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Isolation and molecular detection of *Salmonella Bacteriophage* from Chicken feces and sewage

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ABSTRACT— A total of 170 samples of (chicken poo and sewage) were grown on selective medium for Salmonella isolate identification and bacteriophage isolation (salmonella bacteriophage). Out of 170 samples results gave us the existence of 20 bacteriophage. Salmonella Bacteriophages were found in 11.7% of the samples analyzed and tested against the most commonly isolated of Salmonella isolates. Bacteriophage demonstrated potent lytic activity against Salmonella isolates (enteritidis, typhimurium) by forming a clear and transparent zone in the spot and double layer agar assays, implying that bacterial challenge assays were used to assess the relative ability of Bacteriophage to lyse Salmonella in vitro, producing plaques 3-6 mm in diameter surrounded by halos on a Salmonella lawn. Also using Transmission Electron Microscope to note the shape of the bacteriophage and then diagnosed to which family it belongs, which turned out to be back to the Myoviridae family, revealed the existence of an icosahedral head, 140.20 nm, and a contractile tail, 115.25 nm. Salmonella bacteriophage might encode genes inv-A, sit-C, inv-F from our Salmonella isolates, according to our findings.

KEYWORDS: Salmonella bacteriophage, lytic profile of Salmonella bacteriophage, coding of virulence factor of Salmonella

1. INTRODUCTION

Bacteriophages are bacterial viruses that, unlike antibiotics, precisely and efficiently attack their intended host bacteria. Furthermore, the methods by which antibiotics and bacteriophages kill bacteria are fundamentally different, as are the mechanisms by which bacteria gain resistance to antibiotics or bacteriophages, [1], [2].

Bacteriophages are characterized according to their morphology (three-dimensional, pleomorphic, polyhedral, icosahedral, filamentous, head-tail, or thread-like shaped), nucleic acid (ssRNA, dsRNA, ssDNA, dsDNA), bacterial target, and location. Almost 5000 bacteriophages were studied using electron microscopy, and the vast majority of them (96 percent) possessed tails. Bacteriophages are classified into 15 families and many orders.

The tailed bacteriophages are divided into three families: Siphoviridae (61%), Myoviridae (25%), and Podoviridae (5%). (14 percent). According [3], the vast majority of bacteriophages belong to the Caudovirales group and have isometric heads ranging from 20 to 200 nm, making them 1000 times smaller than the average bacteria 0.5–20 m.

Genetic screens that identified bacterial genes essential for bacteriophage growth as well as host genes associated with delayed or increased host cell death after infection. The identification of new bacterial

genes involved in this process contributes to a better understanding of the molecular processes by which bacteriophages successfully grow in and destroy their host cells. Furthermore, according to [4], [5] we uncovered a novel gene that is essential for bacteriophage infection, and we show that it creates a protein required for the successful transfer of DNA from the virion into the cell.

Bacteriophage virions with double-stranded DNA tails adsorb to certain features on the surface of target cells and then release their genomes into the cytoplasm via a process known as 'injection' or 'ejection.' The host's surface proteins or polysaccharides aid initial adsorption or adhesion, and more host proteins may be required for successful DNA injection [6], [4], [54- 74].

Our objective is to confirm the presence of Salmonella Bacteriophage using a spot test, a. double layer agar assay, and a transmission electron microscope (TEM).

2. Materials and Methods

2.1 Samples collection

The samples were separated into two parts: a) 95 fecal sample from broilers chicken was collected by taking 5 grams of feces in a clean container with disinfectant residue. b) 75 sewage samples of 10 ml were collected from the same sites as (à). The fecal samples were sent to the laboratory to make all the biological and biochemical investigation, therefore the total number of samples was 170, and these samples were collected in various regions of Baghdad between January and September 2021, as shown in table (1).

Table (1): Showed the areas for collecting of samples in this study for isolation of Salmonella Bacteriophage

No.	Name of Places	Type of Samples	No. of Samples
1	Abu Ghraib	(5) gm of feces	40
2	Shisha	(5) gm of feces	55
6	Abu Ghraib	(10 ml) sewage	20
7	shisha	(10 ml) Sewage	25
8	7th of April District/ Amriya	(10 ml) sewage	20
9	Organization Street / Amriya	(10 ml) sewage	10
Total			95+ 75 = 170

2.2 Isolation and Identification of Salmonella Bacteriophage

It was done by taking 5 gm of chicken feces, forming a suspension (1 gram feces: 9 ml physiological saline, pH 7.4), then taking 1.5 ml, putting it in an eppendorf tube, and centrifuged it (14 g for 10 minutes at 4°C). Supernatants were filtered through a 0.22µm-pore-size filter (CHM CA, Spain), and 0.1 ml (100 µL) of these supernatants was combined with 10 ml of nutritional broth (Himedia, indi) After incubation, bacteria were removed by centrifugation (14 g for 10 minutes at 4°C), supernatants were filtered through a 0.22µm-pore-size filter, and Salmonella bacteriophage was isolated from these supernatants using two methods:

(a) Spot assay

We used this assay to ensure the presence of lytic bacteriophages by prepared enriched bacteriophage and filtrates by putting 100 µL of Salmonella bacteriophage supernatant on two plates of nutrient agar (Himedia, india) which contains sodium chloride (NaCL = 5.8/liter) cultured with Salmonella (enteritidis, typhimurium) (300 µL), and (500 µL) 10^8 colony-forming units/mL).

(b) Double Layer agar (DLA) assay

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The double Layer agar allows for localized bacteriophage -bacteria interaction on plates with two levels of agar placed on top of each other (LOT, China, 2020). Bacterial growth medium is created for the bottom layer (for Salmonella, using 1.5 percent agar). The top layer contains the same medium but with a lower concentration of agar (0.5 percent) (Oxoid, UK), considered soft agar, and it is mixed with Salmonella and poured onto the bottom layer, resulting in a so-called lawn, begin: by combining 100 µL of supernatant for Salmonella Bacteriophage (we nominated above) with 300 µL of fresh Salmonella (enteritidis, typhimurium) culture (108 colony forming /ml) in a tube, 5 ml of semi-soft nutritional agar was added. The tube suspensions were combined and sprayed over 30 mL of nutritional agar. The plates were then allowed to firm up at room temperature for 10 minutes before being inverted and incubated at 37 degrees Celsius for 24 hours. Plaques were counted and the number of bacteriophages was calculated as plaque forming unit (per ml) (pfu) in accordance with [7- 13], the (a & b) assays considered a vitro assay for detection of Salmonella bacteriophage.

The concentrated bacteriophage stock was deposited in Eppendorf tubes (Labnet, USA) at a concentration of 108 pfu/ml and stored at 4°C Freeze-dried preparations were stored at -20 C..

2.3 Diagnosis of Salmonella Bacteriophage by Transmission Electron Microscope (TEM)

Transmission electron microscopy(TEM) was used to investigate the morphology of the Salmonella bacteriophage. In summary, 10 µL of concentrated bacteriophages (1010-1011 PFU/mL) were spotted on a 200 mesh carbon-coated copper grid and incubated for 1 minute with a 2 percent uranyl-acetate solution (Sigma, Aldrich, UK). Grids were allowed to dry before TEM (Tescan, France) on 120 kV [14], [5], [15]. We also used TEM to capture the Salmonella bacteriophage that was attacking the Salmonella.

2.4 Salmonella bacteriophage encoded virulence genes

The SaMag Kit was used to extract DNA from bacteriophage (Sacace, Biotechnologies, Italy). Table (2), [16], [4], [17], [15].

Table (2) PCR Primer formula to investigate Salmonella bacteriophage encoded virulence genes of Salmonella isolates

Primers	Sequences (5´---------- 3´)	Product size (bp)	Ref.
Inv-A	´ F: 5´- CTGGCGGTGGGTTTTGTTGTCTTCTCTATT- 3´ R: 5´- AGTTTCTCCCCCTCTTCATGCGTTACCC- 3´	284	(52)
sitC	F: 5´- CAGTATATGCTCAACGCGATGTGGGTCTCC - 3´ R: 5´-CGGGGCGAAAATAAAGGCTGTGATGAAC - 3´	768	(53)
invF	F: 5´- AAGGGATCCATGTCATTTTCTGAAAGCGACAC- 3 R: 5´- GTTGTAGGGAAAGCTTCTCCAGTAATG- 3	918	(52)

Inv-A (invasion factor A). ***
Sit-C (Sal. Iron Transporter C). ***
Inv-F (invasion factor F). ***

3. Results

3.1 Detection of Salmonella bacteriophage in vitro

Between January and September 2021, we collected 95 fecal samples from chickens and 75 sewage samples from different places in Baghdad. In a total of 11.7 percent positive, 9 bacteriophages were isolated using

Salmonella typhimurium as the host bacterium by percentage 1.05 % and 11 bacteriophages were identified using Salmonella enteritidis as the host bacteria by percentage 1.29 %. This was accomplished by employing two approaches for bacteriophage identification. The first, a spot test, established the presence of individual lytic bacteriophages by the formation of clearing zones (lytic spots) in plates coated with corresponding Salmonella isolates.

Bacterial challenge experiments were utilized to evaluate the relative capacity of Salmonella bacteriophage to lyse Salmonella in vitro, producing plaques 3-6 mm in diameter surrounded by halos on a Salmonella lawn. Figures (1 and 2).

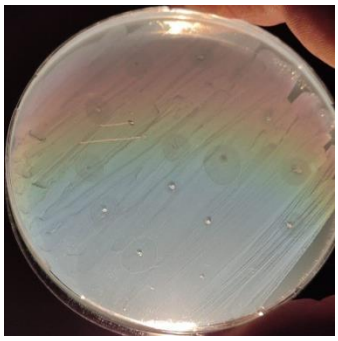

Figure (1) Showed the lytic profile of Bacteriophage to Salmonella isolates

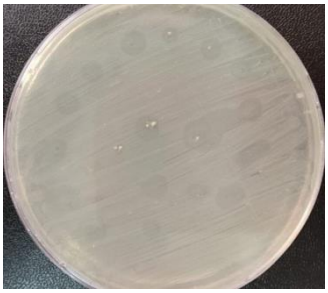

Figure (2) Showed the lytic profile of Bacteriophage to Salmonella isolates

In this study, the bacteriophage showed a high variability, distinguishing some different lysis profiles, forming clear, flat surfaces of varying size with well-defined edges, as shown in Figures (3, 4 and 5), which were defined according to the absence or presence of lysis on Salmonella (enteritidis, typhimurium) as host bacteria.

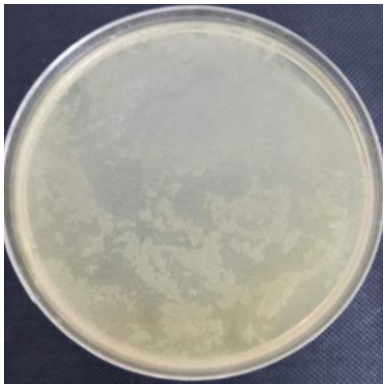

Figure (3) Showed the lytic profile of Bacteriophage to Salmonella isolates

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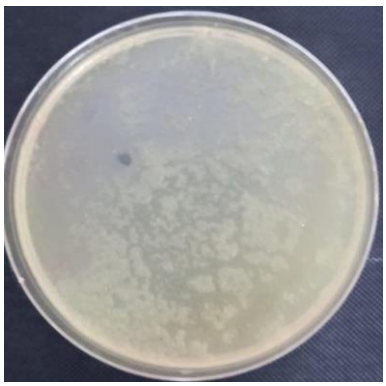

Figure (4) Showed the lytic profile of Bacteriophage to Salmonella isolates

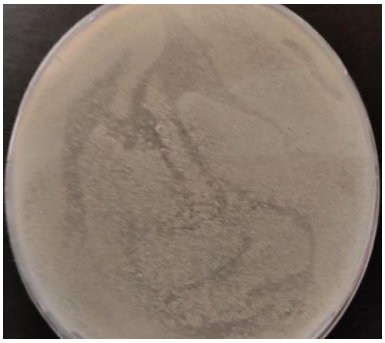

Figure (5) Showed the lytic profile of Bacteriophage to Salmonella isolates

Furthermore, in both diagnostic processes, the majority of the bacteriophages (N=20) displayed a broad lysis profile (spot assay & double layer agar assay).

The (20) isolated bacteriophages in our study showed considerable differences in plaque size and turbidity morphologies.

3.2 Detection of Salmonella Bacteriophages by using Transmission electron microscopy (TEM)

Transmission electron microscopy revealed the existence of an icosahedral head, 140.20 nm, and a contractile tail, 115.25 nm, indicating that it is a member of the Myoviridae family, with scale bars of 100 nm. Figure (6).

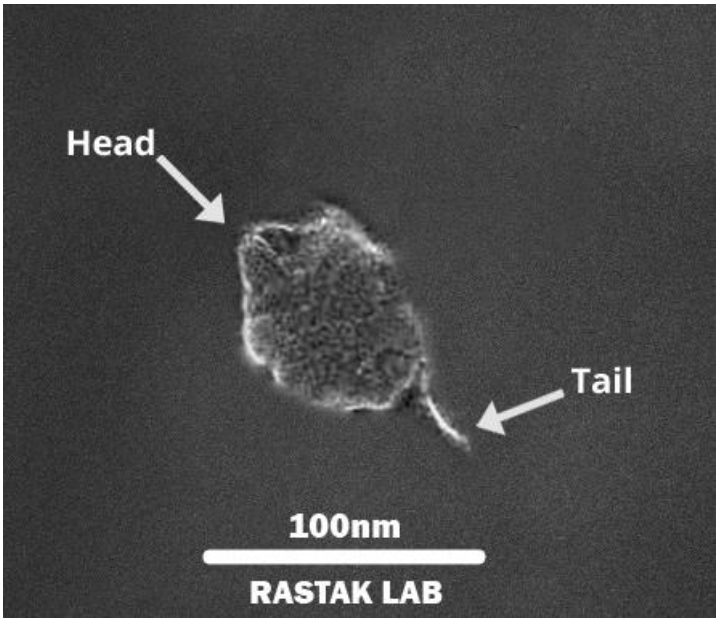

Figure (6) An image showing Salmonella bacteriophage by TEM with scale bars of 100 nm

An image was also captured of the instant when the Salmonella bacteriophage invaded, which was analyzed and photographed using a transmission electron microscope, as shown in Figure (7).

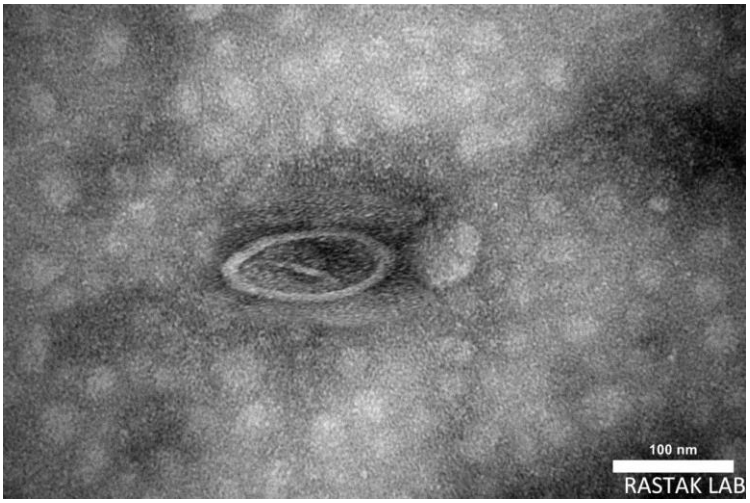

Figure (7) An image showing the moment Salmonella bacteriophage invaded Salmonella by TEM

3.3 Salmonella bacteriophage encoding for virulence genes of Salmonella isolates

According to our results, bacteriophages are capable of encoding Salmonella genes (inv-A, sit-C, inv-F).

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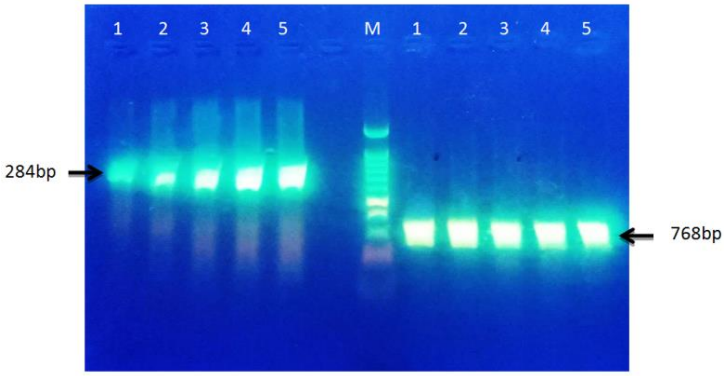

Figure (8) On 1.5 percent agarose gel, the 284 bp amplicon of the inv-A gene was visible, as was the 768 bp amplicon of the Sit-C gene.

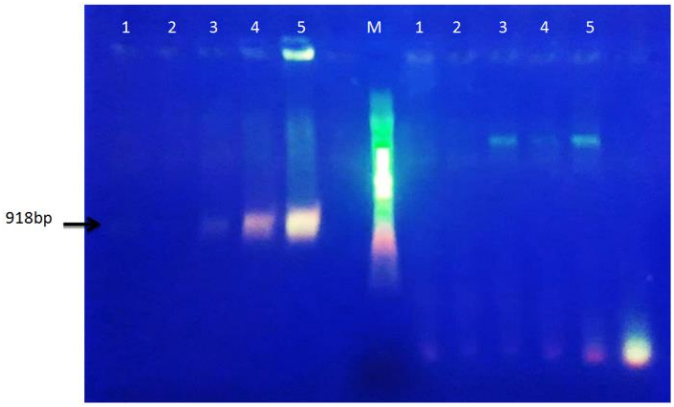

Figure (9) A 1.5 percent agarose gel revealed a 918 bp amplicon of the inv-F gene.

4. Discussion

Bacteriophages (phages) are naturally occurring bacterial population controllers. Bacteriophage proteins are responsible for bacteriophage specificity and pathogenicity. These proteins include: i) adhesins, which recognize specific receptors on the bacterial surface; ii) enzymes, which degrade bacterial cell wall components or bacterial slime; and iii) structural proteins, which comprise the bacteriophage capsid.

Bacteriophage enzymes hydrolyze carbohydrate and protein components, destroying the bacterial cell wall from the inside and the outside. All of these proteins protect bacteriophage genetic material, stimulate bacteriophage proliferation, and assure bacteriophage nucleic acid injection into the bacterial cell [18], [19].

When these bacteriophages were tested using a spot assay and a double layer agar assay, 11.7 percent of the isolated bacteriophages (20 out of 170) produced distinct plaques 3-6 mm in diameter surrounded by halos on a Salmonella lawn and were capable of lysing 17 isolates, according to [20], in the instance of isolation of Staphyloccus aureus bacteriophage from milk in cases of mastitis [21- 23], [5].

Bacteriophage virus must be able to locate and bind to a specific host cell before triggering a mechanism that injects the viral genome (its nucleic acid) into the host cell. The viral nucleic acid is encased in a protein coat known as the capsid, and the majority of bacteriophages have a tail on the capsid via which the viral nucleic acid may be injected into the host cell to infect it [24].

In this investigation, we used lysis profiling to look at the different bacteriophages that infect Salmonella

isolates. The higher efficiency may be attributed to the enrichment isolation procedure, which may have influenced the specific selection of Salmonella and thus of the specific bacteriophages infecting these bacterial isolates; these findings are consistent with [25- 27], [5].

Furthermore, our research demonstrated that diffusion in the two layer agar experiment allows bacteria to completely occupy the grass and bacteriophages to cling to the bacterium [23], [13], [15].

Bacteriophages are self-replicating and self-limiting bacterial viruses that multiply solely at the host's site and are finally k colleagues illed in the absence of host bacteria [28], [1]. Using a lysis efficacy test [29], demonstrated that the bacteriophage may inhibit bacterial growth in the planktonic environment.

Burst size and latency duration have previously been found to be important in defining a bacteriophage's lytic capabilities; moreover, a bacteriophage specific to this large isolate may give a possible precision tool [13], [5].

Double layer agar assay (Plaque assay) One of the most established assays for bacteriophage efficacy determination, is the plaque assay, figures (3, 4, 5). In this method, bacteriophage suspensions are spotted onto bacterial lawns by the double-layer agar assay, and the growth inhibition areas, or plaques, are evaluated. This assay might be well suited for routine and simultaneous testing of multiple candidate bacteriophages and bacterial isolates as it requires only a simple procedure of bacteriophage spotting. Several parameters should be considered when selecting bacteriophages for treatment with the plaque assay. Bacteriophages producing the clearest and largest areas should be favored. Furthermore, expanding plaques can indicate the ability of the bacteriophage to lyse non-dividing cells. However, bacteria colonies within the plaques can indicate pre-existing resistance, and turbid plaques can suggest lysogenic or efficient defence mechanisms of the target bacteria. Final results (ie, plaque-forming units) are usually established within 24 hours or according to the growth rate of the target host. Nevertheless, changes can occur in the plate after evaluation, with the appearance of new plaques or bacterial regrowth [11], [1], [15], [30].

[15] proved, it can only be concluded the bacteriophage has the relatively strict host specificity. The high specificity of bacteriophages is its greatest advantage, and it is also one of the obstacles that limit its practical application. Precise targeting is an important consideration for any prevention and treatment method. The high specificity of bacteriophages is the guarantee of accurate targeting. However, when many different species and interspecies variants need treatment, just like the treatment of typical bacterial infection, the system needs flexibility to target a large part of these variants. Therefore, appropriately broadening and changing the host range of bacteriophage is an important step to realize the application. Although it has been reported that the host spectrum of bacteriophages is changed by engineering methods, but it is limited to several bacteriophages that clarify the mechanism of receptor binding protein adsorption on the host, which is still a long way from practical application.

Generation bacteriophage therapy involves the use of bacteriophage-derived products, such as lytic enzymes. Bacteriophages use lytic enzymes to hydrolyze the cell wall of their host bacteria, allowing for the release of viral progenies. The enzymes, including holins and endolysins, are possible bacteriophage -based pharmaceuticals in the future. In particular, these proteins are seen as potential antimicrobials because of their low-dosage potency and high target specificity similar to bacteriophages. Mass production of lytic enzymes is also easier using traditional recombinant techniques [31]. In recent years, engineered lytic enzymes have been explored to induce highly specific or broad-spectrum cell wall cleavage [30].

Bacteriophage adsorption onto host cells. Understanding the processes involved and the variables influencing them is thus critical for studying host-bacteriophage interactions. This information gives an overview of the bacteriophage, host receptors involved in identification and adsorption, as well as their interactions during attachment. Understanding the entire infection process, beginning with adsorption, can help and speed up our understanding of bacteriophage ecology. To aid with this endeavor, (32), an open access resource that serves as a repository for information on known and newly found bacteriophage receptors [1].

Salmonella bacteriophage morphology was evaluated using TEM, which revealed the presence of an icosahedral head, 140.20 nm, and a contractile tail, 115.25 nm, indicating that it is a member of the Myoviridae family, with scale bars of 100 nm, (figure 6) [14], [5].

Salmonella isolates employ virulence genes to invade cells and begin pathogenicity. Because it comprises sequences that are unique to the genus Salmonella, invasion A (inv A) is one of the most researched virulence factors that is also employed as a biomarker for Salmonella isolate identification. Invasion A is a factor in the outer membrane of Salmonella isolates that is responsible for infection by invading host epithelial cells in the intestines [33].

Were shown to be capable of accurately identifying all Salmonella isolates tested. Salmonella invA gene sequence has been found to be an excellent PCR target with diagnostic potential, and it is fast, sensitive, and accurate for Salmonella identification across a wide spectrum of medical specimens. These results were in line with the conclusions of this investigation. Only pathogenic Salmonella has the virulence invA gene [34]; moreover, [35] said that the invA gene contains unique sequences for the Salmonella genus and has demonstrated to be a viable target for PCR diagnostic purposes.

Salmonella just needs a little amount of nutrients from the host environment to survive and reproduce. Iron is required when Salmonella proliferate rapidly in the body [36]. In vivo, sitC appears to be a major iron transporter [37], along with other iron-containing genes, was thought to have a role in Salmonella pathogenesis by [39], and the sitC gene was found in all isolates [39].

Bacteriophages employed for environmental, industrial, or medicinal objectives should only go through the lytic life cycle to prevent the risk of horizontal virulence gene transmission [29].

It is now possible to evaluate the relevance of integrated viral sequences in bacterial genome diversity. Temperate bacteriophages may, in fact, use site-specific recombination to integrate their host's DNA and transmit vertically to host siblings.

They discovered that an array of factors work synergistically to maintain the microorganism's growth within the host and assist the microorganism in expressing its virulence, which included the sitC gene, which was successfully amplified using PCR in all isolates and plays a role in iron acquisition, in a study on Salmonella enteritidis and Salmonella typhimurium from chicken meat in Egypt.

The findings of this study are similar with prior studies, indicating that these virulence genes are broadly dispersed among Salmonella. The fact that the Salmonella isolates were non-pathogenic may explain this conclusion. This discovery might suggest that sitC was found on virulence plasmids, which are not always present, and that virulence plasmids are serovar-specific, with not all plasmid-bearing serovars including virulence plasmids [40].

Salmonella iron transporter gene (sitC), one of the genes encoding iron acquisition, and other virulence genes [38]. Salmonella isolates that are not virulent have a function in metabolism in the host gut. Stress, environmental changes, and mutation can all cause Salmonella to become virulent, transforming a non-virulent strain into a pathogenic one [41].

A unique Salmonella spp. gene that is essential for the effective entrance of these organisms into cultivated epithelial cells. It is found immediately upstream of the previously described gene invE. Salmonella relies heavily on this gene in non-polar mutations. Salmonella typhimurium relies heavily on this protein to penetrate cultivated epithelial cells. A molecular and functional investigation of the Salmonella typhimurium genetic locus inv,F, which was essential for these organisms to enter cultured mammalian cells, was performed.

This area encodes proteins with strong sequence similarity to proteins from a range of mammalian and plant diseases, all of which can perfectly interact with eukaryotic host cells [42]. According to nucleotide sequence research, invF is the first gene required for Salmonella entrance into cultured epithelial cells. Furthermore, invF regulates the expression of other inv locus members; invF is required for the expression of genus members encoding components of the type secretion apparatus and transcription activators [43].

Furthermore, the positive relationship revealed in this study between resistance and virulence genes shows that antimicrobials may produce selective pressure for co-selection of resistance and virulence determinants [44].

Additionally, the positive association between resistance and virulence genes observed in this study suggests that antimicrobial, may create selective pressure for co-selection of resistance and virulence determinants [44].

Bacteriophages are the most prevalent creatures in the biosphere and a common characteristic of prokaryotic life. Bacteriophages have piqued the interest of scientists as fundamental molecular biology tools, vectors of horizontal gene transfer and drivers of bacterial evolution, suppliers of diagnostic and genetic tools, and potential therapeutic agents. Understanding microbial systems and their exploitation requires.

Lysogenic conversion has long been recognized as contributing additional functionality to the host genome, particularly in bacterial pathogen genomes where prophages are critical for virulence. Despite the fact that bacteriophages are known to play a key role in bacterial fitness and pathogenicity [45], [15].

Several genes encoding components important for DNA replication and nucleotide metabolism are likely implicated in the bacteriophage's ability to infect a wide range of hosts [46], [47].

Transduction efficiencies were examined to rule out whether this bacteriophage could transduce resistance genes to a substantial level, which would have been a major barrier to its employment as a biocontrol agent [13].

In the aim of self-preservation, Bacteriophage has fine-tuned the timing of excision to balance propagation and lateral transduction, promoting host growth through gene transfer and creating infectious bacteriophage particles that are ejected following bacterial cell rupture. When bacterial DNA is wrapped into bacteriophage heads, transducing particles are created as well. This results in a manner of transduction that

provides normal bacteriophage titers while also transferring bacterial chromosomal DNA at significantly higher frequencies than previously recorded for known host gene transfer mechanisms. These discoveries have the potential to radically change our knowledge of the roles bacteriophages play in bacterial evolution, including the development of antibiotic resistance and virulence in clinical strains [48], [5].

Bacteriophages have sparked the interest of scientists as fundamental molecular biology tools, vectors of horizontal gene transfer and drivers of bacterial evolution, providers of diagnostic and genetic tools, and possible therapeutic agents.

Understanding and exploiting microbial systems necessitates studying the biology of bacteriophages and their interactions with their hosts. A different relationship exists between hosts and bacteriophages, in which bacteriophages have a temperate life cycle and contribute to the success of their host. bacterium by encoding useful genes [49- 51].

5. Conclusions

This study indicated the detection of Salmonella bacteriophage from feces of Broiler chickens and sewage were showed the prevalence of Salmonella bacteriophage at (20) out 170 samples of feces from chickens suffering from diarrhea in Baghdad city and sewage by percentage 11.7 % divided to (9) (1.05%) Salmonella typhimurium, (11) (1.29%) Salmonella enteritidis, also Salmonella bacteriophage were tested against the most commonly isolated Salmonella strains. Bacteriophage demonstrated potent lytic activity against Salmonella isolates (enteritidis, typhimurium) by forming a clear and transparent zone in the spot and double layer agar assays, implying that bacterial challenge assays were used to assess the relative ability of Bacteriophage to lyse Salmonella in vitro, producing plaques 3-6 mm in diameter surrounded by halos on a Salmonella lawn.

Using transmission electron microscopy to diagnose salmonella, revealed the existence of an icosahedral head, 140.20 nm, and a contractile tail, 115.25 nm, indicating that it is a member of the Myoviridae family. Salmonella bacteriophage encode genes (inv-A, sit-C, inv-F) of our Salmonella isolates by using PCR assay.

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6. References

[1] Egido JE, Costa AR, Aparicio-Maldonado C, Haas PJ, Brouns SJ. Mechanisms and clinical importance of bacteriophage resistance. FEMS microbiology reviews. 2022 Jan;46(1):fuab048.https://doi.org/10.1093/femsre/fuab048.

[2] Lamy-Besnier Q, Chaffringeon L, Lourenço M, Payne RB, Trinh JT, Schwartz JA, Sulakvelidze A, Debarbieux L. Prophylactic Administration of a Bacteriophage Cocktail Is Safe and Effective in Reducing Salmonella enterica Serovar Typhimurium Burden in Vivo. Microbiology spectrum. 2021 Aug 25;9(1):e00497-21. https://doi.org/10.1128/Spectrum.00497-21

[3] Żbikowska K, Michalczuk M, Dolka B. The use of bacteriophages in the poultry industry. Animals. 2020 May;10(5):872. https://doi.org/10.3390/ani10050872

[4] Bohm K, Porwollik S, Chu W, Dover JA, Gilcrease EB, Casjens SR, McClelland M, Parent KN. Genes affecting progression of bacteriophage P22 infection in Salmonella identified by transposon and single gene deletion screens. Molecular microbiology. 2018 May;108(3):288-305. https://doi.org/10.1111/mmi.13936

[5] Ilyas SZ, Tariq H, Basit A, Tahir H, Haider Z, Rehman SU. SGP-C: A Broad Host Range Temperate Bacteriophage; Against Salmonella gallinarum. Frontiers in microbiology. 2021;12:768931-. https://dx.doi.org/10.3389%2Ffmicb.2021.768931

[6] Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. FEMS microbiology letters. 2016 Feb 1;363(4):fnw002. https://doi.org/10.1093/femsle/fnw002

[7] Adams MH. Methods of study of bacterial viruses. Bacteriophages. Interscience Publishers, New York, 1959; pp. 443-457. https://cir.nii.ac.jp/crid/1571698600607997312

[8] Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. InBacteriophages 2009 (pp. 69-76). Humana Press. https://doi.org/10.1007/978-1-60327-164-6_7

[9] Mohamed A, Taha O, El-Sherif HM, Connerton PL, Hooton S, Bassim ND, Connerton IF, El-Shibiny A. Bacteriophage ZCSE2 is a Potent Antimicrobial against Salmonella enterica Serovars: Ultrastructure, genomics and efficacy. Viruses. 2020 Apr;12(4):424. https://doi.org/10.3390/v12040424

[10] Owen SV, Wenner N, Dulberger CL, Rodwell EV, Bowers-Barnard A, Quinones-Olvera N, Rigden DJ, Rubin EJ, Garner EC, Baym M, Hinton JC. Prophage-encoded phage defense proteins with cognate self-immunity. bioRxiv. 2021 Jan 1:2020-07. https://doi.org/10.1101/2020.07.13.199331

[11] Gelman D, Yerushalmy O, Alkalay-Oren S, Rakov C, Ben-Porat S, Khalifa L, Adler K, Abdalrhman M, Coppenhagen-Glazer S, Aslam S, Schooley RT. Clinical Phage Microbiology: A suggested framework and recommendations for the in-vitro matching steps of phage therapy. The Lancet Microbe. 2021 Oct 1;2(10):e555-63. https://doi.org/10.1016/S2666-5247(21)00127-0

[12] Sattar S, Ullah I, Khanum S, Bailie M, Shamsi B, Ahmed I, Shah TA, Javed S, Ghafoor A, Pervaiz A, Sohail F. Genome Analysis and Therapeutic Evaluation of a Novel Lytic Bacteriophage of Salmonella Typhimurium: Suggestive of a New Genus in the Subfamily Vequintavirinae. Viruses. 2022 Feb;14(2):241. https://doi.org/10.3390/v14020241.

[13] Rivera D, Moreno-Switt AI, Denes TG, Hudson LK, Peters TL, Samir R, Aziz RK, Noben JP, Wagemans J, Dueñas F. Novel Salmonella Phage, vB_Sen_STGO-35-1, Characterization and Evaluation in Chicken Meat. Microorganisms. 2022 Mar 12;10(3):606. https://doi.org/10.3390/microorganisms10030606

[14] Rodwell EV, Wenner N, Pulford CV, Cai Y, Bowers-Barnard A, Beckett A, Rigby J, Picton DM, Blower TR, Feasey NA, Hinton JC. Isolation and characterisation of bacteriophages with activity against invasive non-typhoidal Salmonella causing bloodstream infection in Malawi. Viruses. 2021 Mar;13(3):478. https://doi.org/10.3390/v13030478

[15] Sun Z, Wen H, Ma L, Chen Z. Isolation, characterization and application of bacteriophage PSDA-2

against Salmonella Typhimurium in chilled mutton. PloS one. 2022 Jan 24;17(1):e0262946. https://doi.org/10.1371/journal.pone.0262946.

[16] Diard M, Bakkeren E, Cornuault JK, Moor K, Hausmann A, Sellin ME, Loverdo C, Aertsen A, Ackermann M, De Paepe M, Slack E. Inflammation boosts bacteriophage transfer between Salmonella spp. Science. 2017 Mar 17;355(6330):1211-5. https://doi.org/10.1126/science.aaf8451

[17] Adler BA, Kazakov AE, Zhong C, Liu H, Kutter E, Lui LM, Nielsen TN, Carion H, Deutschbauer AM, Mutalik VK, Arkin AP. The Genetic Basis of phage susceptibility, cross-resistance and host-range in Salmonella. Microbiology. 2021 Dec 15;167(12):001126. https://doi.org/10.1099/mic.0.001126

[18] Sabri JB, Al-Sultan II, Altaif K, Peter S, Saadh MJ. Pathogenesis of Salmonella enterica serovar Albany in experimental infected SPF BALB/c Mice. Iraqi Journal of Veterinary Sciences. 2020 Jun 9;34(2):339-44. http://dx.doi.org/10.33899/ijvs.2019.126269.1282

[19] Brzozowska E, Bazan J, Gamian A. Funkcje białek bakteriofagowych* The functions of bacteriophage proteins. Postepy Hig Med Dosw (online). 2011;65:167-76. https://pubmed.ncbi.nlm.nih.gov/21502693/#:~:text=doi%3A%2010.5604/17322693.936090.

[20] Mahhdii EF, The use of Bacteriophage as Alternative Method to Treat Mastitis Caused by Staphylococcus aureus in Mice. [Master thesis] College of Veterinary Medicine, at University of Baghdad; 2015, 62.

[21] Islam M, Zhou Y, Liang L, Nime I, Liu K, Yan T, Wang X, Li J. Application of a phage cocktail for control of Salmonella in foods and reducing biofilms. Viruses. 2019 Sep;11(9):841. https://doi.org/10.3390/v11090841

[22] Toprak ZT, Şanlıbaba P. Application of Phage for Biocontrol of Salmonella Species in Food Systems. Turkish Journal of Agriculture-Food Science and Technology. 2020 Oct 19;8(10):2214-21. https://doi.org/10.24925/turjaf.v8i10.2214-2221.3689

[23] Shang Y, Sun Q, Chen H, Wu Q, Chen M, Yang S, Du M, Zha F, Ye Q, Zhang J. Isolation and characterization of a novel Salmonella phage vB_SalP_TR2. Frontiers in microbiology. 2021 Jun 21;12:1452. https://doi.org/10.3389/fmicb.2021.664810

[24] Fokine A, Khare B, Sun Y, Rossmann MG. Enhancement of tomogram interpretability using the locked self-rotation function. Journal of structural biology. 2019 Mar 1;205(3):53-8. https://doi.org/10.1016/j.jsb.2019.01.006

[25] Cortes P, Spricigo DA, Bardina C, Llagostera M. Remarkable diversity of Salmonella bacteriophages in swine and poultry. FEMS Microbiology Letters. 2015;362(2):1-7. https://doi.org/10.1093/femsle/fnu034.

[26] Esmael A, Azab E, Gobouri AA, Nasr-Eldin MA, Moustafa M, Mohamed SA, Badr OA, Abdelatty AM. Isolation and characterization of two lytic bacteriophages infecting a multi-drug resistant Salmonella Typhimurium and their efficacy to combat salmonellosis in ready-to-use foods. Microorganisms. 2021 Feb;9(2):423. https://doi.org/10.3390/microorganisms9020423

[27] Malik S, Chauhan R, Laddika L, Qureshi S, Kumar B, Tiwari AK. Isolation and Partial Characterization of Bacteriophage against Salmonella Gallinarum. Journal homepage: http://www. ijcmas. com.;10(03):2021. https://doi.org/10.20546/ijcmas.2021.1003.018

[28] Huh H, Wong S, Jean JS, Slavcev R. Bacteriophage interactions with mammalian tissue: Therapeutic applications. Advanced drug delivery reviews. 2019 May 1;145:4-17. https://doi.org/10.1016/j.addr.2019.01.003

[29] Kwon J, Kim SG, Kim HJ, Giri SS, Kim SW, Lee SB, Park SC. Isolation and characterization of Salmonella jumbo-phage pSal-SNUABM-04. Viruses. 2020 Dec 25;13(1):27. https://doi.org/10.3390/v13010027

[30] Carascal MB, Remenyi R, Cruz MC, Destura RV. Phage Revolution Against Multidrug-Resistant Clinical Pathogens in Southeast Asia. Frontiers in microbiology. 2022:34. https://doi.org/10.3389/fmicb.2022.820572

[31] Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World journal of gastrointestinal pharmacology and therapeutics. 2017 Aug 6;8(3):162. https://doi.org/10.4292%2Fwjgpt.v8.i3.162

[32] Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. FEMS microbiology letters. 2016 Feb 1;363(4):fnw002. https://doi.org/10.1093/femsle/fnw002

[33] El-Sebay NA, Abu Shady HM, El-Rashed El-Zeedy SA, Samy AA. InvA gene sequencing of Salmonella Typhimurium isolated from Egyptian poultry. Asian J. Sci. Res. 2017;10(3):194-202. https://dx.doi.org/10.3923/ajsr.2017.194.202

[34] Karmi M. Detection of virulence gene (invA) in Salmonella isolated from meat and poultry products. Int. J. Genet. 2013;3(2):7-12. https://doi10.5829/idosi.ijg.2013.3.2.82204

[35] Chagas FF, Crispim BA, Oliveira KD, Grisolia AB. Identification and detection of Salmonella strains isolated from chicken carcasses and environmental sources in Dourados, MS, Brazil. Afr J Microbiol Res. 2013;7(25):3222-8. http://dx.doi.org/10.5897/AJMR12.830

[36] Gulig PA. Virulence plasmids of Salmonella typhimurium and other salmonellae. Microbial pathogenesis. 1990 Jan 1;8(1):3-11. https://doi.org/10.1016/0882-4010(90)90003-9

[37] Janakiraman A, Slauch JM. The putative iron transport system SitABCD encoded on SPI1 is required for full virulence of Salmonella typhimurium. Molecular microbiology. 2000 Mar;35(5):1146-55. https://doi.org/10.1046/j.1365-2958.2000.01783.x

[38] Skyberg JA, Logue CM, Nolan LK. Virulence genotyping of Salmonella spp. with multiplex PCR. Avian diseases. 2006 Mar;50(1):77-81. https://doi.org/10.1637/7417.1

[39] 49. Tarabees R, Elsayed MS, Shawish R, Basiouni S, Shehata AA. Isolation and characterization of Salmonella Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. The Journal of Infection in Developing Countries. 2017 Apr 30;11(04):314-9. https://doi.org/10.3855/jidc.8043

[40] Nde CW, Logue CM. Characterization of antimicrobial susceptibility and virulence genes of Salmonella serovars collected at a commercial turkey processing plant. Journal of applied microbiology. 2008 Jan;104(1):215-23. https://doi.org/10.1111/j.1365-2672.2007.03535.x

[41] Mthembu TP, Zishiri OT, El Zowalaty ME. Detection and molecular identification of Salmonella virulence genes in livestock production systems in South Africa. Pathogens. 2019 Sep;8(3):124. https://doi.org/10.3390/pathogens8030124

[42] Ginocchio C, Pace J, Galan JE. Identification and molecular characterization of a Salmonella typhimurium gene involved in triggering the internalization of salmonellae into cultured epithelial cells. Proceedings of the National Academy of Sciences. 1992 Jul 1;89(13):5976-80. https://doi.org/10.1073/pnas.89.13.5976

[43] Darwin KH, Miller VL. The putative invasion protein chaperone SicA acts together with InvF to activate the expression of Salmonella typhimurium virulence genes. Molecular microbiology. 2000 Feb;35(4):949-60. https://doi.org/10.1046/j.1365-2958.2000.01772.x

[44] Fluit AC. Towards more virulent and antibiotic-resistant Salmonella?. FEMS Immunology & Medical Microbiology. 2005 Jan 1;43(1):1-1. https://doi.org/10.1016/j.femsim.2004.10.007

[45] Wahl A, Battesti A, Ansaldi M. Prophages in Salmonella enterica: a driving force in reshaping the genome and physiology of their bacterial host?. Molecular Microbiology. 2019 Feb;111(2):303-16. https://doi.org/10.1111/mmi.14167

[46] Yuan Y, Gao M. Jumbo bacteriophages: an overview. Frontiers in microbiology. 2017 Mar 14;8:403. https://doi.org/10.3389/fmicb.2017.00403

[47] Olszak T, Danis-Wlodarczyk K, Arabski M, Gula G, Maciejewska B, Wasik S, Lood C, Higgins G, Harvey BJ, Lavigne R, Drulis-Kawa Z. Pseudomonas aeruginosa PA5oct jumbo phage impacts planktonic and biofilm population and reduces its host virulence. Viruses. 2019 Dec;11(12):1089. https://doi.org/10.3390/v11121089

[48] Fillol-Salom A, Bacigalupe R, Humphrey S, Chiang YN, Chen J, Penadés JR. Lateral transduction is inherent to the life cycle of the archetypical Salmonella phage P22. Nature communications. 2021 Nov 8;12(1):1-2. https://doi.org/10.1038/s41467-021-26520-4

[49] Clokie MR, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage 1: 31–45. https://doi.org/10.4161%2Fbact.1.1.14942

[50] Saleh Z, Al-Muhana BM, Hamdan K, Jawad MS, Klaif SF. Isolation and identification of Salmonella typhimurium bacteria with detection of type-1 fimbriae coding gene by polymerase chain reaction (PCR) technique. Iraqi Journal of Veterinary Sciences. 2019 Sep 1;33(2):195-9. http://dx.doi.org/10.33899/ijvs.2019.162961

[51] Sharif YM, Tayeb BA. Estimation of limit of detection of Salmonella typhimurium in artificially contaminated chicken meat by cultured-based and polymerase chain reaction techniques. Iraqi Journal of Veterinary Sciences. 2021 Aug 25;35(4):621-5. http://dx.doi.org/10.33899/ijvs.2020.127328.1496

[52] William UD, Hang'ombe B, Victor ZC, King NS. Distribution of virulence genes and antimicrobial susceptibility of Salmonella isolated from dogs and chickens in Zambia. 2012 IJAVMS, 6(5):360- 367. http://dx.doi.org/10.5455/ijavms.170

[53] Tarabees R, Elsayed MS, Shawish R, Basiouni S, Shehata AA. Isolation and characterization of Salmonella Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. The Journal of Infection in Developing Countries. 2017 Apr 30;11(04):314-9. https://doi.org/10.3855/jidc.8043

[54] Jalil, A. T., Kadhum, W. R., Khan, M. U. F., Karevskiy, A., Hanan, Z. K., Suksatan, W., ... & Abdullah, M. M. (2021). Cancer stages and demographical study of HPV16 in gene L2 isolated from cervical cancer in Dhi-Qar province, Iraq. Applied Nanoscience, 1-7. https://doi.org/10.1007/s13204-021-01947-9

[55] Widjaja, G., Jalil, A. T., Rahman, H. S., Abdelbasset, W. K., Bokov, D. O., Suksatan, W., ... & Ahmadi, M. (2021). Humoral Immune mechanisms involved in protective and pathological immunity during COVID-19. Human Immunology. https://doi.org/10.1016/j.humimm.2021.06.011

[56] Moghadasi, S., Elveny, M., Rahman, H. S., Suksatan, W., Jalil, A. T., Abdelbasset, W. K., ... & Jarahian, M. (2021). A paradigm shift in cell-free approach: the emerging role of MSCs-derived exosomes in regenerative medicine. Journal of Translational Medicine, 19(1), 1-21. https://doi.org/10.1186/s12967-021-02980-6

[57] Saleh, M. M., Jalil, A. T., Abdulkereem, R. A., & Suleiman, A. A .(2020). Evaluation of Immunoglobulins, CD4/CD8 T Lymphocyte Ratio and Interleukin-6 in COVID-19 Patients. TURKISH JOURNAL of IMMUNOLOGY, 8(3), 129-134. https://doi.org/10.25002/tji.2020.1347

[58] Turki Jalil, A., Hussain Dilfy, S., Oudah Meza, S., Aravindhan, S., M Kadhim, M., & M Aljeboree, A. (2021). CuO/ZrO2 nanocomposites: facile synthesis, characterization and photocatalytic degradation of tetracycline antibiotic. Journal of Nanostructures. https://doi.org/10.22052/JNS.2021.02.014

[59] Sarjito, Elveny, M., Jalil, A., Davarpanah, A., Alfakeer, M., Awadh Bahajjaj, A. & Ouladsmane, M. (2021). CFD-based simulation to reduce greenhouse gas emissions from industrial plants. International Journal of Chemical Reactor Engineering, 20210063. https://doi.org/10.1515/ijcre-2021-0063

[60] Marofi, F., Rahman, H. S., Al-Obaidi, Z. M. J., Jalil, A. T., Abdelbasset, W. K., Suksatan, W., ... & Jarahian, M. (2021). Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients. Stem Cell Research & Therapy, 12(1), 1-23. https://doi.org/10.1186/s13287-021-02420-8

[61] Jalil, A. T., Shanshool , M. T. ., Dilfy, S. H. ., Saleh, M. M., & Suleiman, A. A. . (2022). HEMATOLOGICAL AND SEROLOGICAL PARAMETERS FOR DETECTION OF COVID-19. Journal of Microbiology, Biotechnology and Food Sciences, e4229. https://doi.org/10.15414/jmbfs.4229

[62] Vakili-Samiani, S., Jalil, A. T., Abdelbasset, W. K., Yumashev, A. V., Karpisheh, V., Jalali, P., ... & Jadidi-Niaragh, F. (2021). Targeting Wee1 kinase as a therapeutic approach in Hematological Malignancies. DNA repair, 103203. https://doi.org/10.1016/j.dnarep.2021.103203

[63] NGAFWAN, N., RASYID, H., ABOOD, E. S., ABDELBASSET, W. K., Al-SHAWI, S. G.,

BOKOV, D., & JALIL, A. T. (2021). Study on novel fluorescent carbon nanomaterials in food analysis. Food Science and Technology. https://doi.org/10.1590/fst.37821

[64] Marofi, F., Abdul-Rasheed, O. F., Rahman, H. S., Budi, H. S., Jalil, A. T., Yumashev, A. V., ... & Jarahian, M. (2021). CAR-NK cell in cancer immunotherapy; A promising frontier. Cancer Science, 112(9), 3427. https://doi.org/10.1111/cas.14993

[65] Abosaooda, M., Wajdy, J. M., Hussein, E. A., Jalil, A. T., Kadhim, M. M., Abdullah, M. M., ... & Almashhadani, H. A. (2021). Role of vitamin C in the protection of the gum and implants in the human body: theoretical and experimental studies. International Journal of Corrosion and Scale Inhibition, 10(3), 1213-1229. https://dx.doi.org/10.17675/2305-6894-2021-10-3-22

[66] Jumintono, J., Alkubaisy, S., Yánez Silva, D., Singh, K., Turki Jalil, A., Mutia Syarifah, S., ... & Derkho, M. (2021). Effect of Cystamine on Sperm and Antioxidant Parameters of Ram Semen Stored at 4° C for 50 Hours. Archives of Razi Institute, 76(4), 923-931. https://dx.doi.org/10.22092/ari.2021.355901.1735

[67] Raya, I., Chupradit, S., Kadhim, M. M., Mahmoud, M. Z., Jalil, A. T., Surendar, A., ... & Bochvar, A. N. (2021). Role of Compositional Changes on Thermal, Magnetic and Mechanical Properties of Fe-PC-Based Amorphous Alloys. Chinese Physics B. https://doi.org/10.1088/1674-1056/ac3655

[68] Chupradit, S., Jalil, A. T., Enina, Y., Neganov, D. A., Alhassan, M. S., Aravindhan, S., & Davarpanah, A. (2021). Use of Organic and Copper-Based Nanoparticles on the Turbulator Installment in a Shell Tube Heat Exchanger: A CFD-Based Simulation Approach by Using Nanofluids. Journal of Nanomaterials. https://doi.org/10.1155/2021/3250058

[69] Mohaddeseh Rahbaran, Ehsan Razeghian, Marwah Suliman Maashi, Abduladheem Turki Jalil, Gunawan Widjaja, Lakshmi Thangavelu, Mariya Yurievna Kuznetsova, Pourya Nasirmoghadas, Farid Heidari, Faroogh Marofi, Mostafa Jarahian, "Cloning and Embryo Splitting in Mammalians: Brief History, Methods, and Achievements", Stem Cells International, vol. 2021, Article ID 2347506, 11 pages, 2021. https://doi.org/10.1155/2021/2347506

[70] Jalil, A.T.; Ashfaq, S.; Bokov, D.O.; Alanazi, A.M.; Hachem, K.; Suksatan, W.; Sillanpää, M. High-Sensitivity Biosensor Based on Glass Resonance PhC Cavities for Detection of Blood Component and Glucose Concentration in Human Urine. Coatings 2021, 11, 1555. https://doi.org/10.3390/coatings11121555

[71] Chupradit, S.; Ashfaq, S.; Bokov, D.; Suksatan, W.; Jalil, A.T.; Alanazi, A.M.; Sillanpaa, M. Ultra-Sensitive Biosensor with Simultaneous Detection (of Cancer and Diabetes) and Analysis of Deformation Effects on Dielectric Rods in Optical Microstructure. Coatings 2021, 11, 1564. https://doi.org/10.3390/coatings11121564

[72] Bokov, D., Turki Jalil, A., Chupradit, S., Suksatan, W., Javed Ansari, M., Shewael, I. H., ... & Kianfar, E. (2021). Nanomaterial by Sol-Gel Method: Synthesis and Application. Advances in Materials Science and Engineering, 2021. https://doi.org/10.1155/2021/5102014

[73] Shabgah, A. G., Al-Obaidi, Z. M. J., Rahman, H. S., Abdelbasset, W. K., Suksatan, W., Bokov, D.

O., ... & Navashenaq, J. G. (2022). Does CCL19 act as a double-edged sword in cancer development?. Clinical and Experimental Immunology, 20, 1-12. , https://doi.org/10.1093/cei/uxab039

[74] Kartika, R., Alsultany, F. H., Jalil, A. T., Mahmoud, M. Z., Fenjan, M. N., & Rajabzadeh, H. (2021). Ca12O12 nanocluster as highly sensitive material for the detection of hazardous mustard gas: Density-functional theory. Inorganic Chemistry Communications,109174. https://doi.org/10.1016/j.inoche.2021.109174