PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 25 (10) 1765-1773 October 2021

Identification and Antibiotic Susceptibility Pattern of *Enterobacteriaceae* Isolated from Gecko (*Hemidactylus frenatus*)

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ABSTRACT: Diseases and infections which are naturally transmitted between animals and humans are of major concern worldwide. Geckos (*Hemidactylus frenatus)* are known to be potential reservoirs of many zoonotic enteropathogens. This study was designed to isolate, identify, and evaluate the antibiotic susceptibility pattern of *Enterobacteriaceae* from Geckos. Using standard microbiological procedures, bacteria were isolated from 138 intestinal samples of *Hemidactylus frenatus* collected from different sampling sites. A total of 20 bacterial species of 9 different genera were identified using automated Colorimetry VITEK 2 system. The percentage occurrences were *Enterobacter aerogenes* (35%), *Proteus mirabilis* (15%), *Salmonella ser paratyphi B* (10%), *Serratia fonticola* (10%), *Enterobacter kobei* (10%), *Raoultella ornithinolytica* (5%), *Sphingomonas paucimobilis* (5%), *Acinetobacter baumannii,* (5%) and *Burkholderia cepacia* (5%). Results obtained from the antibiotic susceptibility pattern according to CLSI guidelines revealed that all the 20 bacterial species have varying rate of resistance with 20 (100%) showing resistance to Ciprofloxacin (CPX), 20 (100%) Pefloxacin (PEF), 19 (95%), Augmentin (AU), 11 (55%) Cotrimoxazole (CXT), 10 (50%) Streptomycin (S), 9 (45%) Chloramphenicol (CH), 6 (30%) Gentamycin (CN), 3 (15%) Ofloxacin (OFX). This study revealed that *Enterobacteriaceae* in the intestine of Geckos are multidrug resistant and are potentially harmful when in contact directly or indirectly with humans. It becomes important to educate people on the importance of personal hygiene in order to eradicate Geckos from our environment.

DOI: **https://dx.doi.org/10.4314/jasem.v25i10.4**

Dates: Received: 22 August 2021; Revised: 17 September 2021; Accepted: 06 October 2021

Keywords: Disease, Antibiotics, *Hemidactylus frenatus, Enterobacteriaceae*

Bacteria are widespread in the environment and have evolved a variety of interactions with animals including those inhabited in human dwellings (Feldhaar, 2011). The presence of insect pests is common and affects all human habitations, creating conditions that are favorable to many pests that can harbor pathogens (Bertone *et al.*, 2016; Leong *et al.*, 2017). Geckos (*Hemidactylus frenatus*) belong to the reptilian family Geckkonidae which can be wild or non-wild type. They are nocturnal animals found within human habitation where they find shelter, heat and food; feeding on other smaller insects and left over food substances (Nwachukwu *et al.*, 2014). Generally, Reptiles have been reported to carry bacteria agents in their digestive tract without manifesting any associated symptom other than serving as sources of contamination and disease vector to humans (Ajayi *et al.*, 2015). Diseases can be transmitted to humans indirectly or directly. The indirect method can be the transmission of pathogens (organisms that causes diseases) through the ingestion of fecal contaminated food and water while the direct method can be from person to person etc. (Whiley *et al.*, 2017). Few researchers who have studied Geckos as a reservoir of pathogens have reported zoonotic enteropathogens such as *Edwardsiella tarda, Citrobacter freundii, Klebsiella pneumonia, Clostridium Intermedius, Erwinia herbicola, Enterobacter cloacae* (Singh *et al.*, 2013, Nwachukwu *et al.*, 2014;), *Shigella sonnei, Enterobacter* species, *Serratia marcescens, Proteus* spp., *Escherichia coli* including non-typhoidal salmonellae (Callaway *et al.*, 2011; Gwen and Saleha, 2013; Arnafia *et al.*, 2016). These microorganisms are Gram-negative bacterium of the *Enterobacteriaceae* family which have been categorized as the major global causes of diarrheal, ulcerative stomatitis, pneumonia, cutaneous lesions, septicemias, caseated abscesses, and are associated with consumption of contaminated food products of animal origin (WHO, 2018; Bjelland *et al.*, 2020). It has been observed that *Enterobacteriaceae* obtained from both food animals and humans shows increasing antibiotic resistance rates (Hakanen *et al.*, 2015). For example, *Salmonella enterica* and *Escherichia coli* have been widely

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reported to have multiple antibiotic resistances of which most of the strains are zoonotic in origin (Ogunleye and Carlson, 2010; Singh *et al.,* 2013). Majority of multiple antibiotic resistance strains acquired their resistance in the food-animal host, causing human infections through the food chain. According to Yakubu *et al.* (2011); Omitola and Taylor-Robinson (2020); approximately 60% of the several infectious microorganisms that causes emerging and re-emerging diseases are confirmed to be zoonotic. However, of all the animals that live in close proximity with humans and liable to harbor pathogens, Geckos (*Hemidactylus frenatus*) are the least studied. In Nigeria, Geckos comes with several myths which prevents their eradication despites their massive invasions of homes. The Common House Gecko have a simple life process of feeding on smaller insects such as cockroach, housefly, weevils, spiders, ants etc as their food, which raises their potentials of being a reservoir for pathogens. Geckos could be vectors of opportunistic bacteria (an organism that was initially a commensal or mutualistic and turns out to cause a disease) or true pathogen, possessing properties that enables them to overcome the body defenses and infect the tissue of a normal healthy subject producing disease. Their frequent excretion of ingested food through their faecal droppings may also serve as a vector for the transmission of enteric pathogens which can be risky to human health. Though earlier studies which focused more on their intestinal droppings were impressive, however, bacteria detected in intestinal tract may not always be present in the excreta probably due to several competitive factors in the posterior part of gastrointestinal tracts, hence, the isolation from intestinal tract in this study. Researchers have reported several other reptiles as host of drug resistant bacteria (Ogunleye and Carlson, 2010; Singh *et al.,* 2013; Ajayi *et al.,* 2015), little has been known of Geckos. However, no research has been able to identify drug-resistant bacteria isolated from Gecko using VITEK 2 system. VITEK 2 system is a user-friendly machine incorporated software with bi-directional interface, epidemiology report module, and comprehensive database used for the identification of bacteria and yeast, and epidemiologic trending and reporting (Maina and Kagotho, 2014). The VITEK 2 system uses an identification technology known as Advanced Colorimetry that enables the identification of routine clinical isolates and provides high discrimination between species (Wani *et al.,* 2016). This study aimed to isolate, characterize and evaluate the antibiotic susceptibility pattern of *Enterobacteriaceae* from the intestinal tract of Geckos (*Hemidactylus frenatus*).

MATERIALS AND METHODS

Study site: Gecko *(Hemidactylus frenatus)* samples for the study were collected randomly from different sites in Abeokuta metropolis, Ogun state, Nigeria. The targeted sites were indoors and outdoors such as Kitchen, Animal House, Corridor, Store, Hospital, Toilet, etc where there are possibilities of direct or indirect contact with Humans. *Hemidactylus frenatus* is known to be carnivorous (insectivorous) and nocturnal animals, so they are captured mostly at nights.

Sample Collection and Storage: A total of one hundred and thirty-eight (138) samples of *Hemidactylus frenatus* were aseptically collected from different sites. The Geckos were placed in a perforated sterile sample bottle to allow enough air-flow for respiration and transferred into sterile plastic bags. Each sample bottle contains one Gecko and then transported to the Microbiology Laboratory, Chrisland University, Abeokuta for further analysis.

Isolation of Microorganisms: *Hemidactylus frenatus* was surface sterilized using iodine, and then 70% ethanol. Dissection for intestinal examination was carried out using sterile dissecting kit. The body cavity was cut open in a ventral longitudinal position to expose the intestinal system. The intestinal tract was carefully separated from the attached tissues. The separated intestine that ends in the cloaca was removed using a sterilized forceps and placed in a sterile swab stick containing already prepared peptone water. The same procedure was carried out for all other samples. All samples were incubated in a shaker incubator for 18 hours at 37^{o}C. After incubation, each sample was streaked on plates containing already prepared MacConkey agar and Eosin Methylene Blue agar respectively. Plates were then incubated at 37^{o}C for 24 hours.

Purification of Bacterial isolates: Pure cultures of the bacterial isolates were obtained by series of sub-culturing on the corresponding medium. Isolates with different morphological appearances were selected and purified by streaking on corresponding medium plates until pure cultures were obtained. All pure cultures of bacterial isolates were inoculated and maintained on the corresponding agar slants and stored at 4^{o}C in the refrigerator.

Phenotypic Characterization of Bacterial isolates: The bacteria isolates were subjected to standard microbiological methods such as morphological characteristics of the colony (colour, surface, margin, and elevation) and Gram staining to differentiate Gram-negative and Gram-positive bacteria.

Biochemical tests, including Catalase test, Citrate utilization test, Voges-Proskaeur test, Urease test, Indole test, Triple Sugar iron test, sugar fermentation test, and methyl-red test were also carried out on the isolates (Fawole and Oso, 1998; Cheesbrough, 2006). The morphological and biochemical characteristics of the isolates were examined according to Bergey's Manual of Determinative Bacteriology.

Antibiotic Susceptibility Testing: The Kirby Bauer disc diffusion agar technique was used to determine the antibiotic susceptibility of the isolated organisms. Mueller-Hinton agar was prepared according to the manufacturer's instruction. An 18-24 hours old test organism was standardized by diluting to 0.5 Mcfarland's standard. A sterile swab stick was inserted into the inoculum and inoculated by spreading evenly onto the sterile Mueller-Hinton agar plate. The inoculated plates were then allowed to dry for few minutes at room temperature with the lid closed. After this, antibiotic-impregnated discs of known concentrations; Cotrimoxazole (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Augmentin (10 µg), Gentamycin (30 µg), Pefloxacin (30 µg), Ofloxacin(10 µg), Streptomycin (30 µg) were carefully seeded on the inoculated Mueller- Hinton agar plates using sterile forceps. The plates were then incubated at 37°C for 18-24 hours. The diameters of the zones of inhibition were measured and interpreted following guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI) (Moses *et al.*, 2018).

VITEK 2 Identification of Bacterial isolates: Bacteria identification were performed using the Vitek Gram-negative card. The card is allowed to come to room temperature before opening the package liner. The Vitek tubes were aseptically filled with 3 mL of sterile Vitek saline. Sterile cotton swabs were used to prepare a homogenous organism suspension by transferring isolated colonies from a pure culture. The suspension was adjusted to 0.5 McFarland turbidity using the densitometer. The suspension was placed in the Vitek cassette and used directly for identification purposes. The straw of the Vitek 2 card was inserted into the inoculated suspension tubes within 30 minutes of suspension preparation. The cassette was placed in the filler box of the Vitek unit and allowed to load. The Vitek 2 machine automatically processed the cards and ejected them into the waste bin collection after the cards had been processed (Ksiazczyk *et al.*, 2016).

Statistical analysis of data: Data were analysed using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS, Chicago IL, U.S.A). The means of the data obtained were analysed by analysis of variance (ANOVA), means were separated using the Student-Newman-Keuls (SNK) test at $\alpha = 0$ (Akintokun and Taiwo, 2016).

RESULTS AND DISCUSSION

The Total Bacterial Counts (TBC) obtained from different samples grown on Eosin Methylene Blue (EMB) and MacConkey agar respectively were shown in table 1. The Total Bacterial Counts grown on both media were significantly different from each other. On EMB medium, bacteria count from MH ($2.25x10^2$) was significantly higher than those obtained from AH ($2.10\ x10^2$), which was significantly higher than AUD ($2.04\ x10^2$). This was followed by CAF ($1.99\ x10^2$) which was significantly higher than ST ($1.97\ x10^2$), SP2 ($1.84\ x10^2$), SP1 ($1.60\ x10^2$) and E3 ($1.38\ x10^2$) respectively. The least bacterial count was obtained in SB2 with a bacterial count of $1.27\ x10^2$. (Table 1). However, bacteria count obtained grown on MAC from LAB ($2.41\ x10^2$) was significantly higher than those obtained from FH2 ($2.09\ x10^2$), which was significantly higher than TS ($2.02\ x10^2$). This was followed by CR3 ($1.73\ x10^2$) which is significantly higher than SB3 ($1.66\ x10^2$), SB1 ($1.50\ x10^2$), E4 ($1.44\ x10^2$), HK1 ($1.23\ x10^2$), SB4 ($1.06\ x10^2$) and E1 ($1.03\ x10^2$) respectively. The least bacterial count was obtained in HP1 with a total bacterial count of $1.02\ x10^2$. (Table 1)

Table 1: Total Bacterial counts isolated from *Hemidactylus frenatus* grown on Eosin Methylene Blue and Mac-Conkey Agar medium

Methylene Blue Agar (MBA)		Mac-Conkey Agar (MCA)	
Sample code	**Bacterial counts (10^2) (CFU/ml)**	**Sample code**	**Bacterial counts (10^2) (CFU/ml)**
SB2	1.27±1.73	SB3	1.66±3.53
AUD	2.04±4.58	SB4	1.06±3.93
SP1	1.60±5.36	SB1	1.50±2.40
MH	2.25±3.52	FH2	2.09±4.70
E3	1.38±6.80	TS	2.02±1.76
CAF	1.99±5.78	LAB	2.41±2.19
AH	2.10±6.65	HK1	1.23±3.18
SP2	1.85±4.33	E4	1.44±3.06
ST	1.97±4.05	E1	1.03±3.18
		CR3	1.73±4.23
		HP1	1.02±2.52

Results are mean values ± standard error of mean for three replicates according to Student Newman-Keuls (SNK) test at $\alpha = 0.05$.

The biochemical characterization of bacterial isolates is shown in table 2. A total of twenty (20) bacteria were isolated and presented for characterization. From the result, all bacterial isolates were Gram-negative with an indication of pink color, while the shape of the bacteria were all rods. The catalase test revealed that all bacterial isolates were catalase-positive indicating the production of the enzyme catalase except GE15 which is catalase-negative. The result from indole test showed that eleven (11) bacterial isolates (GE1, GE2, GE3, GE4, GE6, GE9, GE10, GE11, GE17, GE18, GE20) of the twenty (20) samples were positive while the other nine (GE5, GE7, GE8, GE12, GE13, GE14, GE15, GE16, GE19) were negative. Sixteen (16) isolates with the code GE2, GE3, GE5, GE6, GE7, GE8, GE9, GE10, GE11, GE12, GE13, GE14, GE15, GE18, GE19 and GE20 were all methyl red positive, while the other four isolates (GE1, GE4, GE16, GE17) were negative. Only isolate GE1 was Voges-Proskauer positive while others were negative. Similarly, Only GE15 was able to utilize citrate (positive) while others could not. The results from the sugar fermentation test showed that all bacterial isolates were glucose positive indicating their ability to ferment glucose. For sucrose test, fifteen (15) isolates (GE3, GE4, GE5, GE7, GE8, GE9, GE10, GE12, GE14, GE15, GE16, GE17, GE18, GE19, GE20) were positive while the other five (5) isolates (GE1, GE2, GE6, GE11, and GE13) were sucrose negative. All bacterial isolates also showed their inability to ferment Lactose except GE3, GE7, GE9, GE10, and GE20 which were able to ferment lactose. The result for hydrogen sulfide test (H_2S) showed that all bacterial isolates were positive except GE4, GE12, GE15, GE16, and GE17 which were negative. The test for the production of gas revealed that all bacterial isolates were able to produce gas except GE1, GE4, GE15 and GE17 which were not able to produce gas. All bacterial isolates showed positive for urease test except GE15 which was urease negative (Table 2).

Table 2: Biochemical Characterization of Bacteria Isolates from *Hemidactylus frenatus*

Isolate Code	Gram's	Morphology	Catalase	Indole	Methyl red	Voges-proskauer	Citrate	Glucose	Sucrose	Lactose	H2S	Gas	Urease
GE 1	_	Rod	+	+	_	+	+	+	_	_	+	_	+
GE 2	_	Rod	+	+	+	_	+	+	_	_	+	+	+
GE 3	_	Rod	+	+	+	_	+	+	+	+	+	+	+
GE 4	_	Rod	+	+	_	_	+	+	+	_	_	_	+
GE 5	_	Rod	+	_	+	_	+	+	+	_	+	+	+
GE 6	_	Rod	+	+	+	_	+	+	_	_	+	+	+
GE 7	_	Rod	+	_	+	_	+	+	+	+	+	+	+
GE 8	_	Rod	+	_	+	_	+	+	+	_	+	+	+
GE 9	_	Rod	+	+	+	_	+	+	+	+	+	+	+
GE 10	_	Rod	+	+	+	_	+	+	+	+	+	+	+
GE 11	_	Rod	+	+	+	_	+	+	_	_	+	+	+
GE 12	_	Rod	+	_	+	_	+	+	+	_	_	+	+
GE 13	_	Rod	+	_	+	_	+	+	_	_	+	+	+
GE 14	_	Rod	+	_	+	_	+	+	+	_	+	+	+
GE 15	_	Rod	_	_	+	_	_	+	+	_	_	_	_
GE 16	_	Rod	+	_	_	_	+	+	+	_	_	+	+
GE 17	_	Rod	+	+	_	_	+	+	+	_	_	_	+
GE 18	_	Rod	+	+	+	_	+	+	+	_	+	+	+
GE 19	_	Rod	+	_	+	_	+	+	+	_	+	+	+
GE 20	_	Rod	+	+	+	_	+	+	+	+	+	+	+

KEY: + = Positive result, - = Negative result

The bacterial isolates were presented for a confirmatory identification using VITEK 2 system. Results obtained from the automated method showed that Isolates GE1, GE5 and GE8 were *Proteus mirabilis*, Isolate GE2 was *Raoultella ornithinolytica*, Isolates GE3, GE4, GE9, GE10, GE12, GE17 and GE20 were identified as *Enterobacter aerogenes*, Isolates GE6 and GE11 were identified as *Salmonella ser paratyphi B*, isolates GE7 and GE19 were identified as *Serratia fonticola*, isolates GE13 and GE14 were identified as *Enterobacter kobei.* Isolates GE15, GE16 and GE18 were identified as *Sphingomonas paucimobilis, Acinetobacter baumannii, Burkholderia cepacia* respectively (Table 3).

The prevalence of *Enterobacteriaceae* from different sample sites is shown in Table 4. Three (3) *Enterobacteriaceae* namely *Salmonella ser paratyphi B, Enterobacter aerogenes* and *Acinetobacter baumannii* were isolated from Kitchens (15%) while four (*Proteus mirabilis, Enterobacter aerogenes, Salmonella ser paratyphi B* and *Burkholderia cepacia*)

were isolated from outdoors (20%). *Serratia fonticola* was only isolated from Animal houses (5%) while *Raoultella ornithinolytica* and *Enterobacter kobei* were isolated from Stores (10%). Four (4) *Enterobacteriaceae* namely *Enterobacter aerogenes*, *Proteus mirabilis*, *Sphingomonas paucimobilis* and *Serratia fonticola* were isolated from Hospitals (20%) while *Enterobacter aerogenes* and *Enterobacter kobei* were isolated from Toilets (10%).

Table 3: Identification of Bacterial isolates using VITEK 2 system

Isolate Codes	Identified organisms	Confidence level
GE 1	*Proteus mirabilis*	Good identification
GE 2	*Raoultella ornithinolytica*	Good identification
GE 3	*Enterobacter aerogenes*	Very good identification
GE 4	*Enterobacter aerogenes*	Good identification
GE 5	*Proteus mirabilis*	Good identification
GE 6	*Salmonella ser paratyphi B*	Excellent identification
GE 7	*Serratia fonticola*	Very good identification
GE 8	*Proteus mirabilis*	Good identification
GE 9	*Enterobacter aerogenes*	Very good identification
GE 10	*Enterobacter aerogenes*	Very good identification
GE 11	*Salmonella ser paratyphi B*	Excellent identification
GE 12	*Enterobacter aerogenes*	Very good identification
GE 13	*Enterobacter kobei*	Excellent identification
GE 14	*Enterobacter kobei*	Very good identification
GE 15	*Sphingomonas paucimobilis*	Acceptable identification
GE 16	*Acinetobacter baumannii*	Excellent identification
GE 17	*Enterobacter aerogenes*	Good identification
GE 18	*Burkholderia cepacia*	Very good identification
GE 19	*Serratia fonticola*	Good identification
GE 20	*Enterobacter aerogenes*	Very good identification

Table 4: Prevalence of *Enterobacteriaceae* in different Sample sites

Sample site	Percentage occurrence	Identified Enterobacteriaceae
Kitchen	15%	*Salmonella ser paratyphi B, Enterobacter aerogenes, Acinetobacter baumannii*
Outdoors	20%	*Proteus mirabilis, Enterobacter aerogenes, Salmonella ser paratyphi B, Burkholderia cepacia*
Animal house	5%	*Serratia fonticola*
Store	10%	*Raoultella ornithinolytica, Enterobacter kobei*
Hospital	20%	*Enterobacter aerogenes, Proteus mirabilis, Sphingomonas paucimobilis, Serratia fonticola*
Toilet	10%	*Enterobacter aerogenes, Enterobacter kobei*

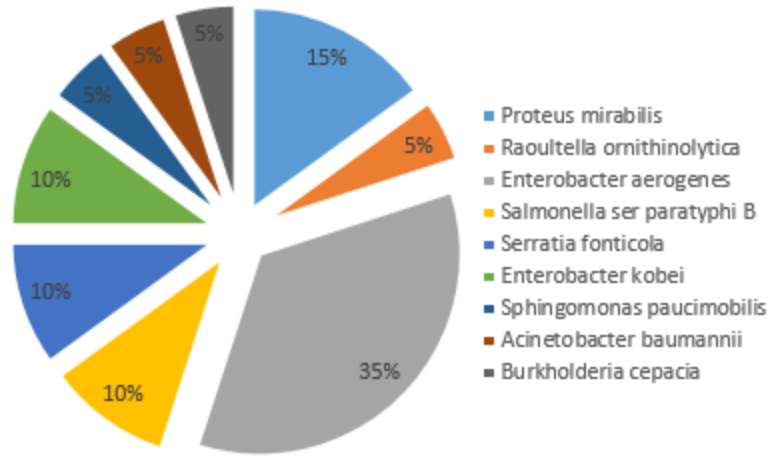

Fig 1: Frequency of *Enterobacteriaceae*

Figure 1 showed the frequency of *Enterobacteriaceae* isolated from Geckos. From the figure presented below, *Enterobacter aerogenes* has the highest frequency of 35%. This was followed by *Proteus*

mirabilis with a frequency of 15%, while *Salmonella ser paratyphi B*, *Serratia fonticola* and *Enterobacter kobei* all has a frequency of 10% each. The least frequency of 5% was each obtained in *Raoultella ornithinolytica*, *Sphingomonas paucimobilis*, *Acinetobacter baumannii*, and *Burkholderia cepacia*.

Evaluation of Enterobacteriaceae against selected Antibiotics: Enterobacteriaceae were tested against Cotrimoxazole (CXT), Chloramphenicol (CH), Ciprofloxacin (CPX), Augmentin (AU), Gentamycin (CN), Pefloxacin (PEF), Ofloxacin (OFX), and Streptomycin (S) using the disk diffusion susceptibility method. *Proteus mirabilis* GE1 was resistant to all antibiotics except Gentamycin and Ofloxacin. *Raoultella ornithinolytica* GE2 was resistant to all antibiotics except Chloramphenicol, Gentamycin, and Ofloxacin. However, *Enterobacter aerogenes* GE3 was resistant to all the antibiotics. *Enterobacter aerogenes* GE4 was resistant to all antibiotics except Cotrimoxazole, Ciprofloxacin, Ofloxacin and Streptomycin**.** *Proteus mirabilis* GE5 was resistant to all antibiotics except Chloramphenicol, Gentamycin and Ofloxacin. *Salmonella ser paratyphi B* GE6 was resistant to all antibiotics except Cotrimoxazole, Chloramphenicol, Gentamycin, Ofloxacin and Streptomycin. *Serratia fonticola* GE7 was resistant to all antibiotics except Gentamycin. *Proteus mirabilis* GE8 was resistant to all antibiotics except Cotrimoxazole. *Enterobacter aerogenes* GE9 was resistant to all antibiotics except Chloramphenicol and Ofloxacin. *Enterobacter aerogenes* GE10 showed resistant to all antibiotics except Ciprofloxacin, Gentamycin and Ofloxacin. *Salmonella ser paratyphi B* GE11 was resistant to all antibiotics except Gentamycin, Ofloxacin and Streptomycin. *Enterobacter aerogenes* GE12 was resistant to all tested antibiotics except Cotrimoxazole, Chloramphenicol, Gentamycin, Augmentin, Ofloxacin and Streptomycin. *Enterobacter kobei* GE13 showed resistance to all antibiotics except Cotrimoxazole, Chloramphenicol, Gentamycin, Ofloxacin and Streptomycin. *Enterobacter kobei* GE14 was resistant to all antibiotics except Cotrimoxazole, Ofloxacin and Streptomycin. *Sphingomonas paucimobilis* GE15 showed resistance to all antibiotics except Cotrimoxazole, Gentamycin, Ofloxacin and Streptomycin. *Acinetobacter baumannii* GE16 was resistant to all antibiotics except Cotrimoxazole, Chloramphenicol, Gentamycin, Ofloxacin and Streptomycin. *Enterobacter aerogenes* GE17 was resistant to all antibiotics except Cotrimoxazle, Chloramphenicol, Ofloxacin and Streptomycin. *Burkholderia cepacia* GE18 was resistant to all antibiotics except Gentamycin, Ofloxacin and Streptomycin. *Serratia fonticola* GE19 showed resistance to antibiotics to all antibiotics except Chloramphenicol, Gentamycin and Ofloxacin. *Enterobacter aerogenes* GE20 was resistant to all antibiotics except Gentamycin and Ofloxacin (Table 5).

Table 5: Sensitivity of Enterobacteriaceae against Antibiotics using CLSI (Clinical and Laboratory Standard Institute) Break points

Bacterial species	**[CXT]**	**[CH]**	**[CPX]**	**[AU]**	**[CN]**	**PEF]**	**[OFX]**	**[S]**
Proteus mirabilis GE1	R	R	R	R	S	R	S	R
Raoultella ornithinolytica GE2	R	S	R	R	S	R	S	R
Enterobacter aerogenes GE3	R	R	R	R	R	R	R	R
Enterobacter aerogenes GE4	S	S	R	R	R	R	S	S
Proteus mirabilis GE5	R	S	R	R	S	R	S	R
Salmonella ser paratyphi B GE6	S	S	R	R	S	R	S	S
Serratia fonticola GE7	R	R	R	R	S	R	R	R
Proteus mirabilis GE8	S	R	R	R	R	R	R	R
Enterobacter aerogenes GE9	R	S	R	R	R	R	S	R
Enterobacter aerogenes GE10	R	S	R	R	S	R	S	R
Salmonella ser paratyphi B GE11	R	R	R	R	S	R	S	S
Enterobacter aerogenes GE12	S	S	R	S	S	R	S	S
Enterobacter kobei GE13	S	S	R	R	S	R	S	S
Enterobacter kobei GE14	S	R	R	R	R	R	S	S
Sphingomonas paucimobilis GE15	S	R	R	R	S	R	S	S
Acinetobacter baumannii GE16	S	S	R	R	S	R	S	S
Enterobacter aerogenes GE17	S	S	R	R	R	R	S	S
Burkholderia cepacia GE18	R	R	R	R	S	R	S	S
Serratia fonticola GE19	R	S	R	R	S	R	S	R
Enterobacter aerogenes GE20	R	R	R	R	S	R	S	R

KEY: *CXT= Cotrimoxazole, CH= Chloranphenicol, CPX= Ciprofloxacin, AU= Augmentin, CN= Gentamycin, PEF= Pefloxacin, OFX= Ofloxacin, S= Streptomycin, R= Resistant, S= Susceptible*

Frequency of Enterobacteriaceae to Antibiotics sensitivity: Figure 2 shows the total number of Enterobacteriaceae that are sensitive (susceptible or resistance) to Antibiotics. From the result presented in figure 3, Cotrimoxazole (CXT) was resistant to eleven (11) Enterobacteriaceae but susceptible to only nine

(9). Chloramphenicol (CH) was resistant to nine (9) Enterobacteriaceae but susceptible to eleven (11). Ciprofloxacin (CPX) was resistant to all the twenty (20) Enterobacteriaceae. Augmentin (AU) was resistant to nineteen (19) Enterobacteriaceae but susceptible to only one. Gentamycin (CN) was resistant to six (6) Enterobacteriaceae but susceptible to the other fourteen (14). Pefloxacin (PEF) was resistant to all the twenty (20) Enterobacteriaceae. Ofloxacin (OFX) was resistant to three (3) Enterobacteriaceae but susceptible to the other seventeen (17). Streptomycin (S) was resistant to ten (10) Enterobacteriaceae but susceptible to the other ten (10).

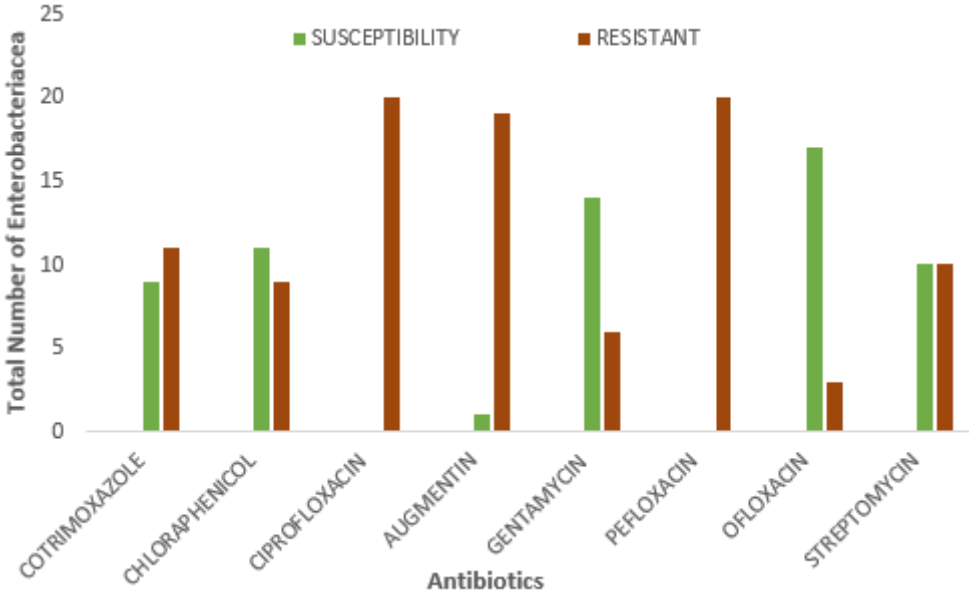

Fig 3: Frequency of Enterobacteriaceae to Antibiotics sensitivity

Geckos (*Hemidactylus frenatus*) are potential reservoirs of enteropathogens and zoonotic important bacteria. In this study, twenty (20) enteric bacteria of nine (9) different species namely *Proteus mirabilis, Raoultella ornithinolytica, Enterobacter aerogenes, Salmonella ser paratyphi B, Serratia fonticola, Enterobacter kobei, Sphingomonas paucimobilis, Acinetobacter baumannii*, and *Burkholderia cepacia* were isolated and identified. Similar bacteria genus had been identified in previous studies involving bacteria associated with Geckos. Singh *et al.* (2013), Nwachukwu *et al.* (2014), Noor *et al.* (2017) and Morrison and Rubin (2020) in their separate studies reported *Salmonella* and *Proteus* to be present in the faecal droppings of Geckos. *Enterobacter* had also been isolated and identified to be associated with Geckos (Singh *et al.*, 2013; Nwachukwu *et al.*, 2014, Casey *et al.*, 2014). Noor *et al.* (2017) and Casey *et al.* (2014) have both reported *Serratia* as a major bacteria harbored by Geckos while Singh *et al.* (2013) had reported *Raoultella*. However, *Sphingomonas, Acinetobacter* and *Burkholderia* isolated in this study have not been previously reported to be associated with Geckos. These Enterobacteriaceae are potential threats to humans. *Sphingomonas paucimobilis* and *Acinetobacter baumannii* have been reported as human pathogens (Howard *et al.*, 2012; Steinberg and Burd, 2015) and typically occur in immunocompromised individuals causing several infections including wound infections, meningitis, catheter-associated bacteremia, ventilator-associated pneumonia, splenic abscess etc (Martino *et al.*, 2010). Similarly, *Burkholderia cepacia* has been reported to be associated to patients who have certain health challenges such as weakened immune systems or chronic lung diseases (Martino *et al.*, 2010).

This study reported genus Enterobacter to have the highest frequency while each of *Raoultella ornithinolytica, Sphingomonas paucimobilis, Acinetobacter baumannii* and *Burkholderia cepacia* have the least frequency. This negates the work of Nwachukwu *et al.* (2014) and Noor *et al*. (2017) who reported Salmonella to have the highest frequency in their respective study on Geckos. *Enterobacter aerogenes and Enterobacter kobei* were recognized for their clinical significance as opportunistic bacteria and have emerged as nosocomial pathogens from intensive care patients (Mezzatesta *et al.*, 2012). In this study, All *Enterobacteriaceae* were resistant to more than one antibiotics evaluated. *Enterobacter aerogenes* GE3 in particular was resistant to all

antibiotics. Antibiotics resistance occurs when microorganisms develop means to defend themselves against the negative effects of specific antibiotics, hence preventing the antibiotics from effectively killing them (Puttaswamy *et al.,* 2018). The multidrug resistance of these *Enterobacteriaceae* are possibly through the development of resistance genes (either intrinsic or acquired) leading to the spread of resistance from one organism to the other (Leonard *et al.,* 2012). Ciprofloxacin (CPX) and Pefloxacin (PEF) have the highest resistance to *Enterobacteriaceae* while Ofloxacin (OFX) has the least resistance. This negates the work of Singh *et al.* (2013) and Casey *et al.* (2014) which showed that Cotrimoxazole (CXT) and Chloranphenicol (CH) have the highest resistance to bacterial species.

Conclusion: Geckos (*Hemidactylus frenatus*) has proven to be potential reservoirs and vectors of enteropathogens and zoonotic bacteria. *Enterobacteriaceae* isolated from this study were resistant to most of the commercially available antibiotics; hence, the need to prevent the contamination of our food and water sources by these Geckos as well as put in place control measures to eradicate them.

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