ARTICLE / INVESTIGACIÓN

Molecular study of *Enterobacteriaceae bacterium* isolated from dishwashers

Aisha W. Al-Omari*, Ikhlas Ramadan Matter, Alaa Hussein Almola

DOI. 10.21931/RB/2022.07.03.28

Department of Biology, College of Sciences, University of Mosul, Mosul, Iraq.
Corresponding author: shsbio124@uomosul.edu.iq

Abstract: The *Enterobacteriaceae* family is considered one of the medically essential families in which there is a continuous change in classification, and new species are added to it. In this study, we obtained a novel strain registered in the NCBI under *Enterobacteriaceae bacterium* strain PRL 4-2, with the accession number MW540823, and the isolate considered unclassified *Enterobacteriaceae*. The isolate was obtained from dishwashers, which is a mine for many new species and strains due to the unique environmental conditions of this habitat, fluctuation in temperature, use of high temperatures, utilization of cleaning materials as well as humidity and the presence of organic materials, especially in case of neglect of cleaning. The isolate was characterized by its slow growth on culture media and its ability to form biofilms and possess some virulence factors. Its resistance to antibiotics was also studied, as it showed resistance to the antibiotics used in this study. The oddness for this strain is that it showed a different diagnosis when using VITEK (The VITEK 2 system has everything healthcare laboratories need for fast, accurate microbial identification and antibiotic susceptibility testing.) compared to diagnosis using 16S rRNA. As for the taxonomic tree, the closest species was *Enterobacter sp.* Strain 188. This study supplements a few other studies of this novel species, isolated from different environments. These researches can be integrated to give an itinerary for other studies on the new species and their different capabilities.

Key words: *Enterobacteriaceae bacterium*, dishwasher, *Enterobacteriaceae bacterium* PRL-2, *Enterobacter sp.*, biofilm.

Introduction

The *Enterobacteriaceae* family is the most extensive and diverse group of Gram-negative bacteria with medicinal significance. Biochemical features, antigenic construction, DNA-DNA hybridization, and 16S rRNA sequencing were used to classify the genera in this family. They are also widely distributed in nature and can be found in water, soil, plants, and the natural microbiota of human and animal intestinal tracts. They are grown aerobically or anaerobically, considered glucose fermenter and many other carbohydrates, convert nitrate to nitrite, positive to catalase, negative to oxidase, and their DNA hold guanine-cytosine (GC) 39% - 59%. The majority of the genus and species in this family are straight Gram-negative and of modest size (0.3 to 1.0x 1.0 to 6,0 m), participate in a well-known antigen, do not create spores, are mobile with 5 pivot flagella in one pivot or lack flagella, and grow on peptone or extract of meat adding sodium chloride or without adding it, and also grow on MacConkey agar. The medical and microbiological importance comes from The deployment of infections, pathogenicity and the abundance of bacteria with multi-resistant to antibiotics employed in therapy[1].

Genetic information technology is used to identify many opportunistic pathogens; most of the opportunistic pathogens may belong to the Enterobacteriaceae[2-6]. *Enterobacteriaceae* family taxonomy is complex, where 28 genera and about 75 species were found in these families, continuously changing with the addition of a novel species[7,8]. Many novel species belonging to the Enterobacteriaceae family were unclassified and undergoing unclassified *Enterobacteriaceae* (miscellaneous), one of these unclassified species *Enterobacteriaceae bacterium*[9].

In 2009, Enterobacteriaceae bacterium "strain FGI 57" was identified in Panama on Pipeline Route from a fungal garden of leaf-cutter ant the Atta colombica. The Leaf-cutter ants are common herbivores in the Neotropics, feeding on plant biomass by cultivating specialized fungal gardens on foliar debris. Instead of feeding directly on the plant material, they are using it as fertilizer to grow specialized garden fungus dominated by the obligate symbiont fungal *Leucoagaricus gongylophorus*. Recent research has revealed that the members within the *Enterobacteriaceae* family and the genus Pseudomonas live in fungus gardens, and metaproteomic studies in those environments have documented proteins of many strains FGI 57, implying that This bacterium is a vital part of the fungal garden's ecosystem[10-12]. In a study on a patient with infection in the biliary, a novel isolate of undetermined taxonomy was found together with the pathogenic *Klebsiella pneumoniae* strain named AF1813. In another study, this species *Enterobacteriaceae bacterium* ENNIH2 isolates from hospital sewage[14].

In our study, a novel species of *Enterobacteriaceae* isolated from an extreme environment with distinct characteristic MW540823.which show differences between biochemical and genetic identification technique. So we focused in this study on some properties of MW540823, such as the ability of biofilm formation, some virulence factors and antibiotic sensitivity.

Citation: A W. Al-Omari, I Ramadan Matter, A Hussein Almola. Molecular study of Enterobacteriaceae bacterium isolated from dishwashers. Revis Bionatura 2022;7(3) 28. http://dx.doi.org/10.21931/RB/2022.07.03.28
Received: 25 January 2022 / **Accepted:** 9 March 2022 / **Published:** 15 Agoust 2022

Materials and methods

The isolation of the *Enterobacteriaceae* bacterium

The isolate was obtained from dishwashers. Swabs were made from multiple areas of dishwashers, then the isolates were cultured on several media (McConkey agar, nutrient agar, EMB agar, blood agar), and the isolates were constantly purified until obtaining pure isolates. Depending on their growth and the shape of colonies on different media, we made smears on the glass slides to determine the shape and types of cells, whether they are gram-negative or gram-positive. To get more information about a unique isolate under study, we used the VITEK 2 system using GN cards to identify Gram-negative bacteria and then a Certain isolate was chosen to diagnose it using 16S rRNA.

16S rRNA sequencing

Total DNA of Enterobacteriaceae bacterium extracted depending on the manufacturer's instruction (intron biotechnology) of the G-spin DNA extraction kit. DNA was detected by agarose electrophoresis. PCR reaction employed in 25 µl final volume as in table 1 and the condition of reaction of 16S rRNA sequencing as in table2. Homologies were done by search in the National Center Biotechnology Information (NCBI) program, and the isolate of *Enterobacteriaceae* bacterium was recorded in NCBI with accession number MW540823.

Bioinformatics of phylogenetic analysis

The partial 16S rRNA sequences for MW540823 isolates were compared with others found in the online NCBI Blast database. The fasta of isolates of higher similarity with MW540823 were selected and downloaded for building a phylogenetic tree. This is done by MEGA 6 program.

The ability of the Enterobacteriaceae bacterium to form biofilm by tube method

Enterobacteriaceae bacteria can form biofilms, hemolysis blood, motility, produce enzymes, and form calcite and antibiotic resistance.

The method of researcher Hassan and his group[15] was adopted to test the ability of Enterobacteriaceae bacterium to form biofilms. The isolate was inoculated on a nutrient medium to activate it and incubated aerobically for 24 hours and 37 ◦C. After the incubation period ended, a group of colonies was transferred to glass test tubes containing tryptic soy broth medium with 1% sterilized glucose added and incubated aerobically for 24 hours and 37 °C.

The tubes used in the experiment are washed with a concentrated saline solution to sterilize them. Samples are poured inside them, and all this is done after the incubation period of the microbe. Then, it is dried and dyed with crystal violet dye at a concentration of 1% for three minutes. The excess dye was poured out and washed with deionized water. Then the tubes were left Dry upside down to notice the formation of biofilms on the inner walls and bottom of the tubes in the form of a violet layer.

The ability of the Enterobacteriaceae bacterium to hemolysis the blood

The blood samples preserved in hospitals, taken from donors earlier, were collected sterilely and in thermal conditions between 2 and 8 degrees Celsius, after which the blood agar medium was prepared. The isolate was activated for 24 hours in a liquid nutrient medium; then, the bacteria were cultured on a blood agar medium to test its ability to hemolysis blood[16].

Surface Motility

A single colony was cultured in LB broth overnight at 37

Material	Volume
Tag PCR Pre Mix	5 µl
Forward primer (5'-AGAGTTTGATCCTGGCTCAG-3')	1 µl
Reverse primer (5'-GGTTACCTTGTTACGACTT-3')	1 µl
Total DNA	1.5 µl
Distill water	16.5 µl

Table 1. Reaction of PCR.

Stage	Cycles	Temperature	Time
Initial denaturation	1	95◦C	5 min
Second denaturation	35	95 ◦C	45 sec
Annealing	35	58◦C	45 sec
Initial extension	35	72◦C	45 sec
Final extension	1	72◦C	7min

Table 2. Condition of 16S rRNA sequencing reaction.

Celsius to detect surface movement. After that, the suspension's optical turbidity was adjusted to match the 0.5McFarland standard, resulting in a roughly 108 CFU/mL concentration. The bacterial suspension of 1µL equivalent to 105 CFU/mL was then inoculated onto a fresh plate of 0.3 % LB-Agar. After 14 hours of incubation at 37°C, the diameter of the diffuse circular shape was determined17.1.4.D. Lipase and lecithinase((which are essential enzymes for this microbial pathogen) are indicated production:

Both lipase and lecithinase are indicated by egg yolk agar. Lipolysis is visible on sold egg yolk media by the creation of a thin glossy (pearly sheet) covering the colonies and a restricted opalescence in the media underneath them, which is best seen after the colonies are scraped .broad areas of opalescence surrounding colonies indicate lecithinase, which is quite intense, more significant than the areas formed by lipolysis. The media of this test consist of Nutrient agar 85 ml, Egg _yolk suspension 15ml. Melt the nutrient agar, cool it to 55°C, and the egg yolk is added to the cooled agar.

The method used to detect the production of lipase and lecithinase by incubating and looking for large areas of opalescence, which indicate lecithinase, and an iridescent film, which indicates lipolysis. Soak the plate first with an aqueous solution of saturated copper sulfate, let it sit for 20 minutes, drain the remaining fluid, then let the plate dry in the incubator for a few minutes. Lipolysis is confirmed by the greenish-blue color of copper washes of fatty acids[18].

Antibiotic sensitivity by Agar disc diffusion method

The agar disc diffusion method, which was first devised in 1940[19], is one of the earliest methods for standard susceptibility testing and, in microbiology, clinical labs considered one of the most widely used techniques for testing the antimicrobial susceptibility[20].

The key benefits include accessibility, repeatability, ease of altering antimicrobial disks, the ability to use as a screening method against a wide range of isolates, minimal cost and many more benefit[21]. A standardized inoculum of the test microorganism is inoculated onto Mueller–Hinton agar plates (90 mm diameter) (equivalent to turbidity at 05 McFarland). On the inoculated agar surface, up to 12 commerce-prepared folious disks (diameter about 6 mm) with suitable doses of the tested substance are arranged. Agar dishes are incubated at 35–37°C for 16–24 hours, depending on the circumstances[22,23]. The diameter of the growth clear zone around every antibiotic disk is then calculated in millimeters (measuring unit), with the disc diameter also included. This is done manually with a ruler or gliding caliper placed on the verso of the reversed agar plate[24].

In this research we used the agar disc diffusion method to study the antibiotic susceptibility, we choose cefixime (CFM 5mcg),pencillin G(P10u),gentamicin(CN 10mcg),vancomycin (VA30 mcg),ceftazidime(CAZ30 mcg),cephalexin(CL30 mcg).

Calcite formation

The ability of bacteria to calcite formation was studied using Luria Bertani acetate medium, consisting of Trypton 1 gm, Yeast extract 0.5 gm, NaCl 0.05 gm, Calcium acetate 1gm, Agar 1.5 gm, D.w. 100 cm3 PH 8, and the medium was sterilized using an autoclave. Then The crystals were chemically examined by adding a few drops of a 10% HCL solution to the calcifications on the plate to observe the formation of bubbles[25,26].

Results

Identification of some characteristics of *Enterobacteriaceae bacterium*

The Colonies of this bacterium appeared on the nutrient agar in a light white color, while on the Macconkey agar, the colonies appeared in a light pink mucoid colony, whereas on the blood agar, its colonies also appeared in white, and it needed a growth period of 48 hours sometimes, and at a temperature of 37 ◦ C as shown in figure (1: A,B,C,D).

The diagnosis of our strain using the colorimetric ID-GN VITEK2 cards system showed pseudomonas stutzeri) microbial pathogen(with a probability of 97% as shown in figure (2).

Result of 16S rRNA sequencing

Universal primers of 16S rRNA sequencing were used for analysis and identification of Enterobacteriaceae bacterium, the band on agarose gel was shown in figure (3).the partial sequence of 16S rRNA was compared with data of NCBI. The percentage of identification is 99.7%, representing standard isolates recorded in NCBI. The comparison result put the isolate to unclassified Enterobacteriaceae as Enterobacteriaceae bacterium with the data recorded in NCBI under accession number MW540823.

Phylogenetic tree analysis of *Enterobacteriaceae bacterium*

The phylogenetic tree of *Enterobacteriaceae bacterium* was built depending on the neighbor-joining relationship and the similarity between the isolate under study and other isolates of Enterobacteriaceae. MW540823 strain (*Enterobacteriaceae bacterium*) was related to MT900997 strain(*Enterobacter sp.* strain 188).

The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the base substitutions per site .this analysis involved 15 nucleotide sequences. The evolutionary tree was conducted in MEGA6. There were 1518 positions in the final dataset, as shown in figure (4).

Enterobacteriaceae bacterium ability to form biofilms, hemolysis blood, motility, produce enzymes, forming calcite and resistance to antibiotics

Our strain MW540823 shows the ability to form a biofilm; we notice the formation of biofilms on the inner walls and bottom of the tubes in the form of a violet layer figure(1: E). At the same time, the strain could not hemolysis the blood (expired human blood) on blood agar media. This bacterium's motility tests revealed a movement pattern in tiny forms with branching colonies figure(1: F).

As for lecithinase and lipase tests, this bacterium was positive for lecithinase and negative for lipase, so broad areas of opalescence surrounding colonies indicate lecithinase. While it showed its ability to form calcite when using Luria Bertani acetate medium figure(1: G), bubbles were formed when conducting a chemical examination of calcifications by adding 10% HCL.

The antibiotic susceptibility was done using the agar disk diffusion method; we chose cefixime (CFM 5mcg), penicillin G(P10u), gentamicin(CN 10mcg), vancomycin (VA30 mcg), ceftazidime(CAZ30 mcg), cephalexin(CL30 mcg), only clear zone of growth appeared around the gentamicin with a diameter of 2.2 cm and a radius of 0.9 cm. In contrast,

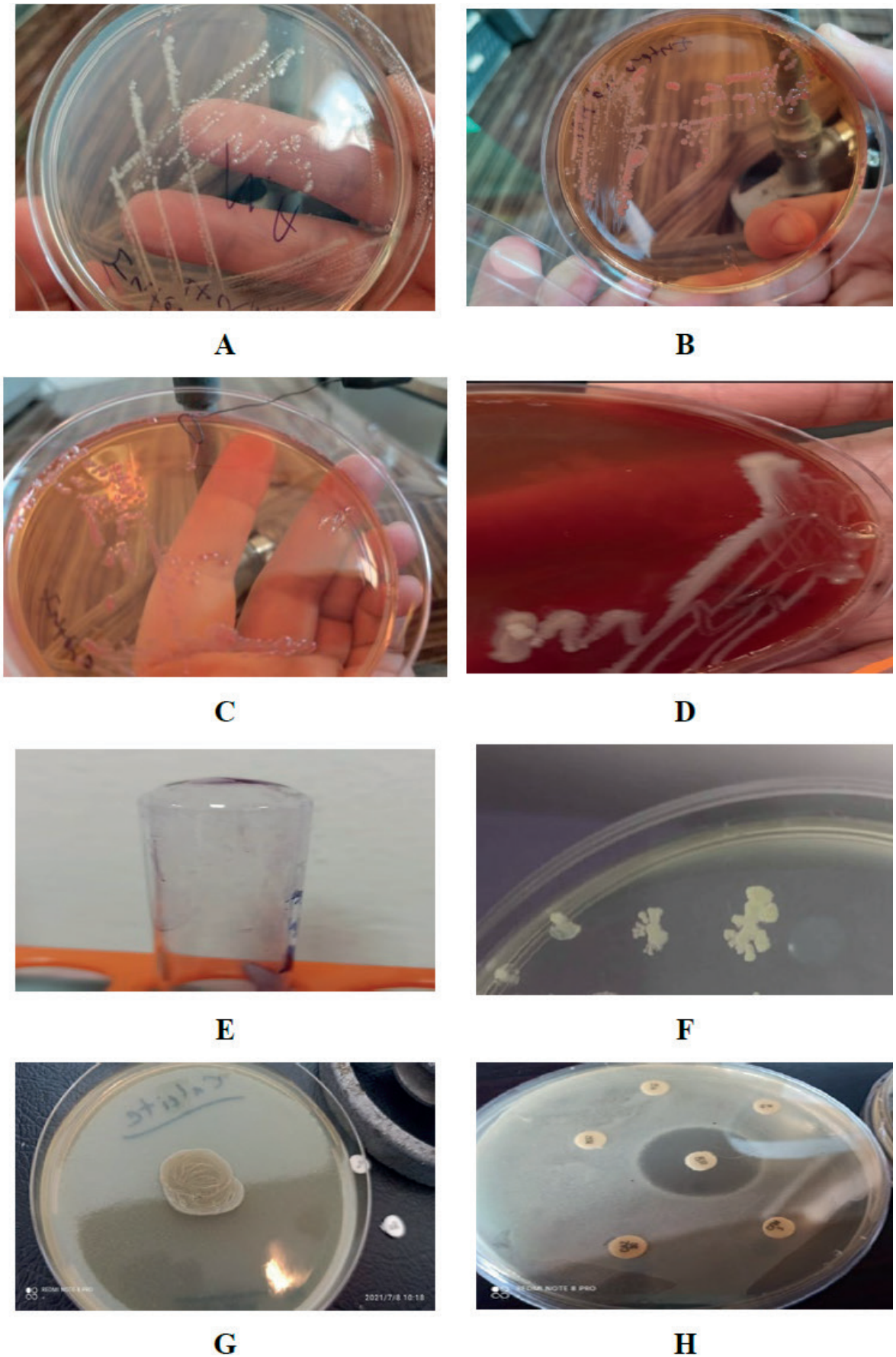

Figure 1. A: *Enterobacteriaceae bacterium* on nutrient agar, B: *Enterobacteriaceae bacterium* on Macconkey agar, C: mucoid colony on MacConkey agar, D: *Enterobacteriaceae bacterium* on Blood agar.E: Biofilm formation by tube method, F: motility pattern, G: calcite formation on Luria Bertani acetate medium, H: Antibiotic susceptibility by disk diffusion method.

] }]

bioMérieux Customer:

DR.RADHWAN ALJAMMAS- LAB
Microbiology Chart Report

Printed Jul 20, 2018 15:35 CDT

Patient Name: Ekhlas Ramathan, .
Location:
Lab ID: 275

Patient ID: lkiklihg
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas stutzeri

Source: swab

Collected:

Comments:	

Identification Information	Analysis Time:	5.80 hours	Status:	Final
Selected Organism	97% Probability Bionumber:	**Pseudomonas stutzeri** 0001001100700042		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-		
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-		
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-		
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-		
40	ILATk	+	41	AGLU	+	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-		
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-		
58	O129R	-	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	+					

Figure 2. Result of VITEK2 system(Standard method).

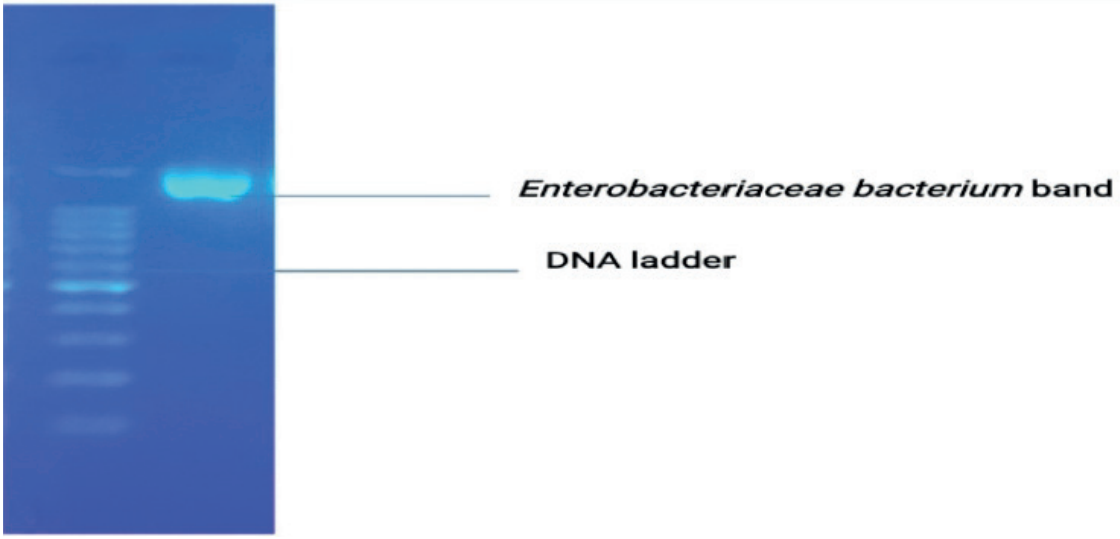

Figure 3. 16S rRNA gene analysis on agarose gel.

the rest of the antibiotic disks did not show any precise zone figure(1: H).

Discussion

Kitchens have the most outstanding richness of extremotolerant bacteria of all the domestic ecosystems examined so far[27-29]. It was only recently discovered that some microorganisms may live and develop in some household appliances, such as dishwashers, even under harsh conditions[30,31]. This environment(dishwasher) is assumed to be one of the extreme environments, which is considered a mine for many new and oddity bacterial strains. In our previous study, two types of boron-tolerant Bacillus were isolated[32].

Microbial communities with poly extreme tolerant traits can thrive in dishwashers, which are severe ecosystems with continually changing conditions. The individual members of the group, and also the entire community, must have critical features that allow them to withstand fluctuating wet and dry cycles, frequent temperature changes during washing cycles (20°C to 74°C), oxidative cleaning agents that raise the pH (6.5 to 12), high organic burden, high concentrations of NaCl, and shearing due to water sprinklers. The rubber, metal, and plastic portions of dishwashers may facilitate the creation and development of combined bacte-

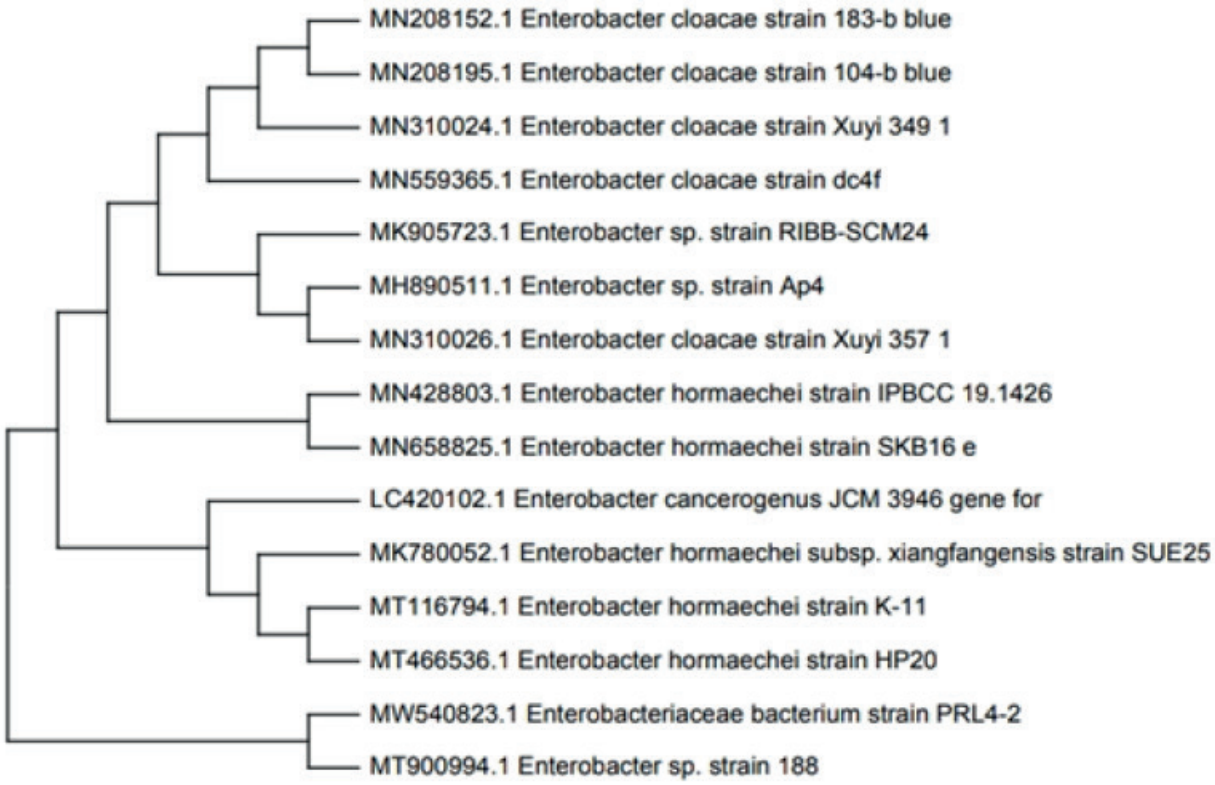

Figure 4. Phylogenetic tree.

rial-fungal populations that are saved by abundant extracellular polymeric substances, which provide extremotolerant features to biofilm populations that go beyond extreme tolerance of individual species[33-35]. When microorganisms are exposed to harsh circumstances in dishwashers, they naturally choose to develop in biofilms, which give protection from external pressures[36]. So these reasons explain the presence of Enterobacteriaceae bacterium in the dishwasher, which can form biofilms. While this strain shows a novel shape of surface motility with tiny branch colonies, this motile ability may help her gather with each other or with other microorganisms and explore the suitable environment to form a biofilm that helps them withstand this harsh environment.

The ability of self-motile bacteria to seek out suitable growth settings by exploration and movement toward resources or away from poisons or by forming complex communities that permit survival is undoubtedly one of their most significant advantages[37]. Because most bacterial species rely on surface interaction, it's only natural that many organisms have evolved motility modes that consider part of surface colonization. The rivalry among species, potential virulence, biogeography, and biofilm growth of a roof-associated community are controlled by motility[38].

Furthermore, our strain shows positive results for lecithinase, negative for lipase, and no hemolysis on human blood agar. The negative result for lipase may be limited to using this medium (egg yolk agar) because there are other media for this examination that may show a different result. In addition, The absence of hemolysis on human blood agar could be related to the age of the red cells in expired human blood, or it could be due to another factor[39]. As well as, the absence of hemolysis maybe mean that this microorganism doesn't have a virulence factor related to blood hemolysis.

The major component of cell membranes is phospholipid lecithin which can be destroyed by lecithinase to create phosphorylcholine and diglyceride, resulting in toxicity. Lecithinase can harm genital tract tissues, produce hemolysis, and rupture cell membranes, resulting in cell lysis[40].

Enterobacteriaceae bacterium shows the ability to grow on McConkey agar with pink mucous colonies, so it considers a lactose fermenter. As well as The *Enterobacteriaceae bacterium* was also slow-growing, which is in agreement with the results of the previous study[13].

Microorganisms have been implicated in biomineral precipitation in various mineral production processes. Several studies have highlighted the function of microorganisms in the mineral deposition in natural habitats. Even though these bioprocesses have been recorded in various environments, biomineralization does not have to be linked to any specific microbial group[41].

Bacterial cells are essential for CaCO3 precipitation because they supply nucleation fields (heterogeneous nucleation) and influence the specific forms of minerals that form. The negative charge groups of The cell surface of Bacteria serve as foragers for divalent cations, e.g., Ca2+, Mg2+ by attaching them to the cell's surface at neutral pH, forming appropriate nucleation fields for calcite accumulation. However, due to their increased ionic selectivity, Ca2+ ions can attach to bacteria's negatively charged cell surface more frequently than Mg2+ ions[42]. Our strain MW540823 shows the ability to form calcite on Luria Bertani acetate medium. This strain showed its ability to form calcite, as this ability is not limited to a specific type of bacteria as mentioned previously as well; as the availability of appropriate conditions in this environment as well as the negative charge of the bacterial cell surface are all factors that contribute to the formation of calcite in these environments. Our strain was identified by the colorimetric ID GN VITEK2 cards system, and the result showed *Pseudomonas stutzeri* with a probability of 97%. And when this strain was analyzed by 16 sRNA, show a different result as *Enterobacteriaceae bacte-*

rium with accession number MW540823 NCBI and a probability of 99% with the uncultured organism (HQ787695.1.).

The results of the VITEK and other diagnostic tests did not match the genetic diagnosis reported in another previous study for the same novel species, *Enterobacteriaceae bacterium*, where this study mentioned that AF18, as well as the other strains with this species, are regarded as uncommon pathogens with opportunistic behavior which having limited documentation, in addition, this study shows that the routine bacterial identification tests aren't always accurate for these novel species[13].

As for its resistance to antibiotics, it showed resistance to the antibiotics used in this study; this resistance may be a result of mutations due to its presence in such extreme environments or its presence in the form of assemblies(biofilms) that may also lead to the transfer of resistance genes between individuals in these environments. In a recent study of this bacterium[13], isolated from a person infected with bile infections, it was found that it was highly resistant to antibiotics. The researcher chose 21 antibiotics, and the bacterium showed resistance to 12 of them.

Conclusions

The extreme environment considers an essential source for novel bacteria because the individuals of these environments are exposed to high stress that may be lead to new characteristics to resistant this extreme condition; also, there is differences between biochemical and molecular test diagnosis of this exotic strain; therefore need more research to know their different capabilities, pathogenicity and resistance to antibiotics.

Funding

Self-funding.

Acknowledgments

In this section, we acknowledge any person who supports us in completing this project.

Conflicts of Interest

There is no conflict.

Bibliographic references

1. os Santos, G. S., Solidônio, E. G., Costa, M. C. V. V., Melo, R., de Souza, I. F. A. C., Silva, G., & Sena, K. X. F. R. Study of the Enterobacteriaceae group CESP (Citrobacter, Enterobacter, Serratia, Providencia, Morganella and Hafnia): a review. The battle against microbial pathogens: basic science, technological advances and educational programs, 2015: 2, 794-805
2. kundayo, T. C., & Okoh, A. I. Pathogenomics of virulence traits of Plesiomonas shigelloides that were deemed inconclusive by traditional experimental approaches. Frontiers in microbiology,2018: 9, 3077
3. Romano-Bertrand, S., Bourdier, A., Aujoulat, F., Michon, A. L., Masnou, A., Parer, S., ... & Jumas-Bilak, E.Skin microbiota is the main reservoir of Roseomonas mucosa, an emerging opportunistic pathogen so far assumed to be environmental. Clinical Microbiology and Infection,2016: 22(8), 737-e1
4. Schürch, A. C., & van Schaik, W.Challenges and opportunities for whole□genome sequencing–based surveillance of antibiotic resistance. Annals of the New York Academy of Sciences,2017: 1388(1), 108-120
5. Hunter, C. J., & Bean, J. F.Cronobacter: an emerging opportunistic pathogen associated with neonatal meningitis, sepsis and necrotizing enterocolitis. Journal of Perinatology,2013: 33(8), 581-585
6. Shin, H., Lee, J. H., Choi, Y., & Ryu, S.Complete genome sequence of the opportunistic food-borne pathogen Cronobacter sakazakii ES15,2013.
7. Adeolu, M., Alnajar, S., Naushad, S., & Gupta, R. S.Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International journal of systematic and evolutionary microbiology, 2016:66(12), 5575-5599
8. Hang, Z., Li, D., Shi, X., Zhai, Y., Guo, Y., Zheng, Y., ... & Gao, Z.Genomic characterization of an emerging Enterobacteriaceae species: the first case of co-infection with a typical pathogen in a human patient. BMC genomics,2020: 21(1), 1-13
9. Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., ... & Karsch-Mizrachi, I. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database, 2020.
10. Aylward, F. O., Burnum, K. E., Scott, J. J., Suen, G., Tringe, S. G., Adams, S. M., ... & Currie, C. R.Metagenomic and metaproteomic insights into bacterial communities in leaf-cutter ant fungus gardens. The ISME journal,2012: 6(9), 1688-1701
11. Aylward, F. O., Currie, C. R., & Suen, G.The evolutionary innovation of nutritional symbioses in leaf-cutter ants. Insects,2012: 3(1), 41-61
12. Aylward, F. O., Tremmel, D. M., Bruce, D. C., Chain, P., Chen, A., Walston Davenport, K., ... & Currie, C. R.Complete genome of Enterobacteriaceae bacterium strain FGI 57, a strain associated with leaf-cutter ant fungus gardens. Genome announcements, 2013:1(1), e00238-12
13. Zhang, Z., Li, D., Shi, X., Zhai, Y., Guo, Y., Zheng, Y., ... & Gao, Z. Genomic characterization of an emerging Enterobacteriaceae species: the first case of co-infection with a typical pathogen in a human patient. BMC genomics,2020: 21(1), 1-13
14. Weingarten, R. A., Johnson, R. C., Conlan, S., Ramsburg, A. M., Dekker, J. P., Lau, A. F., ... & Frank, K. M. Genomic analysis of hospital plumbing reveals diverse reservoir of bacterial plasmids conferring carbapenem resistance. MBio, 2018:9(1), e02011-17
15. Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., & Iqbal, M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Brazilian journal of infectious Diseases, 2011:15(4), 305-311.
16. Russell, F. M., Biribo, S. S. N., Selvaraj, G., Oppedisano, F., Warren, S., Seduadua, A., ... & Carapetis, J. R. As a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in laboratories of developing countries. Journal of clinical microbiology, 2006:44(9), 3346-3351
17. Clemmer, K. M., Bonomo, R. A., & Rather, P. N.Genetic analysis of surface motility in Acinetobacter baumannii. Microbiology, 2011:157(Pt 9), 2534
18. Collee, JG;Mackie,T.J. and McCartney,J.E.Mackie &McCartney Practical Microbiology .New York :Churchill Livingstone,1996.
19. Heatley, N. G. A method for the assay of penicillin. Biochemical Journal,1944: 38(1), 61-65
20. Matuschek, E., Brown, D. F., & Kahlmeter, G.Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection,2014: 20(4), O255-O266
21. Le Page, S., van Belkum, A., Fulchiron, C., Huguet, R., Raoult, D., & Rolain, J. M.Evaluation of the PREVI® Isola automated seeder system compared to reference manual inoculation for antibiotic susceptibility testing by the disk diffusion method. European Journal of Clinical Microbiology & Infectious Diseases, 2015:34(9), 1859-1869

22. CLSI Guideline M02 – Performance Standards for Antimicrobial Disk Susceptibility Tests (13th edn). Wayne,PA: CLSI., 2018a.
23. EUCAST The European Committee on Antimicrobial Susceptibility Testing. Disk Diffusion Method for Antimicrobial Susceptibility Testing – Version 7.0. Vaxjo, Sweden: EUCAST, 2019b.
24. Lalitha, M. K. Manual on antimicrobial susceptibility testing. Performance standards for antimicrobial testing: Twelfth Informational Supplement,2004: 56238, 454-456.
25. Lee, Y. N. Calcite production by Bacillus amyloliquefaciens CMB01. Journal of Microbiology, 2003:41(4), 345-348.
26. Hamilton,W.R.;Woolley,A.R. and Bishop,A.C.Minerals Rocks and Fossils[10].The Hamlyn Publishing Group Limited,Italy, 1986.
27. Ojima M, Toshima Y, Koya E, Ara K, Kawai S, Ueda N.Bacterial contamination of Japanese households and related concern about sanitation. Int J Environ Health Res, 2002:12:41–52. https://doi.org/10.1080/09603120120110040.
28. Sinclair RG, Gerba CP.Microbial contamination in kitchens and bathrooms of rural Cambodian village households. Lett Appl Microbiol, 2011:52:144–149. https://doi.org/10.1111/j.1472-765X.2010.02978.x.
29. Ojima M, Toshima Y, Koya E, Ara K, Tokuda H, Kawai S, Kasuga F, Ueda N.Hygiene measures considering actual distributions of microorganisms in Japanese households. J Appl Microbiol ,92002:3:800–809. https://doi.org/10.1046/j.1365-2672.2002.01746.x.
30. Vilanova C, Iglesias A, Porcar M.The coffee-machine bacteriome: biodiversity and colonisation of the wasted coffee tray leach. Sci Rep, 2015:5:10.1038/srep17163. https://doi.org/10.1038/srep17163.
31. Zalar P, Novak M, de Hoog GS, Gunde-Cimerman N.Dishwashers—a man-made ecological niche accommodating human opportunistic fungal pathogens. Fungal Biol 115:997–1007. https://doi.org/10.1016/ .funbio.2011.04.007
32. Younis, K. M., Matter, I. R., & Al-Omari, A. W.Isolation and Molecular Identification of Bacillus boroniphilus sp. nov., Isolated from Dishwasher. Annals of the Romanian Society for Cell Biology,2021: 5977-5992.
33. Zupanc˘ic˘ J, Babic˘ MN, Zalar P, Gunde-Cimerman N.The black yeast Exophiala dermatitidis and other selected opportunistic human fungal pathogens spread from dishwashers to kitchens. PLoS One 2016:11:e0148166. https://doi.org/10.1371/journal.pone.0148166.
34. Dög˘ en A, Kaplan E, Oksüz Z, Serin MS, Ilkit M, de Hoog GS.Dishwashers are a major source of human opportunistic yeast-like fungi in indoor environments in Mersin, Turkey. Med Mycol ,52013:1:493–498. https://doi.org/10.3109/13693786.2012.738313
35. Imoli DH, Jones CJ, Wozniak DJ.Bacterial extracellular polysaccharides in biofilm formation and function. Microbiol Spectr 3(3)MB-0011-2014.https://doi.org/10.1128/microbiolspec.MB-0011-2014.
36. Burmølle M, Ren D, Bjarnsholt T, Sørensen SJ.Interactions in multispecies biofilms: do they actually matter? Trends Microbiol 22: 84–91. https://doi.org/10.1016/j.tim.2013.12.004.
37. Wei Y, Wang XL, Liu JF, Nememan I, Singh AH, Weiss H, Levin BR.The population dynamics of bacteria in physically structured habitats and the adaptive virtue of random motility. Proc Natl Acad Sci U S A,2011:108:4047–4052. https://doi.org/10.1073/pnas.1013499108.
38. Mattingly, A. E., Weaver, A. A., Dimkovikj, A., & Shrout, J. D.Assessing travel conditions: environmental and host influences on bacterial surface motility. Journal of bacteriology,2018: 200(11), e00014-18.
39. Russell, F. M., Biribo, S. S. N., Selvaraj, G., Oppedisano, F., Warren, S., Seduadua, A., ... & Carapetis, J. RAs a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in laboratories of developing countries. Journal of clinical microbiology,2006: 44(9), 3346-3351.
40. Sharaf, E. F., El-Sayed, W. S., & Abosaif, R. M. Lecithinase-producing bacteria in commercial and home-made foods: Evaluation of toxic properties and identification of potent producers. Journal of Taibah University for science, 2014:8(3), 207-215.
41. Silva-Castro, G. A., Uad, I., Gonzalez-Martinez, A., Rivadeneyra, A., Gonzalez-Lopez, J., & Rivadeneyra, M. A. Bioprecipitation of calcium carbonate crystals by bacteria isolated from saline environments grown in culture media amended with seawater and real brine. BioMed research international, 2015.
42. Anbu, P., Kang, C. H., Shin, Y. J., & So, J. S. Formations of calcium carbonate minerals by bacteria and its multiple applications. Springerplus, 2016:5(1), 1-26.