Journal of Applied Biology & Biotechnology Vol. 9(2), pp. 131-141, Mar-Apr, 2021
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2021.9212

Diversity and susceptibility pattern of medically important bacteria isolated from intestinal tract of *Hemidactylus frenatus* in Ilishan-Remo, Ogun State

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ARTICLE INFO

Article history:
Received on: September 24, 2020
Accepted on: January 06, 2021
Available online: March 10, 2021

Key words:
Antibiotics resistance,
Bacterial diversity,
Geckos,
Fecal droppings.

ABSTRACT

Hemidactylus frenatus (Wall gecko) is reptile of the family Geckkonidae commonly found around human and animal settings. There is a growing concern that this reptile could transmit pathogenic bacteria to humans and animals because of its proximity to the host environment. This study was designed to evaluate the diversity and susceptibility pattern of medically important bacteria obtained from house geckos in Ilishan-Remo. Wall geckos were caught from different households. Bacteria were isolated from intestinal contents and identified based on partial 16S rRNA gene amplicon sequencing. Bacterial sensitivity to selected antibiotic classes was determined by agar diffusion. A total of 163 bacteria were obtained from 51 wall geckos, consisting of five genera; *Hafnia* (46), *Klebsiella* (18), *Salmonella* (43), *Enterobacter* (49), and *Cedecea* (7). *Hafnia*, *Cedecea* and *Klebsiella* species (100%) isolated were resistant to ceftazidime, cefuroxime, ofloxacin, and amoxicillin/clavulanate, *Enterobacter* species (100%) were resistant to ceftazidime, cefuroxime, ofloxacin, amoxicillin/clavulanate, nitrofurantoin, and cefixime while all *Salmonella* species were resistant to cefuroxime and amoxicillin/clavulanate. The results revealed the presence of medically important bacteria in household geckos with high spectrum of resistance to different antibiotic classes. Consequently, contamination of foods and house hold utensils with fecal droppings of wall geckos could be a possible source of bacterial infection to humans.

1. INTRODUCTION

Microbial drug resistance is among the most important public health issues in recent time [1], but its implication in public health is not well emphasized in many countries [2]. Several pathogens of public health importance have developed ability to resist many drugs they were originally susceptible to [3]. This is as a result of the frequent use and in addition, abuse of antibiotics in community, hospitals, and relatedsettings [4]. Bacteria capable of resisting different antibiotics have been found in both hospital and community settings; thus, there is now no localization of reservoirs of drug resistance gene to a particular setting [5,6]. The natural driving forces responsible for the dissemination of bacteria with multi-resistance property world-wide include migrant birds, household animals, travellers, as well as movement of commercial food across the globe [7,8].

Geckos (*Geckonidae*) are reported as reservoirs of zoonotic bacteria and are considered an avenue for infection to humans through fecal contamination [8]. The zoonotic pathogens in geckos are mostly intestinal commensals such as non-typhoidal *salmonellae*, *Citrobacter freundii*, *Shigella sonnei*, *Serratia marcescens*, *Klebsiella pneumonia*, and *Escherichia coli* to mention a few. There are over 100 million cases of enteropathogenic illnesses reported globally each year with significant hospitalizations and mortalities [9]. Geckos constitute a great threat by the indiscriminate littering of human habitat with their droppings. Hence, the contamination of the environment and spread of medically important pathogens could be inevitable sources of infection to humans and animals. Besides, severe clinical problems associated with enteropathogenic bacteria may increase mortality rate especially in developing countries where there are limited therapeutic options for infected patients. In Nigeria only pocket of studies investigated isolation of bacteria in wall geckos [10-12] with no reference to their resistance patterns. Thus, there is a dearth in information on susceptibility pattern of gecko-associated pathogens in this region. This study was, therefore, design to investigate the diversity and antibiotic resistance pattern of medically important bacteria isolated from household gecko in Ilishan-Remo Ogun State.

2. MATERIALS AND METHODS

2.1. Study Areas

The sample collection points for this study were households in Ilishan-Remo. Ilishan-Remo is a small town located within Irepodun district

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in Ikenne Local Government Area, Ogun State. It is situated in South Western Nigeria within Latitude 6.8932 E and Longitude 3.7105 N.

2.2. Sample Collection and Bacterial Isolation

Wall geckos were caught from different households with nets and were sacrificed within glass jars by the help of chloroform as described elsewhere [13]. The samples were transported to the Department of Microbiology Laboratory, Babcock University, in clean polythene bags for further processing. The body surfaces of the geckos were sterilized with 70% alcohol and dissected to get the intestines. With the help of sterilized laboratory gadgets such as forceps and scissors, the intestines were aseptically obtained and transferred into test tubes containing nutrient broth (Oxoid, UK). The samples were homogenized and then incubated at 37°C for 24 h. Isolation was carried out as described elsewhere [13].

2.3. Antibiotic Susceptibility Test

The antibiogram was performed by agar diffusion and interpreted following criteria recommended by recognized body [14]. Antibiotics used were manufactured by Abtek, Biologicals Ltd which include ceftazidime (30 µg); cefuroxime (30 µg); gentamicin (10 µg); ofloxacin (5 µg); amoxicillin/clavulanate (30 µg); nitrofurantoin (30 µg); cefixime (5 µg); ciprofloxacin (5 µg); ceftriaxone (30 µg); erythromycin (5 µg); and cloxacillin (5 µg).

2.4. Molecular Characterization of Bacterial Isolates

Based on the preliminary tests (selective agar media, Gram staining, and biochemical tests), 12 bacteria were picked for sequencing. Bacterial DNA was extracted using Qick-DNA™ miniprep plus kit (Zymo research, Biolab, USA. Agarose electrophoresis was used to check the quality of the DNA before polymerase chain reaction. The bacterial 16S rRNA was amplified by PCR with forward primer 341: 5'-CCTACGGGAGGCAGCAG-3'and reverse primer R806 5'-GGACTACHVGGGTWTCTAAT-3'. Amplification reaction was achieved using GeneAmp PCR9700 system(Applied Biosystems) with the program set up: Initial denaturation at 94°C for 3 min, denaturation 94°C for 30 s, annealing 50°C for 30 s, extension 68°C for 30 s, and final extension 68°C for 5 min. The program was set for 30 cycles. The amplicons were sent to Inqaba Biotech (South Africa) for sequencing. The sequence reads trimmed and contigs assembled with help of BioEdit (version 7.2.5.0) [15]. Neighbor-Joining method was used to infer the evolutionary history [16]. Jukes-Cantor method was used to calculate the evolutionary distances [17]. Gaps and missing data were eliminated. MEGA6 was used for evolutionary analyses [18].

2.5. Data Analysis

Data (susceptibility) were analyzed descriptively using SPSS Statistics for Windows, Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp).

3. RESULTS AND DISCUSSION

3.1. Occurrence of Bacterial Isolates and Susceptibility Pattern

A total of 163 bacteria were obtained from 51 samples of *Hemidactylus frenatus.* Molecular characterization, indicated that the bacteria isolated were made up of 5 genera; *Hafnia, Klebsiella*, *Salmonella*, *Enterobacter,* and *Cedecea* [Table 1]. All the sequenced data have been deposited in GenBank under the accession numbers: MT271752-MT271763. *Enterobacter* species had the highest occurrence and closely, followed by *Hafnia* species. *Cedecea* species had the least occurrence. The phylogenetic relationship between the species identified and those in database is shown [Figure 1]. All the species identified fell within the same cluster with 99% identity except *Enterobacter hormaechei* subsp. *hormaechei* (73%).

The species isolated showed varying susceptibility patterns to the selected antibiotics. *Hafnia* species were resistant to ceftazidime, cefuroxime, ofloxacin, amoxicillin/clavulanate, nitrofurantoin, and cefixime with 65.2% (30) and 54.3% (25) of the organisms resistant to gentamicin and ciprofloxacin, respectively [Table 1]. *Klebsiella* species were not different in resistance pattern as they were resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, ofloxacin, and amoxicillin/clavulanate with half of the species resistant to gentamicin [Table 1]. The trend in resistance pattern of *Enterobacter* and *Cedecea* species was similar to other species described above. *Enterobacter* species isolated were resistant to ceftazidime, cefuroxime, ofloxacin, amoxicillin/clavulanate, nitrofurantoin, and cefixime. Likewise, *Cedecea* species were resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxicillin, ofloxacin, and amoxicillin/

Table 1: Species distribution and susceptibility pattern of bacteria obtained from intestinal track of wall geckos.

Antibiotics	Name of bacteria and % resistance to different antibiotics				
	***Hafnia* spp *n*=46**	***Klebsiella* spp *n*=18**	***Salmonella* spp *n*=43**	***Enterobacter* spp *n*=49**	***Cedecea* spp *n*=7**
CAZ (30 µg)	46 (100)	18 (100)	31 (72.1)	49 (100)	7 (100)
CRX (30 µg)	46 (100)	18 (100)	43 (100)	49 (100)	7 (100)
GEN (10 µg)	30 (65.2)	9 (50)	28 (65.1)	46 (93.9)	3 (42.9)
OFL (5 µg)	46 (100)	18 (100)	41 (95.3)	49 (100)	7 (100)
AUG (30 µg)	46 (100)	18 (100)	43 (100)	49 (100)	7 (100)
NIT (30 µg)	46(100)	0	38 (88.4)	49 (100)	0
CXM (5 µg)	46 (100)	0	35 (81.4)	49 (100)	0
CPR (5 µg)	25 (54.3)	0	13 (30.2)	6	0
CTR (30 µg)	0	18 (100)	0	0	7 (100)
ERY (5 µg)	0	18 (100)	0	0	7 (100)
CXC (5 µg)	0	14 (77.8)	0	0	7 (100)

n: Sample size, CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, OFL: Ofloxacin, AUG: Amoxicillin/clavulanate, NIT: Nitrofurantoin, CXM: Cefixime, CPR: Ciprofloxacin, CTR: Ceftriaxone, ERY: Erythromycin, CXC: Cloxicillin, 0: Not tested

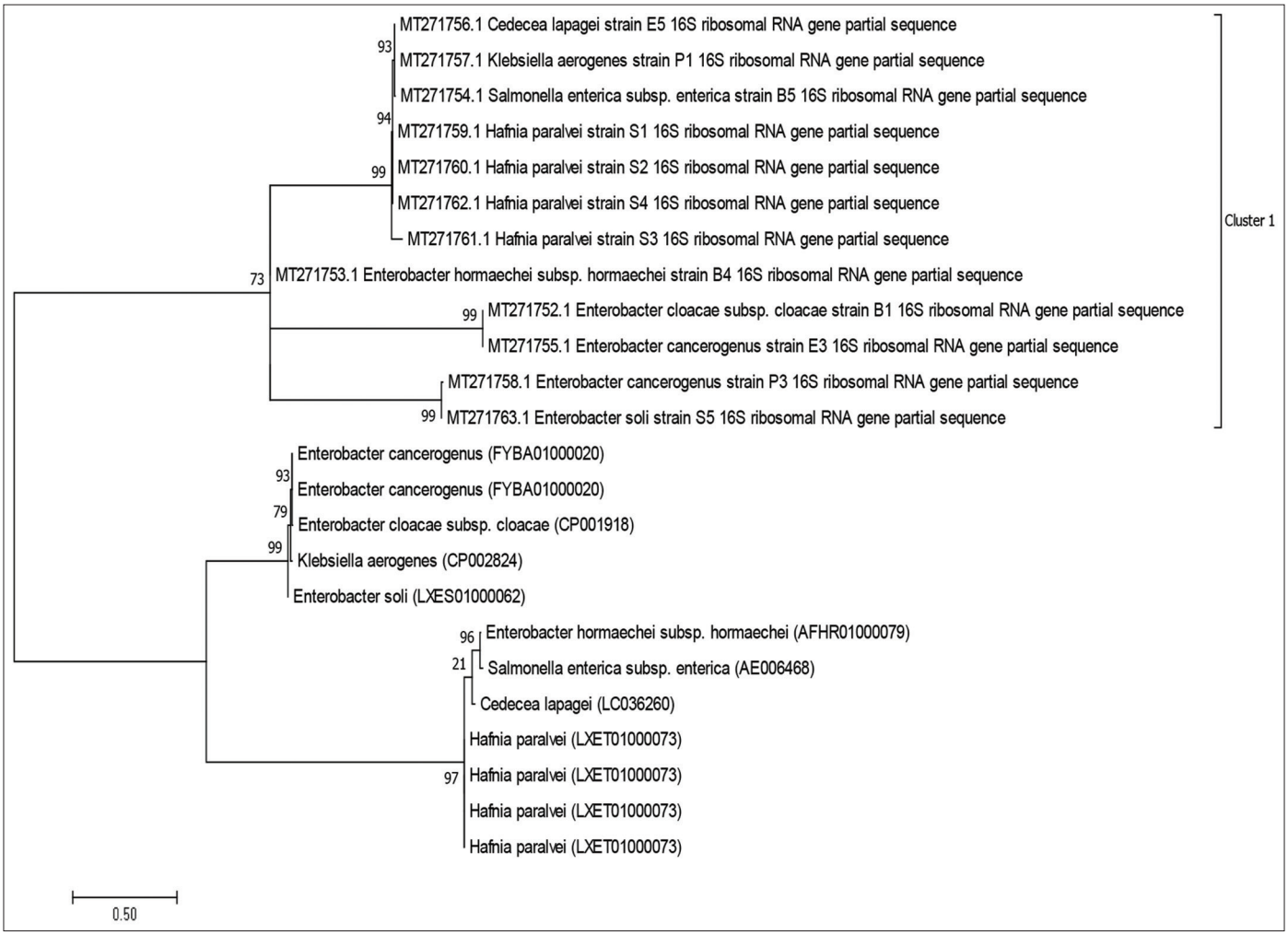

Figure 1: Phylogenetic relationship of the species identified with close relatives in database. The evolutionary history was inferred using the Neighbor-Joining method and distances were computed using the Jukes-Cantor method. Cluster 1: *Cedecea lapagei* strain E5, *Klebsiella aerogenes* strain P1and *Salmonella enterica* subsp. *enterica* strain B5 had 93% identity, but were 94% close to *Hafnia paralvei* strain S1, *H. paralvei* strain S2, *H. paralvei* strain S3, and *H. paralvei* strain S4 (99%). Close family: *Enterobacter hormaechei* subsp. *hormaechei* strain B4, *E. cloacae* subsp. *cloacae* strain B1, *Enterobacter cancerogenus* strain E3, *E. cancerogenus* strain P3 and *E. soli* strain S5 were 73% identical to the aforementioned species.

clavulanate. More than 50% of *Salmonella* species were resistant to seven antibiotics tested [Appendix 1-5].

The family Enterobacteriaceae is predominantly diverse within the intestinal tract of geckos. The phylogenetic tree revealed that all the species obtained were within this family with close species having 99% identity, thus explaining the reason for one cluster group obtained. A review by Janda and Abbott [19] suggested that 16S rRNA gene sequencing provides genus identification in most cases (>90%) but less so with regard to species (65 to 83%). A more stringent boundary for species delineation was proposed to increase the accuracy of identification [20]. Pairwise nucleotide similarity values for the species in this study were within the acceptable range for species identification, thus, stressing the importance of sequencing method in microbial taxonomy.

Studies on geckos in indicated that they are reservoirs for bacteria such as *Salmonella*, *Citrobacter, Erwinia, Shigella, Edwardsilla, Enterobacter, Serratia, Proteus, Klebsiella,* and *Escherichia coli* [11,21]. This study has indeed strengthened the fact that geckos pose serious risk to human population especially the rural dwellers. The isolation of pathogenic bacteria in this study (*Hafnia* species*, Klebsiella* species, *Cedecea* species, *Salmonella* species, and *Enterobacter* species) was consistent with previously studies on geckos and closely related species [21,22]. Food contamination due to non-typhoidal *Salmonella* is a significant reason for both irregular gastroenteritis and epidemic universally. In sub-Saharan Africa, community-acquired infection due to *Salmonella enterica*, *Salmonella Typhi,* and non-typhoidal *Salmonella* has been reported [23]. *Salmonella* causes intestinal-associated sicknesses such as diarrhea and vomiting with graded fever as well as life-threatening septicemia [24]. The infection is severe especially in elderly, children, and immunocompromised patients. The species of *Salmonella* relevant to human infections are the *S. enterica*, (*S. ser*. *Typhimurium* and *S. ser. Enteritidis*).

Antimicrobial drug resistance in *Salmonella* infections has been a growing concern in the treatment and management of patients. In this study, *Salmonella* species were multidrug-resistant (>50%) and this was comparable to 76% report by Elkenany *et al*. [25]. According to EU summary report on antimicrobial resistance, *Salmonella* species were among the multidrug-resistant zoonotic pathogens [26]. The importance of *Salmonella* infections cannot be overemphasized.

Salmonella infection was estimated to be 185 diseases per 100,000 populace for each year in Australia [27]. Specifically, *S. enterica* infection results in 450 death with an estimate of 24, 000 hospitalization annually within United States [28].

The isolation of medically important bacteria from geckos such a *Hafnia* species is of great concern. For instance, *Hafnia alvei* has been implicated as cause of various diseases such as bacteremia, pneumonia, gastroenteritis, meningitis, nosocomial wound infections, endophthalmitis, and a buttock abscess [29]. Diarrhea-associated *H. alvei* has been reported to cause acute gastroenteritis in children [30,31]. Acute gastroenteritis and diarrhea were reported in 16% of Finnish tourists who visited Morocco [32].

The multi-drug resistance observed among the *H. alvei* in this study may complicate patients' recovery leading to high mortality in the event of infection. However, previous report showed that this species were highly susceptible to most antibiotics [29]. The cause of the disparity in susceptibility pattern of this species in this study compared to the former is unknown. However, it might not be unconnected with increasing antibiotic pressure that is prevalent in the environment in recent times. It is can also be postulated that gene exchange might have been occurring among the intestinal microbiota in the host. Besides, geographical location, differential power of techniques, batches of antibiotics used and strains encountered might have contributed significantly to this variation.

Wall geckos are "innocent" in appearance, but "guilty" as they are reservoirs of zoonotic pathogens such as *Enterobacter*, *Klebsiella,* and *Cedecea* species. *Enterobacter* species causes serious infection in patients, particularly to individuals on mechanical ventilation [32]. Two species: *E. hormaechei* and *Enterobacter cloacae* are most encountered in human infections. *E. cloacae* was reported as the most widely recognized *Enterobacter* species causing nosocomial diseases and scores of data regarding their resistance to antibiotics have been highlighted [33]. The pathogenic instruments contributing the sickness related to *E. cloacae* are unclear. However, its capacity to form biofilms and to produce different cytotoxins is significant for its virulence potentials. While this species remain as commensal microflora in the intestinal tracts of living creatures and pathogenic in plants and creepy crawlies, notable nosocomial infections (bacteremia, endocarditis, septic joint pain, osteomyelitis, and skin/delicate tissue diseases), as well as lower respiratory tract-urinary tract and intra-abdominal infections have been reported [34]. *E. cloacae* in general taints different medical gadgets [35,36] and there has been its recurrent in neonatal units with a few episodes of disease reported [37,38]. The two species of *Enterobacter* reported in this study have been associated with infections. *E. hormaechei,* for example, is commonly considered a causative pathogen for human infection and it does not usually cause diseases in animals. However, it was first found to be associated with respiratory disease in unweaned calves in China as well as respiratory and blood stream infections among premature infants in intensive care nursery at the Hospital of the University of Pennsylvania [39,40].

Among the species reported in this study, *Cedecea* has less prevalence. However, it is an opportunistic pathogen commonly isolated from immunocompromised patients and has been linked to infections such as bacteremia, scrotal abscess, chronic renal, and heart diseases, pneumonia [41-45]. The first case fatality due to *Cedecea lapagei* infection was reported in a 52-year-old Mexican who developed septic shock and multiple organ failure [46].

Klebsiella species are universally widespread in nature and presumably have two regular natural surroundings (surface water, sewage, soil, and plants) and the mucosal surfaces of animals (horses, humans, and swine), for habitation [47]. Nosocomial infections associated with *Klebsiella* species include chronic pulmonary obstruction, neonatal sepsis, urinary tract infections, septicemia, and wound infections [47,48]. In this study, nearly all the *Klebsiella* species were resistant to antibiotics used. The previous cases of *Klebsiella aerogenes* in different hospitals were reported to be multidrug-resistant strains [49,50] and studies indicated that its resistance against carbapenem involves over-expression of AmpC or ESBLs enzymes together with mutations that disrupt membrane permeability [33].

4. CONCLUSION

This study revealed that household geckos harbor pathogenic bacteria with spectrum of resistance to different antibiotic. Hence, exposure to fecal droppings of wall geckos could be a possible source of bacterial infection that may be difficult to treat. This study recommends that general public should maintain high level of domestic hygiene to avoid contamination of food and food stuffs with fecal droppings of wall geckos.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

6. FUNDING

There is no funding to report.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

Not applicable.

9. PUBLISHER'S NOTE

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How to cite this article:
Ogbodogbo OF, Ezeamagu CO, Barns JN. Diversity and susceptibility pattern of medically important bacteria isolated from intestinal tract of *Hemidactylus frenatus* in Ilishan-Remo, Ogun State. J App Biol Biotech. 2021;9(2):131-141. DOI: 10.7324/JABB.2021.9212

APPENDIX I

Inhibition zone produced by *Hafnia* spp against selected antibiotics												
ORGANISM	**CAZ (30 µg)**	**CRX (30 µg)**	**GEN (10 µg)**	**CXM (5 µg)**	**OFL (5 µg)**	**AUG (30 µg)**	**NIT (30 µg)**	**CXM (5 µg)**	**CPR (5 µg)**	**CTR (30 µg)**	**ERY (5 µg)**	**CXC (5 µg)**
H1	8	-	16	-	8	-	7	2	17	0	0	0
H2	11	-	-	-	-	-	-	7	-	0	0	0
H3	13	-	-	-	-	-	5	-	5	0	0	0
H4	13	-	-	-	-	-	7	-	7	0	0	0
H5	14	-	-	-	-	-	-	2	-	0	0	0
H6	-	-	-	-	-	-	-	4	6	0	0	0
H7	14	5	-	-	-	-	-	7	-	0	0	0
H8	-	-	-	-	2	-	10	-	19	0	0	0
H9	12	-	18	-	5	-	-	8	17	0	0	0
H10	10	3	17	12	10	-	11	10	19	0	0	0
H11	14	-	-	-	9	-	-	7	17	0	0	0
H12	10	-	-	-	2	-	-	3	16	0	0	0
H13	9	-	.	-	10	-	-	4	16	0	0	0
H14	10	-	-	-	-	-	-	5	-	0	0	0
H15	-	-	-	-	2	-	-	-	17	0	0	0
H16	12	-	17	-	10	-	12	5	16	0	0	0
H17	11	8	-	-	5	-	9	3	17	0	0	0
H18	13	-	-	-	9	-	-	6	17	0	0	0
H19	13	-	17	-	7	-	10	5	13	0	0	0
H20	12	-	16	-	4	-	-	8	16	0	0	0
H21	-	-	-	-	-	-	5	-	18	0	0	0
H22	11	-	17	-	8	-	-	3	19	0	0	0
H23	12	-	-	-	-	-	-	7	-	0	0	0
H24	13	-	16	-	7	-	9	6	13	0	0	0
H25	-	-	-	-	3	-	-	-	-	0	0	0
H26	-	-	-	-	-	-	-	-	17	0	0	0
H27	10	-	-	-	2	-	-	4	19	0	0	0
H28	-	-	-	-	-	-	-	-	-	0	0	0
H29	9	-	17	-	5	-	12	3	16	0	0	0
H30	-	-	18	-	10	-	6	2	19	0	0	0
H31	12	-	12	-	7	-	-	6	17	0	0	0
H32	13	-	17	-	6	-	12	4	19	0	0	0
H33	9	4	-	-	-	-	-	2	13	0	0	0
H34	13	-	-	-	-	-	5	7	12	0	0	0
H35	10	-	-	-	-	-	-	2	17	0	0	0
H36	-	-	-	-	-	-	-		16	0	0	0
H37	-	-	-	-	-	-	-		-	0	0	0
H38	-	-	-	-	-	-	-		-	0	0	0
H39	-	-	18	-	10	-	-	-	18	0	0	0
H40	-	-	-	-	-	-	-	-	-	0	0	0
H41	-	-	19	-	5	-	8	-	17	0	0	0
H42	12	-	16	-	-	-	-	4	9	0	0	0
H43	-	-	-	-	-	-	-	-	-	0	0	0
H44	-	-	-	-	2	-	-	-	-	0	0	0
H45	12	-	16	-	-	-	10	7	10	0	0	0
H46	9	-	17	14	9	-	-	3	19	0	0	0

APPENDIX II

Inhibition zone produced by *Klebsiella* spp against Selected antibiotics											
ORGANISM	**CAZ (30 µg)**	**CRX (30 µg)**	**GEN (10 µg)**	**OFL (5 µg)**	**AUG (30 µg)**	**NIT (30 µg)**	**CXM (5 µg)**	**CPR (5 µg)**	**CTR (30 µg)**	**ERY (5 µg)**	**CXC (5 µg)**
K1	5	9	17	-	11	0	0	0	12	7	16
K2	-	-	-	-	-	0	0	0	-	-	-
K3	2	-	17	-	10	0	0	0	12	-	-
K4	-	-	-	-	-	0	0	0	-	-	-
K5	-	-	18	-	13	0	0	0	10	5	15
K6	-	-	-	-	-	0	0	0	-	-	-
K7	7	10	18	-	12	0	0	0	7	9	15
K8	-	-	10	-	-	0	0	0	-	-	-
K9	4	-	19	-	13	0	0	0	9	-	10
K10	-	-	17	-	9	0	0	0	-	-	8
K11	-	-	-	-	8	0	0	0	-	-	-
K12	-	-	-	-	-	0	0	0	-	-	-
K13	-	-	18	-	12	0	0	0	7	6	15
K14	-	-	-	-	-	0	0	0	-	-	-
K15	-	-	-	-	-	0	0	0	-	-	-
K16	-	-	17	-	12	0	0	0	6	10	11
K17	-	-	-	-	-	0	0	0	-	-	-
K18	7	6	17	-	13	0	0	0	7	9	13

APPENDIX III

Inhibition zone produced by *Salmonella* spp against selected antibiotics											
ORGANISM	**CAZ (30 µg)**	**CRX (30 µg)**	**GEN (10 µg)**	**OFL (5 µg)**	**AUG (30 µg)**	**NIT (30 µg)**	**CXM (5 µg)**	**CPR (5 µg)**	**CTR (30 µg)**	**ERY (5 µg)**	**CXC (5 µg)**
S1	20	13	13	-	-	17	18	17	0	0	0
S2	-	-	-	-	-	-	-	8	0	0	0
S3	11	-	18	13	11	-	19	18	0	0	0
S4	21	-	19	24	-	-	17	19	0	0	0
S5	20	13	18	12	-	-	19	17	0	0	0
S6	21	-	18	17	-	14	18	18	0	0	0
S7	12	-	-	-	-	-	10	17	0	0	0
S8	10	-	-	12	-	-	7	19	0	0	0
S9	22	12	19	-	-	-	19	17	0	0	0
S10	-	-	-	-	-	-	-	5	0	0	0
S11	-	-	-	-	-	-	-	7	0	0	0
S12	11	-	-	13	-	-	-	16	0	0	0
S13	-	-	-	-	-	-	-	-	0	0	0
S14	19	14	22	11	-	18	17	17	0	0	0
S15	22	-	20	12	-	-	19	18	0	0	0
S16	24	13	13	8	-	13	7	19	0	0	0
S17	11	-	-	-	-	-	-	16	0	0	0
S18	23	-	19	11	-	17	7	17	0	0	0
S19	13	-	-	13	-	-	10	19	0	0	0
S20	31	-	18	13	-	-	11	17	0	0	0
S21	-	-	-	-	-	-	-	-	0	0	0
S22	-	-	-	-	-	-	-	-	0	0	0
S23	11	-	-	-	-	-	-	16	0	0	0
S24	-	-	14	10	-	13	10	18	0	0	0
S25	22	-	-	11	-	11	11	17	0	0	0
S26	20	-	18	14	-	-	7	-	0	0	0
S27	-	-	18	9	-	-	11	17	0	0	0
S28	8	-	19	-	-	-	-	19	0	0	0
S29	-	-	18	-	-	-	-	16	0	0	0
S30	-	-	17	13	-	19	7	16	0	0	0
S31	-	-	-	-	-	-	-	-	0	0	0
S32	-	-	-	-	-	-	-	-	0	0	0
S33	10	-	-	-	-	-	11	19	0	0	0
S34	9	-	-	-	-	-	6	17	0	0	0
S35	-	-	-	-	-	-	-	-	0	0	0
S36	12	-	-	-	-	-	3	16	0	0	0
S37	-	-	-	-	-	-	-	-	0	0	0
S38	-	-	19	-	-	-	7	18	0	0	0
S39	-	-	-	-	-	-	-	-	0	0	0
S40	-	-	-	-	-	-	-	-	0	0	0
S41	-	-	13	-	-	-	-	17	0	0	0
S42	13	-	-	-	-	-	-	17	0	0	0
S43	-	-	12	10	-	18	8	19	0	0	0

APPENDIX IV

Inhibition zone produced by *Enterobacter* spp against selected antibiotics											
ORGANISM	**CAZ (30 µg)**	**CRX (30 µg)**	**GEN (10 µg)**	**OFL (5 µg)**	**AUG (30 µg)**	**NIT (30 µg)**	**CXM (5 µg)**	**CPR (5 µg)**	**CTR (30 µg)**	**ERY (5 µg)**	**CXC (5 µg)**
E1	-	-	-	-	-	-	-	7	0	0	0
E2	-	-	-	-	-	-	-	2	0	0	0
E3	-	-	-	-	-	-	-	16	0	0	0
E4	11	2	18	11	-	-	2	19	0	0	0
E5	-	-	-	2	-	-	-	17	0	0	0
E6	-		-	-	-	-	-	15	0	0	0
E7	-	-	-	7	-	-	-	18	0	0	0
E8	-	-	-	-	-	-	-	17	0	0	0
E9	-	-	-	3	-	-	-	16	0	0	0
E10	-	-	-	-	-	-	-	10	0	0	0
E11	-	-	-	-	-	-	-	16	0	0	0
E12	-	-	-	7	-	-	-	16	0	0	0
E13	-	-	-	11	-	-	-	17	0	0	0
E14	-	-	-	8	-	-	-	19	0	0	0
E15	-	-	-	-	-	-	-	11	0	0	0
E16	11	5	17	12	-	-	5	19	0	0	0
E17	-	-	-	-	-	-	-	10	0	0	0
E18	-	-	-	10	-	12	-	17	0	0	0
E19	-	-	-	7	-	-	-	19	0	0	0
E20	-	-	-	-	-	-	-	-	0	0	0
E21	-	-	-	11	-	-	-	17	0	0	0
E22	-	-	-	2	-	-	-	-	0	0	0
E23	-	-	-	10	-	-	4	18	0	0	0
E24	-	-	-	3	-	-	-	17	0	0	0
E25	-	-	-	-	-	-	-	16	0	0	0
E26	12	7	-	10	-	-	7	19	0	0	0
E27	-	-	-	-	-	-	-	16	0	0	0
E28	-	-	-	10	-	-	-	17	0	0	0
E29	-	-	-	-	-	-	-	16	0	0	0
E30	-	-	11	11	-	-	-	19	0	0	0
E31	-	-	-	10	-	-	-	17	0	0	0
E32	-	-	-	11	-	-	-	17	0	0	0
E33	-	-	-	-	-	-	-	18	0	0	0
E34	-	-	-	-	-	-	-	16	0	0	0
E35	-	-	10	10	-	-	-	19	0	0	0
E36	-	-	-	-	-	-	-	17	0	0	0
E37	-	-	-	8	-	-	-	17	0	0	0
E38	-	-	-	6	-	-	-	20	0	0	0
E39	-	-	-	-	-	-	-	18	0	0	0
E40	-	-	-	-	-	-	-	17	0	0	0
E41	-	-	-	-	-	-	-	16	0	0	0
E42	-	-	-	13	-	-	-	17	0	0	0
E43	-	-	-	10	-	-	-	18	0	0	0
E44	12	2	-	11	-	-	-	17	0	0	0
E45	-	-	-	-	-	-	-	19	0	0	0
E46	-	-	-	9	-	-	-	17	0	0	0
E47	-	-	17	10	-	-	-	17	0	0	0
E48	-	-	-	-	-	-	-	17	0	0	0
E49	-	-	11	9	-	-	7	19	0	0	0

APPENDIX V

Inhibition zone produced by *Cedecea* spp against selected antibiotics											
ORGANISM	**CAZ (30 µg)**	**CRX (30 µg)**	**GEN (10 µg)**	**OFL (5 µg)**	**AUG (30 µg)**	**NIT (30 µg)**	**CXM (5 µg)**	**CPR (5 µg)**	**CTR (30 µg)**	**ERY (5 µg)**	**CXC (5 µg)**
C1	-	-	17	7	-	0	0	0	-	2	-
C2	-	-	19	-	-	0	0	0	13	8	-
C3	-	-	-	9	-	0	0	0	9	10	-
C4	-	-	-	-	-	0	0	0	5	-	-
C5	-	-	12	-	-	0	0	0	11	6	-
C6	-	-	17	1o	-	0	0	0	7	-	-
C7	-	-	19	7	-	0	0	0	9	-	-