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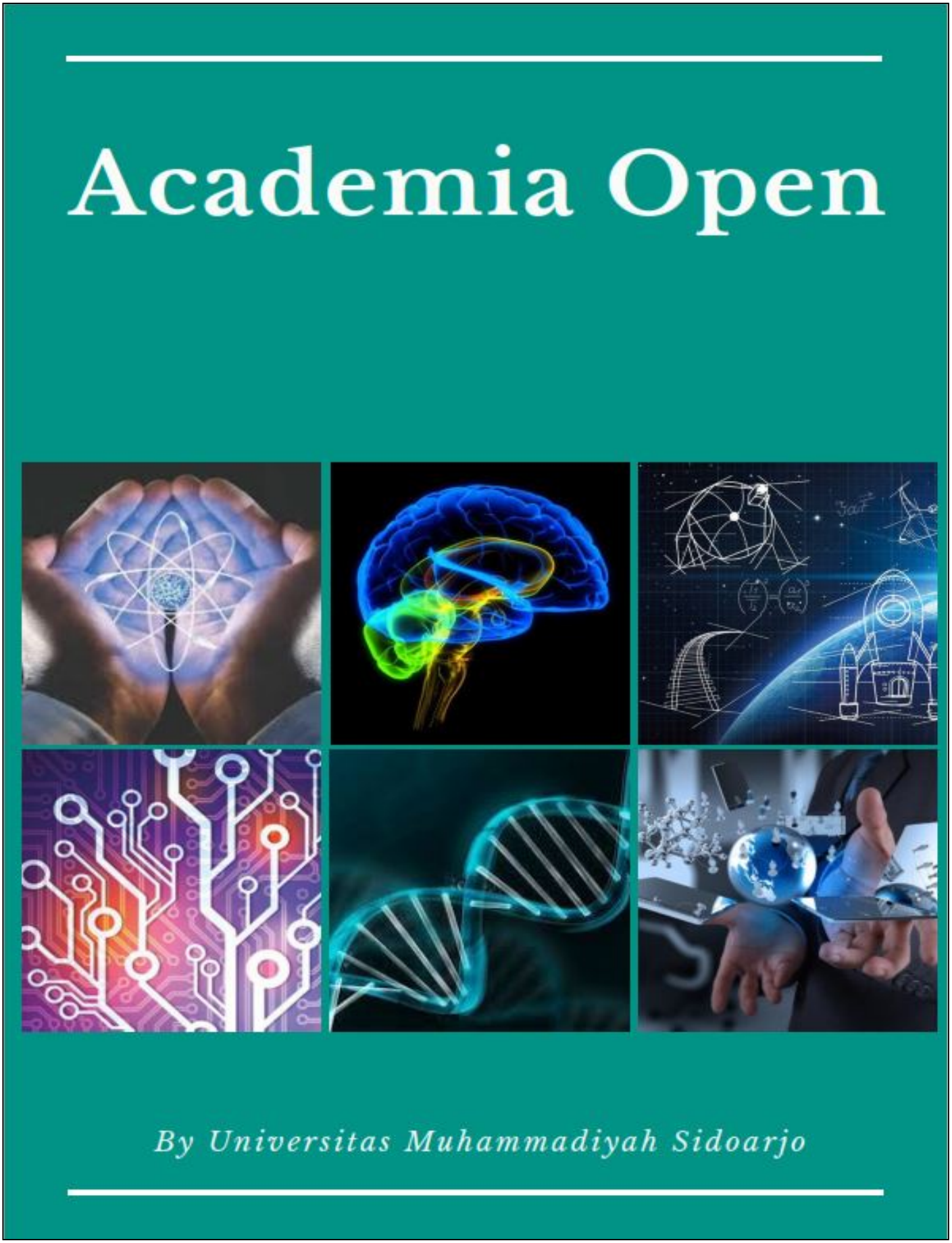

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Genetic Stability and Public Health Implications of Plasmid-Free Vibrio cholerae

Stabilitas Genetik dan Implikasi Kesehatan Masyarakat dari Vibrio cholerae Bebas Plasmid

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Abstract

Background: Cholera, caused by Vibrio cholerae, is a significant global health concern, particularly in regions with poor sanitation. **Specific Background:** This study focuses on V. cholerae strains isolated from cholera patients in Al-Nasiriyah city, Iraq, where limited genetic studies have been conducted. **Knowledge Gap:** Despite extensive research on V. cholerae, little is known about the genetic makeup of strains from Al-Nasiriyah city and their plasmid content, which traditionally correlates with antibiotic resistance and virulence. **Aims:** This research aimed to characterize the genetic and antibiotic resistance traits of V. cholerae strains from cholera patients in Al-Nasiriyah city and to determine the presence of plasmids using advanced molecular techniques. **Results:** Contrary to expectations, our study identified V. cholerae strains devoid of plasmids and exhibiting a stable genetic profile across two chromosomes, with sizes ranging from 1 to 3 million base pairs. **Novelty:** Our findings challenge conventional wisdom by demonstrating the absence of plasmids in local V. cholerae strains and highlighting their unique genetic architecture. **Implications:** This study underscores the importance of genomic diversity studies in understanding V. cholerae epidemiology and guiding public health strategies. Future research should explore larger strain cohorts and conduct comparative genomic analyses to elucidate the broader implications of genetic variability on cholera outbreaks and treatment strategies.

Highlights:

1. Genetic Stability: V. cholerae strains exhibit stable genetic profiles.
2. Plasmid Absence: No plasmids found, challenging antibiotic resistance assumptions.
3. Public Health Impact: Crucial for effective cholera management and prevention strategies.

Keywords: Vibrio cholerae, genetic diversity, antibiotic resistance, plasmid absence, public health implications

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Introduction

Genetic research of the vivo cholera revealed that it has two rod-shaped chromosomes instead of a typical single chromosome [1]. This simultaneous presence of extra chromosomes in different bacterial genera is unclear yet and has been reported in other bacterial genera such as Brucella melitensis (2) for example. Gene expression for cholera toxin (CT) synthesis, cell division, gene transcription, protein translation, DNA replication, and cell wall crafting also requires large polymer of about three million base pair genes present on a single chromosome. The data include large pathogenic genes that encode for adhesion proteins (TCP) and toxins (TOXR) as well [2, 3]. However, the second chromosome is comparatively small, like only one-third of the given one chromosome, possessing nearly one million base pairs. The genetic material also contains key genes useful for routine cellular functions, such as ribosomal protein L20 and L35 that assist in making vital proteins, metabolic components that ensure the metabolic activity, and antibiotics resistance gene like chloramphenicol acetyltransferase, glutathione transferase and phosphomycin resistance protein [4]. [5] However, plasmids are generally thought to accompany the chromosome of the cholera strains rather than on their own, and [6] on the contrary, the chromosome of cholera strains has been reported to be offered in two main sizes that do not contain any plasmids whatsoever. It is possible that the smaller chromosome began when it was a huge plasmid, known as a megaplasmid, thrived in the bacterium’s environment. Along the way the plasticity occurred that was followed by transportation of large number of highly significant genes from bigger chromosome into plasmid[7]. It is also important to mention that there are other vibrios of cholera strains that are not the only two antibiotics in the chromosome. The research revealed that Vibrio Parahaemolyticus has got two types of chromosomes one of them huge and one of them small [8].The findings show that cholera vibrio and eukaryotic associates have amazing complex genetic makeup which explain the vibrio's evolution and adaptability. The pathogenic version of V. vulnificus in humans, which causes diseases by consuming contaminated sea food, or by by injury of the tissue, consists of two chromosomes, as well as a plasmid that is about 508.48 base pairs long. A 2018 report by Schwartz et al. describes an advanced electrical device that in addition to separating large molecules through two electric fields oriented oppositely during migration can also move particles and micelles through the migration chamber under the influence of an applied ultrasonic wave. If we apply one electrical field the particles move with less force and cannot reach the tank. Using such method, scientists sucessfully isolated kilo base pairs long yeast chromosomes. Because the DNA molecules are set to relax in the gel when their movement is transferred from the first electric field to the second electric field, in the process something else was observed, that is, a DNA molecule stretching when exposed to the second field of electricity. Among his contributions is the pulse field electrophoresis gel technique (PFGE) that he is the first to describe and gave it its name [2, 3]. PFGE has a better separate genes fragments that range from 10kb to 1000kb, unlike the conventional electrophoresis technique wherein fragments with length of 30kb to 50kb are limited in range [4].

Principle of PFGE:

The concept of Pulse Field Gel Electrophoresis (PFGE) is as follows:The concept of Pulse Field Gel Electrophoresis (PFGE) is as follows:

As the first part of our experiment, we develop the bacterial culture than not more than 24 hours old. In this process, the DNA is separated without being disturbed because it is the buffer that can kill the bacteria. Deterioration of cellular protein is carried out by using protease-SDS blend and SDS is denatured substances working in this process. In here, a plug is built into the DNA; therefore, the cell passes down into the agarose matrix and then penetrates into the electrophoresis. In the decontamination process, these filters are put through a thorough washing in order to remove the plant cell residues and the left over enzymes. In the following step, the DNA chain is chopped in two pieces where the restriction enzymes play role as enzyme that can recognize the individual DNA sequences with eight or more base pairs and fragment the DNA molecule into smaller pieces. The final step is putting of the beads onto special agarose gel wells and using electrophoresis, which is a method that leads to separation of the DNA fragments through the application of a specified voltage. The step that comes after is of paramount importance to examination of genetic factors. PFGE, as a preferred technique for genomic sequencing teams, involves a long duration of prominent DNA fragments shifted. Moreover, an electric current is used to apply it in different directions to the hydrogel so that the motions of the particles proceed along the serpentine until they reach a certain point [9].This technology, which brings about the significant altering of the DNA analysis and its processing, is what has continuously led to the unprecedented discoveries in genetic and molecular research due to its the innovative effect. One of the researches most commonly used in laboratory research practice in is the Pulse Field Gel Electrophoresis (PFGE) technique which pinpoints the location of the gene, evaluates fast the content of the genetic material, tags along chromosomal defects or variations, and counts the number of chromosomes on bacteria. The Trucksis and his team were also successful in tracking down that particular genetic element that was responsible for the development of fluoroquinolone antibiotic resistance which was on the Staphylococcus aureus bacterium chromosome. In 1998, the researcher again applied PFGE method which was quite exhaustive and it was used to assess the genetic material of cholera microbes. The experimentatic data demonstrated the presence of the material of cholera in two chromosomes, one of them being a significantly large and the other is small, by the PFGE method with certain dissimilarities [10]. Another study (Pulinti et al., 2005) by Okada and his groupfrom Japan showed PFGE that were used in the study demonstration that the isolated strain of cholera had two ring chromosomes instead of one [11]. Modern investigation of the genetic makeup of Vibrio cholerae demonstrated two circluar chromosomes among the members of this bacterium by researchers.

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Chromosomes often gave the same merged pattern among Bacteria and other bacterial phyla, which shows the stability of their organizational form. The Vibrio cholerae possesses two chromosomes called small and large chromosome that is one-third and two times the size of large chromosome respectively. The small chromosome of Vibrio cholerae, which carries essential genes, is needed for the normal cellular function including antibiotic resistance [12]. The eubacteria's large chromosome responsible for a production of cholera toxin and other processes which are crucial for cell viability that detected in isolates from different continents and from different localities. Whether plasmids mean the most in v. cholera - the mainstay of the discussion in the field - is what still preoccupies minds amidst a lot of new scientific findings in the branch. But also some of the other researches of the plasmid lack so and also there is another ones which pointed that the plasmids are in the near-by chromosomes. [13]This variety of pathogens depicts the need for a whole genome sequencing approach for cholera pathogens in order to establish the timing of Vibrio cholerae strains and similar contenders. This specific work, which include whole genomic profile through PFGE and other labs sharing the data with national institutes, is quite a worthy one. The primary purpose of the experiment is to find plasmids, learning the structure of the chromosomes and the consequences of the anomalies of a bacterium on it, and steepening our knowledge of bacterial genetics in relationship with the antibiotic resistance and pathogenicity.

Methods

Testing and data collection took place between April 1, 2023, and October 1, 2023. The last day marks the end of the six-month study period.The latter day marks the end of our study period which consists of six months. 1,152 stool samples were collected from patients who had recent symptoms of diarrhea, whether mild or severe.. A clinical trial was conducted by patients who were already suffering from the disease by sending their samples to hospitals located in community centers. . During a disease state of cholera outbreak, 36% of cholera serogroup O1 isolates at the central public laboratory were conformed to be found in the sample in the study reported. The bacteria from the was grown in nutrient medium for six to eight hours, during which the growth was alternate aerobic and anaerobic type (anaerobic–aerobic metabolism). After the incubation period, the bacterial culture segment was transferred to three different types of culture media: growing colonies of bacteria (of Vibrio cholera group) on the blood agar medium,McConkey agar medium, and a composition (TCB) of the medium with nutrients that allow the bacteria to grow fast. The Viruses incubated at 37°C continually the temperature the overnight. For the next series of experiments, other samples were used together. Doing a gel electrophoresis of agarose, for the ability to view DNA, 0.7 grams of agarose should be dissolved in 100 milliliters of TBE buffer. The heated mixture should then be boiled and allowed to cool at 45-50 degrees Celsius. Pour the prepared liquid on the tray with the well comb. After the pouring, leave it to harden; eject the comb and put it in the electrophoresis machine. In this solution, added mixed DNA was mixed with a loading buffer that had been added. 7 volts of an electric field per square centimeter were applied to the solution within the wells. Then, the results of the ultraviolet light were visualized. For the extraction of PFGE for cholera bacteria, the growth of the bacteria in TSB should be cultured until they obtain optical density (O.D.) at a wavelength of 600 nm and 0.9 measurement. After centrifugation and washing, the precipitate is resuspended in the EU buffer. Material with molds is allowed to solidify, and then the cells are split by an EC buffer with lysozyme and RNase. After that, ESP buffer is first shot in, and the second shot was TE-PMSF buffer. If not urgently required, store in TE buffer. Subsequent to application of the ethidium bromide spot and imaging under ultraviolet light, the electrophoresis of the plugs is done using a Pulse Field Gel Electrophoresis (PFGE) apparatus at a voltage of 200 volts with an agarose gel concentration of 1.3%.

Result and Discussion

Bacterial Diagnosis Based on Colony Characteristics

The colonies had standard colonial characteristics of colonial morphology that was noted. The cholera colonies showed characteristics typical in nature, such as being rounded, moderate in size, and having a smooth, glossy texture due to fermentation, as seen in Fig.1 on medium TCBS agar. The character of the environment allowed retaining the development of bacteria not giving rise to cholera. The colonies of bacteria causing cholera were looking rather weak on the McConkey agar medium. On the other hand, these isolates had black colonies with distinct β-hemolysin zones.

Pulsed-Field Gel Electrophoresis (PFGE) Analysis

The chromosomal identification in Fig.2 has confirmed through the PFGE data that all isolated strains of Vibrio cholerae have two chromosomes. The larger of the two chromosomes averaged about 2 million base pairs in length, whereas the other ranged in size from 1 million to 1.125 million base pairs. In addition, a predicted genetic fragment was also verified in size-matched cholera vibrios with a chromosomal yeast marker reference to compare the predicted size.

Plasmid analysis

None of the isolates harbored plasmids for the study. It suggested that there was no direct relationship between

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plasmid content, bacterial pathogenicity, and antibiotic resistance by these strains.

Observations in the context of previous studies Data obtained are indicative of the whole-genome nucleotide sequence of the classical V. cholera strain O395, which was consistent with the two-chromosomal structure of the cholera vibrio and, apart from that, fully negated plasmids' presence in these bacteria from very original deduction.

Criteria for the diagnosis of bacteria according to the characteristics of colonies: The identification of bacteria was based on a culture process, and due consideration was given to the size, morphology, pigmentation, and texture of the colony. The colonies on the selective TCBS agar medium of Vibrio cholerae were characterized as round, medium-sized, smooth, flat, glossy. All these characters developed from the sucrose fermentation. These characters are displayed in the fig.1. It has an alkaline nature of pH 8.6 and combines with rather high proportions of sodium chloride and thiosulfate salts, being highly effective for the inhibition of growth among the majority of other bacterial species.

Because of the fact that the vibrios do not ferment lactose, the colonies were pale in color, formed on MaConkey agar medium. But when the isolates were grown on blood agar medium, most of them formed black-colored colonies with characteristic recognizable area of hemolysis. This strain of bacteria has the potential to lyse the blood completely due to the production of the hemolytic enzyme (β-hemolysin).

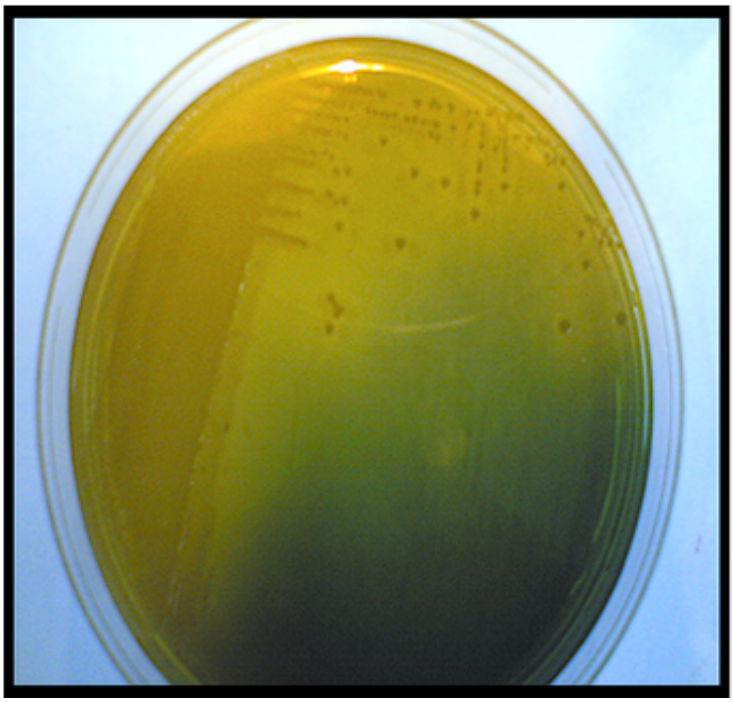

Figure 1. *cholera colonies cultured on TCB medium*

The hue of the cholera colonies, which were observed ON TCB media, is yellow, their morphology is flat, and their size is mostly large.

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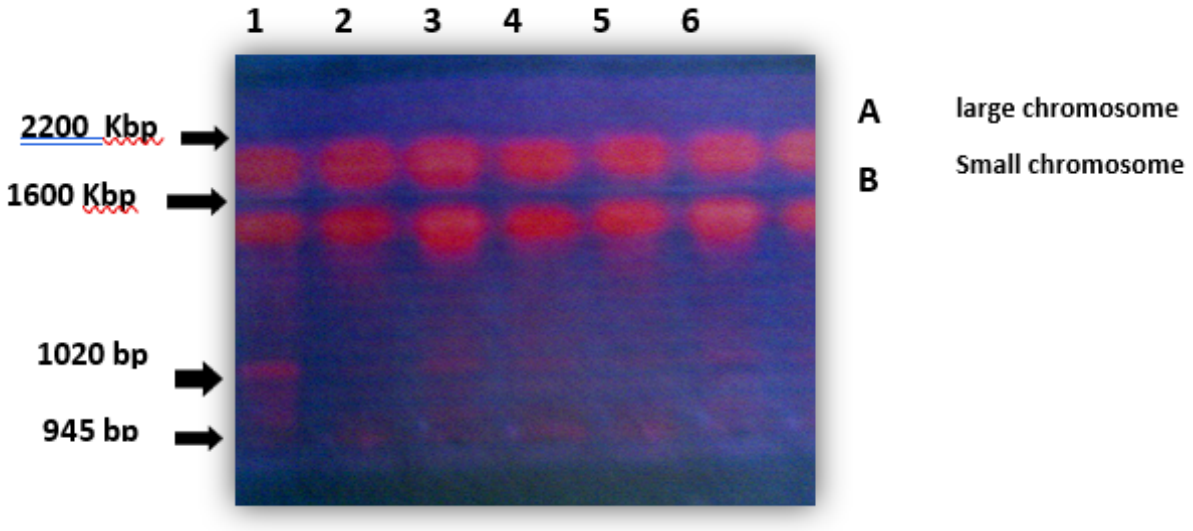

Figure 2. *Pulsed-Field Gel Electrophoresis (PFGE) Results*

1. The chromosome is large, 2200 base pairs and the smaller chromosome contains 1600 kbp A B. The letter used is “B”. There are also 16 chromosomes, each of which has 1,020 base pairs, and 15 chromosomes, each of which has 945 base pairs.

2. This image shows the result of pulsed-field gel electrophoresis.. This picture shows the outcome of Pulse Field Gel Electrophoresis.

The left bar contains a yeast chromosomal marker whose fragments’ sizes are given in kilobase pairs kbp. The 2 nd , 3 rd , and 4 th columns display the clone composition of cholera vibrio Inaba serotype. The two left columns baptized Vibrio cholerae serotype Ogawa; they are found in columns 5 and 6. is “The big chromosome, Base pair is 2,200 bp.” The size-limited chromosome B Chromosome is small. The text of the user is just “B.” “Throughput” of one component is 1600 kilobits.the other chromosome have Base pair is 1020 bp and also last chromosome Base pair is 945 bp. The results of PFGE are shown in Fig.2. The yeast chromosomal marker is contained in the left column; the size of the fragments of the marker is expressed in kilobase pairs . Columns 2, 3, and 4 contain data on the basis of the genetic composition of cholera vibrio of serotype Inaba. In columns 5 and 6 of Fig.2, data on the basis of the genetic composition of cholera vibrio ogawa serotype are shown. “The Big Chromosome, Part A . B: Size-limited chromosome. One of the possible results of PFGE is shown here. The sizes of the fragments of the yeast chromosome marker, measured in kilobasepairs, are present in the left column. The image improves the visual representation of PFGE results, as well as increases the scientific rigor and clarity of the results.

Discussion

These studies confirm that the genetic material of Vibrio cholerae remains the same throughout all isolates obtained. This statement contradicts all other study results, as they all show the presence of plasmids. However, this statement solidifies the earlier concept since plasmids cannot direct antibiotic resistance and pathogenicity among these isolates. There is no information available about the presence of bacterial cells that possess more than one chromosome in recent data. As per the researcher, this situation has been reported in several studies conducted in the past concerning bacterial cells in taxonomic groups such as Rhodobacter sphaeriods, Rhizobium meliloti, Agrobacterium tumefaciens, and Brucella melitensis, who were the first scientists to use Pulse Field Electrophoresis Gel (PFGE) to determine the whole genetic constituent of cholera vibrios. When they applied this method, they observed that the cholera vibrios had further scattered their genetic material across conditions that comprised more than one ring chromosome instead of one larger ring chromosome. Therefore, in this experiment, PFGE used to investigate the Inaba and Ogawa serotypes’ cholera isolates obtained locally to confirm the existence of more than one chromosome. Fig.2 demonstrates that all isolates possess two chromosomes. To estimate their sizes, comparisons were made using molecular weight standards, which are fragments of yeast chromosomal DNA. The larger chromosome, distributed near the agarose gel loading wells, is estimated to contain about 2 to 3 million base pairs (2,000 to 3,000 kilobase pairs). It appeared to cover a wide range of possibilities. The smaller chromosome, situated below the larger one, appeared as a thinner band when viewed without magnification, with an estimated size ranging from 1,000 to 1,125 kilobase pairs. The Inaba serotype of cholera bacteria consists of two ring chromosomes, one considerably large at about 3 million base pairs and a smaller one at about 1 million base pairs. These results align with those presented in previous studies[14]. Our current findings are consistent with earlier conclusions, suggesting that cholera vibrios house a genetic content of approximately 4 million base pairs distributed across two chromosomes, one large and one small our study confirms the presence of two different chromosomes in cholera isolates. The larger chromosome has about 2 million base pairs, whereas the smaller

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chromosome ranges from 1 million to 1.125 million base pairs. Unlike previous studies that included plasmid-mediated traits, the present study did not identify any plasmids in the strains studied. This indicates the existence of an alternative mechanism for both antibiotic resistance and virulence. Thus, it highlights the genetic diversity present among Vibrio cholerae populations.

Future studies should focus on conducting a thorough analysis of plasmid variability in more strains of Vibrio cholerae. In addition, the study of the functional roles of the identified genomic structures and the conduct of comparative genomic studies on strains from different regions will provide valuable information on evolutionary dynamics and adaptation to the environment. The importance of this finding cannot be overstated to understand the full extent of the public health impact of the genetic makeup of Vibrio cholerae, especially in areas where outbreaks of diarrhea are common.

Conclusion

Based on our study, we have characterized Vibrio cholerae strains isolated from cholera patients in Al-Nasiriyah city, Iraq, revealing a unique genetic profile. Our findings indicate that these strains lack plasmids traditionally associated with antibiotic resistance and virulence, challenging conventional assumptions. Instead, the strains exhibit a consistent genetic makeup across two chromosomes, with sizes ranging from 2 to 3 million base pairs and 1 to 1.125 million base pairs, respectively. This genomic stability suggests an alternative mechanism for adaptation and pathogenicity in Vibrio cholerae populations. These results underscore the need for further research into the genetic diversity and functional roles of these genomic structures, particularly in understanding their evolutionary dynamics and environmental adaptation. Future studies should prioritize exploring plasmid variability in larger strain cohorts and conducting comparative genomic analyses across different geographic regions to elucidate the broader public health implications of Vibrio cholerae genetic diversity.

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