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TOXICOLOGICAL IMPLICATIONS OF DICHLORVOS ON THANATOMICROBIOME PROFILES AND ABUNDANCE FOR POST MORTEM INVESTIGATIONS

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Abstract: This research studied the bacterial and fungal microbial ecologies on dichlorvos-treated carrions and their effects on post-mortem microbial clock for post-mortem interval (PMI) estimation during death investigations. Carrion-soil, skin, oral and rectal samples were aseptically collected, cultured and microbes isolated from both dichlorvos-treated and control pig (*Sus scrofa*, Linnaeus) carrions at the fresh, early and advanced stages of decomposition. The microbes were morphologically identified using microscopy and biochemical characteristics of catalase, oxidase, indole and citrate utilization. A total of seven bacterial species (*Bacillus* sp., *Escherichia coli, Pseudomonas* sp., *Staphylococcus* sp., *Enterobacter* sp., *Clostridium* sp. and *Enterococcus* sp.) and two fungal species (*Aspergillus* sp. and *Fusarium* sp.) were isolated and identified from both carrion groups. Lesser microbial community abundance of 26 (46.43%) was recorded from the dichlorvos-treated carrions when compared with the control carrions with higher abundance of 30 (53.57 %). *Bacillus* sp. and *Aspergillus* sp. were the dominant bacteria and fungi from the dichlorvos-treated and control carrions respectively. Also, there was more microbial fauna abundance from the carrion soil samples of both carrion groups. The study provided good comparative information between the microbiome identities and successions on dichlorvos-treated and control carrions that can aid the resolution of medico-legal cases.

Keywords: Bacteria, Carrion decomposition, Dichlorvos, Fungi, Microbiome, PMI, Toxicology.

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INTRODUCTION

Post-mortem microbiome (PMM), a term also indicatively referred to as necrobiome, epinecrobiome or thanatomicrobiome are communities of microorganisms associated with the decaying body (Javan *et al.*, 2016). The combined interplay of microbial degradation and insect activities constitute the bedrock upon which the decomposition of carrions or dead remains is driven (Gunn and Pitt, 2012; Hyde *et*

al., 2013; Metcalf *et al.,* 2013; Pechal *et al.,* 2013). Decomposition is an enzymatic biochemical process which leads to the breakdown of dead body as a result of microbial, invertebrates and vertebrates' invasions of the dead body (Janaway, 2009; Pechal *et al.,* 2014). The decomposition of corpse after the death is facilitated by microorganisms (bacteria, fungi, protozoa), insects or animal scavengers and non-biological factors linked to the environment such as temperature, humidity, precipitation, oxygen, soil types, weather and climate (Guo *et al.,* 2016; Habtom *et al.,* 2019). Carrion decomposition occurs in stages which include the fresh, bloat, active decay, advanced decay and dry decomposition (Catts and Goff, 1992; Anderson and Van Vanlaerhoven, 1996). The fresh stage is associated with the release and breakdown of simple to macromolecules due to microbial activities. The early and advanced decay release various gaseous compounds, which cause the bloating and consequent rupture of the dead body and subsequent skeletonisation (Mondor *et al.,* 2012; Metcalf *et al.*, 2013; Hyde *et al.,* 2013).

Like insects and other arthropods, microbial succession on carrion shows a pattern that is predictable (Hauther *et al.,* 2015; Javan *et al.,* 2016; DeBruyn and Hauther, 2017; Deel *et al.,* 2020; Lutz *et al.,* 2020; Scott *et al.,* 2020). The postmortem microbiome community associated with the decomposition of carrions is made up of the thanatomicrobiome and the epinecrotic microbial community. The thanatomicrobiomes are prokaryotic and eukaryotic microorganisms found in the internal body tissues and organs such as blood, liver, lungs, brain and heart. The overall epinecrotic community include the microorganisms that are found on carrion surfaces such as skin and gut orifices (Audrey *et al.,* 2022). The complex microbial communities associated with decaying bodies such as the proteobacteria, firmicutes, bacteroidetes, actinobacteria and different fungal species have been utilized as biological clock and fingerprints for estimating the post-mortem interval (PMI) during forensic investigations (Gunn and Pitt, 2012; Finley *et al.,* 2015; Pechal *et al.,* 2014; Burcham *et al.,* 2016; Guo *et al.*, 2016; Metcalf *et al.,* 2016; Javan *et al.,* 2017; Metcalf *et al.,* 2017; Liu *et al.,* 2020; Huan *et al.,* 2021; Hafz *et al.,* 2021). It is also useful in the determination of causes and manner of death, identification of individuals, detection of crime locations, trafficking, corpse relocation (Clarke *et al.,* 2017; Huiya *et al.,* 2023), drowning (Racz *et al.,* 2016; Lee *et al.,* 2017; Wang *et al.,* 2020) and poisoning (Butzbach, 2010; Han *et al.,* 2012; Butzbach *et al.,* 2013; Sastre *et al.,* 2017).

The microbial community succession on carrion differs in accordance with the stage of decomposition, body collection site and sampling location. Bacterial and fungal communities possess an extremely geographical and site-specific profile (Kong and Segre, 2012; Grantham *et al.,* 2015; Hyde *et al.,* 2015; Deel *et al.,* 2020; Yang *et al.,* 2021). Also, the microbial species and diversity found on decomposing body is grossly influenced by the prevailing weather and environmental conditions such as temperature, humidity, precipitation, soil type, diseases, nutritional factors and chemical toxins (Costello *et al.,* 2009; Turnabaugh *et al.,* 2009; Knight *et al.,* 2017; Lax *et al.,* 2015) and this over time causes an alteration in the community composition of the microbiome (Can *et al.,* 2014; Javan *et al.,* 2016).

The presence of chemical toxins has a gross effect on the species composition, diversity and community abundance of microbiomes on a decomposing body. Some microbial species such *as Bacillus cereus, Staphylococcus epidermidis, Clostridium perfringens* and *Bacteroides fragilis* are known to be responsible for bioconversion of some drugs, and so pre-mortem drug intake and chemical use can affect the postmortem microbial activity with a consequent alteration of information necessary for the interpretation of the cause and manner of death (Drummer, 2004; Butzbach, 2010; Skopp, 2010; Butzbach *et al.,* 2013; Gunn and Pitt, 2012; Castle *et al.,* 2017; Sastre *et al.,* 2017).

In Nigeria and other parts of the world, the illicit use of dichlorvos, some drugs and other chemicals have been implicated in many suicidal deaths. The World Health Organization (WHO) has implicated the use of dichlorvos and other

pesticides as leading cause of suicide worldwide (Kora *et al.,* 2011; Martin *et al.,* 2020). Dichlorvos or DDVP (2, 2-dimethyl dichlorovinyl phosphate) is an organophosphate insecticide used to control insects (USEPA, 2007). Dichlorvos poisoning usually occurs through ingestion, inhalation or penetration through the skin (Owoeye *et al.,* 2012; Razwiedani and Rautenbach, 2017). Dichlorvos poisoning poses a lot of negative systemic and health effects and on severe cases could lead to death (Michael *et al.,* 2008).

The present work was aimed to study the bacterial and fungal microbial ecologies on dichlorvos-treated carrions and their effects on postmortem microbial clock for post-mortem interval estimation during death investigations.

MATERIALS AND METHODS

Study area

The study was conducted in Uburu, Ohaozara Local Government Area in southern region of Ebonyi State, Nigeria (fig. 1). Uburu is bounded by Mpu, Nkerefi, Oduma and Okpanku communities in Enugu State and Isu, Okposi, and Onicha communities in Ebonyi State. Uburu is the administrative headquarters of Ohaozara Local Government Area with an area of 312 km^2. It lies within the coordinates 6° 2' 48" N and 7°45' 18" E with an altitude of 50m/164.04 ft. The climatic condition of Uburu is that of a humid tropical climate marked with two distinct seasonal periods i.e. the dry and wet seasons. The wet season begins in March and ends in October with annual rainfall ranges between 270mm to 2250mm, while the dry season begins in November and ends in February. The temperature condition of Uburu ranges from 25 ^{0}C to 33 ^{0}C in the dry season and 23 ^{0}C to 27 ^{0}C in the wet season (Makwe and Okobia, 2020). Uburu is a salt mining town inhabited by mostly civil servants, traders, artisans and farmers. The study site is an open farmland made up of trees such as African oil bean tree (*Pentaclethra macrophylla*), mango (*Mangifera indica*), oil palm (*Elaeis guineensis*), shrubs and grasses.

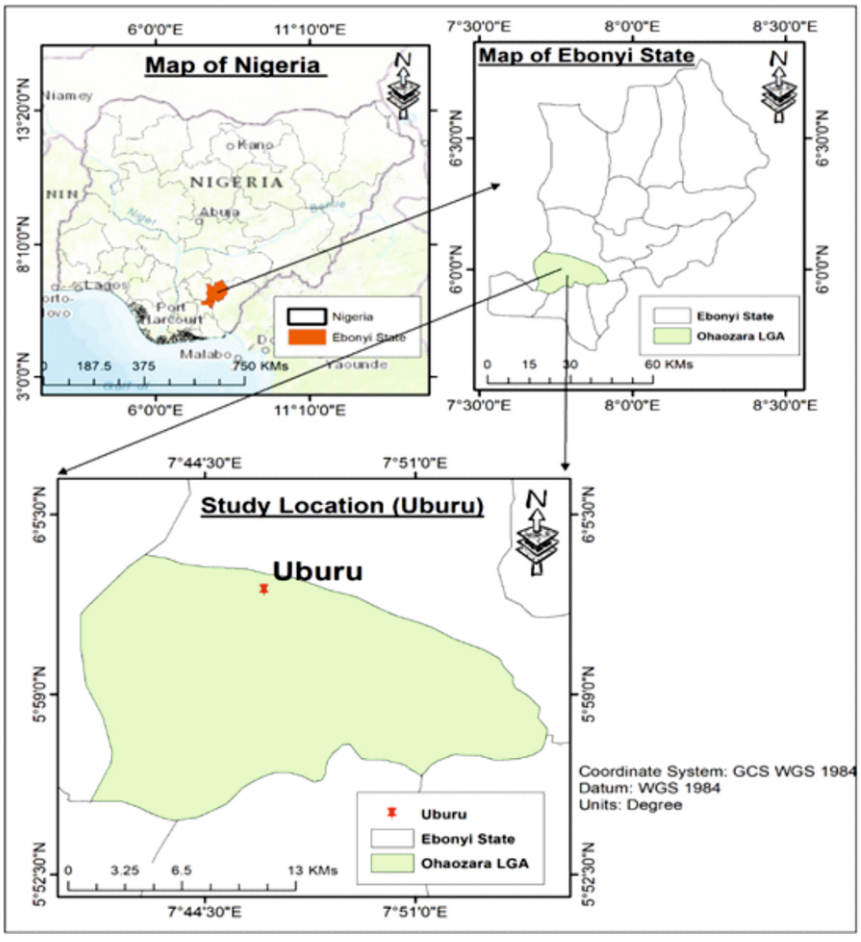

Fig. 1: Map of the study area.

Ethics approval
All the Ethics and Regulations guiding the use of research animals as approved by the Ethical Board, Enugu State Ministry of Health with the Number: MH/MSD/REC23/0016 dated 17th May, 2023 was obtained and duly followed (S1).

Experimental animals and handling
Pigs (*Sus scrofa* Linnaeus) were used as the animal model for the study (Catts and Goff, 1992; Anderson *et al.,* 2001; Dickson *et al.,* 2011). Four (4) domestic pigs: two dichlorvos-treated and two control pigs weighing between 25 kg and 27 kg were purchased from piggery farm in Uburu in Ohaozara LGA Ebonyi State and used for the study. The control pigs were sacrificed by asphyxia and the dichlorvos-treated pigs by gastric tube oral injection of lethal dose of dichlorvos in accordance with the methods of Abd El-Bar *et al.* (2016) and Fatma *et al.* (2022). The pigs, immediately after death, were bagged and transported to the study sites and placed at different locations within the study area set at 120 m apart to minimize an overlapping effect of organisms attracted to the carrions as demonstrated by Tullis and Goff (1987). The pig carrions were secured with a wire mesh cage (60cm x 45cm x 30cm) which prevented vertebrate scavengers.

Ecological data collection
Internal temperature of the carrion, maggot mass temperature, carrion-soil interface temperature, soil temperature and soil pH were taken with temperature and pH digital probe (CE-ABS: China). Ambient temperature, relative humidity and precipitation data were recorded daily throughout the study from the nearest weather station in Ake Eze, Ivo Local Government Area, Ebonyi State.

Microbial sample collection
Microbial samples were aseptically collected with sterile cotton-tipped swabs at three points over the decomposition process and placed directly in individual sterile swab container to avoid cross contamination by microbes from other sources as described by Metcalf (2019). Microbial samples for each carcass were collected from three different body regions: the oral cavity (internal region), the skin (superior side exposed to the environment), and the soil-carrion interface at the fresh decay stage (day 1), early decay stage (day 5) and advanced decay stages (day 10) according to the methods of Audrey *et al*. (2022). Soil samples were collected from under the carcasses at three different regions- the head, abdomen and anal regions at a depth of 0-3cm for microbial sampling after placement. Samples collected were stored at -20^{0}C until subsequent analysis of the microbial post-mortem communities was done as stated by Cobaugh *et al.* (2015). Soil sampling was conducted for the fresh, early decay and advanced decay stages of the decomposition proces.

Microbial isolation and identification of bacteria
The isolation of microbes was done aseptically by standard pour plate technique in accordance with work of Uzeh *et al.* (2009). The swabs were immersed in 10ml phosphate buffer saline (PBS) and vortexed for 2 minutes to extract the microbes. With a sterile pipette, tenfold, three serial dilutions were made by adding 1ml of the extracted solution into 9ml sterile water and vortexed. This was done for the swab samples and for the soil samples, a soil suspension was made by diluting 1gram of soil sample with 50ml sterile water in a sterile bottle and vortexed to release the microbes. With a sterile pipette, tenfold, three serial dilutions were made by adding 1ml of the soil solution into 9ml sterile water and vortexed for culture. Gram stain was performed for all isolated colonies according to the standard procedure by Kohinur *et al.* (2017) and Emmanuel *et al.* (2017) and observed under the microscope (Olympus Cx23: China) using x10 and x40 objectives and the bacteria were identified according to Bergey Manual of Systematic Bacteriology (Garrity *et al.,* 2004). Other different biochemical tests for individual identification were done using biochemical characteristics of catalase, oxidase, indole and citrate utilization.

Microbial Isolation and Identification of fungi
Isolation of fungi was done by standard pour plate technique as reported in Uzeh *et al.* (2009). The swab sticks were immersed in 10ml

phosphate buffer saline (PBS) and vortexed for 2 minutes to extract the microbes. Three (3) tenfold serial dilution were made by adding 1ml of the extracted solution into 9ml sterile water and vortexed. For the soil, a soil suspension was made by diluting 1gram of soil sample with 50ml sterile water in a sterile bottle and vortexed to release microbes. Tenfold serial dilution was made by adding 1ml of the soil solution into 9ml sterile water and vortexed. 1ml of each sample from the swab and soil suspension was transferred into the center of well labeled petri plates respectively. Cooled (45°c), molten Saboroud Dextrose Agar (SDA) medium was poured into the petri dishes. The plates were rotated gently to ensure uniform distribution of cells in the medium. The medium was allowed to solidify and the plates incubated at 37°c and observed daily for a period of 4 days. Two drops of Lactopherol Cotton blue were placed on a clean glass slide and a small tuft of fungus with spore bearing structures were transferred unto the lactopherol cotton blue on the slide using a flamed, cooled needle. The stain and the mold structures were gently mixed using the straight wire. A cover glass was placed over the preparation and observed under the microscope using x40 objective and the fungi were identified as demonstrated by Garrity *et al.* (2004) and Cheesbrough (2005).

Statistical analysis

Data obtained from this study were analysed using IBM SPSS Statistics 26 and Microsoft Excel 2016 softwares and presented as relative abundances and percentages (%). The microbial counts were presented as mean and standard deviation. The mean total abundance of microbes isolated and identified from the control and dichlorvos-treated carrions were compared using the T Test. All the statistical analyses were performed at $p < 0.05$ level of significance.

RESULTS

Records of ecological parameters

The mean daily ecological conditions during the study include maximum temperature of 29.83± 0.30 °C; minimum temperature of 24.15± 0.12°C; relative humidity of 93.38 ± 0.82%; precipitation of 8.95 ± 5.8 mm/day and soil pH 7.20 ± 0.06 and 8.17 ± 0.03 for dichlorvos-treated and control carrions soils (soil-carrion interphases). The total carrion decomposition period lasted 50 days for the dichlorvos-treated carrions and 65 days for the control carrions.

Microbial growth and colony count

There were bacterial growths for the dichlorvos-treated carrions at the fresh, early and advanced decay stages of decomposition for the soil sample; the skin sample showed microbial growth at the fresh and early decomposition stages but no growth at the advanced stage. The oral sample manifested microbial growth only at the fresh decay stage but no growth at the early and advanced decomposition stages. Microbial growth was seen at fresh and early decomposition stages for the rectal sample while there was no available sample for collection at the advanced stage due to total rectal decomposition (table 4). For the control carrions there were bacteria growth for the fresh, early and advanced decay stages of decomposition for the soil and skin samples; the oral sample showed microbial growth at the fresh and early decomposition stages but no growth at the advanced stage while the oral sample showed microbial growth at the fresh decay stage only but no growth at the early and advanced decomposition stages due to total rectal decomposition (table 5). There were fungal growth for all the samples collected at the three different decomposition stages on the dichlorvos-treated and control carrions (tables 4 and 5).

The mean bacterial and fungal colonies counts for the soil, skin, oral and rectal samples for both the dichlorvos–treated and control carrions show no significant difference ($p < 0.05$). The soil and rectal samples from the dichlorvos–treated carrions had the highest and lowest mean bacteria count of 103176.67 $Cfuml^{-1}$ and 4100 $Cfuml^{-1}$ respectively. While the skin and rectal samples from the control carrions had the highest and lowest mean bacteria count of 55433.33 $Cfuml^{-1}$ and 5100 $Cfuml^{-1}$ respectively (table 1). On the other hands, the soil and rectal samples from the dichlorvos–treated carrions had the highest and lowest mean fungal count of 64333.33 $Cfuml^{-1}$ and 5730 $Cfuml^{-1}$ respectively. While the soil and rectal samples from the control carrions had the highest and lowest mean fungal count of 146620 $Cfuml^{-1}$ and 2500 $Cfuml^{-1}$ respectively (table 2).

Table 1: Bacterial count of microbes on dichlorvos-treated and control carrions.

	SOIL		SKIN		ORAL		RECTAL	
	D	C	D	C	D	C	D	C
FD	3.4,000	30,200	42,000	94,000	16,200	48,000	3,900	5,100
ED	100,030	32,000	55,000	12,000	NG	8,520	4,300	NS
AD	175,500	21,900	18,000	60,300	NG	NG	NS	NS
MEAN	103176.67	28033.33	38333.33	55433.33	16200.00	28260.00	4100.00	5100.00
STD	70802.46	5387.33	18770.54	41216.06	-	27916.58	282.84	-

D = Dichlorvos-treated carrions, C = Control carrion, FD = Fresh Decay, ED = Early Decay, AD = Advanced Decay, STD = Standard Deviation

Table 2: Fungal count of microbes on dichlorvos-treated and control carrions.

	SOIL		SKIN		ORAL		RECTAL	
	D	C	D	C	D	C	D	C
FD	44,000	216,000	2,400	40,000	21,000	4,080	9,400	2,500
ED	126,000	222,100	1,900	32,000	17,000	2,920	2,060	NS
AD	23,000	1,760	3,800	15,000	6,000	960	NS	NS
MEAN	64333.33	146620.00	2700.00	29000.00	14666.67	2653.33	5730.00	2500.00
STD	54427.32	125489.51	984.89	12767.15	7767.45	1577.00	5190.16	-

D = Dichlorvos-treated carrions, C = Control carrion, FD = Fresh Decay, ED = Early Decay, AD = Advanced Decay, STD = Standard Deviation

Microbial isolation and identification

A total of nine (9) microbes including bacteria (7 species) and fungi (2 species) were isolated and identified from the dichlorvos-treated and control carrions. These include *Bacillus* sp., *Escherichia coli*, *Pseudomonas* sp., *Staphylococcus* sp., *Enterobacter* sp., *Clostridium* sp., *Enterococci* sp., *Aspergillus* sp. and *Fusarium* sp. All the microbial species isolated and identified were common for the control carrions while the other species except the *Enterobacter* sp., *Clostridium* sp., *Enterococci* sp. were associated with the dichlorvos-treated carrions (table 3). The microbial community of the dichlorvos-treated carrion yielded an abundance of 26 ($t = 2.63$; $p < 0.05$) and a total percentage value of 46.43 % (table 4), while the control carrions had microbial community abundance of 30 ($t = 6.637$; $p < 0.05$) and a total percentage value of 53.57% (table 5).

Based on the location of sample collection and their characteristic microbes on the dichlorvos-treated carrions, the soil sample presented *Bacillus* sp., *Escherichia coli, Pseudomonas* sp., *Aspergillus* sp. and *Fusarium* sp. with a highest total microbial abundance of 10 (38.46%); the skin sample presented *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Aspergillus* sp. and *Fusarium* sp. with a total microbial abundance of 7 (26.92%); the oral sample presented *Bacillus* sp., *Escherichia coli* and *Aspergillus* sp. with a total microbial abundance of 5 (19.23%); while the rectal sample presented *Bacillus* sp., *Staphylococcus* sp. and *Aspergillus* sp. with a least total microbial abundance of 4 (15.38%) (table 5).

For the control carrions, the soil sample presented *Bacillus* sp., *Escherichia coli, Staphylococcus* sp., *Enterobacter* sp., *Clostridium* sp., *Aspergillus* sp. and *Fusarium* sp. with a total microbial abundance of 10 (38.46%); the skin sample the presented *Bacillus* sp., *Escherichia coli, Pseudomonas* sp., *Staphylococcus* sp., *Aspergillus* sp. and *Fusarium* sp. with a total microbial abundance of 9 (30.00%); the oral sample presented the *Bacillus* sp., *Enterococci* sp., *Aspergillus* sp. and *Fusarium* sp. with a total microbial abundance of 8 (26.67%); while the rectal sample presented *Bacillus* sp., *Staphylococcus* sp. and *Aspergillus* sp. with a total microbial abundance of 3 (10.00%) (table 4).

The microbiome signatures from all the samples collected based on the three stage decomposition criteria – the fresh, early and advanced decay stages studied for the dichlorvos-treated carrions showed that *Aspergillus* sp. had the highest abundance of 10 (38.46%) and was present at all the stages of decomposition of the dichlorvos-treated and control carrions. *Bacillus* sp. had the second highest abundance of 6 (23.08%). The abundances of *Escherichia coli*, *Fusarium* sp., *Pseudomonas* sp. and *Staphylococcus* sp. were 3 (11.54%), 3 (11.54%), 2 (7.69%) and 2 (7.69%) respectively (table 4). The control carrions showed also that *Aspergillus* sp. had the highest abundance of 9 (10.00%). *Bacillus* sp. also had the second highest abundance of 7 (23.33%) followed by *Fusarium* sp., *Staphylococcus* sp., *Escherichia* coli, *Pseudomonas* sp., *Enterococci* sp., *Clostridium* sp. and *Enterobacter* sp. with abundances of 5 (16.67%), 3 (10.00%), 1 (3.33%), 1 (3.33%), and 1 (3.33%) respectively (table 5).

Bacteria gram reaction revealed different gram positive and gram negative rods, cocci and bacilli which were made up of mainly lactose and non-lactose fermenting colonies. The fungal colonies featured blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores (table 6).

Table 3: Composition of microbes on dichlorvos-treated and control carrions.

DICHLORVOS-TREATED		CONTROL	
BACTERIA	**FUNGI**	**BACTERIA**	**FUNGI**
Bacillus sp.	*Aspergillus* sp.	*Bacillus* sp.	*Aspergillus* sp.
Escherichia coli	*Fusarium* sp.	*Escherichia coli*	*Fusarium* sp.
Pseudomonas sp.		*Pseudomonas* sp.	
Staphylococcus aureus		*Staphylococcus aureus*	
		Enterobacter sp.	
		Clostridium sp.	
		Enterococci sp.	

Table 4: Sample composition of microbes on dichlorvos-treated carrions during decomposition.

SAMPLE		BACTERIA							FUNGI		ABUNDANCE
		Bacillus sp.	*Escherichia coli*	*Pseudomonas* sp.	*Staphylococcus* sp.	*Enterobacter* sp.	*Clostridium* sp.	*Enterococci* sp.	*Aspergillus* sp.	*Fusarium* sp.	
Carrion-soil Swab	F D	+	+	+	-	-	-	-	+	-	4 (15.38%)
	E D	+	+	-	-	-	-	-	+	-	3 (11.54%)
	A D	+	-	-	-	-	-	-	+	+	3 (11.54%)
TOTAL (%)											10 (38.46%)
	F D	-	-	-	+	-	-	-	+	-	2 (7.69%)
Skin Swab	E D	+	-	+	-	-	-	-	+	+	4 (15.38%)
	A D	NG	NG	NG	NG	NG	NG	NG	-	+	1 (3.85%)
TOTAL (%)											7 (26.92%)
Oral Swab	F D	+	+	-	-	-	-	-	+	-	3 (11.54%)

	E D	NG	NG	NG	NG	NG	NG	NG	+	-	1 (3.85%)
	A D	NG	NG	NG	NG	NG	NG	NG	+	-	1 (3.85%)
TOTAL (%)											5 (19.23%)
Rectal Swab	F D	-	-	-	+	-	-	-	+	-	2 (7.69%)
	E D	+	-	-	-	-	-	-	+	-	2 (7.69%)
	A D	NS	NS	NS	NS	NS	NS	NS	NS	NS	- 4 (15.38%)
ABUN-DANCE		6 (23.08%)	3 (11.54%)	2 (7.69%)	2 (7.69%)	-	-	-	10 (38.46%)	3 (11.54%)	26 (100%)

FD = Fresh decay, ED = Early decay, AD = Advanced decay, NG = No growth, NS = No sample, + = Present, - = Absent

Table 5: Sample composition of microbes on control carrions during decomposition.

SAMPLE		**BACTERIA**							**FUNGI**		**ABUN-DANCE**
		***Bacillus* sp.**	***Escherichia coli***	***Pseudo-monas* sp.**	***Staphy-lococcus* sp.**	***Entero-bacter* sp.**	***Clostridium* sp.**	***Enterococci* sp.**	***Aspergillus* sp.**	***Fusarium* sp.**	
Carrion-soil Swab	F D	+	-	-	-	+	-	-	+	-	3 (10.00%)
	E D	+	-	-	+	-	-	-	+	-	3 (10.00%)
	A D	-	+	-	-	-	+	-	+	+	4 (13.33%)
TOTAL (%)											10 (33.33%)
Skin Swab	F D	+	-	-	+	-	-	-	+	-	3 (10.00%)
	E D	+	-	+	-	-	-	-	+	-	3 (10.00%)
	A D	-	+	-	-	-	-	-	+	+	3 (10.00%)
TOTAL (%)											9 (30.00%)
Oral Swab	F D	+	-	-	-	-	-	+	+	+	4 (13.33%)
	E D	+	-	-	-	-	-	-	+	+	3 (10.00%)
	A D	NG	NG	NG	NG	NG	NG	NG	-	+	1 (3.33%)
TOTAL (%)											8 (26.67%)
Rectal Swab	F D	+	-	-	+	-	-	-	+	-	3 (10.00%)
	E D	NS	NS	NS	NS	NS	NS	NS	NS	NS	-
	A D	NS	NS	NS	NS	NS	NS	NS	NS	NS	- 3 (10.00%)
ABUN-DANCE		7 (23.33%)	2 (6.67%)	1 (3.33%)	3 (10.00%)	1 (3.33%)	1 (3.33%)	1 (3.33%)	9 (30.00%)	5 (16.67%)	30 (100%)

FD = Fresh decay, ED = Early decay, AD = Advanced decay, NG = No growth, NS = No sample, + = Present, - = Absent

Table 6: Morphological features microbes on dichlorvos-treated and control carrions.

SAMPLE	GRAM REACTION	MICROBIAL MORPHOLOGY	ORGANISMS	
			BACTERIA	FUNGI
DICHLORVOS - TREATED				
Carrion-Soil	Gram positive and Gram negative rods	Lactose fermenting colonies, Confluent, Large mucoid and tiny non lactose fermenting colonies.	*Bacillus* sp., *Pseudomonas* sp., *Escherichia coli*	
		Blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores.		*Aspergillus* sp., *Fusarium* sp.
Skin Swab	Gram positive cocci in cluster, Gram positive bacilli, Gram negative bacilli	Small lactose fermenting, colonies, Non lactose fermenting colonies.	*Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp.	
		Blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores.		*Aspergillus* sp., *Fusarium* sp.
Oral Swab	Gram positive and negative rods	Small to large reddish colonies.	*Escherichia coli, Bacillus* sp.	
		Blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores.		*Aspergillus* sp., *Fusarium* sp.
Rectal Swab	Gram positive cocci, Gram positive bacilli	Large pinkish colonies, Lactose fermenting colonies.	*Staphylococcus aureus, Bacillus* sp.	
		Erect and long non-septate blastoconidia and conidiophores.		*Aspergillus* sp.
CONTROL				
Carrion-soil Swab	Gram positive bacilli, Gram negative bacilli, Gram positive cocci	Large and small lactose fermenting colonies, Confluent non lactose fermenting colonies.	*Enterobacter* sp., *Bacillus* sp., *Staphylococcus* sp., *Escherichia coli, Clostridium* sp.	
		Blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores.		*Aspergillus* sp., *Fusarium* sp.
Skin Swab	Gram positive coccii, Gram positive and negative bacilli	Large lactose fermenting colonies, Confluent non lactose fermenting colonies.	*Staphylococcus* sp., *Bacillus* sp., *Escherichia coli, Pseudomonas* sp.	
		Blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores.		*Aspergillus* sp., *Fusarium* sp.
Oral Swab	Gram positive cocci, Gram positive bacilli	Large, smooth lactose fermenting colonies.	*Enterococci* sp., *Bacillus* sp.	
		Blastoconidia, cylindrical to ovoid conidia, septate and non-septate conidiophores.		*Aspergillus* sp., *Fusarium* sp.
Rectal Swab	Gram positive cocci, Gram positive bacilli	Small lactose fermenting colonies.	*Staphylococcus* sp., *Bacillus* sp.	
		Erect and long non-septate blastoconidia and conidiophores.		*Aspergillus* sp.

DISCUSSION

The necrotic association between necrobiomes, thanatomicrobiomes, and decomposing bodies have been of immense utility in the determination of post mortem interval (PMI) during investigation of the cause and manner of death and this has been demonstrated by recent studies (Carter *et al.,* 2015; Hyde *et al.,* 2015; Guo *et al.,* 2016; Johnson *et al.,* 2016; Javan *et al.,* 2016, 2017; Debruyn and Hauther, 2017; Bell *et al.,* 2018; Lutz *et al.,* 2020; Scott *et al.,* 2020; Wang *et al.,* 2020).

The present study x-rayed the epinecrotic microbial community successions and composition on dichlorvos-treated and control carrions using soil (from carrion-soil interface), skin, oral and rectal samples at the fresh, active and advanced decay stages of decomposition in line with previous works associated with mammalian cadaver decomposition (Cobaugh *et al.,* 2015; Hyde *et al.,* 2015; Metcalf. *et al.,* 2016; Finley *et al.,* 2015; Javan *et al*., 2017).

There were marked non microbial growth seen from some of the samples collected for both carrion groups especially at the advanced decomposition stage and it could be as a result decreased autolytic and necrotic processes with the attendant decreased vertebrates, invertebrates and microbial activities. Again, the unavailability of rectal samples at some stages of decomposition happened due to rectal rupture and deterioration because of advanced and dry decomposition of the carrions. The soil samples recorded the highest mean bacterial count (103176.67) and fungal count (4100) from the dichlorvos-treated and control carrions respectively while the rectal samples recorded the lowest mean bacterial count of 4100 from the dichlorvos-treated and lowest mean fungal count from the control carrions. The difference in the microbial counts recorded could be due to environmental factors (such as temperature, humidity, precipitation and pH), soil organic matter content, presence of poison, arthropods and other invertebrates' activities that could facilitate or mar the proliferation of microorganisms (Johnson *et al.,* 2016; Ameh and Kawo, 2017; Metcalf, 2019).

Seven (7) species of bacteria of the taxa firmicutes and proteobacteria together with two (2) fungal species were isolated and identified from both the dichlorvos-treated and control carrions. Finley *et al.* (2015; Guo *et al.* (2016); Metcalf *et al.* (2016) and DeBruyn and Hauther (2017) in their previous microbial succession studies on carrion also observed the dominance of firmicutes (*Bacillus* sp., *Staphylococcus* sp., *Clostridium* sp.), proteobacteria (*Escherichia coli, Pseudomonas* sp.) and other bacterial phyla of Bacteroidetes, Actinobacteria and Acidobacteria.

The highest abundance and occurrence of fungi of the generaAspergillus and Fusarium in both the dichlorvos-treated and control carrions is indicative of the aerobic nature of fungi and their ability to withstand and thrive even under harsh environmental conditions such as poor nutrients status, low pH and extreme temperature (Ameh and Kawo, 2017).

The observed lesser abundance of microbial community on the dichlorvos-treated carrions 26 (46.43%) compared to the control carrions with microbial community abundance of 30 (53.57%) and the absence of *Enterobacter* sp., *Clostridium* sp., and *Enterococci* sp. in the dichlorvos-treated carrions might be as a result of the poisonous effect of dichlorvos on the cadavers as some studies have proven that drugs and poisons affect microbial clock and post-mortem interval (Han *et al.,* 2012; Butzbach *et al.,* 2013; Sastre *et al.,* 2017; Metcalf, 2019).

The differences in microbial signatures associated with the sites of sample collection and the different stages of decomposition is in agreement with the works of (Kong and Segre, 2012; Hyde *et al.,* 2013, 2015; Javan *et al.,* 2016; Deel *et al.,* 2020; Yang *et al.,* 2021). It was reported by Grantham *et al.* (2015) that bacterial and fungal communities possess an extremely site-specific profile. Clarke *et al*. (2017) revealed that microbial community succession on carrion differs in accordance with the stage of decomposition, body collection site, sampling geographical location and climatic conditions. Also, Guo *et al.* (2016) pointed out other factors that could affect the microbial community

structure to include; the first microbial communities to invade the cadaver, the cadaver environment and the method of sample collection. As decomposition progresses, microbial communities' diversity decreases relative to a decrease in nutrients until skeletonization with only a few genera becoming dominant and a shift from an aerobic bacteria-based community to an anaerobic bacteria based community (Damann, 2017; DeBruyn and Hauther, 2017).

The study revealed the early microbial communities as bacteria in the phylum firmicutes (*Bacillus* sp. *and Escherichia coli*) in contrast to the work of Benbow *et al.* (2015) where bacteria in the phylum Proteobacteria dominated the early decomposer communities and the Firmicutes dominated the later decomposer communities. Proteobacteria was the most abundant bacterium phylum in grave soil samples with decreased abundance of acidobacteria and increased Firmicutes in surface cadaver-soil communities, while the microbial community composition remained rather constant in buried soil communities (Finley *et al,.* 2015, 2016). In another study by Hyde *et al.* (2013), it was shown that communities from the mouth and oral cavity differ in pre-bloat and end of bloat stages of decomposition. The firmicutes and actinobacteria were predominant in the fresh stage, while the clostridiales and bacillaceae were the dominant firmicutes from bloat to advanced decay stages of decomposition.

Recent studies have identified the skin and the oral cavity as good sites to build an accurate microbial clock (Kodama *et al.,* 2019; Roy *et al.,* 2021). However, this study observed more microbial fauna abundance from the soil and skin samples and also bacterium phyla firmicutes (*Bacillus* sp. and *Staphylococcus* sp.) and proteobacteria (*Esherichia coli*) and fungal genera of *Aspergillus* and *Fusarium* as the most abundant microbial species on dichlorvos-treated and control carrions thereby suggesting the soil and skin samples, the firmicutes (*Bacillus* sp. and *Staphylococcus* sp.), proteobacteria (*Escherichia coli*) and fungal genera of *Aspergillus* and *Fusarium* as markers and resorts to establishing accurate microbiome profiles for post mortem investigations.

In conclusion, the study identified unique microbiomes of dichlorvos-treated and control carrions which are potential markers to establishing accurate microbiome profiles for post mortem investigations. It has also provided good comparative information between the microbiome identities and successions on dichlorvos-treated and control carrions for the study location that can be useful in the resolution of medico-legal cases in relation to dichlorvos and other suicidal or poisonous chemical agents. Since microbial forensics works are still in the infancy stage in this study location and Nigeria at large, this study stands as a curtain raiser for more studies that will take microbial identification beyond the traditional microbial culture method to the use of modern technology of genome wide sequencing of informative gene markers.

CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest in relation to the current article.

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