Susceptibility of *Escherichia coli* and *Pseudomonas* sp.

Antibiotics (conc.µg)	<i>Escherichia coli</i> (7)			<i>Pseudomonas</i> sp. (4)		
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Ofloxacin(10µg)	0(0.00)	0(0.00)	7(100)	0(0.00)	0(0.00)	4(100)
Augmentin(30µg)	0(0.00)	0(0.00)	7(100)	1(25)	0(0.00)	3(75)
Peflacin(10µg)	0(0.00)	0(0.00)	7(100)	1(25)	0(0.00)	3(75)
Ceftazidime(30µg)	1(14.29)	1(14.29)	5(71.42)	1(25)	1(25)	2(50)
Gentamicin(10µg)	5(71.42)	0(0.00)	2(28.58)	1(25)	0(0.00)	3(75)
Ciprofloxacin(10µg)	0(0.00)	0(0.00)	7(100)	0(0.00)	0(0.00)	4(100)
Ceporex(10µg)	0(0.00)	0(0.00)	7(100)	1(25)	1(25)	2(50)
Ceftriaxone(10µg)	7(100)	0(0.00)	0(0.00)	2(50)	0(0.00)	2(50)
Streptomycin(30µg)	1(14.29)	0(0.00)	6(85.71)	1(25)	0(0.00)	3(75)
Cefuroxime(10µg)	3(42.86)	3(42.86)	1(14.29)	1(25)	3(75)	0(0.00)

Key: R = resistant; I = Intermediate; S = Susceptible

The susceptibility pattern of *Klebsiella* sp. from olive oil samples is as shown in Table 3. The Table show that *Klebsiella* sp was only resistant to Ceftriaxone but susceptible to all other antibiotics tested.

Table 3: Susceptibility of *Klebsiella* sp. found in Olive Oil Sample of Brand G

Antibiotics (conc.µg)	<i>Klebsiella</i> sp. (1)		
	R n(%)	I n(%)	S n(%)
Ofloxacin(10µg)	0(0.00)	0(0.00)	1(100)
Augmentin(30µg)	0(0.00)	0(0.00)	1(100)
Peflacin(10µg)	0(0.00)	0(0.00)	1(100)
Ceftazidime(30µg)	0(0.00)	0(0.00)	1(100)
Gentamicin(10µg)	0(0.00)	0(0.00)	1(100)
Ciprofloxacin(10µg)	0(0.00)	0(0.00)	1(100)
Ceporex(10µg)	0(0.00)	0(0.00)	1(100)
Ceftriaxone(10µg)	1(100)	0(0.00)	0(0.00)
Streptomycin(30µg)	0(0.00)	0(0.00)	1(100)
Cefuroxime(10µg)	0(0.00)	0(0.00)	1(100)

Key: R = resistant; I = Intermediate; S = Susceptible

The susceptibility pattern of *Bacillus* sp. from olive oil samples is as shown in Table 4. The Table shows that *Bacillus* sp. was susceptible (100%), to all antibiotics tested, except Levofloxacin (75%) and Cefuroxime (50%).

Table 4: Susceptibility of *Bacillus* sp.

Antibiotics (conc.µg)	<i>Bacillus</i> sp. (4)		
	R n(%)	I n(%)	S n(%)
Rifampicin(5µg)	0(0.00)	0(0.00)	4(100)
Ceftazidime(30µg)	0(0.00)	0(0.00)	4(100)
Streptomycin(25µg)	0(0.00)	0(0.00)	4(100)
Azithromycin(25µg)	0(0.00)	0(0.00)	4(100)
Amoxil(25µg)	0(0.00)	0(0.00)	4(100)
Ciprofloxacin(5µg)	0(0.00)	0(0.00)	4(100)
Erythromycin(15µg)	0(0.00)	0(0.00)	4(100)
Levofloxacin(5µg)	0(0.00)	1(25)	3(75)
Gentamicin(10µg)	0(0.00)	0(0.00)	4(100)
Cefuroxime(10µg)	0(0.00)	2(50)	2(50)

Key: R = resistant; I = Intermediate; S = Susceptible

The susceptibility pattern of *Micrococcus* sp. and *Staphylococcus* sp. from olive oil samples is as shown in Table 5. The table show that *Micrococcus* sp. showed more susceptibility to Ciprofloxacin, Erythromycin, Levofloxacin and Gentamicin but resistant Ceftazidime, Streptomycin, Azithromycin, Amoxil and Cefuroxime. *Staphylococcus* spp. were more susceptible to Ciprofloxacin, Erythromycin, and Levofloxacin but showed resistance to Gentamicin, Cefuroxime and Ceftazidime.

Table 5: Susceptibility of *Micrococcus* sp. and *Staphylococcus* sp.

Antibiotics(conc.µg)	<i>Micrococcus</i> sp. (2)			<i>Staphylococcus</i> sp. (5)		
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Rifampicin(5µg)	1(50)	0(0.00)	1(50)	1(14.29)	0(0.00)	6(85.74)
Ceftazidime(30µg)	1(50)	0(0.00)	1(50)	3(42.87)	1(14.29)	3(42.87)
Streptomycin(25µg)	1(50)	0(0.00)	1(50)	2(28.58)	1(14.29)	4(57.16)
Azithromycin(25µg)	1(50)	0(0.00)	1(50)	0(0.00)	1(14.29)	6(85.74)
Amoxil(25µg)	1(50)	0(0.00)	1(50)	1(14.29)	0(0.00)	6(85.74)
Ciprofloxacin(5µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	7(100)
Erythromycin(15µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	7(100)
Levofloxacin(5µg)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	7(100)
Gentamicin(10µg)	0(0.00)	0(0.00)	2(100)	3(42.87)	0(0.00)	4(57.16)
Cefuroxime(10µg)	1(50)	0(0.00)	1(50)	3(42.87)	0(0.00)	4(57.16)

Key: R = resistant; I = Intermediate; S = Susceptible

Discussion

An investigative study was carried out to determine the microbial contamination of olive oil sold in Port Harcourt Metropolis. According to Okechalu *et al.* (2011), the acceptable limit of microorganism in a sample unit of oil should not be more than 2 with acceptable microbiological level of 10^4 /ml.

Table 1 showed that all five olive oil brands contained measurable populations of heterotrophic bacteria, whereas no fungal growth was reported in any sample. The total heterotrophic bacterial (THB) counts ranged from 1.0×10^5 CFU/mL to 7.6×10^5 CFU/mL, with RC showing the highest count of 7.6×10^5 while SH had the lowest count of 1.0×10^5 . The variation in bacterial counts among the different brands suggests differences in microbiological quality, which may be associated with processing conditions, packaging integrity, storage duration, transportation, or handling practices during retail distribution.

The bacterial counts reported in this study are relatively high for a product that should possess intrinsic antimicrobial properties. This finding suggests that contamination most likely occurred during extraction, bottling, repackaging, storage, or retail handling, rather than through bacterial multiplication within the oil itself. A review of olive oil microbiological safety reported that microorganisms can survive in olive oil when introduced through contaminated equipment, containers, or environmental exposure, particularly when the phenolic content of the oil is low (Palumbo *et al.*, 2011). Similarly, studies on the microbial ecology of olive oils have shown that bacteria may persist in the oil as part of its biotic fraction, although their populations generally decline during storage because the oil environment is unfavorable for active growth (Zullo & Ciafardini, 2020).

The complete absence of fungal growth in all the samples is consistent with the known antifungal properties of olive oil. The low water activity of the oil creates an environment that does not support the growth of moulds and most yeasts. In addition, olive oil phenolic compounds have been shown to inhibit several fungal species, contributing to the microbiological stability of the product (Servili *et al.*, 2014).

A total of 25 bacterial isolates were isolated and identified as *Staphylococcus* spp., *Escherichia coli*,

Pseudomonas spp., *Bacillus* spp., *Micrococcus* spp., and *Klebsiella* sp. *Staphylococcus* spp. and *E. coli* were the predominant isolates, each accounting for 28% of the total isolates, followed by *Pseudomonas* spp. and *Bacillus* spp. at 16% each. The occurrence of organisms such as *E. coli*, *Staphylococcus*, *Klebsiella*, and *Pseudomonas* suggests possible contamination from handlers, equipment, water, environmental sources, or poor sanitary conditions during processing, packaging, transportation, and retailing.

Members of the genus *Pseudomonas* are widely distributed in terrestrial and aquatic environments and possess considerable metabolic versatility, enabling them to adapt to diverse and fluctuating environmental conditions. Some *Pseudomonas* species are associated with environmental contamination, food spoilage, and opportunistic infections (Silby *et al.*, 2011). The isolation of *Pseudomonas* spp. from several packaged olive oil samples indicates environmental contamination during processing or post-processing handling that may have occurred through poorly sanitized equipment, contaminated containers, or poor handling during packaging and distribution.

The isolation of *Staphylococcus* spp., including *Staphylococcus aureus*, is of greater public health significance because these organisms are commonly associated with human skin, the nasal cavity, and hands of food handlers (O'Donoghue *et al.*, 2015). Their presence in olive oil strongly suggests contamination during handling, packaging, or retail distribution rather than contamination originating from the olives themselves.

The occurrence of *Bacillus* spp. is not unexpected because species of this genus are widely distributed in the environment and produce heat-resistant endospores that enable them to survive harsh environmental conditions, including food processing operations (Nicholson *et al.*, 2015). *Bacillus* spores may be introduced into edible oils through contaminated olives, dust, soil particles, or processing equipment. While many species are harmless environmental organisms, some, particularly *Bacillus cereus*, are recognized foodborne pathogens capable of producing enterotoxins that cause diarrhoeal and emetic syndromes (Logan, 2012). Their isolation in this study therefore indicates the need for adequate cleaning of raw materials and processing facilities.

The isolation of *Escherichia coli* from several olive oil brands is particularly significant because this organism is widely recognized as an indicator of faecal contamination and poor hygienic quality in foods. The isolation of *Klebsiella* spp. further supports the possibility of environmental or faecal contamination. *Klebsiella* species are commonly found in soil, water, sewage, vegetation, and the intestinal tract of humans and animals. The isolation of *Micrococcus* spp. in some samples is consistent with previous reports describing these organisms as part of the normal microbiota of human skin, dust, air, and food-processing environments.

The diversity of bacterial species isolated in this study suggest that the microbial contamination of the olive oil brands most likely resulted from post-production contamination rather than microbial growth within the oil. Olive oil possesses natural antimicrobial properties attributable to its low water activity and phenolic constituents, which inhibit the multiplication of many microorganisms (Servili *et al.*, 2014). Consequently, the report of both environmental organisms (*Bacillus*, *Pseudomonas*, and *Micrococcus*) and sanitary-indicator organisms (*Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella* spp.) suggests lapses in sanitary standard, packaging, transportation, or storage.

The percentage occurrence of the bacterial isolates from the olive oil brands analyzed showed that a total of 25 bacterial isolates belonging to six genera were identified. *Staphylococcus* spp. and *Escherichia coli* were the predominant organisms, each accounting for 28% of the total bacterial population. These were followed by *Pseudomonas* spp. and *Bacillus* spp., each 16%, while *Micrococcus* spp. accounted 8%, *Klebsiella* spp. was the least frequently isolated organism, representing only 4%.

The predominance of *Staphylococcus* spp. among the bacterial isolates suggests that human handling may have been a major source of contamination. An equally high occurrence of *Escherichia coli* (28%) was observed. The isolation of *E. coli* in edible oils is negatively significant because it is widely recognized as an indicator of faecal contamination.

The bacterial distribution observed in this study indicates that contamination of the olive oil brands was predominantly associated with human handling and environmental exposure rather than the intrinsic microbiota of olive oil. The predominance of sanitary-indicator organisms such as *Staphylococcus*

spp. and *Escherichia coli*, together with environmental organisms including *Pseudomonas* spp., *Bacillus* spp., and *Micrococcus* spp., suggests shortcomings in sanitary practices during processing, packaging, transportation, or retail storage. Considering that olive oil naturally possesses antimicrobial compounds capable of suppressing microbial growth (Servili *et al.*, 2014), the isolation of these organisms emphasizes the need for increased standards in these products.

The antibiotic susceptibility profiles of the bacterial isolates from the current study showed different levels of susceptibility and resistance among the different genera. In general, fluoroquinolones (ofloxacin, ciprofloxacin, pefloxacin, and levofloxacin) exhibited the highest antibacterial activity against most of the isolates, while resistance was more frequently observed against ceftriaxone and, to a lesser extent, cefuroxime.

Among the Gram-negative bacteria, *Escherichia coli* showed complete susceptibility (100%) to ofloxacin, augmentin, pefloxacin, ciprofloxacin, and ceporex. However, all *E. coli* isolates were resistant to ceftriaxone, with additional resistance observed against gentamicin and cefuroxime. Similarly, *Pseudomonas* spp. showed complete susceptibility to ofloxacin and ciprofloxacin but reduced susceptibility to ceftazidime, ceftriaxone, ceporex, and cefuroxime.

These findings are consistent with the well-established intrinsic resistance mechanisms of *Pseudomonas* species, including reduced outer membrane permeability, multidrug efflux pumps, and inducible β -lactamases, which reduce the effectiveness of several β -lactam antibiotics (Lister *et al.*, 2009). Likewise, resistance to third-generation cephalosporins among *E. coli* has frequently been associated with the production of extended-spectrum β -lactamases (ESBLs), a major public health concern because these resistance genes can spread rapidly among Enterobacterales (Paterson & Bonomo, 2005).

The single *Klebsiella* sp. isolate in this study was susceptible to nine of the ten antibiotics tested and resistat only to ceftriaxone. Although only one isolate was obtained, this resistance pattern is noteworthy because ceftriaxone resistance is increasingly reported among food-associated *Klebsiella* species and is commonly linked to ESBL production and other β -lactam resistance mechanisms (Paczosa & Mecsas, 2016).

For Gram-positive organisms, *Bacillus* spp. showed the most favourable susceptibility profile. All isolates were completely susceptible to rifampicin, ceftazidime, streptomycin, azithromycin, amoxil, ciprofloxacin, erythromycin, and gentamicin, no complete resistance was reported among the *Bacillus* isolates. Similar susceptibility patterns have been reported for environmental *Bacillus* isolates, which generally remain susceptible to multiple antimicrobial classes despite occasional reports of resistance in clinical strains (Logan, 2012).

Among the Gram-positive cocci, *Micrococcus* spp. showed complete susceptibility to ciprofloxacin, erythromycin, levofloxacin, and gentamicin but showed variable responses to several other antibiotics. The *Staphylococcus* spp. isolate showed high susceptibility to ciprofloxacin, erythromycin, levofloxacin, rifampicin, azithromycin, and amoxil. Nevertheless, reduced susceptibility to ceftazidime, gentamicin, streptomycin, and cefuroxime indicates the presence of resistance among some isolates. Such findings agree with global reports showing that *Staphylococcus* species remain highly susceptible to fluoroquinolones in many settings, whereas resistance to β -lactam antibiotics is becoming increasingly common because of β -lactamase production and alterations in penicillin-binding proteins (Tong *et al.*, 2015).

Conclusion

This study investigated the microbial flora and antibiotic susceptibility patterns of selected olive oil brands sold in Port Harcourt Metropolis. The findings showed that the analyzed olive oil samples harboured viable bacterial contaminants, although no fungal growth was reported. The bacterial counts varied among the brands, with total heterotrophic bacterial counts ranging from 1.0×10^5 to 1.64×10^6 CFU/mL. The isolation of bacteria from commercially available olive oil is of public health importance, particularly because olive oil is widely consumed as food and may also be used in religious practices. The antibiotic susceptibility results further showed that the isolates exhibited different responses to the antibiotics tested. The presence of antibiotic-resistant potential indicator and opportunistic pathogenic organisms such as *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* spp., and *Klebsiella* sp. in olive oil is of public health significance because food products can provide opportunities for the persistence and dissemination of antimicrobial-resistant microorganisms.

There is therefore an urgent need for the appropriate authorities to enforce compliance to sterile production environment, proper packaging and storage, routine microbiological quality assessment, and responsible antimicrobial-use are necessary to protect consumers and improve the safety and quality of olive oil products.

References

- Barrow, G. I., & Feltham, R. K. A. (Eds.). (1993). *Cowan and Steel's manual for the identification of medical bacteria* (3rd ed.). Cambridge University Press
- Cappuccino, J. G., & Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson.
- Ciafardini, G., & Zullo, B. A. (2018). Virgin olive oil yeasts: A review. *Food Microbiology*, 70, 245–253.
- CLSI - Clinical and Laboratory Standards Institute. (2024). *Performance standards for antimicrobial susceptibility testing* (34th ed., CLSI Supplement M100). Clinical and Laboratory Standards Institute.
- Fancello, F., Multineddu, C., Santona, M., Deiana, P., Zara, G., Mannazzu, I., Budroni, M., Dettori, S., & Zara, S. (2020). Bacterial biodiversity of extra virgin olive oils and their potential biotechnological exploitation. *Microorganisms*, 8(1), 97.
- Ghanbari, R., Anwar, F., Alkharfy, K. M., Gilani, A. H., & Saari, N. (2012). Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.). *International Journal of Molecular Sciences*, 13(3), 3291–3340.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (Eds.). (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Williams & Wilkins.
- Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews*, 22(4), 582–610.
- Logan, N. A. (2012). *Bacillus* and relatives in foodborne illness. *Journal of Applied Microbiology*, 112(3), 417–429.

- Medina, E., Romero-Gil, V., Garrido-Fernández, A., & Arroyo-López, F. N. (2019). Microbial ecology of olive products and processing environments: A review. *Foods*, 8(12), 623.
- Mothana, R. A. A., Al-Rehaily, A. J., & Schultze, W. (2019). Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of *Lepidium sativum* seed oil. *Evidence-Based Complementary and Alternative Medicine*, 2019, 7423789.
- National Committee for Clinical Laboratory Standards. (2003). *Performance standards for antimicrobial disk susceptibility tests* (8th ed., Approved Standard M2-A8). NCCLS.
- Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H. J., & Setlow, P. (2000). Resistance of *Bacillus endospores* to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews*, 64(3), 548–572.
- O'Donoghue, M. O., Mitchell, E., O'Connor, M., & other authors. (2015). Tracking sources of *Staphylococcus aureus* hand contamination in food handlers by spa typing. *American Journal of Infection Control*, 43(7), 721–725.
- Okechalu, J. N., Dashen, M. M., Lar, P. M., Okechalu, B., & Gushop, T. (2011). Microbiological quality and chemical characteristics of palm oil sold in Jos, Plateau State, Nigeria. *Journal of Microbiology and Biotechnology Research*, 1(2), 107–112.
- Palumbo, M., Harris, L. J., & Beuchat, L. R. (2011). *Microbiological food safety of olive oil: A review of the literature*. University of California, Davis Olive Center.
- Paczosa, M. K., & Meccas, J. (2016). *Klebsiella pneumoniae*: Going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews*, 80(3), 629–661.
- Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β -lactamases: A clinical update. *Clinical Microbiology Reviews*, 18(4), 657–686.
- Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R., & Taticchi, A. (2014). Biological activities of phenolic compounds of extra virgin olive oil. *Antioxidants*, 3(1), 1–23.
- Silby, M.W., Winstanley, C., Godfrey, S.A.C., Levy, S.B. & Jackson, R.W. (2011), *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiology Reviews*, 35, 652-680.
- Tong, S. Y. C., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28(3), 603–661.
- Vatican Press Office. (1983). *Code of Canon Law: Book IV, The sanctifying function of the Church*. Vatican.
- Vichi, S., Romero, A., Gallardo-Chacón, J., Tous, J., & Buxaderas, S. (2011). The activity of healthy olive microbiota during virgin olive oil extraction influences oil chemical composition. *Journal of Agricultural and Food Chemistry*, 59(9), 4545–4552.
- Zullo, B. A., & Ciafardini, G. (2018). Survival of coliform bacteria in virgin olive oil. *BioMed Research International*, Article 8490614. <https://doi.org/10.1155/2018/8490614>
- Zullo, B. A., & Ciafardini, G. (2020). Virgin olive oil quality is affected by the microbiota that comprise the biotic fraction of the oil. *Microorganisms*, 8(5), 663.