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MODERN PHAGE MODULATION STRATEGY ON VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) RECOVERED FROM MASTITIS IN BAGHDAD

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Abstract : Potential pattern of foodborne bacteriophages against multidrug-resistant pathogens was a promising hygienic strategy module. Post-antibiotics era becomes evident due to emerging of dramatic episodes of infectious foci harboring biofilm and multidrug-resistant pathogens transferred mainly throughout food chain. Vancomycin-resistant enterococci (VRE) were struggling among these new infectious emergencies. Phenotypic epigenetic transit tolerant drift cascaded by genetic resistant shift behaviors of recalcitrant VRE forbidden clones recovered from mastitis cases in Cows from territories of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya in Baghdad ecosystem were combated by redirected lytic bacteriophages cocktails recovered from the same raw-milk ecosystem. Modified and verified techniques were dependent for this enrollment. Plaques-forming techniques (PFU.ml^{-1}) posted by Vega3 scanning electron microscopy confirm recovery and potency of unveiled phages. Frequency and distribution pattern of VRE clones unveiled 27 strains (30 %) out of 90-pooled raw-milk samples, as 19 strains (21.11 %) of *E. faecalis* versus 8 strains (8.88 %) of *E. faecium*. Resistance profile index reveal diverse degree of resistance to beta-lactams and vancomycin of all recovered clones. Diverse and intelligent phage profile lytic and bactericidal index was present among recovered VRE clones in which, verified (5-9) logs PFU.ml^{-1} reduction load noticed according to degree of potency for both recovered clones and phages cocktails. Milieus with new active bio-preservative and matrixed nano biosensor lytic foodborne phages against multidrug-resistant entities of enterococci located and borne in the same niche ecosystem reveal a promising tool for healthy lifestyle in Baghdad.

Key words : Bacteriophages, cows, enterococci, mastitis, raw milk, vancomycin-resistance.

INTRODUCTION

Frequency and distribution of emergent antimicrobial resistance can adversely increase morbidity and mortality hazards and so on threats in management of infectious diseases (O'Neill, 2016). Global Antimicrobial Surveillance System recovered and posted the highest levels of these modified problems in *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Salmonella* (Organization, 2018). Natural glycopeptides like vancomycin since 1986 in France becomes a verified choice for treatment of these multidrug resistant infectious foci as alternative milestone strategy (Dhanda *et al*, 2018). Clever combating of emergent opportunistic vancomycin-resistant *Enterococcus faecalis* and *E. faecium* by bacteriophages provides a crucial step in decreasing these recalcitrant biofilm problems due to these quorums considered as a bridge for transferring resistance memory to another microbiome (Ahmed and Baptiste, 2018; Lossouarn *et al*, 2019). Newer classes and derivatives of glycopeptides and semisynthetic generations of antibiotics with enhanced binding affinity, membrane disruption ability, and lipid-binding properties have unveiled favorable consequences against Gram-positive and Gram-negative pathogens. Recaptured and verified hazards analysis and critical control points plans by many associations and governments in order to legislate modified strategies against these resistance threats resulting in approval of these newer generations of antimicrobial agents like lipopeptides (daptomycin), oxazolidinones (linezolid), pleuromutilin (retapamulin), macrocyclic antibiotics (fidaxomicin) and diarylquinoline (bedaquiline) (Domingo-Calap *et al*, 2018). Growing problems of developed resistance to newer generations of antibiotics might verified module to other nano strategies to overcome dramatic concerns in nosocomial acquired infections and so on reducing ecosystem hazards (Tommasi *et al*, 2015).

Potential pattern of foodborne bacteriophages against multidrug-resistant pathogens was a promising hygienic strategy module. Post-antibiotics era become evident due to emerging of dramatic episodes of infectious foci harboring biofilm and multidrug resistant pathogens transferred mainly throughout food chain. Vancomycin-

resistant enterococci were struggling among these new infectious emergencies. New live nanoscale therapy strategies with bacteriophages as bacterial lytic lysosomal eaters with high selective toxicity against prokaryotic microbes and no side effects on human and animal cells (cell receptor module) in association with old-new antibiotics can alleviate the developed problem[7]. Radical changing of these situations occurs throughout decades as a result in rapid development of antibiotics resistance coupled with the decline in verified and novel antimicrobial agents (Perros, 2015; Collineau *et al*, 2019). To date avoidance or limitation in the risk of emergence of multidrug-resistant pathogens due to developed genes sharing strategies associated with the development of newer therapy weapons are incomplete silent. So that, post antibiotics era must be verified to encourage using of lytic phages as an associated module in hurdle society for hygienic lifestyle module (Yosef *et al*, 2015; Principi *et al*, 2019). Phages represented the most common biological entity. They present in soil, seawater, oceanic, terrestrial surfaces and extreme environments, such as those categorized by very high or very low temperatures. Moreover, they recovered from hospitals, wastewater, environment, tissues and so on (Clokie *et al*, 2011). Recovered phages are classified according to their morphological behaviors, their nucleic acid content, the site where they can mostly be found and the bacterial species that they can kill. Classification module divides tailed phages according to their entity ecosystem (Fauquet and Pringle, 2000; Van Regenmortel, 2007). Lytic or virulent and lysogenic or temperate bacteriophages identified. Their biological cycles implies attachment to and invasion of the bacterium. However, to initiate binding, phages structures have to match strain-specific variants phenotypes of bacterial receptors. Considering continuous changing in phages and resident opportunistic pathogens, a single phage can affect limited strains (Young and Phage, 2013). This explains the specificity of phages. Once the phages enter the cell, the bacterial synthetic machinery is redirected to the production of viral genome and proteins. Finally, assembly and packing of phages occur and cells be lysed with the release of new virions that can infect other bacterial cells. However, these behaviors varies with the degree of interconnected relationships among phages, bacterial clones and environment (Weinbauer, 2015). In contrast, the lysogenic cycle posted by integration of viral genome into the host DNA and during division, the transmission of virus chromosomes to daughter cells. When the viral genome detached by bacterial DNA or through CRISPR module, it enters the lytic stage. Specificity and sensitivity of redirected lytic phages support their applications against invading microbes in our ecosystem (Salmond and Fineran, 2015).

Non-directed phages have narrow spectrum of activity linked with antibiotics efficacy, *i.e.*, the influence on the entire microbiome with elimination of potential microflora, overgrowth of secondary opportunistic pathogens and emergence of resistant clones. Concluded several pros of phages in comparison to antibiotics cons including safer and better tolerated, as they replicate only in the target bacterium but cannot infect mammalian cells (Kakasis and Panitsa, 2019). Moreover, easier administration provides no need for repeated administrations shortly after one another over several days, as commonly required for antibiotics because they can remain in the human body for relatively prolonged periods, *i.e.*, up to several days. Generally, nano doses supporting their concentration in the site of infection after the initial administration. Contrarily to antibiotics, their effect is limited to the site of infection that is reached, even when bacteria situated in a body organ or system in which antimicrobials can hardly penetrate (Bogovazova *et al*, 1991; Rather *et al*, 2012). Modified DNA technologies verified engineered and redirected phages to be able to overcome some limitations of antibiotics. The verified evidence of biofilm dispersion by phages, a barrier that makes infections difficult to eradicate with standard antibiotic therapy even if bacteria are sensitive to the administered drug, give us a new module. Effective anti-biofilm phages against *E. coli* to express biofilm-degrading enzymes was verified (Lu and Collins, 2007). Modified regimes by insertion of *rpsl* and *gyrA* genes in lysogenic phages for streptomycin and nalidixic acid, change and reduce the sensitivity module of resistant bacterial clone dramatically to low inhibitory levels (Edgar *et al*, 2012). Custom therapy design without modification of the microbiota by phages noticed in mice after oral administration of four T_4-like phages that redirected to pathogenic *E. coli* did not prime to any injury and loss of non-pathogenic bacteria of the same species (Chibani-Chennoufi *et al*, 2004). Finally, phages might be less expensive than that of antibiotics targeting multidrug-resistant pathogens like surveillance encountered in hospitalized patients suffering from methicillin-resistant *Staphylococcus aureus* infection (Weber-D[1] browska and Górski, 2007).

Potential emergency of resistance against phages was possible, as bacteria possess or can develop several mechanisms to prevent viral infections as genetically modified defense barrier clustered regularly interspaced short palindromic repeats (CRISPR-CAS). Among these strategies hiding, change or loss of receptor, secretion of

substances that prevent phage adhesion to the bacterial pathogen, activation of modules for blocking phage DNA injection into the cell and inhibition of phage replication and release (Seed, 2015). Alteration or loss of receptor for membrane protein modifications has revealed for *E. coli* (Riede and Eschbach, 1986), *S. aureus* (Nordström and Forsgren, 1974), *Bordetella bronchiseptica* (Liu *et al*, 2002) and *Vibrio cholera* (Seed *et al*, 2012). Secretion of extracellular polymeric substances and glycol-conjugates has described for *Pseudomonas* spp. and *Enterobacteriaceae* (Drulis-Kawa *et al*, 2012), respectively. Cocktails of phages reduce these developed problems especially when associated with antibiotics. If phages kill pathogens faster than they can replicate, the problem could be solved. However, all these outcomes indicate selection of cocktails will reduce these post exposed and predicted problems (Torres-Barceló, 2018). Consequently, transduction power of inserted framework of lysogenic phages inside bacterial genome might be vehicles for horizontal genes transfer and guideline the diffusion of antibiotic resistance genes (O'Shea and Boyd, 2002; Brabban *et al*, 2005; Maiques *et al*, 2007). Moreover, phage inducers can encourage diffusion of these genes in the environment, i.e., expression of prophage gene products or lead to the excision and spread of temperate ones. Huge phages carrying genes associated with antibiotic resistance recovered from secretions and tissues of patients suffering from recurrent contaminations due to multidrug resistant pathogens and treated with antimicrobial drugs[35]. Processing of wastewater with EDTA or sodium citrate activates the lytic cycle of lysogenic phages and leads to the generation of new phages that can lyse large numbers of microbiota thus purifying ecosystem (Fancello *et al*, 2011; Colomer-Lluch *et al*, 2014).

The objective of this study was to verify new strategy against multidrug-resistant foodborne pathogens in Iraqi ecosystem as alternative or combo tool with antibiotics to overcome the dramatic increase in resistance problems leading as to verify recovery of phages cocktail against vancomycin-resistant enterococci as a potential hygienic module.

MATERIALS AND METHODS

Sampling

Totally ninety congregated cows raw milk samples collected from variant territories in Baghdad ecosystem, throughout February until June 2019. Thirty samples pooled from each Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya in which, six integrated units collected per month from each territory. Pooled samples translocated sonly to Food Microbiology Hygiene Laboratories posted by Public Health Department at Baghdad Veterinary College. Samples units processed according to modified and verified techniques in food microbiology (Pincus, 2006; Hemraj *et al*, 2013).

Processing and recovery

Pooled normal, subclinical and clinical mastitic milk samples were examined by screening test. The test was performed by commercial CMT kit (Immucell California mastitis Test Kit, Portland) was dependent in which, a leukocyte DNA dependent reaction with CMT reagents indicate degree of hygienist milk and predict healthy status of Cows. Clear to viscous milk with dye reduction time reflect the positive and negative scores and predict the shifting curve of neutrophils. All tested milk units from all quarters subjected to recovery protocol. Modified recovery firmware were dependent for isolation-confirmation series strategy. Modified double strengthen powered Tryptone-Soya Yeast-Extract Enriched Broth (Oxoid) cascaded by double selective-differential-enrichment Sodium Azide Tryptone-Soya Yeast-Extract Blood Agar (Oxoid) was a primary combo recovery tool unit. Resuscitation enrollment of 25 ml from each mixed milk units was diluted by ten parts of double strengthen powered Tryptone-Soya Yeast-Extract Enriched Broth and incubated at 37°C for 24 hours. Droplet and pour plate techniques were enrolled for culturing strategy in which, dual replicates culturing on double selective-differential-enrichment Sodium-Azide Tryptone-Soya Yeast-Extract Blood Agar at 37°C for (24-48) hours encourage a recovery scheme (Pincus, 2006; Hemraj *et al*, 2013).

Confirmation

Phenotypic assessment initiated by Gram stain and catalase (Oxoid) reaction followed by segregation firmware. Lancefield's serological grouping (Oxoid) confirm entity group D enterococci. Virulence markers arrays index of recovered vancomycin-resistant enterococci assessed by modified biofilm-slim assay and Vitek2® biosensor. Vitek2® (bioMérieux's-Software Series) index assets in segregation and certification of clone diversity (*E. faecalis* versus *E. faecium*) and antibiotics susceptibility profile. The Vitek2® algorithm system customs a fluorogenic policy for entity identification and a turbid metric scheme for susceptibility testing using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number. Automated species identity with resistance index to a series of tested β-lactams and vancomycin according to kit reagents,

scanned laser beamed readings and computerized reference guide index drive segregate clone diversity. Tolerant growth profile index on McConkey agar (MC, Oxoid) linked to antibiotics resistance profile aid in segregation of tolerant VRE clones grown on MC agar (MC positive) and MC negative sensitive clones none grown on MC agar. Modified Congo Red Agar (Oxoid) designed for biofilm-formation sensor and phenotypic segregation of plasmid-index strain strategy (Pincus, 2006; Hemraj *et al*, 2013).

Phage

Designed, modified and verified formulated buffered phage by Al-Shammary (2019) was precisely (specificity and sensitivity) support recovery of phages cocktail (bacteriophages Ø) from the same samples units. Lytic concept of recovered foodborne bacteriophages (*Caudauvirales* nano active bullets: Thick and large, contractile tailed *Myoviridae*, non-contractile long tailed *Siphoviridae* and short tailed *Podoviridae* subunits) was confirmed by modified recycled specific recovery-plaques forming techniques (PFU.ml^{-1}) posted by Vega3 scanning electron microscopy. Mix one part milk (30 ml) replicates with ten parts (270 ml) phage buffer-76 enrolled by incubation overnight inside a refrigerator at 4°C. Propagation strategy enrolled by freshly prepared broth of enterococci (VRE series) adjusted at 9 logs CFU.ml^{-1} be mixed with prepared cooled phages cocktail by formula 0.1 ml VRE broth to 100 ml prepared phages, mixed well and incubated at 37°C for 24 hours. Purification by centrifugation at 10000 rpm for 30 minutes and supernatant sieved by filter papers until collection of most dripped phages. Modified spot-plaques technique was dependent for checking susceptibility pattern in which, a 5 logs CFU.ml^{-1} McFarland broth from each VRE clone was swapped according to Kirby-Bauer technique of antibiotics sensitivity test on Muller-Hinton agar and adsorbed medium after 10 minutes was inoculated by droplet technique of Miles-Misra by 20 micron (0.02 ml) from each phages according to brand of milk from which it prepared, lifted for another 10 minutes for absorption then incubated at 37°C for (24-48) hours for checking degree and number of lytic zones appeared with their size in millimeter, clarity of turbidity and morphology. Another modified technique enrolled by mixing 0.1 ml concentrated phages microbiome with 0.1 ml freshly prepared 5 logs CFU.ml^{-1} McFarland VRE broth, incubated at 37°C for one hour, then mixed and poured into Tryptone-Soya Yeast-Extract Agars, incubated at 37°C for (24-48) hours for checking stepwise series of appeared lytic zones demography. Titration and cut-off threshold values for each phages cocktail calculated as RTD-PFU ml^{-1} units by tenfold dilution formula with PBS and modified pour plate-spot technique. Purified phages were spotted in clear one-centimeter designed glass slides, lifted to concentrated and dry, then subjected to trans-scanning by Vega3 electron microscopy for demonstration of recovered phages and their lytic actions on VRE clones (Pincus, 2006; Hemraj *et al*, 2013).

Data analysis

Biostatistician analysis by Chi-square throughout SPSS software (version 25, 2019) was dependent on significance levels ($p \leq 0.05$) among encountered results.

RESULTS

Certified data index posted by frequency and distribution pattern of VRE clones declare recovery of 27 strains (30%) out of 90 pooled subunits. Ecomap segregation recovery profile declare phenotypically 19 *E. faecalis* strains (21.11%) versus 8 *E. faecium* strains (8.88%). Territory profile index reveal recovery of 18 strains (20%) from Abu-Ghraib followed by 7 strains (7.78%) from Al-Sadrya and 2 strain (2.22%) from Al-Fudhaliyah. Frequency and distribution pattern of diverse clones according to territory reveal segregation ecosystem profile into 13 clones (14.44%): *E. faecalis* phenotypes versus 5 clones (5.55%) as *E. faecium* phenotypes from Abu-Ghraib region. 4 clones (4.44%) as *E. faecalis* phenotypes versus 3 clones (3.33%) as *E. faecium* phenotypes from Al-Sadrya region. 2 clone (2.22%) as *E. faecalis* phenotype versus none *E. faecium* (0%) phenotype from Al-Fudhaliyah region. California mastitis recovery index profile segregate phenotypes according to examined milk brand into 3 in 2 bridge as *E. faecalis* bridge: 11 clones (12.22%) from mastitic milk followed by 6 clones (6.67%) from subclinical cases and 2 clone (2.22%) from normal milk units, while *E. faecium* bridge: 5 clones (5.55%) from mastitic milk versus 3 clones (3.33%) from subclinical cases. Haemolysis pattern (β-clear) noticed in most isolates. Tables 1-3 and Fig. 1 illustrates this dogmatism.

Phenotypic assessment initiated by Gram stain and catalase reaction followed by segregation firmware. Lancefield's serological grouping confirm entity group D enterococci. Virulence markers arrays index of recovered vancomycin-resistant enterococci assessed by modified biofilm-slim assay and Vitek2® biosensor. Resistance profile index reveal diverse degree of resistance to â-lactams and vancomycin of all recovered clones. Resistance ecomap segregate clones into 20 strains (74.07%) as β-lactams, methicillin and vancomycin resistant enterococci (BLRE, MRE and VRE) variants:

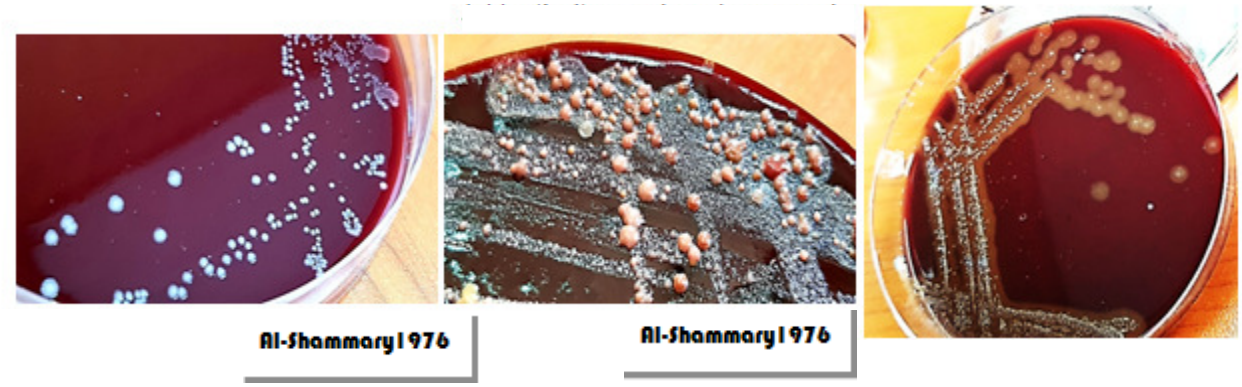

Fig. 1 : Growth profile and haemolysis pattern of enterococci on modified sodium azide blood agar.

Fig. 2 : Biofilm-Plasmid (BP) and Biofilm (B) bridged profile index of enterococci on modified Congo red agar.

Fig. 3 : Lytic clear, small and large plaques or zones of inhibitions on Muller-Hinton and tryptone soya yeast extract agars of recovered phages cocktails on VRE clones.

12 strains (44.44%) of *E. faecalis* VRE versus 8 strains (29.63%) of *E. faecium* VRE and 7 strains (25.93%) as intermediate *E. faecalis* VRE variants. Biofilm-Plasmid (BP) bridged profile index divulge segregation of diverse clones into 20 (74.07%) bridged virulent BP dark-red to black colonies of both phenotypes: 15 (55.55%) *E. faecalis* bridge versus 5 (18.52%) *E. faecium* bridge, and 7 (25.93%) moderate red-pink colonies as 4 (14.82%) *E. faecalis* versus 3 (11.11%) *E. faecium* Bridge producing matrixed biofilm-slim stratum. All recovered strains were tolerate to growth on MC agar in diverse degree (generation time until appearance of visible colonies) according to degree of antibiotics resistance among phenotypes (positive correlation). Tables 4-6 and Figure 2 illustrates cross-tabulated module.

Diverse and intelligent phage profile lytic and bactericidal index was present among recovered VRE clones in which, verified (5-9) logs $PFU.ml^{-1}$ reduction load noticed according to degree of virulence for both

Table 1 : Recovery module of enterococci according to territory.

Territory	Number of Samples	*E. faecalis* %	*E. faecium* %	Total Ecomap %
Abu-Ghraib	30	13 (14.44) ^{Aa}	5 (5.55) ^{Ab}	18 (20) ^{A}
Al-Sadrya	30	4 (4.44) ^{Ba}	3 (3.33) ^{Aa}	7 (7.78) ^{B}
Al-Fudhaliyah	30	2 (2.22) ^{Ba}	0 ^{Bb}	2 (2.22) ^{C}
Total	90	19 (21.11) ^{a}	8 (8.88) ^{b}	27 (30)

A, B, C: Indicate clinically and bio statically significant differences vertically within territory at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

Table 2 : Segregation Ecomap of enterococci according to CMT bridge.

CMT Brand	*E. faecalis* %	*E. faecium* %	Total Bridge %
Mastitis	11 (12.22)^{Aa}	5 (5.55)^{Ab}	16 (17.77)^{A}
Subclinical	6 (6.67)^{Ba}	3 (3.33)^{Ab}	9 (10)^{B}
Normal	2 (2.22)^{Ca}	0^{Bb}	2 (2.22)^{C}
Total	19 (21.11)^{a}	8 (8.88)^{b}	27 (30)

A, B, C: Indicate clinically and bio statically significant differences vertically within CMT Brand at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

Table 3 : Segregation Ecomap of enterococci according to month.

Month	Samples	*E. faecalis* %	*E. faecium* %	Recovery %
February	16	9 (10) ^{Aa}	5 (5.55) ^{Ab}	14 (15.5) ^{A}
March	16	6 (6.67) ^{Ba}	1 (1.11) ^{Bb}	7 (7.78) ^{B}
April	16	2 (2.22) ^{Ca}	1 (1.11) ^{Ba}	3 (3.33) ^{C}
May	16	1 (1.11) ^{Ca}	1 (1.11) ^{Ba}	2 (2.22) ^{C}
June	16	1 (1.11) ^{Ca}	0 ^{Cb}	1 (1.11) ^{C}
Total	90	19 (21.11) ^{a}	8 (8.88) ^{b}	27 (30)

A, B, C: Indicate clinically and bio statically significant differences vertically within month at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

Table 4 : Resistance module within clones.

Resistance module	*E. faecalis* %	*E. faecium* %	Resistance Ecomap %
Resistant *VRE* variants	12(44.44)^{Aa}	8(29.63)^{Ab}	20(74.07)^{A}
Intermediate *VRE* variants	7(25.93)^{Ba}	0 ^{Bb}	7(25.93)^{B}
Total	19(70.37)^{a}	8(29.63)^{b}	27(100)

A, B: Indicate clinically and bio statically significant differences vertically within resistance at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

recovered clones and phages cocktail. Titration and cut-off threshold values for each phages cocktail calculated as RTD-PFU.ml^{-1} units by tenfold dilution formula with PBS and modified pour plate-spot technique. Recaptured data revealed powerful efficacy of lytic phages cocktail

Table 5 : Biofilm-Plasmid bridged profile index on modified Congo red agar.

Biofilm-plasmid bridge	*E. faecalis* %	*E. faecium* %	Bridge Ecomap %
Positive phenotype (BP)	15(55.55)^{Aa}	5(18.52)^{Ab}	20(74.07)^{A}
Moderate phenotype (B)	4(14.82)^{Ba}	3(11.11)^{Ab}	7(25.93)^{B}
Total	19(70.37)^{a}	8(29.63)^{b}	27(100)

A, B: Indicate clinically and bio statically significant differences vertically within BP bridge at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

Table 6 : Growth tolerance curve on McConkey (MC) agar.

MC tolerance	*E. faecalis* %	*E. faecium* %	Tolerance Ecomap %
Positive	12 (44.44) ^{Aa}	8 (29.63) ^{Ab}	20 (74.07) ^{A}
Moderate	7 (25.93) ^{Ba}	0 ^{Bb}	7 (25.93) ^{B}
Total	19 (70.37) ^{a}	8 (29.63) ^{b}	27 (100)

A, B: Indicate clinically and bio statically significant differences vertically within MC tolerance at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

recovered from clinical cases of mastitis than other milk brands on both clones. Modified dual plaques technique reveal the presence of more than 3 logs (≥ 300) circular to irregular, small to large, turbid to clear spots from all recovered cocktails with virulent efficacy against VRE clones. In other words, we calculate the mean log counts of appeared plaques or spots after dual culturing by modified droplet and pour plate techniques. Modified formula practical by mean number of spots on cultured plate x a reciprocal of dilution factor x 50 CFU/ml. Routine test dilution (RTD) titer for each cocktail reveal diverse efficacy from 9 $\log_{10}$ PFU.ml^{-1} for mastitis phages to 5 $\log_{10}$ PFU.ml^{-1} for normal milk phages. Vega3 scanning electron microscopy reveals attachment and processing of broad-spectrum *Caudauvirales* nanoscale's subunits on matrixed biofilm and cell membranes of virulent VRE clones. Tables 7 and 8 and Figs. 3 and 4 illustrate these events.

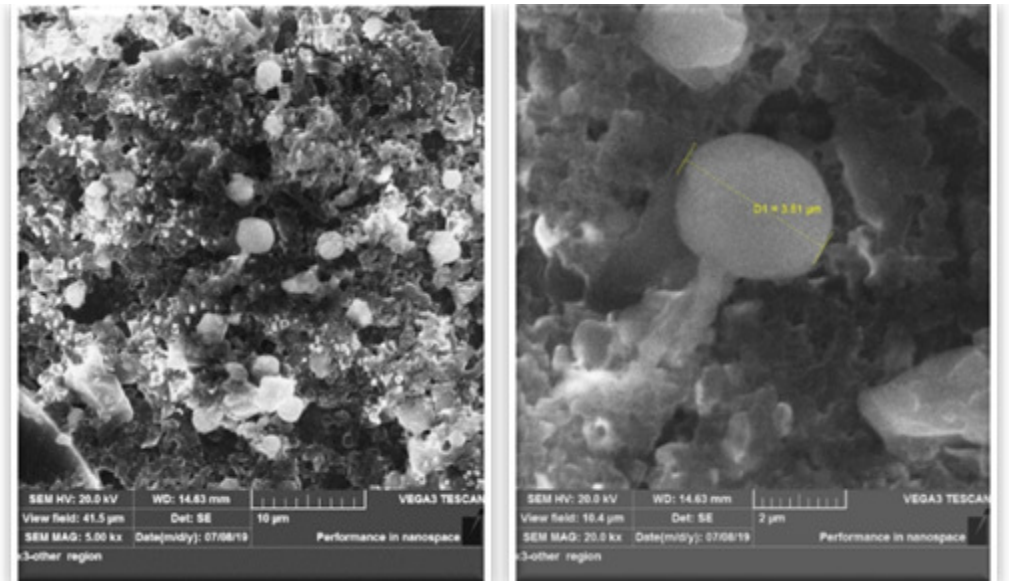

Fig. 4 : Scanning electron micrographs of VRE phages by VEGA 3 TESCAN microscopy (10 mμ).

Table 7 : Phages efficacy module (mean log count) on VRE clones.

Phages cocktail	Plaques mean log count (PFU^{-1} ml)		Spots Morphology
	VR *E. faecalis*	VR *E. faecium*	
Mastitis	7.397 ^{Aa}	6.518 ^{Aa}	Large clear spots
Subclinical	4.301 ^{Ba}	3.361 ^{Aa}	Small-large, turbid-clear spots
Normal	1.176 ^{Ca}	1.113 ^{Aa}	

A, B, C: Indicate clinically and bio statically significant differences vertically within Phages efficacy at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

Table 8 : RTD virulence titers for each cocktail.

Phages cocktail	RTD virulence titers for each cocktail	
	VR *E. faecalis*	VR *E. faecium*
Mastitis	Log 5 ^{Aa}	Log 3 ^{Ab}
Subclinical	Log 3 ^{ABa}	Log 2 ^{ABa}
Normal	Log 1 ^{Ba}	Log 1 ^{Ba}

A,AB, B: Indicate clinically and bio statically significant differences vertically within RTD titers at level (p≤0.05).
a, b: Indicate clinically and bio statically significant differences horizontally within clones at level (p≤0.05).

DISCUSSION

Deciphered strange, accumulative and emergent behaviors present among man, food-producing animals and food chain triangle inside Iraqi ecosystem. Dogmatic increase of modified old and even new unknown cases of variable modules ending with the appearance of dangerous infectious foci here and there. Our study reveal recovery of reciprocal powers of life represented by phages and death represented by multidrug-resistant enterococci from the same niche as opposite module. "ALLAH", most gracious, most merciful, set of scales between corrupt and decent surrounded by stimuli stressors ending with different choices and destiny. Sophisticated dramatic changes in genetic material of opportunistic translocated microorganisms governed by environmental ecosystem module and symposium with other microbiota encourage in transferring of new characters from one to another. So on segregated selective disease changed from narrow- to broad-spectrum with the appearance of new clever enemies own their developed strategies guided by quorum brain-like machine, genes sharing strategies, CRISPR-CAS module and even chimera. In the same house, nanoscale powerful antimicrobial bacteriophages were present to neutralize or buffering the sequels. Controversial opinions present among applications of phages in our ecosystem in food, feed, for therapy, as bio-preservatives (cold pasteurization), with antibiotics, etc. due to safety issues and guarantee priorities in consumers. Lytic vs lysogenic lifecycle cocktail, some adverse effects on good microflora, sensitivity of our immune defense barriers due to foreign behavior and so on the fear from uncontrolled impulsivity unbalanced extra enthusiasm leading us to letdown the new era of our clean and safe life with these

good nano biota against extra levels of modified struggling emergencies. Verified references encounter measures these struggling events (Merabishvili *et al*, 2009).

Our data recapture different events for increased cases of bovine mastitis not because contaminated and polluted environment only with other interconnected sources of infections like infected active and passive series of carriers (workers and animals), contaminated and polluted water and containers, flies, dust, etc. but also due to negligence, carelessness, struggling with life stressors, uncontrolled importation and entrance of new foreign infectious genetic materials and germs from outside the Iraqi ecosystem inserted and developed accumulatively within locally low virulent strains and so on changed the formula to cascaded level of hazards like in Chinese Coronavirus that developed suddenly. Different series of accumulative researches satisfied with the appearance of new emergent infectious foci of multidrug resistant foodborne pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA) in our ecosystem after 2003 and be continued and increased until now with biofilm-plasmid clones of vancomycin-resistant enterococci (Al-Shammary, 2015). The problem was the dramatic change in harboring host with adaptation strategies of pathogens from man to animals or animals to operate and so on to food chain and vice versa with zoonosis-reverse zoonosis interconnected cycles. These sophisticated intelligent behavioral movements cannot be done by our local strains unless presence of foreign inserted genetic material from different resources like importation. Complex Iraqi life throughout wars might encourage the appearance of these emergencies. Variable territory environment might indeed create a link among outbreaks in regions like Abu-Ghraib, Al-Sadrya and Al-Fudhaliyah but the recovery of new multidrug-resistant enterococci from mastitic milk by these numbers indicate dangerous changing module of infection from narrow-spectrum low virulent to broad-spectrum high virulent one. Verified references encounter measures these struggling events (Al-Shammary, 2015).

All these events open the gate of old-new post antibiotics epoch represented by applications of new strategies to overcome these increased variables like discovered combo new antibiotics, bacteriocins, plants extracts, probiotics, biofilm inhibitors, lytic bacteriophages and others. Our data revealed recovery of phages cocktails from the same milk units that destroy these plasmid and biofilm-encapsulated infectious foci of VRE clones. Mastitic encountered phages were the strongest and virulent bactericidal lytic operators with high affinity to inserted and disrupt the barriers of biofilm layers and cell membranes of encapsulated clones recaptured by plaques-spots denominators and scanning electron microscopy. Activated phages phenomenon might encounter these behaviors, as there was recaptured indirect clues refers to silent or dormant phages might be linked by encapsulated Nano rooms and tubules with the host microbiome like a symposium of baby with their mother by the umbilical cord but when these ligands were cutouts for any reason, this will activate these phages to begin sonly other intracellular life cycle or die, thus one of the drawbacks of recovered phages was short life cycle and lacking of repair machine that needs new host to attached or anchored and so on to lived. These encountered phages in our ecosystem from different sources as sewages (Hamasalih, 2017) can helps many diseased peoples to cure from of struggling behaviors and purifies our food chain ecosystem from different hidden and developed problems like in our study plethora or placebo. Verified references encounter measures these struggling events (Merabishvili *et al*, 2009).

To date, ultimate strategies designed for estimation of the efficacy of recovered phages trials verified. Modified protocols were justified for recovery and redirection of phages cocktails against new developed module of antibiotics resistance without elicitation of phages resistance module. In addition, the mechanisms concerning coevolution between phages and bacteria are unknown. Further trails specifically devoted to solve these problems desired before phages certified in humans, animals, food chains and environment (Principi *et al*, 2019). Interesting in phages therapy module increased with the increasing of antibiotics resistance problems. Overcoming new enemies by verified phages encounters different hindrances such as delivery barriers into the living matrix and developing of bacterial resistance to phages. So that, modified delivering a programmable DNA nuclease by redirected phages against clustered regularly interspaced short palindromic repeats associated (CRISPR-CAS) to reverse antibiotic resistance and eliminate the transfer of resistance among strains was a promising module. Combination of CRISPR-CAS delivery with lytic phage selection of antibiotic-sensitized bacteria. This strategy may reduce the prevalence of antibiotic-resistant bacteria in treated surfaces and on skin of medical personnel, as it uses phages in a unique way that overcomes many of the hurdles encountered by programed phages. A proof of concept for a genetic strategy that aims to sensitize bacteria to antibiotics and selectively kill antibiotic-resistant bacteria by using temperate phages to deliver a functional CRISPR-CAS redirected ecosystem into the genome of antibiotic-

resistant bacteria. The delivered CRISPR-CAS redirected ecosystem destroys both antibiotic resistance-conferring plasmids and genetically modified lytic phages. This linkage between antibiotic sensitization and protection from lytic phages is a key feature of the strategy. It allows programming of lytic phages to kill only antibiotic-resistant bacteria while protecting antibiotic-sensitized bacteria. Phages designed according to this module can assist in verified style for ending of increased antibiotics resistance problems in our ecosystem and certified on hospital surfaces and hand sanitizers to facilitate the replacement of antibiotic-resistant pathogens with sensitive ones (Yosef *et al*, 2015).

In conclusion, struggling with new active bio-preservative and matrixed nano biosensor lytic foodborne phages against multidrug-resistant enterococci located and borne in the same niche ecosystem reveal a promising tool for healthy lifestyle in Baghdad. In the last twenty years, Iraqi ecosystem changed dramatically with accumulative stressors to unhygienic media due to different known and unknown causes. Foodborne pathogens modified strangely without any insults or signs of resistance behaviors. Unsolved human-animals syndromes affect stressfully on peoples with continuous hazards. Foodborne bacteriophages or enterococci eaters can assist in remodeling and buffering the effect of dangerous infectious foci for multimedia ecosystems: Prophylactics', therapy, feed-food and even environment sprayers, etc.

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Conflict of interest

The authors declare they do not have any conflict of interest.

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