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ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH DECOMPOSING PIG (*Sus scrofa*) CARCASS

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ABSTRACT

The emerging field of forensic biology has attempted to solve certain problems encountered when estimating post-mortem interval (PMI) by using predictable changes in the microbial and arthropod community structure. Pig (*Sus scrofa*) carcasses are widely used as animal models in clinical human studies. The objective of this study was to identify bacteria from the skin surface of pig carcass for possible use in forensic investigation.
Three pigs (a suitable human substitute) were collected from a local farm and killed by suffocation and further place in a bush land for decomposition. 24hours later skin samples were collected and transported to the laboratory for the isolation of bacteria using standard pour plate techniques and identified using Bergey's manual of systemic bacteriology. The experiment was conducted in February 2019 during the dry season of the year with an average temperature of 23.5^{0}c and relative humidity of 60.8%
A total of fourteen (14) isolates were gotten from the pig carcass samples out of which four (4) were Gram-positive bacteria and the remaining ten (10) were Gram-negative. *Staphylococcus* spp. (28.6%) was the most abundant while *Salmonella* sp., *Serratia* sp., *Klebsiella* sp., *Citrobacter* sp. and *Proteus* sp. occurred at 14.3% each. This study focus on the type of bacteria communities during a decomposition process which will help provide baseline information in the application of forensic biology to determination of nature of death, abuse or neglect.

Keywords: Bacteria, Decomposition, Forensic, Identification, Isolation

INTRODUCTION

The decomposition of a dead body starts through the action of microorganisms, such as fungi and bacteria, followed by the action of a series of arthropods **(Huntington *et al.*, 2008)**. Over the years, carrion insects have been extensively studied for their role in decomposition. It is in this light that recent studies focus on the microbial community structure during the decomposition process in effort to complement forensic entomology by providing another tool for post-mortem interval estimation by following the same principles of forensic entomology. It is a known fact that microorganisms work hand in hand with arthropods to bring about decomposition. Our current understanding shows that soil, host-associated, and pig carcass-associated microbial communities change over time as decomposition progresses
A study that focused on specific taxa of the human gut microbiome found that over the course of decomposition, certain taxa could be tracked over time. Specifically, *Bacteroides* sp., *Lactobacillus* sp. and *Bifidobacterium* sp. were quantified via qPCR during the decomposition of human cadavers as putative quantitative indicators of PMI (Kathleen *et al.*, 2015) others on the overall intestinal microbiota of piglets with and without diarrhoea **(Guo *et al.*, 2008; Hermann-Bank *et al.*, 2013)**.
To further understand the role of microbes in the decomposition process of carrion and how it pertains to the post-mortem interval estimation, the present research provides an insight into bacteria as primary decomposers. To observe changes in the microbial community, pig carcasses were used as analogues for human remains. This is because pig carcasses have been widely used as animal models in clinical human studies **(Nikolaidis *et al.*, 2004; Kyparos *et al.*, 2005)**, generating a level of replication and destructive sampling that is easier to achieve compared to human samples. In addition, pig have been successfully used in previous forensic experiments **(Huntington *et al.*, 2008; Guo *et al.*, 2016)**, as the decomposition process and microbial composition of a pig carcass is comparable to a human cadaver **(Parkinson, 2009; Guo *et al.*, 2016)**. Bacteria are the first colonizers of decomposing carrion because these microorganisms are present at death. During putrefaction, bacteria and other microorganisms proliferate and play a vital role in the recycling of carrion through enzymatic degradation of tissues **(Pechal *et al.*, 2013; Crippen and Singh, 2015; Cobaugh *et al.*, 2015; Metcalf *et al.*, 2016)**
Bacteria are also responsible for many aspects of decomposition (fresh bloat active advance decay and skeletonization), hence they appear to manipulate the behaviour of insects to attract species that benefit their survival while repelling those that are detrimental to them **(Crippen and Singh, 2015)**. Additionally, the presence of bacteria species may be necessary for proper development of many fly species **(Crooks *et al.*, 2016; Tomberlin *et al.*, 2017)**. The knowledge of the types of bacteria succession and function during decomposition is important to understand the downstream effects on decomposition rates and patterns which will further strengthen the entomology aspect of forensic research. Therefore this study aims at Isolating and identifying bacteria from the skin surface of pig carcass for possible use in forensic investigation.

MATERIALS AND METHODS

Carcass Placement and Sample Collection

Three pigs (*Sus* scrofa Linneaus) of between 23 to 25 kg were collected from a local farm in Iwo suburb and killed by suffocation. One of each was placed inside an iron welded scavenger-proof cage and allowed to decompose in a bushland near Bowen University, Iwo Osun State for 24 hours. Atmospheric temperature and relative humidity were recorded with a weather station located approximately 1km from the bushland. Using sterile knife, a section of the skin was scraped into a sterile polythene bag and taken to the laboratory for further investigation.

Isolation and Identification of Bacteria from Pig Carcass

Bacteria were isolated from the pig carcass by standard pour plate techniques. Tenfold serial dilution was prepared; using 9 mL sterile distilled water in test-tubes and aseptically plated on Nutrient agar (NA). The plates were incubated overnight at 37^{o}C for 24 h. discrete colonies were picked from the plates and repeated streaking was done to obtain pure cultures. Various biochemical tests were carried out on the bacterial isolates, such as Gram staining, catalase test, methyl red, Voges-Proskauer, indole, citrate utilization and sugar fermentation and identified as described by Bergey's Manual of Systematic Bacteriology **(Garrity *et al.*, 2004)**.

RESULT

Weather and Decomposition

Iwo is located in the tropics and has a yearly temperature that typically varies from 18.9°C to 35°C and is rarely below 15.6°C or above 37.8°C. There was no record of rainfall because the study was conducted during the dry season. Average atmospheric temperature during the study was 23.5°C and an average relative humidity of 60 .8 %. Decomposition was still at the fresh stage as there was only a slight difference in the appearance (*no discernable odour, little fluid leakage from the eyes and nostrils with some blood discharged from the nostrils*) of the carcass at the time of placement and the time of collection of sample
Table I presents the bacteria isolated from the skin of pig carcass showing the morphological characteristics including appearance, colony, size, surface colony and optical characteristics. Table 2 shows the biochemical characteristics of the bacteria isolated from the pig carcass. Based on the biochemical tests, the isolates

were identified as *Staphylococcus* sp., *Citrobacter* sp., *Serratia* sp., *Proteus* sp., and *Klebsiella* sp. Isolates A, B, C, D, and E were obtained from skin while isolates F, G, H, I, J, K, L, M, and N were obtained from the flesh. Isolates A, C, G, and K were identified as *Staphylococcus* spp. They were cocci in shape. Isolates B, K were identified as *Salmonella* spp. and were rod in shape. Isolates E and I were identified as *Serratia* spp. and were also rod in shape. Isolates H and M were identified as *Proteus* spp., while isolates D and N were identified as *Klebsiella* spp. Isolates F and L were identified as *Citrobacter* spp.

Table 3 shows the percentage occurrence of the bacteria isolated. Based on the percentage occurrence of the bacteria isolated from pig carcass, the most common was *Staphylococcus* sp. (28.6%), followed by other bacteria genera, which include *Citrobacter* sp., *Serratia* sp., *Proteus* sp., and *Klebsiella* sp. which had the same percentage occurrence (14.3%).

Table 1 Morphological characteristics of bacteria isolated from flesh and skin of pig carcass

Isolates Code	Appearance	Colony	Size	Surface Colony	Optical Characteristics	Part of pig
A	Cocci	Clusters	Large	Rough	Opaque	Skin
B	Rod	Clusters	Small	Rough	Opaque	Skin
C	Cocci	Clusters	Large	Rough	Opaque	Skin
D	Rod	Clusters	Tiny	Smooth	Translucent	Skin
E	Rod	Clusters	Small	Rough	Translucent	Skin
F	Rod	Clusters	Small	Rough	Opaque	Flesh
G	Cocci	Clusters	Large	Smooth	Translucent	Flesh
H	Rod	Clusters	Tiny	Smooth	Opaque	Flesh
I	Rod	Clusters	Small	Rough	Opaque	Flesh
J	Rod	Clusters	Small	Smooth	Opaque	Flesh
K	Cocci	Clusters	Large	Rough	Translucent	Flesh
L	Rod	Clusters	Small	Rough	Translucent	Flesh
M	Rod	Clusters	Small	Rough	Opaque	Flesh
N	Rod	Clusters	Small	Rough	Translucent	Flesh

Table 2 Biochemical characteristics of bacteria isolated from pig carcass

Isolates code	Gram's Reaction	Morphology	CAT	CIT	IND	MR	VP	LAC	SUC	MAL	GLU	Probable organism
A	+	Cocci	+	+	-	+	+	AG	AG	AG	AG	*Staphylococcus* sp.
B	-	Rod	+	-	-	+	-	-	-	AG	AG	*Salmonella* sp.
C	+	Cocci	+	+	-	+	+	A	AG	A	AG	*Staphylococcus* sp.
D	-	Rod	+	+	-	-	+	AG	A	AG	AG	*Klebsiella* sp.
E	-	Rod	+	+	-	-	+	-	AG	AG	AG	*Serratia* sp.
F	-	Rod	+	+	-	+	-	AG	AG	AG	AG	*Citrobacter* sp.
G	+	Cocci	+	+	-	+	+	A	AG	A	AG	*Staphylococcus* sp.
H	-	Rod	+	+	-	+	-	-	A	-	AG	*Proteus* sp.
I	-	Rod	+	+	-	-	+	-	AG	A	AG	*Serratia* sp.
J	-	Rod	+	-	-	+	-	-	A	AG	AG	*Salmonella* sp.
K	+	Cocci	+	+	-	+	+	A	AG	AG	AG	*Staphylococcus* sp.
L	-	Rod	+	+	-	+	-	AG	A	A	AG	*Citrobacter* sp.
M	-	Rod	+	+	-	+	-	-	-	A	AG	*Proteus* sp.
N	-	Rod	+	+	-	-	+	AG	A	A	AG	*Klebsiella* sp.

KEY: + = Positive, - = Negative, AG= Acid and gas production, NA= Nutrient agar, CIT= citrate utilization, IND= indole, CAT= catalase, MR= methyl red, VP= Vogues Proskauer, LAC= lactose, SUC= sucrose, MAL= maltose, GLU= glucose

Table 3 Percentage occurrence of bacteria isolated from pig carcass

S/N	Probable organisms	Number of isolates	Percentage occurrence (%)
1	*Staphylococcus* sp.	4	28.6
2	*Salmonella* sp.	2	14.3
3	*Klebsiella* sp.	2	14.3
4	*Serratia* sp.	2	14.3
5	*Citrobacter* sp.	2	14.3
6	*Proteus* sp.	2	14.3
	Total	15	100

DISCUSSION

A total of fourteen (14) bacteria species were isolated in this study. Four (4) were Gram-positive while the remaining ten (10) were Gram-negative. The bacteria isolates were *Staphylococcus* sp., *Salmonella* sp., *Klebsiella* sp., *Serratia* sp., *Citrobacter* sp. and *Proteus* sp. *Staphylococcus* sp. had the highest percentage occurrence (28.6%). *Salmonella* sp., *Klebsiella* sp. and *Serratia* sp., *Citrobacter* sp., and *Proteus* sp. all had the same percentage occurrence of 14.3%. The *Staphylococcus* species were found on the skin of the pig samples while others, such as *Serratia* sp., *Proteus* sp., *Citrobacter* sp., *Salmonella* sp., *Klebsiella* sp., were found on the flesh of the pig samples.

In a similar study on the carcass of pig, **Epling *et al.* (1993)** isolated *Salmonella* sp. (12% - 20%) from swabbed ham surfaces of freshly slaughtered pork carcasses while **Bo-Min Ki *et al.* (2017)** in their findings reported that the most dominant bacteria associated with decomposing pig carcass in south korea were Arthrobacter (10.9%) and Lysobacter (10.3%) in the early stage of decomposition. In previous study **Hyde *et al.* (2013)** observed a wide range of aerobic bacteria to anaerobic bacteria in all body sites sampled from human cadaver **(Janaway, Percival & Wilson, 2009; Vass, 2001; Payen *et al.*, 1988)**

from the onset to end of the bloat stage of decomposition with *Staphylococcus* and Enterobacteriacae being the dominant anaerobic bacteria present and (*Clostridia* and *Bacteroides*) been aerobic. This agrees with this study to an extent indicating a shift in the community structure of bacteria as the bacteria from this study were isolated during the pre-bloat stage of decomposition. This findings is not unlikely as variable conditions surrounding decomposition of each cadaver or carcass could greatly influence diversity of intrinsic and extrinsic bacterial communities and therefore could have an impact on the overall process of decay.

CONCLUSION

This study confirmed the presence of Gram-positive and Gram-negative bacteria on the pig carcass which maybe pathogenic showing the diversity of microbial organism in pig carcass. The understanding of microbial population, taxonomic and functional succession can provide significant insight into the decomposition process and can serve as a useful tool in forensic examinations .

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