



Supplementary Materials

Characterization of Bacterial Communities and Antibiotic Resistant Genes from Houseflies Inhabiting Hospices in South Africa

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Abstract: Houseflies are alleged reservoirs as well as vectors of human and animal pathogens, including bacteria, because they frequently have contact with animal excreta and decaying organic substances. The rapid adaptation process of ingested microbes in the insect gut may involve gene transfer, including antibiotic resistant determinants among different bacterial strains. As such, this study characterized bacterial communities harboured by houseflies collected from hospices using next-generation sequencing (NGS) platform and further sought to detect antibiotic resistance traits by gene-specific PCR assays. Gene sequence analysis revealed that the most abundant phyla detected with variable abundance observed among all houseflies were Proteobacteria, followed by Firmicutes, Bacteroidetes, and Actinobacteria. Furthermore, NGS data revealed the presence of multiple bacterial genera, including *Providencia*, *Enterobacter*, *Dysgonomonas*, *Escherichia-Shigella*, *Klebsiella*, *Pseudomonas*, and *Streptococcus*, which are known to harbour potentially pathogenic species of animals and humans. Antibiotic resistant genes detected from the housefly DNA in this study included *ermB*, *tetA*, *blaSHV*, and *blaTEM*. Furthermore, these genes are associated with resistance to erythromycin, tetracycline, and beta-lactams antibiotics respectively. The presence of bacterial pathogens and detection of antibiotic resistant genes from houseflies collected from the hospices indicates the possible health risk to patients in hospices and the surrounding community. Therefore, it is imperative to keep high standards of hygiene, food preparation, safety, and control of houseflies in hospices.

Keywords: bacterial communities; 16S *Rrna*; houseflies, resistance genes

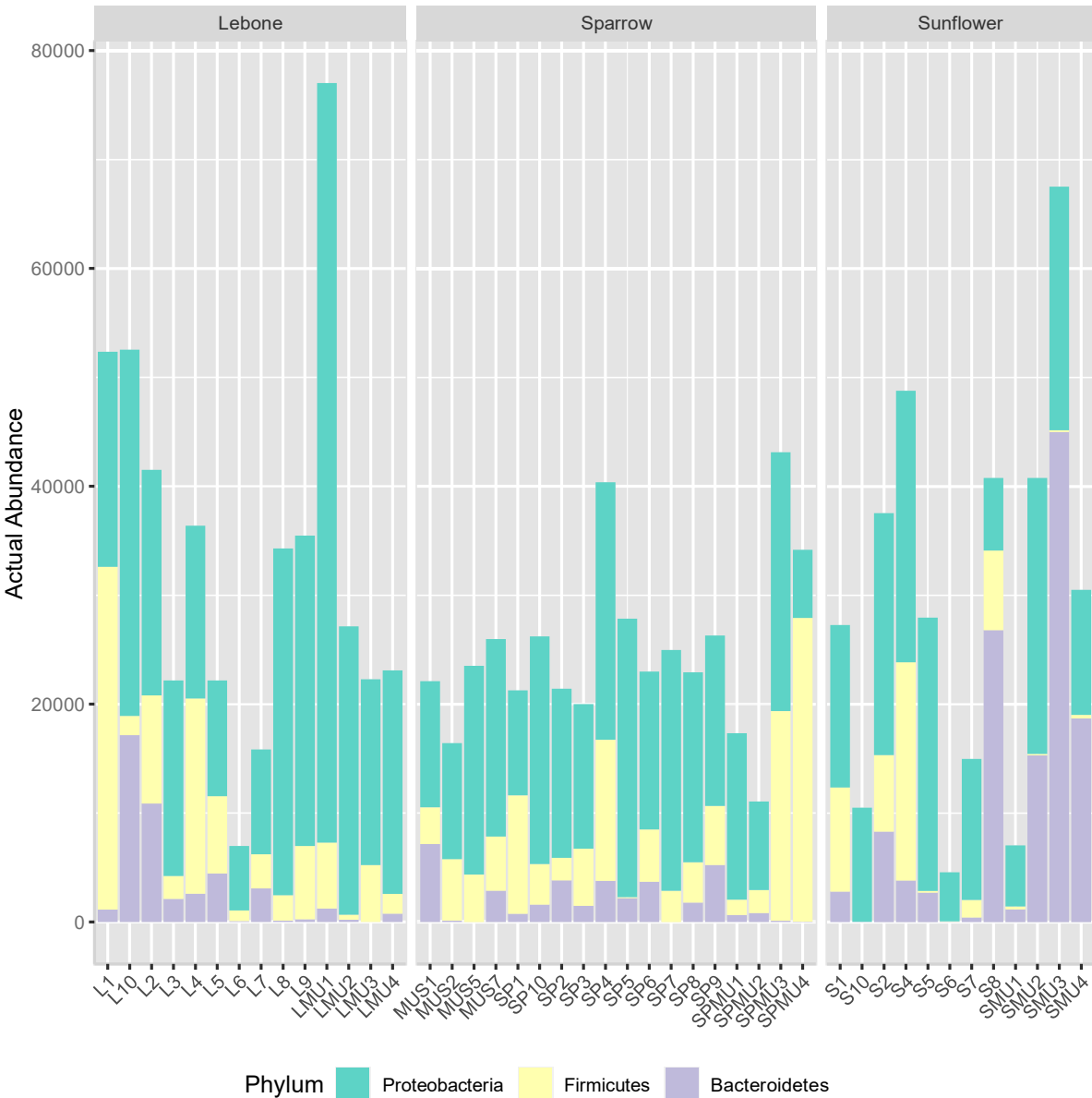


Figure S1. A stacked bar plot showing relative abundance of bacterial phyla detected from houseflies collected from three hospices in Bloemfontein and Johannesburg cities.

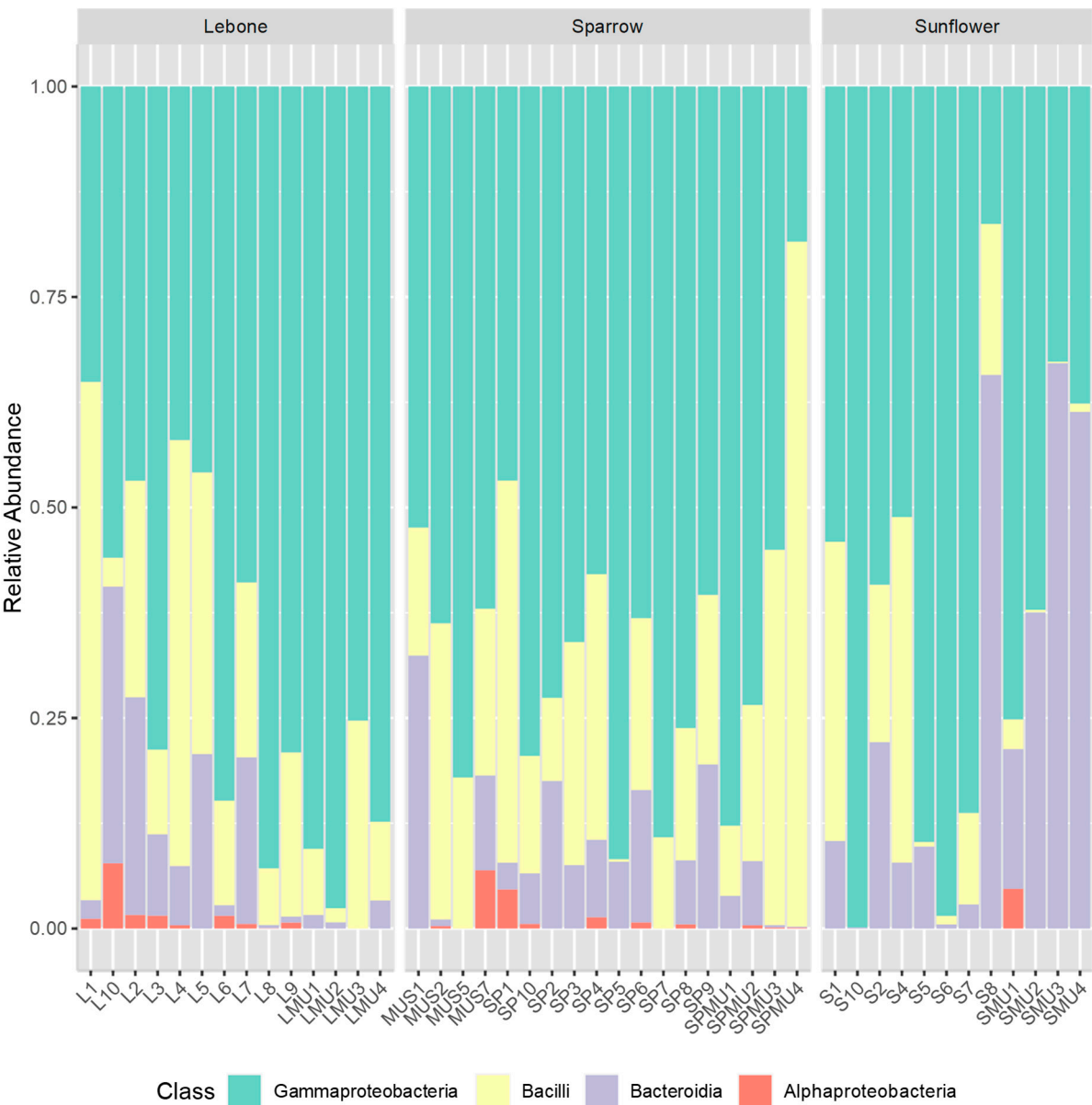


Figure S2. A stacked bar plot showing relative abundance of bacterial class detected from houseflies collected from three hospices in Bloemfontein and Johannesburg cities.

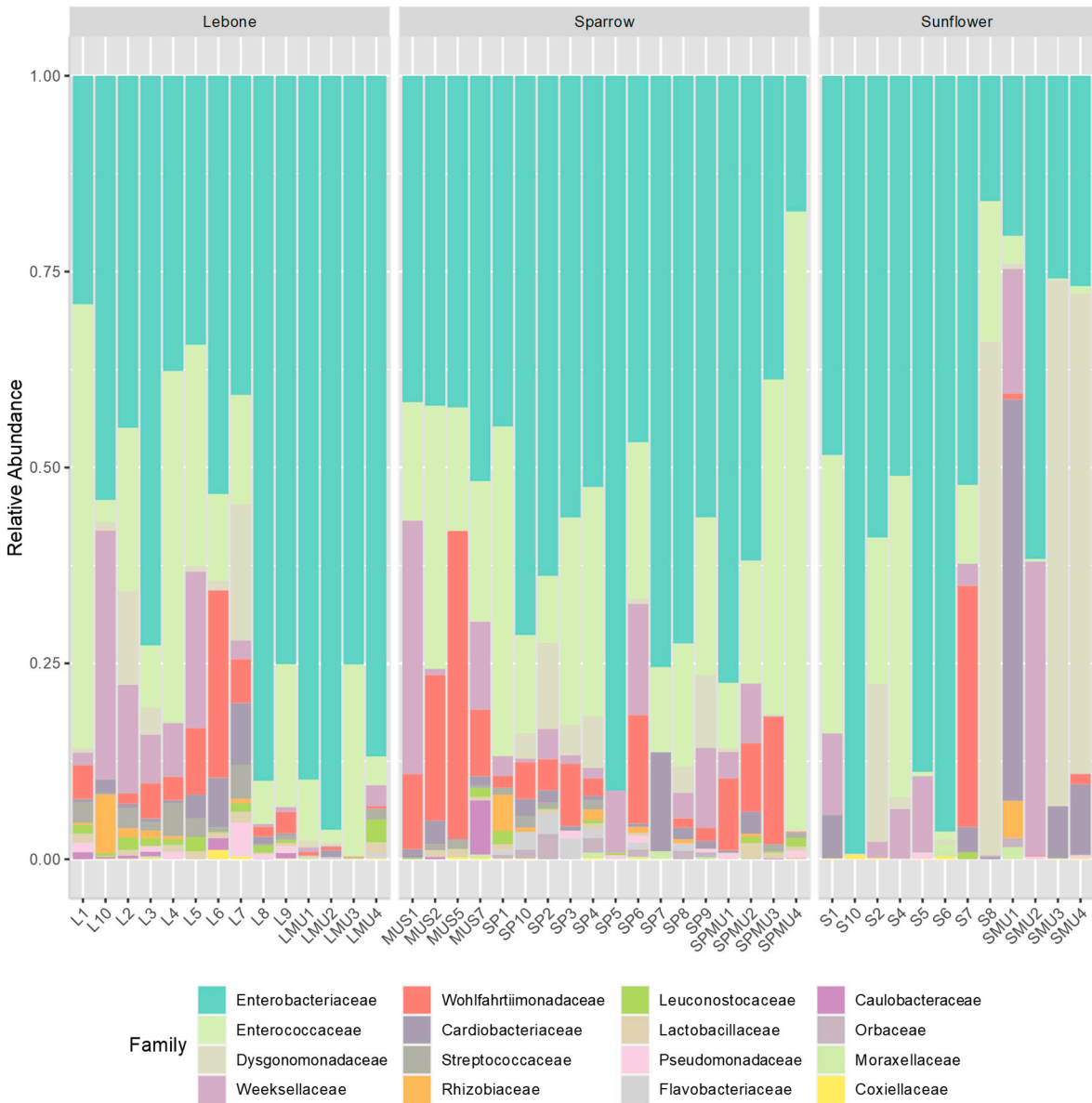


Figure S3. A stacked bar plot showing relative abundance of bacterial families detected from houseflies collected from three hospices in Bloemfontein and Johannesburg cities.

Table S1. Primer sequences specific to different antimicrobial resistant determinants in housefly DNA extracts

Resistant Gene De- tected	Primer Direc- tion	Sequence (5'–3')	Annealing Temperature (°C)	Amplicon Size (bp)	References
Macrolide antibiotic-resistant genes					
<i>ermB</i>	F	GCATTTAACGACGAAACTGGCT	61	573	[28]
	R	GACAATACTTGCTCATAAGTAATGGT			
<i>mecA</i>	F	TAATAGTTGTAGTTGTCGGGTTTG	61	733	[28]
	R	TAACCTAATAGATGTGAAGTCGCT			
<i>vanA</i>	F	GAAATCAACCATGTTGATGTAGCA	61	572	[28]
	R	TTTGCCGTTTCCTGTATCCGT			
β-lactam antibiotic-resistant genes					
CARB	F	CAAGTACTTTYAAAACAATAGC	46	534	[28]
	R	GCTGTAATACTCCKAGCAC			
TEM	F	AAAGATGCTGAAGATCA TTTGG-	44	425	[28]
	R	TATGGCTTCATTC			
SHV	F	GCGAAAGCCAGCTGTCGGGC	62	304	[28]
	R	GATTGGCGGCGCTGTTATCGC			
Tetracycline antibiotic-resistant genes					
<i>tetA</i>	F	GCGCTNTATGCGTTGATGCA	62	387	[28]
	R	ACAGCCCGTCAGGAAATT			
<i>tetW</i>	F	GAGAGCCTGCTATATGCCAGC	64	168	[28]
	R	GGGCGTATCCACAATGTTAAC			
<i>tetX</i>	F	CCGACACGGAAGTTGAAGAA CCTT-	60	468	[28]
	R	GGTGAGATGCCATTAGC			
Integrans					
<i>intI</i>	F	GATGCCTGCTTGTTCTAC	55	558	[60]
	R	GCCTTGCTGTTCTTCTAC			
<i>intII</i>	F	GTAGCAAACGAGTGACGAAATG	62	788	[61]
	R	CACGGATATGCGACAAAAAGGT			
16S <i>rRNA</i>					
16S <i>rRNA</i>	F	ATGGCTGTCGTCAGCT	-	440	[62]
	R	ACGGGCGGTGTGTAC			

Virulence

genes

<i>invA</i>	invA	GTGAAATTATCGCCACGTTTCGGGCAA	53	284	[63]
	139	TCATCGCACCGTCAAAGGAACC			
	invA				
	141				
<i>sefA</i>	S1	GCCGTACACGAGCTTATAGA	53	250	[63]
	S4	ACCTACAGGGGCACAATAAC			
<i>fliC</i>	Fli15	CGGTGTTGCCAGGTTGGTAAT	53	620	[63]
	Typ04	ACTGGTAAAGATGGCT			
<i>uge</i>	F	TCT TCA CGC CTT CCT TCA CT	54	534	[64]
	R	GAT CAT CCG GTC TCC CTG TA			
<i>magA</i>	F	GGT GCT CTT TAC ATC ATT GC	59	1280	[64]
	R	GCA ATG GCC ATT TGC GTT AG			
<i>kfu</i>	F	GAA GTG ACG CTG TTT CTG GC	54	797	[64]
	R	TTT CGT GTG GCC AGT GAC TC			