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Dynamic Efficacy of Cloves Against Pluripotent *Acinetobacter baumannii* Recovered from Raw Milk of Mastitis Cow

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Abstract | A pluripotent *Acinetobacter baumannii* isolate was recovered from the milk of a mastitis cow and confirmed using the California mastitis test (CMT). Antibiotic sensitivity assays demonstrated that multi-stress resistant isolates from raw milk were extensively resistant to antibiotics (XDR phenotypes) as augmented vis Vitek2, and extremely tolerant to highly doses of type C ultraviolet irradiation cold pasteurization regime using forbidden diffused electromagnetic clouds of recalcitrant biofilm stratus, and dual drive microtiter plate assay as DNA dependent and Congo red plate assay as plasmid dependent. These evolutionary mastitis foci pathogens with Methicillin Resistant *Staphylococcus aureus* MRSA clones and extended spectrum beta lactamase resistant *Escherichia coli* ESβL clones inside a profuse biofilm barrier. Locally cloves extracts produced and tested clove extracts, prepared fresh with a highly aromatic flavor were found to terminate these evolutionary entities as a healthy, hygienic remedy with a dynamic efficacy potentiation module that exceeds that of tested antibiotics. Taken together, Snyder's evolutionary clones of *A. baumannii* with multi-stress hardening cover prohibited behaviors to diverse and versatile selected antibiotics and an extra UVC irradiation decontamination strategy was terminated intelligently with the cheapest health extracts of *Syzygium aromaticum* (Cloves).

Keywords | *A. baumannii*, Multidrug resistant XDR, UVC irradiation tolerant, Cloves

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INTRODUCTION

Cascaded pollution by multidrug-resistant microbes, such as the predominant *Staphylococcus aureus* (MRSA) and *Escherichia coli* lineages (ESβL), especially those evolving into extensively drug-resistant (XDR) clonal ancestral strains within food chain, signifies a public health emergency priority. The dairy chain represents an important reservoir and bridge for the transformation of these entities and their genes. Conjugated plasmids bridges, foreign external DNA acquisition transformation

and transduction with forbidden CRISPR/Cas prophages represents the predominant evolutionary trails for construction of these chimeras (Almutairy, 2024; Dan and Talapan, 2024; Machado *et al.*, 2024; Aljohni *et al.*, 2025; Daruka *et al.*, 2025; Farrukh *et al.*, 2025; Machado *et al.*, 2025; Ng *et al.*, 2025; Rastmanesh *et al.*, 2025; Wang *et al.*, 2025). Catastrophic sequels cascaded from the pollution of dairy chain by an emergent foreign biohazard mirror entity, well equipped with stress genes expressed as chimaeras covered by a biofilm barrier (Al-Shammary and Abdul Mounam, 2023). *A. baumannii*, an emergent topic

priority verified and certified as an ESKAPE chainsaw puzzle mirror cascaded pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), a group of pathogens with a high rate of antibiotic resistance that are responsible for the majority of nosocomial infections. Colloquially, *A. baumannii* is referred to as “Iraqi bacter” due to its sudden emergence in military treatment facilities during the Iraq War. Multidrug -resistant *A. baumannii* and even remarkably or extensively drug-resistant clones XDR ancestral patterns have spread to civilian hospitals post -epidemic, in part due to the transport of infected soldiers through multiple medical facilities. During the COVID-19 pandemic, coinfection with *A. baumannii* secondary to SARS-CoV-2 infections has been reported multiple times in the literature (Charm, 2023; Al-Yais and Al-Shammmary, 2024;; Al-Yais and Al-Shammmary, 2024; Dorgham *et al.*, 2024; Mellace *et al.*, 2024; Savin *et al.*, 2024; Traglia *et al.*, 2024).

The dynamic efficacy of plant extracts and special topic cloves extracts (*Syzygium aromaticum*) offers powerful antimicrobial remedies and safe cytotoxic active ingredients (Al-Shammmary and Abdul Mounam, 2017; Liu *et al.*, 2017; Al-Shammmary and Dakheel, 2020; Faujdar *et al.*, 2020; Razooqi and Al-Shammmary, 2020b; Mahmoud *et al.*, 2021; Al-Shammmary and Abdul Mounam, 2022; Maggini *et al.*, 2024; Zhao *et al.*, 2024). Complementary to antibiotics, lytic bacteriophages have the potential to act as bio-preservative strategies and have been extensively documented and deciphered against multidrug -resistant pathogens. However, these strategies are lacking against multi-stress evolutionary mirrors, such as XDR and UVC-tolerant clones recovered from mastitis raw milk. Therefore, this study was designed and executed to decipher the antimicrobial potential overcoming the emergency problems of recovered clonal isolates of *A. baumannii*.

MATERIALS AND METHODS

Diverse and versatile frequency patterns of *A. baumannii* were observed in mastitis milk collected from retail markets. Isolation and confirmation tests were performed on positive California mastitis test (CMT) samples, which were enriched with a selective and differential *A. baumannii* medium. The enrichment broth and agar were supplemented with tryptone and yeast extract. Timelines seasonal episodes were dependent on the collection of samples, extended from January to September (2024), in which two hundred seventy (n= 270) samples were presented as thirty (n= 30) samples from all regions monthly (deposited as ten (10) per region monthly). All samples were tested for a positive CMT score, and then cultured periodically after resuscitation, enriched

via TSBYE overnight at 37 °C. Incubated broths were cultured on selective and differential *A. baumannii* chrome agar overnight at 37 °C. Augmented colonies were picked up, filtered, and purified as the original seeds on TSBYA for processing with tissue culture microtiter and Congo red plate assays for biofilm detection, Vitek 2 test kits for dual biochemical identification, and a segregation regime based on antibiotic susceptibility patterns. The identified isolates were selected based on resistance patterns to proceed with 16S rRNA polymerase chain reaction (PCR). In this process, resistant PCR-documented isolates were challenged for tolerance against an ultraviolet irradiation decontamination regime, followed by susceptibility to lytic bacteriophages. Only isolates that resist both antibiotics and UVC irradiation, with abnormal potency remodelling to lytic bacteriophages expressed and deciphered as evolved prophages, were selected for the antimicrobial properties of the oily cloves extract.



Figure 1: Clove extracts are imported as oil (A) and fresh raw leaves (B).

Built-in dual drive trails were dependent on *A. baumannii* patterns, which augmented biodiverse biofilm electromagnetic polysaccharide clouds. This was verified by a modified roll-cup TSBYE microtiter tissue culture plate assay for direct assessment of chromosomally built-in slime and a verified Congo Red agar for indirect assessment of plasmid-dependent slime potency. Built-in Sensitivity and Specifically Pattern equivocally Slime Virulence Index was documented as a verified mean log of both interconnected built-in growth pattern techniques represented by generation time and log curve primordial built-in design (Al-Shammmary, 2009). Built-in quorum sensing, a trusted platform module, overtone, via a cascaded chain of multi-stress hardening, genetically hidden sophisticated bionetworks, augmented within the *A. baumannii* denominator lineage, shifts an emergent problem to the endpoint of recalcitrant views. Antimicrobial susceptibility testing (AST 17 GN ID panels series) is a significant bio-identification technique for recovered clinical isolates

(Al-Yais and Al-Shammmary, 2024). The results are cast off for the selection of the accurate drug of choice for *in vitro* determination of the responses of *A. baumannii* to antimicrobial agents. Based on authorized supervisor experience and instructions of national committee of clinical laboratory standards (NCCLS) formerly clinical laboratory standards institute (CLSI, 2024) guidelines followed in this account of the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966) study the sensitivity and susceptibility patterns of recovered *A. baumannii* isolates to selected and grouped antibiotics (HiMedia®, India, 2024): A dose dependent ecomaps gradient concentrated discs distributed as cascaded with selective pressure presented upstairs were dependent for this augmented torment as in detailed manual instructions of company legislation leaflet publisher (HiMedia, 2024).

A 16S ribosomal RNA subunit-dependent enrollment verifies the biodiversity of primed recovered *A. baumannii* isolates from local raw milk samples versus adapted food chain ancestors. A phylogenetic interconnected genotypic relationship exists between host-specific isolates and their linked ancestral tree. Primed cascaded experimental methods and workflow built-in analysis on sequences and confirmation of microorganism's homogeneity data using rRNA database (NCBI) after amplification of targeted denominator ribosomal RNA. All processes, including special topic gDNA extraction, PCR amplification, sequencing, and assembly. For *A. baumannii*, PCR on 16S rRNA using 27F and 1492R primers, yielding 1,300 bp or more of sequencing data, illustrates and deciphers this dogma. Dependent protocol UPGMA dendrogram was a reciprocal relationship between local *A. baumannii* isolates in terms of genetic sequence and worldwide bank isolates recorded within NCBI databases in which, represented as a chi square cube cascaded by increase similarity matching score with the decrease calculated numbers among cubes and vice versa with increasing dissimilarity index with increase the differences numbers among cubes (Shah *et al.*, 2019). Ultraviolet (UV) is a form of electromagnetic radiation with wavelengths shorter than those of visible light, but longer than X-rays. The UV region covers the wavelength range 100-400 nm and is divided into three bands: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm). The mechanisms of UVGI on microorganisms are uniquely vulnerable to light at wavelengths of 253.7 nm or near this value because the maximum absorption wavelength of a DNA molecule is 260 nm. After UV irradiation, the DNA sequence of microorganisms can form pyrimidine dimers, which can interfere with DNA duplication, as well as lead to the destruction of nucleic acids and render the viruses noninfectious. Microbial death induced by UVC has been attributed to DNA mutations, including the formation of cyclobutyl-type dimers (pyrimidine dimers) and pyrimidine adducts. Furthermore, the overproduction

of reactive oxygen species (ROS) induced by UV radiation can oxidize membrane lipids and inhibit critical cellular enzymes; therefore, Gram-negative bacteria are more susceptible than Gram-positive bacteria. 1 Gray (Gy) = 1 Joule/kilogram = 100 rad (radiation adsorption density). Grey can be used for any type of radiation (e.g., alpha, beta, neutron, gamma), but it does not describe the biological effects of different types of radiation. Biological effects of radiation are measured in units of "sievert" (or the older designation "rem") (AL-Salihi and Al-Shammmary, 2024).

Absolute measurement exposed the amount of UVC energy emitting power on the target per unit area, expressed in milliwatts or millijoule second per square centimeter (mW-s or mJ-s/cm²) in which, standardized inside biosafety cabinet by 40 watts mercury fluorescent lamp to a one-meter distance vertical area via exposing emitting dose dependent power 125 mw mJ.s/cm² to a cumulatively reach to 30 KGy (3 x 10⁶ rad) in about 12.5 minutes that's fair enough to disinfect the area under the hood (2 x 2 meter distance of stainless steel hood bench surrounded by special corning glass). Most susceptible microorganisms, either RNA or DNA, single-stranded or double-stranded genetic material, can be affected and killed at a range between (1-10) UVC Gy. Targeted experimental module exposing these forbidden multidrug resistant recovered isolates directly on agar and indirectly within contaminated milk or water inside a biosafety cabinet for one hour period to determining whether susceptibility patterns (tolerance vs resistance) despite disinfecting protocol in which, radiation dose reach approximately (4-5) folds original standardized dose (30 KGy) i.e., (120-150 KGy). Most Bacteria and viruses are sensitive to (1-10) Gy UVC for (5-15) secs. "UV dose (μW·s/cm²) = UV intensity (μW/cm²) × exposure time (seconds)" (AL-Salihi and Al-Shammmary, 2024). The inactivation efficiency of bacteria was analyzed by calculating log inactivation using Eq. (Hobbs *et al.*, 2022): "Log inactivation = Log (N₀/N) = k×D" in which, N₀ and N were the colony counts (CFU/mL) before and immediately after disinfection, k was the UV inactivation rate constant (cm²/mJ), and D was the dosage of UV treatment received by the bacteria (mJ/cm²) (Wang and Zhang, 2023).

Orchestrated multi-stress behaviors were recognized in XDR isolates of *A. baumannii*, characterized by cascaded and sophisticated built-in epigenetic tolerance drift and genetic resistance shift quorum sensing interconnected mechanisms. An *in vitro* dual-drive UVC light irradiation susceptibility experimental model (Direct irradiation of inoculated TSAYE and Indirect irradiation of UHT white whole milk tetra packs experimentally polluted with eight logs of *A. baumannii*) was initiated to assess the resistance pattern. The radiation-irradiation experimental project was

enrolled in Department of Veterinary Public Health/Milk Hygiene lab for postgraduates in which, a selected multidrug resistant PCR primed genotypes of *A. baumannii* were freshly enriched and boosted with dsTSBYE overnight at 37 °C, then titrated and standardized with MacFarland opacity tubes throughout counting series of droplet and roll-pour plate techniques to be dose dependent curve of eight logs 10⁸ CFU.ml⁻¹ for each isolate. A biosafety cabinet supported by a 15-W mercury vapour 254-nm germicidal lamp with a total emitted pooled adjusted dose-dependent radiation beam of 25 J/cm². Freshly cultured plates on dsTSAYE were exposed to these wavelengths at approximately fifty cm distance (critical checkpoint plates were opened, not covered, due to the UV light beam not penetrating the covers). According to the radiology field, UVC lights act as a bactericidal decontamination regime with this range of wavelength in a specified period, not exceeding twenty minutes, in comparison to laser beams of infrared irradiation protocol (fifteen seconds to two minutes). Control positive inoculated not irradiated cultures and control negative irradiated not inoculated cultured plates and polluted UHT white whole milk tetra packs (twenty ml of UHT milk decanted in a serial disposable fifty ml cups polluted and mixed thoroughly with one-hundred micron of freshly prepared and titrated half McFarland eight logs *A. baumannii*) were enrolled in this verified achievement. Primordial triplicates for each iconic culture stone were exposed to UV for a sixty-minute interval following the supervisor's vision and experience. The intensity of radiation was designed to flood approximately a fifteen ft² area under the hood. Irradiated cultures were covered with their covers inside a hood and then incubated at 37°C for 24 hours. The radiation susceptibility index for selected isolates was compared to the standard zones of inhibition of Recommended Antibiotics, selected and standardized as a control parameter following updated CLSI instructions and standard tables of antibiotics (CLSI, 2024). All UV light processed units with alternate pairs of control positive and negative trials were cultured directly in the case of plates and indirectly from processed polluted UHT milk units and replicates cascaded by MM droplets and roll-tube pore plate techniques, followed by incubation at 37 °C for 18-24 hours. According to irradiation strategies, a direct DNA breakdown occurred, resulting in the death of *A. baumannii* or the verification of tolerance versus resistance behaviours. Indirect hurdling with cooling processed polluted UHT milk units inside a refrigerator at 4 °C, then repeated culturing on TSAYE upstairs either assist in termination or killing of recovered isolates mix indirectly during activation of free radicals from waters or abnormally resuscitate dormant forbidden tolerant vs resistant clones of *A. baumannii* overall total, three times episodes of culturing with replicates, then recording counting results in mean logs with critics, phenotypic changing of colonies and isolates after direct

and indirect impedance to UVC light regimes with aid of scanning electron microscopic micrographs (SEM).

Colloquially, lytic bacteriophages susceptibility pattern verified via phage ø buffer WYVERN76 (NaCl 6 g + MgSO₄ 2 g + CaCl 2 g + Tris 8 g + Gelatin 0.1 g) in 1 litre distilled water deciphered in the downstream potentiation unit. The hot-cold regime was dependent (Al-Naseri and Al-Shammary, 2020a; Al-Shammary and Abdul Mounam, 2020-2023) on the local *in situ* fresh preparation of lytic bacteriophage cocktails, which involved mixing one part yoghurt with four parts buffer (250 ml yoghurt to 1 litre buffer). Overnight incubation inside a refrigerator at 4 °C. Propagating of lytic phages by mixing freshly prepared 10 logs MacFarland broth of *A. baumannii* in TSBYE as 1 ml to 250 ml of overnight yogurt upstairs, then incubation overnight at 37 °C. Centrifugation and filtration by filter paper drop by drop and the decanted residual water like suspension is a raw bacteriophages cocktail. Plaques Spots technique enrolled via mixing 0.1 MacFarland *A. baumannii* with 0.1 ml concentrated Ø incubation at 37 °C for one hour (Resident A). The modified verified roll pour plate technique was employed by mixing melted temperate TSAYE in a cup containing resident A for a minute, followed by rolling and then pouring into a plate to solidify. The plate was then incubated overnight at 37°C. The appearance of holes, spots, or plaques was observed. Verification via Scanning Electron Microscope. A VIP critical augmented redirected processing enrollment was conducted by adding freshly prepared 0.1 mL McFarland dsTSBYE *A. baumannii* to assigned 50 mL volume plastic cup containers (ten logs CFU/mL), followed by decantation and mixing 25 mL of UHT tetra packs of white complete cream milk units (experimental pollution regime). Add freshly prepared 0.1 mL McFarland dsTSBYE lytic bacteriophage cocktail, recovered and redirected from fresh, ropy, pooled yogurt (ten logs PFU/mL). Incubate for two hours at 37 °C to accommodate the specific receptor reaction. Segregate two subunits (direct and resuscitation indirect). First culturing with modified verified roll-cup pore plate technique. The second overnight incubation of phages processed UHT milk units was performed inside a refrigerator at 4 °C. Subculturing was carried out using the cooled subunit with the upstairs roll-cup pore plate technique (Al-Shammary and Abdul-Mounam, 2023; Al-Salihi, 2024).

Cloves extracts (*Syzygium aromaticum*) were prepared and tested for their efficacy active ingredients by cross comparability versus compatibility modules in which, an imported oily extract from Pakistan was purchased with new production date (freshly synthetic) while locally purchased cloves were prepared freshly in two directions (Nzeako *et al.*, 2006) firstly, 40 g fresh cloves aromatic flower buds powders were mixed with 100 ml fresh cold distilled water thoroughly via vortex for fifteen minutes,

then left settling inside a refrigerator at 4 °C for (24-48) hours, after that settled bottled tubes transferred to shaker water baths with continuous mixing at 37-40 °C gradient temperatures for (30-45-60) minutes, then cooling at lab temperature for modified Kirby-Bauer impregnated concentrated discs diffusion method (discs immersed with fresh extract for five minutes) to checking susceptibility patterns of XDR-UVC tolerant and prophages impedance isolates to both extracts in comparison to standardized authorized drugs of choice antibiotics that resist to them either actively or passively as biofilm persisters. Active ingredients are prepared oppositely, freshly from local cloves using a reciprocal method, as a result of a water bath hot step followed by a cold state phase. Dual driver techniques were employed for the extraction of watery versus oily aromatic active ingredients from fresh, locally purchased cloves. Ciprofloxacin in dose dependent curve concentration (CIP 5 µ) as a standard control (as disc's for Kirby-Bauer technique and as complementary formulated Vitek AST) to check susceptibility efficacy index of tested cloves extracts with their decimal reduction times (D-values) required to inhibit or inactivate *A. baumannii* growth patterns even their in dormant biofilm persisters state phase, cascaded by minimum inhibitory concentrations (MICs) as the lowest concentration of CIP that will inhibit the visible growth of a target after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of CIP that will prevent the growth of target after subculture on Muller-Hinton versus dsTSAYE agars. So that, only recovered XDR isolates that resistant to CIP (according to CLSI antibiotics tables (2024) reference zone of inhibition for CIP 5 µ (mm): 29 mm ≤ Resistant versus 38 mm ≥ Susceptible) were selected as a control group for determination of efficacy values of tested imported versus locally synthesized cloves extracts.

RESULTS AND DISCUSSION

Biostatistical integration was a predominant tool for deciphering and matching displayed calculated cascaded results series at confidence intervals 95 and 99 % in which, all observed results were analyzed by statistical analysis system (SAS, 2018) software program throughout interconnected values of significant and non or insignificant probability index of $p \leq 0.05$ via dependent analysis of variance (ANOVA) with least significant difference (LSD) cascaded by Chi square (χ^2) to understanding normal distribution nature of samples and their replicates. Not always non or insignificant results means they were not important clinically or scientifically in accordance to pairs of null and alternative experimental hypothesis design but this dependent primarily on virulence indices cascaded by evolved isolate i.e., their genetic makeup in terms of genetically modified microorganisms as priority bioterrorism special topic issue within food chain with

other interconnected predisposing factors and ecosystems in terms of zone of infection cascaded by episodes times intervals. In conclusion, Clinical observed trials were significant, although their statistical values were not significant in some situations.

Emergent microbial pollution violence: Unhygienic predominant pollution of dairy chain cascaded by series of frequency and distribution microbial growth epidemiological patterns of genetically modified, highly infectious prohibited predominant foci of *A. baumannii* derived from resident portals of entrance throughout cascaded bridges to be deposited within Baghdad retail markets specifically among raw milk with continuous stress adaptation, response and hardening accommodation strategies. This predominant, violent, biohazard-targeted topic denominator was genetically well-equipped entities augmented to survive harsh environments with other infectious and contagious foci inside a recalcitrant barrier of electromagnetic clouds of biofilm to establish an unsafe and sophisticated struggling hygienic problems with diverse and versatile, multi-stressors impendency, drift-shift antigenic transformations behaviours ending with banned sequels (Al-Shammary, 2009; Kanaan and Al-Shammary, 2013; Al-Shammary, 2019; Al-Naseri and Al-Shammary, 2020b; Al-Salihi and Al-Shammary, 2024; Al-Yais and Al-Shammary, 2024). Pollution of food chain by antimicrobial resistance genes across sophisticated interconnected mechanisms of vertical and horizontal gene transfer in which, "vertical multiplication and proliferation of tolerant versus resistant denominators" cascaded via "horizontal scenario deciphered as a genetic material can be transferred either by conjugated plasmids or transformation from external forbidden genetic materials of killed and lysed extensively drug resistant (XDR) phenotypes (temporarily epigenetic tolerant drifts) versus genotypes (permanent genetically mutants resistant persisters) within environmental ecosystems of food, water and air chains, and via transduction forbidden prohibited lysogenic prophages. These scenarios of emergent bioterrorism are overwhelmed with recalcitrant biofilm (Sagar *et al.*, 2020).

Below mentioned (Tables 1, 2, 3) and (Figures 2, 3) decipher the special topic workflow:

Boosting synergistic potentiation efficacy, the bactericidal module displayed among watery extracts of cloves and ciprofloxacin, while no or slow bacteriostatic inhibitory effect of this type of extract was observed when subculturing a loop or swab from small inhibitory zones of the extract in Muller-Hinton agar on dsTSAYE. Various diverse and versatile interconnected issues, influences, features and reasons presents and displayed between local and imported cloves extracts as clinically significant efficacy in fresh local in comparison to no or slow action potential bacteriostatic

Table 1: Susceptibility index for selected antibiotics against recovered *A. baumannii* isolates from positive CMT milk samples in Baghdad retail markets.

Region	Recovered isolates	Resistant phenotypes	CIP resistant	XDR phenotypes
Al-Fudhaliyah	7 (2.592 %)	6 (2.222 %)	4 (1.481 %)	1 (0.370 %)
Abu-Ghraiib	9 (3.333 %)	7 (2.592 %)	4 (1.481 %)	2 (0.740 %)
Al-Sadrya	9 (3.333 %)	8 (2.962 %)	4 (1.481 %)	2 (0.740 %)
Total	25 (9.259%)	21 (7.777 %)	12 (4.444 %)	5 (1.851 %)
Chi-square test: χ^2	0.3618			
P-value	0.999992			
	Significant differences at * (P≤0.05).			

Table 2: Cloves extracts susceptibility pattern against recovered *A. baumannii*.

XDR isolates	MacFarland Pollution	Zone of Inhibition (mm) for CIP	Zone of Inhibition (mm) for Cloves	
			Local	Imported
Al-Fudhaliyah	8 Logs (10 ⁸ CFU.ml ⁻¹)	20 Resistant	45-55 Susceptible	8-25 Resistant
Abu-Ghraiib		15 Resistant	40-50 Susceptible	6-10 Resistant
Al-Sadrya		10 Resistant	40-45 Susceptible	15-25 Resistant
Chi-square test: χ^2	7.215			
P-value	0.124956			
	Significant differences at * (P≤0.05).			

Table 3: Cloves D-values against XDR *A. baumannii* (MIC-MBC verges).

XDR isolates	MacFarland pollution	Logs reduction fold (D-values)	
		Local	Imported
Al-Fudhaliyah	8 Logs (10 ⁸ CFU.ml ⁻¹)	(6-7) Logs ^A	(1-2) Logs ^B slow
Abu-Ghraiib		excellent	efficacy
Al-Sadrya		efficacy	

A, B: Significant differences (ANOVA) among local and imported Cloves extracts reduction logs of XDR *A. baumannii*. The excellent efficacy of local clove extracts in terminating XDR *A. baumannii*, including persisters, is bactericidal, whereas imported clove extracts exhibit no or slow bacteriostatic antimicrobial activity.

module in imported; might be to differences in the type or species of cloves, storage conditions, freshness or duration (time) of preparation, variation in active ingredients and their potency and concentrations between both types of extracts, type of isolates and their genotypes differences in susceptibility patterns to both extracts, types of samples in which they recovered with active and dormant biofilm persisters associated with other host defense barriers present in mastitis like antibodies, bacteriocins, lytic bacteriophages against CRISPR-Cas XDR *A. baumannii*, idiosyncrasy effects between *in vitro in situ* and *in vivo* efficacy with host defense barriers, etc. Al-Shammary and Abdul Mounam (2017) investigated the efficacy of extracts from oak, cinnamon, oregano, and thyme on biofilm-producing ESβL *Klebsiella pneumoniae*. Al-Shammary and Dakheel (2020) determined cut-off values for blackcurrant, London plane, and pine buds about

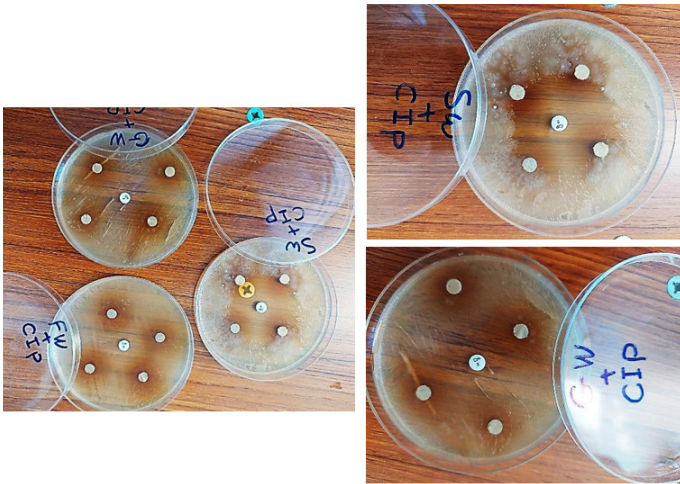


Figure 2: Clarification efficacy module of local watery extracts of cloves (periphery infused discs) against XDR *A. baumannii* in comparison to the middle disc of standard control antibiotic CIP. Apparent bactericidal terminatory effect of these fresh local extracts when subculturing a loop or swab from large inhibitory zones of extracts in Muller-Hinton agar on dsTSAYE and boosting synergistic potentiation efficacy of the bactericidal module displayed among watery extracts of cloves and ciprofloxacin.

vancomycin-resistant *Enterococci* (VRE clones) isolated from mastitis milk in Baghdad. Al-Shammary and Mounam (2023) deciphered the “Exodia” phenomenon of foodborne “Mycophages” cocktails against chimeric strains of *Candida albicans* (Azoles resistant ARCA clones) recovered from dairy chain ecosystems in Baghdad. Synergistic potentiation efficacy displayed between local

cloves extracts and ciprofloxacin as resident in [Figure 2](#) against XDR *A. baumannii* with complementary targeting of fresh cloves active ingredients to combating biofilm defense barriers of forbidden isolates with sensitization activation of dormant state persisters within recalcitrant biofilm to transformed from lag to log phase cascaded with inhibition of their house keeping stress hardening strategies and so on potentiation bactericidal activity of ciprofloxacin against resident denominators.

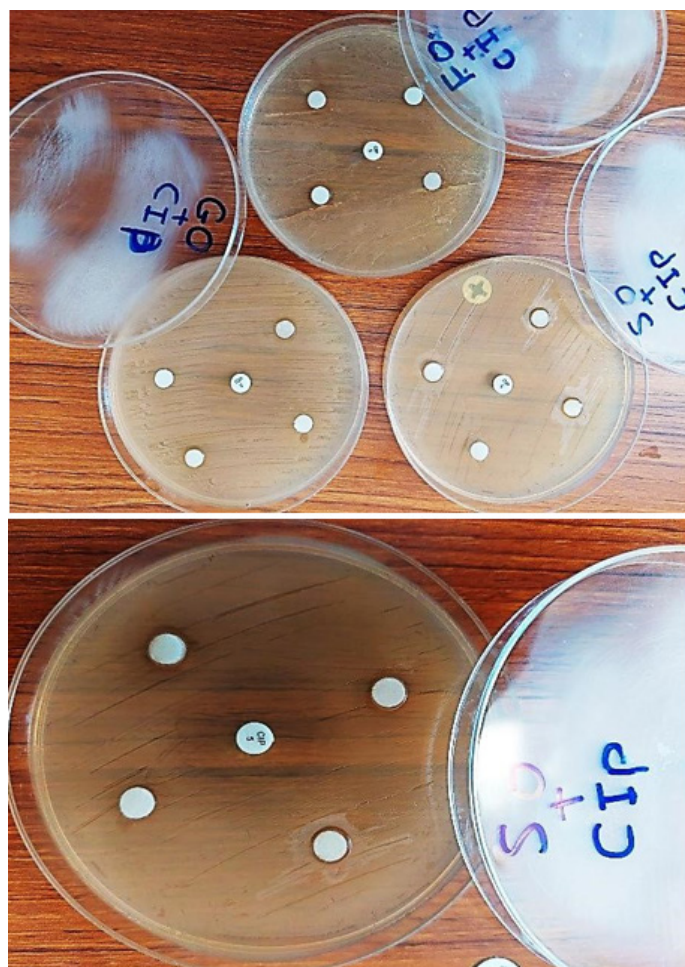


Figure 3: Clarification efficacy module of imported oily alcoholic extract of cloves (periphery infused discs) against XDR *A. baumannii* in comparison to the middle disc of standard control antibiotic CIP. No or slow bacteriostatic inhibitory effect of this type of extract when subculturing a loop or swab from small inhibitory zones of the extract in Muller-Hinton agar on dsTSAYE.

Conservative and semiconservative microbial defence mechanisms represent a battle bridge arms race between bacterial immune ecosystem and eco-friendly lytic bacteriophages, deciphered as diverse cyclic oligonucleotide-based antiphage signaling system (CBASS) and pyrimidine cyclase system for antiphage resistance (Pycsar), a brain cell-suicide machine in diverse bacteria that use cyclic nucleotide signals to induce cell death and prevent viral propagation. Diverse, intelligent

CBASS and Pycsar cascaded components, in association with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Thoreis ecosystems, represent the main antiphage defence strategies to overcome or combat viral invasion. CBASS is chelated in a cascaded, dependent manner to activate different pathways, including programmed cell death and induced apoptosis, thereby preventing phage propagation through a series of DNA degradation, membrane destruction, and nicotinamide dinucleotide (NAD) depletion, ultimately preventing the readout of viral machinery molecules. While others, such as CRISPR-CAS and Thoreis, tolerate and resist viral infection and replication. In mammals, viral infection activates remote modelling cellular DNA operons to initiate interferons, especially gamma isotypes, to protect other normal, non-infected cells throughout remote modelling of DNA, so preventing viral integration into genomic material, ending with death of the virus. CBASS ecosystems cascaded throughout activation of CD-NTase to synthesize cyclic oligonucleotides and regulation of cyclic adenosine and guanosine monophosphate (cGAS axis) to production of death associated proteins (CAPs) to execute cell death thereby inhibit phage action ([Łobocka et al., 2021](#); [Hobbs et al., 2022](#); [Wang and Zhang, 2023](#); [Kimchi et al., 2024](#); [Maestri et al., 2024](#); [Patel et al., 2024](#); [Wang and Leptihn, 2024](#); [Wu et al., 2024](#); [Xu and Gu, 2024](#); [Niault et al., 2025](#); [Tesson et al., 2025](#)).

Synergistic combinations among plant extracts, lytic bacteriophages, and antibiotics, deciphered in various derivatives of associated papers in this special topic issue, for combating multidrug-resistant microbes. [Vazquez-Ucha et al. \(2020\)](#) demonstrated an increased susceptibility to colistin in treating multidrug-resistant *A. baumannii* and *Klebsiella pneumoniae* when combined with *Syzygium aromaticum* (clove) essential oil versus *Thymus zygis* (thyme) essential oil. [Malczak and Gajda \(2023\)](#) displayed potentiation remedy between plants active ingredients and antibiotics (to reduce their toxicity) with potent selective toxicity integrated with Terpenes, Thymol, Carvacrol, Citral, Pentacyclic triterpenoids, Clerodane diterpenes, Alkaloids, Capsaicin, Piperine, Berberine, Caffeine, Flavonoids, Diosmin, Silibinin, Baicalein, Licoricidin, Quercetin, Luteolin, Hesperetin and naringenin, Tannins, Corilagin, Tellimagrandins, Punicalagin, Gallic acid, Sulfoxides (Allicin, DMSO), etc. [Sagar et al. \(2020\)](#) and [Al-Tawalbeh et al. \(2025\)](#) demonstrated the antibacterial effects of Cloves, Eucalyptus, and ginger, as well as the synergistic combination of clove extracts and imipenem, versus Augmentin (amoxicillin/clavulanic acid) in combating clinical *Pseudomonas aeruginosa*. [Elwakil et al. \(2023\)](#) demonstrated the effects of natural products, such as curcumin and honey, against multidrug-resistant *A. baumannii*, which is a resident in the UK and Egypt. [Hazzaa](#)

and Al-Shammary (2022c) demonstrated a potentiation-selective regime of pasteurization cascaded by propolis processing versus multi-stress-resistant *Mycoplasma pneumoniae*. Hazaa and Al-Shammary (2022c), Razooqi and Al-Shammary (2020b) demonstrated the biodegradation of recalcitrant foodborne clones of *Candida albicans* by harnessing the pasteurization potential of a peppermint processing ecosystem in Baghdad, Iraq. Paneri *et al.* (2023) displayed the efficacy of modules of cinnamon, ginger, Coriander, Bael, Kiwi, Oregano, and Eucalyptus red river gum in combating carbapenem-resistant *A. baumannii*. Wang *et al.* (2023) displayed a synergistic combination of Ciprofloxacin and Polymyxin B for combating multidrug-resistant *A. baumannii*.

Iraqibacter chimaera *A. baumannii* with multi-stress hardening resistance to antibiotics and ultraviolet irradiation, as displayed by Al-Yais and Al-Shammary (2024), illustration versus deciphering colloquially as “food chain bioterrorism represented via atomic Iraqibacter chimaera *A. baumannii* could struggle to accelerate termination of lifestyle as a constructed new forbidden AI cascaded war. A “Denji” phenomenon of an emergent bioterror biofilm recalcitrant infectious foci of *A. baumannii* recovered from local dairy chain and Human UTI cases in Baghdad evolved as a multi stress hardening denominator with broad-spectrum genetic plasticity that catastrophic resistance to versatile and diverse antibiotics cascaded quorum sensing stress stimuli to proceed it to epigenetic tolerance behavior to ultraviolet irradiation across sophisticated intelligent brain like machines networks”. Chimera patterns of *Escherichia coli* lineage with multi stress hardening resistance to antibiotics and ultraviolet irradiation displayed by Al-Salihi *et al.* (2024) and Al-Salihi and Al-Shammary (2024) illustration versus deciphering colloquially as “Frequency and distribution patterns of diverse and versatile *E. coli* lineage with dynamic pluripotent virulency to different potent microbicidal stressors like pasteurization regimes cascaded by well-equipped genetic plasticity to evolved to a chimeras with forbidden prohibited resistance to different antibiotics and tolerance to ultraviolet irradiation cold pasteurization were resident within tap waters chain especially within purification stations covered and protected by electromagnetic clouds barriers of biofilm in Baghdad as an emergent bioterrorism hazard catastrophically threatening lifestyles in this mess. These emergent entities unveiled in this torment could be terminated by lytic bacteriophages later as a new combating pathway for hurtling decontamination cascaded by sterilization efficacy dynamic module”. Albndar (2025) and Al-Bndar and Al-Shammary (2025) displayed “The mirror entity of XDR phenotypes stress hardening *Staphylococcus aureus* versus *Escherichia coli* patterns recovered from milk ice creams in

retail markets of Baghdad” deciphered as an AI algorithm quorum sensing special topics in pollution epidemiological patterns, overwhelming resistant complex recalcitrant biofilm engaged embedded within electromagnetic clouds of chimeras, that behaves likes a forbidden sophisticated emergent biohazard biofilm entity as Extremely Drug-Resistant Mirrors Entities (XDR-MEs) evolution equipped with stress hardening genes resident within milk ice creams chain.

These evolutionary mirrors entities were partially eliminated when exposed to redirected recovered cocktails of ropy yogurt lytic bacteriophages to be non lysed forbidden clonal colonies presented via plaques plate technique, that developed from continuous hosts variation lifestyle to be a forbidden sophisticated clones initiated via interconnected portals of conjugated bridges with transformation DNA extended to evolve with lysogenic prophage as documented in this torment. Locally produced and tested clove extracts, prepared fresh with a highly aromatic flavour, were found to be effective in terminating these evolutionary mirrors as a healthy, hygienic remedy with a dynamic efficacy potentiation module, exceeding that of tested antibiotics. In conclusion, Mastitis Snyder’s evolutionary pollution clones of *A. baumannii* with multi-stress hardening cover prohibited behaviors of diverse and versatile selected antibiotics, and extra UVC irradiation decontamination strategy was terminated intelligently with the cheapest health extracts of *Syzygium aromaticum* (Cloves).

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NOVELTY STATEMENT

The findings provide novel insights for the treatment and processing of food where Mastitis Snyder evolutionary pollution clones of *Acinetobacter baumannii* with multi stress hardening cover prohibited behaviors to diverse and versatile selected antibiotics.

AUTHOR'S CONTRIBUTIONS

Authors contributed all efforts in designing, management and processing all cascaded parts of workflows from experimental design to title to collection and analyzing the data, to paraphrasing the manuscript. All cascades were processed by Assist. Prof. Dr. Ali Al-Shammary and Lecturer Dr. Iman Hazaa.

The authors declare that no Genrative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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