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Pathogens of Medical Importance Isolated from *Phaenicia* (*Lucilia*) *sericata* (Diptera:Calliphoridae) in Benin City, Nigeria

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Abstract: Adult flies of *Phaenicia sericata* were collected, from three different locations, using sweep net. Sterile vials were used in transferring collected specimens from the sweepnet to the laboratory for further analyses of the microbial content on their external body surfaces. Several bacteria were isolated from their external body surface. A prevalence within the neighborhood of 50% of all bacteria isolated, were *Staphylococcus aureus* and *Lactobacillus* species. *Escherichia coli* was an ubiquitous species across the three locations studied. *Corynebacteria dipththeria*, *Klebsiella* sp. and *Corynebacteria* sp. were the least abundant bacteria 'isolates' of relative abundance of 2.94% each. *Aspergillus* and *Fusarium* species were among the isolated fungal species. This study re-emphasizes the need for maintainance of high sanitary standards around human dwellings. The health implications of the isolated bacteria and fungi were discussed.

Key words: *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Klebsiella*, *Escherichia*

INTRODUCTION

The green bottle fly, *Phaenicia* (*Lucilia*) *sericata* (Diptera:Calliphoridae), is among the cohort of flies that frequently visit filthy sites within and around human dwellings. The vectorial competences of such flies in passive or mechanical transmission of pathogens have been widely documented (Kobayashi *et al*., 1999; Sukontason *et al*., 2007).

Although, the external surface of the insect's body (e.g., wings, spines, etc.), regurgitation of salivary contents and feacal deposits have been highlighted as the probable routes of pathogen transmission among flies (Rosef and Kapperud, 1983; Nazni *et al*., 2005), the most important route still remains controversial. This might explain why diarrhea, food poisoning and other preventable infections are still plaguing man especially in the developing countries and could be ascribed to the indiscriminate dumping of (domestic) waste, which serves as breeding grounds for these flies and pathogens (Holt *et al*., 2007).

Given the relatively low level of hygiene around human dwellings in developing nations and its attendant health effects, the crux of this study was to identify the cohort of microbes associated with *P. sericata* in Benin City and to discuss the possible health implications of such microbes.

MATERIALS AND METHODS

The specimens used for this study were collected from three locations namely: abattoir in the city centre; restaurant and hostel both within the precinct of the University of Benin, Ugbowo campus. These specimens were collected using an aspirator and transferred to sterile vials for *ex situ* studies. Crystal violet, distilled H_2O, Lugol's iodine, Safranin dye and H_2O_2 were some of the reagents used. Glass wares were cleaned and sterilized at 160-170°C for 45-60 min. Inoculating loops were heat-sterilized over a bursen burner until red hot before use. Nutrient agar, McConkey agar, potato-dextrose agar and blood agar (autoclaved for 15 min at 120°C and cooled to 50°C). The media were aseptically dispensed into the Petri dishes containing the samples. The Petri dishes were gently rotated for evenness and homogenous spread to prevent confluence growth. The media were left to solidify and incubated at 37°C for 24 h.

Isolation and enumeration of bacteria: Using pour plate method, the total viable bacteria were enumerated. A stock sample was prepared by the introduction of an adult *L. sericata* into normal saline and 1 mL of this solution was serially diluted into 1:10, 1:100 and 1:1000. The serial dilutions of 1:100 were then plate on nutrient agar, McConkey agar, blood agar and potato dextrose agar. All

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cultures were incubated at 37°C for 48 h. Using streak techniques, bacteria colonies were purified by sub-culturing on fresh nutrient agar.

Isolation and enumeration of fungi: Isolates were established using Pour Plate method on potato dextrose agar. To discourage the growth of bacteria 50 mg mL^{-1} of streptomycin was added to the medium.

Sample identification

Bacteria: Isolates were identified using biochemical and morphological tests as described in Berger's manual of determinative bacteriology (Buchaman and Gibbons, 1974).

Fungi: Spores staining carried out on a heat fixed smear of lactophenol cotton and blue green reagent was added for allowed to stand for a minute before rinsing with distilled water. Then 95% ethanol was added for 30 sec and rinsed. Sample was then identified using microscopic and macroscopic features of the hyphal mass and morphology of cells and spores.

Gram staining: On glass slides, smear of each of the bacteria isolate was made and heat fixed. Then treated with crystal violet (0.3% w/v) and rinsed off with distilled H_2O after 1 min; in similar manner, the isolates on the slide were also treated with iodine (0.4% w/v) and rinsed, followed by ethanol (95% w/v) for 30 sec and then stained with a secondary stain, Safranin (0.4% w/v) for a minute, rinsed and air dried. Slides were then examined under oil immersion lens (magnification, x100).

Oxidase test: This was used to differentiate between *Pseudomonas* and other gram -ve rod shaped bacteria. Each isolate from the culture was smear on filter paper using sterile wire loop. A drop of oxidase reagent (1.0% aqueous tetramethyl-B-phenylenediamine dihydrochloride) was added. The appearance of purple colour within 10 sec is indicative of positive oxidase.

Citrate-utilization test: Simmon's citrate medium was used to identify microbes utilizing citrate as their sole source of energy for growth. Twenty three gram of Simmon's citrate agar was dissolved in 1 L of distilled H_2O and dispensed in a sterile McCartney bottle, followed by sterilization at 120°C for 15 min. The test organism was introduced into the medium and incubated at 37°C for 24 h. A colour change from light green to bright blue indicates a positive result, while turbidity in the medium and no colour change indicate a negative citrate test.

Catalase test: This was used to delineate catalase-producing bacteria from non-catalase-producing bacteria by adding 3 mL of H_2O_2 (3% v/v) into a test tube. Using sterile glass rod, colonies of the test organism were introduced into the H_2O_2 solution. Immediate release of air bubbles indicates a positive test (*Staphylococcus* species) and no bubbles shows negative test (*Streptococcus* species).

Fungal identification: Spore staining was carried out on a heat-fixed smear of lactophenol cotton; blue green reagent was added and rinsed after a minute. Ethanol (95%) was then added and rinsed after 30 sec. Sample was then viewed under the microscope.

RESULTS AND DISCUSSION

Different bacteria were isolated from the external body parts of *Phaenicia sericata*. The bacteria were *Micrococcus* species, *Klebsiella* species, *Lactobacillus* species, *Staphylococcus aureus*, *Escherichia coli*, *Corynebacteria* species and *Streptococcus pyogenes* (Table 1). Some bacteria were present on *P. sericata* across three different sampling sites. *Lactobacillus* sp., *Staphylococcus aureus* and *Escherichia coli* were such ubiquitous microbes whereas *Corynebacteria* sp. and *Micrococcus* sp. were only isolated from *P. sericata* collected around the restaurants.

Staphylococcus aureus and *Lactobacillus* spp. were the most abundance species found on *P. sericata* with incidence of 35.29 and 20.57%, respectively and collectively had an incidence of >50% (Table 2). *Corynebacteria dipththeria*, *Klebsella* sp. and *Corynebacteria* sp. were relatively low with an incidence of 2.94% each (i.e., 8.82% collectively).

Eight genera of fungi were isolated from *Phaenicia sericata*. Of these fungi, *Aspergillus* species, *Trichoderma*, *Geotrichum* were ubiquitous across the studied sites. Others are *Fusarium* species, *Penicillium* species and *Rhizopus* species (Table 3).

In a study carried out in 2008 by Vazirianzadeh *et al.* (2009) the most abundant microbes isolated from the external surfaces of *Musca domestica* were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp., whereas earlier, (Olsen and Hammack, 2000) isolated *Salmonella enteritidis*, *S. infantis*, *S. heidelberg* from housefly. Cases of dermatophytes such as *Microsporium gypseum* and *Trichophyton mentagrophytes* have also been recorded (Zarrin *et al.*, 2007). *Staphylococcus aureus* is generally recognized as one of the causative

Table 1: Morpho-characteristics and identity of bacteria isolated from *Phaenicia sericata* in three different sites in Benin city

		Isolates								
Characteristics	Array	*Corynebacteria* sp.	*Lactobacillus* sp.	*Micrococcus* sp.	*Staphylococcus aureus*	*Escherichia coli*	*Micrococcus* sp.	*Corynebacteria* sp.	*Streptococcus* sp.	*Klebsiella* sp.
Colour	Cream	+	+	+		+			+	+
	Pale				+		+	+		
Edge of colony	Smooth	+							+	+
	Rough		+	+	+	+	+	+		
Elevation	Raised	+				+		+	+	+
	Flat		+	+	+		+			
Surface	Dry	+	+	+	+	+	+		+	+
	Moist							+		
Gram reaction	Positive	+	+	+	+		+	+	+	
	Negative					+				+
Cell shape	Rod	+	+			+		+		+
	Cocci			+	+		+		+	
Catalase	Positive	+		+	+	+	+	+		+
	Negative		+						+	
Oxidase	Positive		+		+	+			+	+
	Negative	+		+			+	+		
Citrate	Positive				+				+	+
	Negative	+	+	+		+	+	+		
Coagulase positive		+			+				+	
	Negative		+	+		+	+	+		+
Total	Restaurant	+	+	+	+	+	+	+	-	-
	Abattoir	-	+	-	+	+	-	-	+	-
	Hall	-	+	-	+	+	-	-	+	+

+: Present, -: Absent

Table 2: Abundance and prevalence of bacteria recovered from *P. sericata* in Benin city

	Site						
	Hall		Abattior		Restaurant		
Species	No.	%	No.	%	No.	%	Prevalence (%)
Staphylococcus aureus	4	33.33	5	41.67	3	30.00	35.29
Streptococcus pyogenes	1	8.33	2	216.67	0	0.00	8.82
Streptococcus pnemonia	2	16.67	1	8.33	0	0.00	8.82
Corynebacteria diphtheria	0	0.00	0	0.00	1	10.00	2.94
Lactobacillus sp.	3	25.00	3	25.00	1	10.00	20.57
Micrococcus sp.	0	0.00	0	0.00	3	30.00	8.82
Klebsella sp.	1	8.33	0	0.00	0	0.00	2.94
Escherichia coli	1	8.33	1	8.33	1	10.00	8.82
Corynebacteria sp.	0	0.00	0	0.00	1	10.00	2.94
Total per site		12		12		10	

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Table 3: Fungi isolated from *Phaenicia sericata* in Benin city

Isolated fungi	Colour of colony	Location*
Aspergillus niger	Black	1, 2, 3
A. flavus	Dark green	1, 2, 3
Geotrichum sp.	Brown	1, 2, 3
Trichoderma sp.	Yellow	2, 3
Fusarium sp.	Light yellow	3
Penicillium sp.	Green	2, 3
Rhizopus sp.	Floxy white	3

*1: Within the precinct of the hall or hostel, 2:Abattoir, 3: Restaurant

agent of food poisoning while *Streptococcus* species are mainly human pathogen associated with local systemic invasion and post streptococcal disorder.

In congruent with other findings, *Phaenicia sericata* contributes to the epidemiology of pathogenic infections around cafeteria and hostels within the precinct of the university and also abattoir. And the presence of *E. coli* on *P. sericata* collected from these sites, with an incidence of 8.8%, is symptomatic of an environment lade with human feacal deposits. *Escherichia coli* has also been isolated from the body surface of *Musca domestica* (Sasaki *et al*., 2000), a frequent visitor of filthy sites.

In a comparative account houseflies and American cockroaches have been reported to have similar vectorial competence in the mechanical transmission of pathogens (Lamiaa *et al*., 2007). This study highlights the nuisance value of *P. sericata* in and around the studied sites with 8 genera of bacteria isolated, precisely a total of nine species.

Aspergillus niger has been isolated from *Cochliomyia megacephala* (Kontoyiannis and Lewis, 2010). Similarly, our findings revealed that *Phaenecia sericata* can also transmit *A. niger*. Worthy of note is the medical importance or pathogenic status of *A. niger* in nosocosmic infections (Kontoyiannis and Lewis, 2010). *Aspergillus fumigates* has been incriminated in all forms of invasive and non-invasive aspergillosis. Although, acclaimed to be cosmopolitan, the fungus, *Fusarium* species are also regarded as rare opportunistic fungi, which have been implicated in cutaneous and sub-cutaneous infections (Srivoramas *et al*., 2012).

CONCLUSION

Conclusively, in order to circumvent the possible health issues that might arise from infections associated with these pathogens, the environment should be kept clean; and the agencies and/or departments in charge of the sanitary conditions of these places should brace up to their responsibilities.

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