Plant Archives Volume 20 No. 2, 2020 pp. 5583-5590 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DAIRY CHAIN PHAGES COCKTAILS AGAINST EXTENDED SPECTRUM BETA LACTAMASE RESISTANT *STAPHYLOCOCCUS AUREUS*

Jinan Sahib Abdulnabi Al-Naseri* and Ali Hassan Ahmed Al-Shammary

Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract

New augmented and redirected shifted reality to nano bio preservation of food cascaded by nano therapy module against super established entities of clustered regularly interspaced short palindromic repeats, biofilm producing and multidrug resistant foodborne pathogens and their toxins and enzymes unveiled massive requirements for new directed potential hygienic policy and so on food safety and public health. Naturally foodborne bio life and bio sense God molecules directed toward recalcitrant pathogens, represented by cocktails of lytic bacteriophages recovered from dairy series ecosystem in Baghdad shifted the battle to another side as a spirit of wisdom in the heart of the sea. Receptors specific custom combo decorum regime was dependent to verify recovered phages cocktails in which, plaques-spots Ø pattern morphology assay was dependent with modified molecular scanning crystallography micrographs dependent VEGA 3 TESCAN laser electron microscopy for confirmation of specificity and sensitivity of recovered phages against encapsulated biofilm strains of *S. aureus*. Frequency and distribution pattern unveiled segregation of twenty-two phages out of sixty samples (36.67 %) in which, ultra-one was recovered from fresh yogurt in Abu-Ghraib. In conclusion, terminating recalcitrant foodborne pathogens needs new potential strategies such as natural antimicrobial resources from food chain.

Key words : Dairy chain, Bacteriophages, Multidrug-Resistance, *Staphylococcus aureus*.

Introduction

Many of the remaining challenges in infectious disease control involve pathogens that fail to elicit long-lasting immunity in their hosts. Antigenic variation is a common reason for this failure and a contributor to the complexity, and often correctly, invoked to explain antigenic variability in pathogens. However, there is a wide variety of patterns of antigenic variation across space, time within, and between hosts, and we do not yet understand the determinants of these different patterns. This project describes such patterns of drifting and shifting mechanisms in extended spectrum beta lactamase resistant strains (ESβLs) that might occurs during symposia ecosystem interconnected interactions among *S. aureus* with environment, host and other Eco biota. Pathogen-specific explanations for these patterns of diversity are critically evaluated, and the patterns are compared against predictions of theoretical models for antigenic diversity. Major remaining challenges highlighted, including the identification of key protective antigens in study model, the design of vaccines to combat antigenic variability for shifting and drifting like in influenza viruses and the development of more systematic explanations for patterns of antigenic variation (Lipsitch and Hagan, 2007 and Pal *et al.,* 2020). Stress adaptation in genetically and phenotypically modified, diverse and versatile clones of methicillin and vancomycin resistant *S. aureus* lineage-complex ancestral denominators due to dual phased quorum sensing and sigma factors interconnected with acquisition of extra sharing forbidden sets of genes and antibiotics resistance profile for extended spectrum beta lactamases and carbapenemases associated with sophisticated clustered regularly interspaced short palindromic repeats throughout conjugation bridged plasmids, transduction prophages and transformation with environmental DNA strands throughout a years in Iraqi food chain ecosystem represent

***Author for correspondence :** E-mail: Jinan.shb79@gmail.com

dangerous emergency policy in healthy lifestyle. Molecular remarks for translocation from one environment to another give us direct and indirect arrays about regulation and responding to different insults of stressors. Translocation of stress-adapted foodborne pathogens from food ecosystem to human body might regulate cross response sense genes and proteins to re-modulate and accommodate their behaviors in new ecosystem. Broad-spectrum elasticity of the genome of these opportunistic pathogens with versatile and diverse nature of acquisition of new genes from environment by genes sharing mechanisms regulated by quorum sensing and sigma factors as expressed under one protective encapsulated biofilm tent or pavilion might exaggerate the verification steps of HACCP strategy to reduce their emergent risks (Labrie *et al.,* 2010; Kanaan and Al-Shammary, 2013; Xia and Wolz, 2014; Begley and Hill, 2015; Rath *et al.,* 2015; Al-Shammary, 2019 and Pal *et al.,* 2020).

Bacteriophages and pathogenicity islands inside clonal selection lineage of *S. aureus* accounts for the most genotypic and phenotypic differences between and within their isomers. Acquired behaviors of antigenic drift and shift in these clones (like in influenza or corona covid-19 entities) by these translocated genetic elements provide the transformed clone into the case of forbidden gadget-match for their diversity to adapt to different ecosystems throughout coding for diverse virulence factors such as Panton-Valentine leukocidin, staphylokinase, enterotoxins, chemotaxis-inhibitory proteins, and exfoliative toxins. Switching to highly coordinated manner for transferring of different genes as a primary vehicle for chromosomal and extra milieu by vertical versus horizontal shifting terminate the final destiny of verified phages. Self-replicating entities of foodborne lytic phages cocktails in association with antibiotics brands constitute a new module strategy for fighting these developed chimeras. Diversity of these interconnected cocktails of phages driven by their dynamic adaptation when facing selective pressure such as phage resistance mechanisms, which are widespread in bacterial hosts. When infecting bacterial cells, phages face a range of antiviral mechanisms, and they have evolved multiple tactics to avoid, circumvent or subvert these mechanisms in order to thrive in most environments. Combo verified modules were shifting to fighting the recalcitrant biofilm electromagnetic cloud-barrier of these entities, as they are inherently refractory to many types of antibiotics sequel with resistance problems versus residues drawbacks. Hazards successful foodborne S. aureus shift the battle toward the persistent behavior causing serious checkerboard points to consumer and food industry. Reduce efficacy of antibiotics with emergence of resistant clones and phenotypes has limited the opportunities for controlling them in food commodities and treating food borne infections. Therefore new hurdle technology module with living and safe bio nano molecules and their derivatives will open the framework about new verified era of hygiene (Labrie *et al.,* 2010; Rio *et al.,* 2010; Li and Zhang, 2014; Sangha *et al.,* 2014; Xia and Wolz, 2014; Abdulamir *et al.,* 2015; Ali and Jamalludeen, 2015; Jensen *et al.,* 2015; Rath *et al.,* 2015; Rasool *et al.,* 2016; Abata Angelo *et al.,* 2017; Barrera-Rivas *et al.,* 2017; Malik *et al.,* 2017; Premarathne *et al.,* 2017; Hill *et al.,* 2018; Alvarez *et al.,* 2019; Dakheel *et al.,* 2019; Dickey and Perrot, 2019; Moller *et al.,* 2019).

The gadget match of this structural milieu was to investigate the potential module of recovered bacteriophages (specificality and sensitivity) from dairy series on recalcitrant biofilm producing and multidrug resistant *S. aureus* recovered from the same niche ecosystem in Baghdad.

Materials and Methods

Module 1

Thesis dependent guidelines under Supervisor authority module was facilitate recovery pattern of denominator. Deciphered checkerboard pattern of biofilm producing and multidrug resistant clones of *S. aureus* from programmed ecomap segregation dairy chain ecosystem of Cows raw and mastitic milk, fresh homemade and purchased soured yogurts from markets, and fresh homemade and brined purchased soft cheeses with its whey from markets. Sixty predisposed pooled samples of six brands were dependent for isolation, identification and confirmation of denominator with tested developed behaviors of biofilm entity and multidrug resistance sensed power of isolates with confirmatory module as setup plan in thesis under Supervisor guidelines. Verified scheduled worksheet cascaded by workstation methodology architecture were dependent for recovery module in which, modified tryptone-soya yeast-extract broth support enrollment enriched strategy cascade by modified mannitol salt agars with modified tryptone-soya yeast-extract agar facilitate recovery module of targeted clone. Modified tissue culture microtiter plate assay declared by Christensen *et al*., (1985) with two stains was dependent for assaying ability of isolate to produce and secrete biofilm-slim layers with score forming unit. Based on authorized supervisor experience and instructions of national committee of clinical laboratory standards (NCCLS) formerly clinical laboratory standards

institute (CLSI, 2013-2019) guidelines followed in this account of the Kirby-Bauer disc diffusion method (Bauer, 1966) study the sensitivity of isolates to selected and grouped antibiotics (33 antibiotics) to ensure phenotypically epigenetic drift tolerance or genetically shifted CRISPR-CAS resistant entities with biofilm persisters clones behaviors module. Selected zones within specified episodes were dependent in Baghdad in which, twenty series of dairy brands were considered from different markets and locations in Al-Fudhaliyah, Al-Sadrya and Abu-Ghraib as two units from each verified brand per month within January to June (2019). IMMUCEL California mastitis periodic test and table was dependent for screening of normal from mastitic milk units.

Extended spectrum beta lactamase or Carbapenemases resistance activity was determined in food laboratory for recovered isolates by modified double diffusion inhibition technique or Oxoid Cefpodoxime Combination Kit (Rahma, 2017 and Oxoid-Remel, 2019). Combination discs were a blend of cephalosporin and clavulanic acid on a single disc, which are, used in conjugation with a plain cephalosporin for *in vitro* detection of ESBLs strains that do not produce inducible AmpC enzymes. The kit contains the following: Cefpodoxime/Clavulanic acid (CD01) 10/1µg and Cefpodoxime (CPD10) 10µg. The presence of Clavulanate enlarged the inhibition zones for all of genetically modified ESβL-producing *S. aureus* lineage complex clones by $\geq$ 5 mm, whereas inhibition zones for Cefpodoxime-susceptible isolates and Cefpodoxime-resistant isolates with AmpC and K1 β-lactamases were enlarged by $\leq$ 1 mm. Good discrimination was achieved with either the NCCLS (CLSI) or British Society for Antimicrobial Chemotherapy (BSAC Standardized Disc Sensitivity Testing Method) (Carter *et al.,* 2000; NCCLS, 2000; Gheldre *et al.,* 2003; Livermore and Brown, 2005). Combination discs should be used by qualified personnel trained to handle category 2 resistant pathogens, and be competent in basic microbiological techniques including antibiotic susceptibility testing. The discs need to be placed on sensitivity media (Muller-Hinton agars) with sufficient space between the discs to allow the formation of clearly defined zones of inhibition and combination of them. A freshly prepared standardized inoculum 0.5 McFarland from each isolate on TSBYE was used in test procedure (4-5 logs equivalent to 104-105 $CFU.ml^{-1}$). Zone diameters were measured to the nearest millimeter. A difference of $\geq$ 5 mm between the zones of the CD01 (10- plus 1-mg) and CPD (10-mg) disks was taken to indicate ESBL production, as advocated by the manufacturer. Ceftazidime (CAZ) (30-mg) disks (Oxoid) were tested in parallel as a control discs with kit components in comparison with the susceptibility tables of CLSI (2019) and antibiotics resistance profile index overhead ladders. Alternatively semiconservative protocol was dependent in which, Double-Disc Synergy Test (DDST) elucidate discs containing cephalosporin (cefotaxime or ceftriaxone, ceftazidime, cefepime) were applied next to a disc with clavulanic acid, amoxicillin + clavulanic acid or ticarcillin + clavulanic acid. Positive result was indicated when the inhibition zones around any of the cephalosporin discs were augmented in the direction of the disc containing clavulanic acid. The distance between the discs was critical and 20 mm center-to-center has been found to be optimal for cephalosporin 30 µg discs; however, it may be reduced (15 mm) or expanded (30 mm) for strains with very high or low resistance level, respectively.

Module 2

Candidate trial model was dependent during preparation and propagation of foodborne bacteriophages cocktails from dairy series in selected regions. Modified dual thermal modules (Cold-Hot regime Al-Shammary (2019) used for recovery of phages (LYTIC Ø WYVERN76) from soft cheese-yogurt ecosystem and from cases of mastitis. Inspired patented prepared phage buffer designed by Al-Shammary (2019) was dependent. Dairy samples were mixed with Ø buffer by dilution formula one part sample (100 ml or g) to ten parts (1 liter) Ø buffer, and then incubated overnight inside a refrigerator at 4 °C for resuscitation, enrich and enrichment of phages and to dissociate them from attachment from sample microbiota. Propagation step followed by inoculation of freshly prepared and counted strain clones of *S. aureus* lineage complex (Chimera CRISPR-CAS ESβL extra complex biofilm strain) according to modified McFarland technique as log 8-10 $CFU.ml^{-1}$ (0.1 ml) to 100 ml prepared Ø cocktails, mixed well and incubated overnight at 37 °C. If receptor specific lytic phages were present for Chimera CRISPR-CAS ESβL extra complex biofilm strain, then it will eat them by cascaded lifecycle and proliferated to new virions. Purification of Ø cocktails by qualitative and quantitavely centrifugation and filtration several times until clear concentrated raw Ø cocktails were collected and preserved. Custom modified combo decorum regime was dependent to verify recovered phages cocktails. Modified spot-plaques Ø pattern morphology assay was dependent by verifying Kirby-Bauer antibiotics sensitivity diffusion and McFarland Log modulation assay throughout Muller-Hinton agars. Standardized formula for swabbing

calculated clones was dependent in which, absorbed inoculated plates dropped and distributed by twenty micron from each recovered Ø. Overnight incubation at 37 °C followed by mean log calculating and enumeration of spots-plaques (lytic zones like inhibition zones of antibiotics) as plaques forming units PFU.ml^{-1} with checking morphology, size and purity or degree of clearance or turbidity for each examined spot. Versus modified technique was dependent for checking recovered Ø cocktails. Verified enrolled pour plate technique was dependent in which, 0.1 ml concentrated Ø were mixed with 0.1 ml standardized clone log, and incubated at 37 °C for one hour. Remixed enrollment with melted freshly prepared TSA-YE inside sterile cups with circular vortex for one minute, then poured into the plates and incubated overnight at 37 °C followed by same calculated style upstairs. Titration to determination of threshold cut-off values by modified routine test dilution assay of Tbilisi (Ø RTD). Dual tenfold and mixed tenfold cascaded twofold steps of dilutions with formula PBS was dependent plus upstairs plaques techniques to calculate the suitable titer log for each recovered Ø as MIC and MBC with detected phenotypic criteria. Modified molecular scanning three dimensional crystallography micrographs was dependent to confirmation strategy in which, concentrated spotted mixture of freshly prepared combo (Phage vs ESβL) on special circular or cubic one cm sterile glasses designed for electron microscopy. VEGA 3 TESCAN laser electron microscopy was dependent for improvement of Ø recovery module with direct clues features of specificity and sensitivity of recovered Ø on attachment and lysis of recalcitrant biofilm-capsule layers of chimera with insertion of Ø DNA inside the target and termination of Terminator X module by Exodia module as unveiled upstairs by plaques techniques. Verified references were derived and dependent for redesigning and redirection techniques (Garcia *et al.,* 2007; Center for Phage Technology, 2011; Abdulamir *et al.,* 2015; Ali and Jamalludeen, 2015; Mohammed and Jamalludeen, 2015; Nasser *et al.,* 2019; Zhao *et al.,* 2019).

Statistical analysis

Bio statistically dependent software of Statistical Package for the Social Sciences (SPSS, version 25, 2019), including t-test and Chi-square for glance significance variations among evoked data.

Results and Discussion

Focusing issue was emphasized mainly and primarily on recovery and action of bacteriophages on recalcitrant clones of *S. aureus* and their biofilm. Phenotypically ecomaps segregation chain unveiled recovery of nano entities of *Caudovirales* family (tailed *Myoviridae*, *Podoviridae*, and *Siphoviridae* bacteriophages) from raw milk, mastitis units replicates, fresh-soured yogurt ecosystem and fresh-brined soft cheese- ecosystem under the Baltimore classification scheme as group I viruses with double stranded DNA genomes. Totally, recovered bacteriophages (Ø) as integrated redirected cocktails displayed twenty-two out of sixty assembled trials (22 from 60: 36.67 %). Recovery scheme ecomap module revel strongest lytic phages (Anti CRISPR-CAS ESβL CAZ Chimera strains (Ultra) from yogurt ecosystem especially fresh homemade brand, from mastitic milk units and from raw milk especially in Abu-Ghraib territory cascaded and followed by according to degree of plaques forming unis into intermediate virulent lytic phages from soft cheese-whey ecosystem disintegrated to those recovered from fresh soft cheese and brined brands in Al-Fudhaliyah, Al-Sadrya and Abu-Ghraib territories consequently and respectively. Detailed illustrated tables 1-9 with Figs. 1 and 2 post these cascaded deciphered events.

Combo illustration depending on the authorized experience and visions guidelines of supervisor researches on multidrug resistant foodborne microorganisms and on linked or associated previous researchers on methicillin and vancomycin resistant clones of *S. aureus* lineage-complex. Upgraded electromagnetic bridges of biofilm entity in these developed and genetically well-equipped

Table 1: Bacteriophages cocktails ecomap recovery module from raw milk units replicates.

Territory	Brands No.	Recovery Ecomap %			
		Clinical	Normal	20	60
Abu-Ghraib	20	1 ^{Aa}	1 ^{Aa}	10	3.33
Al-Fudhaliyah	20	1 ^{Aa}	0 ^{Aa}	5	1.67
Al-Sadrya	20	1 ^{Aa}	0 ^{Aa}	5	1.67
Total	60	3	1	20	6.67

A: Vertically indicate non-significant differences among territories at level ($P \leq 0.05$).

a: Horizontally indicate non-significant differences within territory at level ($P \leq 0.05$).

Table 2: Recovery mean log count of Ø (PFU.ml^{-1}) from CMT posted raw milk units replicates.

Raw Concentrated Ø Cocktail	Plaques mean log count (PFU.ml^{-1})	Plaques-Spots Denominator	
		Inhibition Zones (mm)	Morphology
Normal Raw Milk	4.301 ^{B}	1-2	Turbid and Irregular
Clinical Mastitis	9.653 ^{A}	3-5	Clear and circular

A, B: Vertically indicate significant differences among plaques cocktails brands at level ($P \leq 0.05$).

Table 3: Tbilisi Routine Test Dilution Titer (Ø RTD Cutoff Values).

Raw Concentrated Ø Cocktail	Ø RTD Cutoff Values
Normal Raw Milk	Log 6^{B}
Clinical Mastitis	Log 13^{A}

A, B: Vertically indicate significant differences among Ø RTD cutoff values at level ($P \leq 0.05$).

Table 4: Bacteriophages cocktails ecomap recovery module from yogurt ecosystem.

Territory	Brands No.	Recovery Ecomap %			
		Fresh	Soured	20	60
Abu-Ghraib	20	3 ^{Aa*}	1 ^{Ab}	20	6.67
Al-Fudhaliyah	20	1 ^{Ba}	1 ^{Aa}	10	3.33
Al-Sadrya	20	1 ^{Ba}	1 ^{Aa}	10	3.33
Total	60	5	3	40	13.33

A,B: Vertically indicate significant differences among territories at level ($P \leq 0.05$).

a,b: Horizontally indicate significant differences within territory at level ($P \leq 0.05$).

*: Indicate highest isolation ratio from fresh yogurt from Abu-Ghraib.

Table 5: Recovery mean log count of Ø (PFU.ml^{-1}) from yogurt ecosystem.

Raw Concentrated Ø Cocktail	Plaques mean log count (PFU.ml^{-1})	Plaques-Spots Denominator	
		Inhibition Zones (mm)	Morphology
Fresh Yogurt	10.740^{A}	2-3	Turbid to clear and Irregular to circular
Soured Yogurt	6.591^{B}	1-2	

A, B: Vertically indicate significant differences among plaques cocktails brands at level ($P \leq 0.05$).

Table 6: Tbilisi Routine Test Dilution Titer (Ø RTD Cutoff Values).

Raw Concentrated Ø Cocktail	Ø RTD Cutoff Values
Fresh Yogurt	Log 11^{A}
Soured Yogurt	Log 6^{B}

A, B: Vertically indicate significant differences among Ø RTD cutoff values at level ($P \leq 0.05$).

Table 7: Bacteriophages cocktails ecomap recovery module from soft cheese-whey ecosystem.

Territory	Brands No.	Recovery Ecomap %			
		Fresh	Brined	20	60
Abu-Ghraib	20	1 ^{Aa}	1 ^{Aa}	10	3.33
Al-Fudhaliyah	20	3 ^{Ba*}	2 ^{Aa}	25	8.33
Al-Sadrya	20	2 ^{ABa}	1 ^{Aa}	15	5
Total	60	6	4	50	16.67

A, B, AB: Vertically indicate significant differences among territories at level ($P \leq 0.05$).

a,b: Horizontally indicate significant differences within territory at level ($P \leq 0.05$).

*: Indicate highest isolation ratio from fresh yogurt from Al-Fudhaliyah.

chimeras reflect their potential hazard policy for man, animals, food, water and environment. Uncontrolled importation of contaminated feeds-foods recycled contaminated Iraqi environment with foreign clones carrying strong and intelligent defense barriers strategies called CRISPR-CAS immune system cascaded and regulated cleverly by quorum after 2003. All these scenarios with other obscure causes leading to emergency of these resistant and recalcitrant persisters biofilm clones. Acquisition and accumulation of inserted foreign genetic material rather than mutation in Enterotoxigenic *S. aureus* Iraqi clones due to symposium relationship among and within this Eco biota in the same niche ecosystem through years leading to sophisticated genes sharing strategies either by conjugation plasmids bridges or by transduction with pathogenic prophage or throughout transformation by residual forbidden genetic material (environmental or eDNA) cascaded by abnormalities in intentional redirected genetic engineering and programed penicillin binding proteins of mecA genes of staphylococcal pathogenicity islands versus staphylococcal chromosome cassette islands regulated by di headed chaperone accessory gene regulator and Sigma factors linked with acquisition of foreign sets of antibiotics resistance represented by extended-spectrum beta lactamase and Carbapenemase from Gram negative

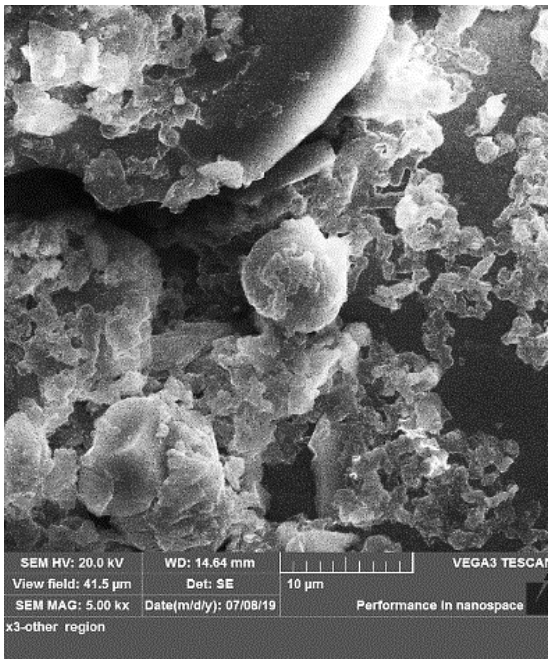

Fig. 2: Electron micrograph of recovered bacteriophage (Ultra Ø) integrated on biofilm-cell wall of *S. aureus* as specific receptor lytic action (3.5 nanometer scale size at 5 million MPX).

Fig. 1: Apoptotic ***S. aureus*** plaques by recovered foodborne lytic bacteriophages Cocktails (**Ø ΩΨςEPN76**).

Table 8: Recovery mean log count of Ø (PFU.ml^{-1}) from soft cheese-whey ecosystem.

Raw Concentrated Ø Cocktail	Plaques mean log count (PFU.ml^{-1})	Plaques-Spots Denominator	
		Inhibition Zones (mm)	Morphology
Fresh soft cheese-whey	8.518^{A}	2-3	Turbid to clear and
Brined soft cheese-whey	5.342^{B}	1-2	Irregular to circular

A, B: Vertically indicate significant differences among plaques cocktails brands at level (P≤0.05).

Table 9: Tbilisi Routine Test Dilution Titer (Ø RTD Cutoff Values).

Raw Concentrated Ø Cocktail	Ø RTD Cutoff Values
Fresh soft cheese-whey	Log 9^{A}
Brined soft cheese-whey	Log 5^{B}

A, B: Vertically indicate significant differences among Ø RTD cutoff values at level (P≤0.05).

pathogens versus interphases positively-negatively charged zwitterion capsule ending with transformation to armamentarium entity of ESβL.

In contrast, natural defense barriers displayed in the same ecosystem like microflora and their natural antimicrobial products (Bacteriocins) associated with foodborne lytic bacteriophages. Recently encapsulated armamentarium redirected and augmented phages with antibiotics could solve the problems of new emergent multidrug resistant pathogens as dual antimicrobial regime. Cocktails of phages sprayer might hurdles with other bio preservation technologies to inshore biosafety of food. Cold pasteurization by bacteriophages was now an urgent priority needs due to these discriminated safe nanoscale biomolecules could lyse receptor specific CRISPR-CAS foodborne pathogens throughout three-dimensional attachment to invaders or predators and lyses their biofilm clouds then penetration with insertion of their DNA material ending with replication inside the target and proliferation to kill it. Not always, this was a true situation because a CRISPR-CAS machine inside a developed pathogen can stop these events. So that, we need to recovery of upgraded super phage to face off these struggling entity as in our work scheme, which enthroned by discovery of developed phages cocktails from dairy ecosystem especially fresh homemade yogurt called Ultra or Exodia to face off the Chimera Medusa as intelligent secret weapon on my hands of Supervisor Ali Al-Shammary. Transduction *S. aureus* phages might carry genes coding for diverse virulence factors such as Panton-Valentine leukocidin, staphylokinase, enterotoxins, chemotaxis-inhibitory proteins and exfoliative toxins. Phages also mediate the transfer of pathogenicity islands in a highly coordinated manner and are the primary vehicle for the horizontal transfer of chromosomal and extra-chromosomal genes. These might causing adaptation of *S. aureus* lineage-complex to broad-spectrum hosts and food ecosystems with the generation of diverse and versatile antigenic drift and shift in new redesigned forbidden clones with new emergent and hazardous capacity to remodeling their homeostasis with stress hardening and ballooning theory to overcome or resist different encountered environmental harsh modules (Rowan and Anderson, 1998; Al-Shammary, 2009; Xia and Wolz, 2014). Understanding clever switching between drift and shift behavior under accessory gene regulator quorum sensing mechanisms could illustrate some struggling *in vivo*, *in vitro* and even *in situ* of these recovered creatures.

References

AbatAngelo, V., N. Peressutti Bacci, C.A. Boncompain, A.A. Amadio, S. Carrasco and C.A. Suarez (2017). Broad- range lytic bacteriophages that kill *Staphylococcus aureus* local field strains. *PLOS ONE*, **12(7):** e 0181671.

Abdulamir, A.S., A.A. Sabah, S.A. Jassim, R.R. Hafidh and F. AbuBakar (2015). The potential of bacteriophage cocktail in eliminating methicillin-resistant *Staphylococcus aureus* biofilms in terms of different extracellular matrices expressed by PIA, cia A-D and FnBPA genes. *Ann. Clin. Microbiol. Antimicrob*., **14:** 49.

Ali, M.N.M. and N.M. Jamalludeen (2015). Isolation and Characterization of Bacteriophage against Methicillin Resistant *Staphylococcus aureus*. *J. Med. Microb. Diagn*., **5 (1):** 1-6.

Al-Shammary, A.H. (2009). Detection of *Listeria monocytogenes* in Milk and some Dairy Products. PhD. Thesis. Dept. of

Vet Public Health, Univ. of Baghdad, Iraq. (In Arabic).

Al-Shammary, A.H.A. (2019). Run-off Patterns of Vancomycin Resistant *Enterococci* (VRE clones) in Cows Raw Milk and Imported Milk Powders at Baghdad Markets. *The Iraqi Journal of Veterinary Medicine*, **43(2):** 61 – 66.

Alvarez, A., L. Fernandez, D. Gutiérrez, B. Iglesias, A. Rodriguez and P. Garcia (2019). Methicillin resistant *Staphylococcus aureus* in hospitals: latest trends and treatments based on bacteriophages. *J. Clin. Microbiol*., **57:** e01006-19.

Barrera-Rivas, C.I., N.A. Valle- Hurtado, G.M. Gonzalez-Lugo, V.M. Baizabal-Aguirre, A. Bravo-Patino, M. Cajero-Juarez and J.J. Valdez-Alarcon (2017). Bacteriophage Therapy: An Alternative for the Treatment of *Staphylococcus aureus* Infections in Animals and Animal Models.

Begley, M. and C. Hill (2015). Stress Adaptation in Food borne Pathogens. *Annu. Rev. Food Sci. Technol*., **6:** 9.1-9.20.

Carter, M.W., K.J. Oakton, M. Warner and D.M. Livermore (2000). Detection of extended-spectrum β-lactamases in *Klebsiellae* with the Oxoid Combination Disk Method. *Journal of Clinical Microbiology*, **38:** 4228-32.

Center for Phage Technology (2011). https:// agrilifetoday.tamu.edu/2011/11/03/phages-confront-antibiotic-crisis/phage-work/

Christensen, G.D., W.A. Simpson and J.J. Yonger (1985). Adherence of coagulase-negative *Staphylococci* to plastic tissue culture plates: a quantitative model for the adherence of *Staphylococci* to medical devices. *J. Clin. Microbiol.*, **22:** 996-06.

CLSI (2019).Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI Document M100es20. Wayne, PA: CLSI.

Dakheel, K.H., R. Abdul Rahim, V.K. Neela, J.R. Al-Obaidi, T.G. Hun, M.N. Matlsa and K. Yusoff (2019). Genomic analyses of two novel biofilm- degrading methicillin-resistant *Staphylococcus aureus* phages. *BMC Microbiology*, **19:** 114.

Dickey, J. and V. Perrot (2019). Adjunct phage treatment enhances the effectiveness of low antibiotic concentration against *Staphylococcus aureus* biofilms in vitro. *PLOS ONE*, **14(1):** e0209390.

García, P., C. Madera, B. Martínez and A. Rodríguez (2007). Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. *Int. Dairy J.*, **17:** 1232–9.

Gheldre, Y.D., A. Veronique, C. Berhin, D. Michel and G. Youri (2003). Evaluation of Oxoid combination discs for detection of extended- spectrum beta-lactamases. *Journal of Antimicrobial chemotherapy*, **52:** 591-597.

Hill, C., S. Mills' and R.P. Ross (2018). Phages and antibiotic resistance: are the most abundant entities on earth ready for a comeback? *Future Microbid.*, **13(6):** 711-726.

Jensen, K.C., B.B. Hair, T.M. Wienclaw, M.H. Murdock and J.B. Hatch (2015). ''Isolation and Host Range of Bacteriophage with Lytic Activity against Methicillin-Resistant *Staphylococcus aureus* and Potential Use as a Fomite Decontaminant''. *PLOS ONE*, **10:** e0131714.

Kanaan, M.H.G. and A.H.A. Al-Shammary (2013). Detection of Methicillin or multidrug resistant *Staphylococcus aureus* (MRSA) in locally produced raw milk and soft cheese in Baghdad markets. *The Iraqi Journal of Veterinary Medicine*, **37(2):** 226-231.

Labrie, S.J., J.E. Samson and S. Moineau (2010). Bacteriophage resistance mechanisms. *Nature Reviews Microbiology*, **8(5):** 317-27.

Li, L. and Z. Zhang (2014). Isolation and characterization of a virulent bacteriophage SPW specific for *Staphylococcus aureus* Isolated from bovine mastitis of lactating dairy cattle.

Lipsitch, M. and J.J. O'Hagan (2007). Patterns of antigenic diversity and the mechanisms that maintain them. *J. R. Soc. Interface*, **4(16):** 787–802.

Livermore, D.M. and D.F.J. Brown (2005). Detection of β-Lactamase-mediated resistance. Fax: +44-20-8200-3292. Email: dlivermore @phls.uk.

Malik, D.J., I.J. Sokolov, G.K. Vinner, F. Mancuso, S. Cinquerrui and F. Vladisavljevic (2017). Formulation, Stabilization and encapsulation of bacteriophage for phage therapy. *Adv. Colloid Interface Sci.*, **249:** 100-133.

Mohammed-Ali, M.N. and N.M. Jamalludeen (2015). Isolation and Characterization of Bacteriophage against Methicillin Resistant *Staphylococcus aureus*. *J. Med. Microb. Diagn*., **5:** 213.

Moller, A.G., J.A. Lindsay and T.D. Read (2019). Determinants of phage host range in *Staphylococcus* species. *Appl. Environ. Microbiol*., **85:** e00209-19.

Nasser, A., R. Azizian, M. Tabasi, J.K. Khezerloo, F.S. Heravi, M.T. Kalani, N. Sadeghifard, R. Amini, I. Pakzad, A. Radmanesh and F.A. Jalilian (2019). Specification of Bacteriophage Isolated against Clinical Methicillin-Resistant *Staphylococcus aureus*. *Osong Public Health Res. Perspect.*, **10(1):** 20–24.

NCCLS (2000). National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard M2–A7 (M100–S10). NCCLS, Wayne, Pa.

Oxoid-Remel (2019). Laboratory Manual for Media and Diagnostic Kits.

Pal, M., G.B. Kerosa, L.M. Marami and V. Kandi (2020). "Epidemiology, Pathogenicity, Animal Infections, Antibiotic Resistance, Public Health Significance, and Economic Impact of *Staphylococcus aureus*. A comprehensive Review." *American Journal of Public Health Research*, **8(1):** 14-21.

Premarathne, J., A.S. Anuar, T.Y. Thung, D.A. Satharasinghe, N.N. Jambari and N.A. Abdul-Mutalib (2017). Prevalence

and antibiotic resistance against tetracycline in *Campylobacter jejuni* and *C. coli* in cattle and beef meat from Selangor, Malaysia. *Frontiers in Microbiology*, **8:** 1-9.

Rasool, M.H., R. Yousaf, A.B. Siddique, M. Saqalein and M. Khurshid (2016). Isolation, Characterization, and Antibacterial Activity of Bacteriophages against Methicillin-Resistant *Staphylococcus aureus* in Pakstan. *Jundishapur J. Microbiol*., **9(10):** e36135.

Rath, D., L. Amlinger, A. Rath and M. Lundgren (2015). The CRISPR-Cas immune system: Biology, mechanisms and Applications. *Biochimie.*, **117:** 119-28.

Rio, D.B., M.C. Martin, V. Ladero, N. Martinez, D.M. Linares, M. Fernandez and M.A. Alvarez (2010). Bacteriophages in Dairy Industry: PCR Methods as Valuable Tools. Instituto de Productos Lacteos de Asturias, IPLA- CSIC, Spain.

Rowan, N.J. and J. Anderson (1998). Effects of above-optimum growth temperature and cell morphology on thermos-tolerance of *Listeria Monocytogenes* cells suspended in bovine milk. *Appl. Environm. Microbiol*., **64(6):** 2065-2071.

Sangha, K.K., B.V. Kumar , R.K. Agrawal, D. Deka and R. Verma (2014). Proteomic Characterization of Lytic Bacteriophages of *Staphylococcus aureus* Isolated from Sewage Affluent of India. *Int. Sch. Res. Notices*, 265-298.

SPSS (2019). Statistical Package for the Social Sciences, Version 25, Users' Guide SPSS Inc., Chicago III, USA. Website. http://WWW.SPSS.Coml.

Xia, G. and C. Wolz (2014). Phages of *Staphylococcus aureus* and their impact on host evolution. *Infect. Genet. Evol*., **21:** 593–601.