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The Mirror Entity of XDR Phenotypes Stress Hardening Staphylococcus Aureus Versus Escherichia Coli Patterns Recovered From Milk ice Creams in Retail Markets of Baghdad.

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ABSTRACT

An artificial intelligence 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 Existents (XDR-MEs) evolution equipped with stress hardening genes resident within milk ice creams chain in retail markets of Baghdad. An emergent hazard analysis critical control points (HACCP) policy for pollution of randomized collected samples of milk ice creams with multidrug resistant biofilm Staphylococcus aureus and Escherichia coli contaminants investigated recovery frequency and distribution isolation patterns from retail markets of Al-Fudhaliyah, Al-Sadrya and Abu-Ghraib within Baghdad. Molecular arrayed genes confirmation for biofilms antibiotics resistance phylogenetic tree relationships documented and recorded via Staph. aureus mecA antibiotics resistance & fib biofilm genes mapping cascaded by E. coli ESβL antibiotics resistance & fimA biofilm genes mapping. Ecomap's segregation prevalence frequency & distribution patterns of targeted special topic denominators S. aureus & E. coli from retail milk ice creams ecosystems in Baghdad within specified timelines episodes from June to September (2024) unveiled phenotypic isolation, biochemical segregation & molecular 16S rRNA PCR confirmation of thirty-four isolates (34: 28.33 %) out of proceeds processed one hundred twenty (120) traditional versus commercial ice creams from retail markets of Al-Fudhaliyah, Al-Sadrya & Abu-Ghraib within Baghdad province. In Conclusions: Pollution of collected retail milk ice creams samples during the workflow period within Baghdad city with an emerging biohazard multidrug resistant S. aureus and E. coli lineages as prohibited contaminants entities with violent outcome for health and safety of individuals and food chain.

Keyword: *Staphylococcus aureus, Escherichia coli, Stress Adaptation, XDR, Milk Ice Creams*

1. INTRODUCTION

Ice creams represent one of the famous desserts for all consumers especially in warm seasons. Contamination of these products can occurs in different scenarios series of interconnected events from active ingredients like raw or powder milk, additives and flavors, ice containers, manufacturing ecosystems (unclean and unhygienic environments), ice machines, shipment transformation, preservation storage conditions (critical point) within refrigerators and freezers, atmospheric pollution with mixed food products, workers especially carriers, polluted and contaminated waters suppliers, dust and flies, and even now the highly dangerous incremental intentionally contamination via bioterror's in order to catastrophic destruction of infrastructural components with panic waves of societies (WHO Bacterial Priority Pathogens List, 2024; Abdullah, 2023; Nalbone et al., 2022; Sohel et al., 2022; Yan et al., 2022; Microbiology of Ice-cream & Frozen Desserts 2020; Fadihl et al., 2019; Samir et al., 2018).

For these genes evolutionary scenarios of extensively drug-resistant forbidden recovery dogma, aim investigation epidemiological patterns of multidrug resistant S. aureus & E. coli in milk ice creams with their biofilm & resistance genes from Baghdad retail markets.

2. METHODOLOGY

A primordial experimental design was initiated as a core plan cascades of scheduled pilot scheme throughout series of cross-sectional testing models encoded and enrolled in dependent of authorized guidelines in which, projected and

recommended

indices were established before roadmap cascaded series chain proceeds from June to September (2024) at Milk Hygiene Lab \ Department of Veterinary Public Health, cascaded by processing proceeds until concluding of MSc workflow project.

HiCrome™ Staph Selective Agar M1931 and HiCrome™ E. coli Agar M1295 with BioMérieux's VITEK®2 compendium kits system (Kit 1 Biochemical ID 64 panel tests plus Kit 2 Antibiotics ID 27 GP and 17 GN panel tests) and Polymerase Chain Reaction (16s rRNA PCR) system (kits, primers and instruments) segregate (according to NCBI, USA) confirmatory recovery patterns of special topics targeted denominators. Microtiter plate assay displayed confirmation of blue biofilm formation and slim secretion from recovered isolates. Molecular arrayed genes confirmation for biofilms antibiotics resistance phylogenetic tree relationships documented and recorded via Staph. aureus mecA antibiotics resistance & fib biofilm genes mapping cascaded by E. coli ESβL antibiotics resistance & fimA biofilm genes mapping. Colloquially, randomized experimental design was dependent for scheduled collection and processing of samples in which, segregation roadmap proceeds patterns were epidemiologically dependent for targeted denominators (Staph. aureus & E. coli) & scanned markets within Abu-Ghraib, Al-Fudhaliyah & Al-Sadrya. Verified purchased as one hundred twenty (120) milk ice cream samples from different markets in which, forty (40) samples from each territory (ten (10) samples per month from each zone markets & entirely thirty (30) samples from all three sectors markets monthly) for four (4) months from June to September (2024) processed proceeds. Segregated labeled pooled quantities of samples were collected via sterile disposable hygienic durable plastic bags in which, population formulas as verified locally and imported versus traditional and commercial with different taste and odour and different types and flavors from sticks to cups to cones, and from milk to mixed cacao to vanilla to fruits purchased from different randomly retail stores and immediately translocated and transported freshly cooled preserved with ice box to workflow lab, mixed & homogenized well during accommodation directly preserved

under 4 C during processing proceeds. Colloquially, taken a representative unit from fully homogenized original sample as double replicates in size volume of twenty ml or gram per each. These units were resuscitated indirectly via doubled strengthen powered an ATP dependent enriched tryptone soya yeast extract broth (TSBYE), vortexes well via Rotomixer upon incubation period at 37 C overnight.

Modified verified dilution formulas were enrolled as one part sample to five- or ten-part decimal diluent TSBYE. Culturing overnight inoculated units on surface of HiCrome™ M1931 Staphylococcus aureus selective and differential agar, and HiCrome™ M1295 Escherichia coli selective and differential agar (HiMedia, 2024) including buffering dilution culturing five droplets technique of Miles & Misra (Miles et al., 1938) with or without three lines swabbing procedure in which, each cascaded line from concerted mixed decanted droplets (twenty microliter each for tenth ml whole) to titrated one represent approximately one valid log for each unit replicate. Synchronously, verified roll tube pour plate technique (Van Soestbergen & Lee, 1969) was dependent for comparative metering's if facultative anaerobic respiration needed for resuscitation of sublethal targeted denominator. False positive cascaded by false negative results might reduce dramatically upon recovery dogmas. Clarified colonies numbers cascaded concurrently with positive samples by equivocal procedures in which, counting of viable visible colonies or colloquially colony forming units per ml or g of original sample paraphrased as means log counts (CFU.ml-1 versus g-1) reflect the nature and type conditions of microbial load either contamination or clinical infection or both. Decimal dilution techniques manually or automatically as with colony counters or bio sensitive chips tensors probes represented in the known volume of a culture to verify and estimate its concentration in original one. A dose dependent curve was calculated for enrollment of adapted and improved dual decimal or logarithmic procedure reducing the aerobic and anaerobic environment for missing colonies and so counting errors. The mean log count of recovered lineage-complex was dependent on colonial phenotypes variants like structures fingerprints and colonial biofilm behavior of isolates. The augmented reality of targeted topics load log recovery titers calculated via mean number of colonies on cultured plate x a reciprocal of dilution factor x 50 CFU.ml-1 versus g-1 (Ali Al-Shammary, 2009). Noticeable mucous biofilm overproduction in boosted ATP dependent TSBYE cascaded by enrollment of other interconnected virulence biomarkers.

A 16s ribosomal RNA subunit dependent enrollment verify biodiversity of primed recovered Staph. aureus versus E. coli isolates from local and imported milk ice creams versus adapted food chain ancestors. A phylogenetic interconnected genotypic relationship presents 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 homogenic data using rRNA database (NCBI) after amplification of Bacteria or Fungi's ribosomal RNA. All processes including Bacteria/Fungi gDNA extraction, PCR amplification, sequencing, and assembly. For Bacteria, PCR on 16S rRNA using 27F and 1492R primers; and yielding of 1,300bp or more sequencing data. Flowchart (3.1) illustrate and deciphered this dogma. Dependent protocol UPGMA was a reciprocal relationship between local Madyan 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. In this context, two terms have to be defined (Borriss et al., 2011).

A built-in triple drive trails were dependent for pattern recognition of augmented five stages biofilm or electromagnetic

polysaccharides clouds of slime, in which verified versatile biofilm matrix diversity enrolled throughout modified roll-cup TSBYE76, augmented gold standard microtiter or tissue culture plates assay for direct assessment of slime bounds mucous via modified chromosomal built-in Tissue Culture Assay (Microtiter plate, MTCA) and a verified Congo Red medium for indirect assessment of Plasmid dependent twisters of slime equipped 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 (Naves et al., 2008; Dadawala et al., 2010; Hassan et al., 2911; Panda et al., 2016; Nosrati et al., 2017; Kirmusaoglu, 2019; Fokt et al., 2022; 2022A; Kudinha and Kong, 2022; Sanchez et al., 2022; Zhou et al., 2022).

Qualitative and Quantitavely assessment throughout a MTP (TCP) verified technique in which, a modified double strengthen TSBYE76 was primed by a fresh McFarland active log dependent culture (CFU.ml-5) on a large negatively charged concave wells enrolled rectangular plate as described by Christensen et al. (1985). Alternatively, modified Congo red agar (Freeman et al., 1989) was dependent for detection of biofilm-producing phenotypes cascaded with the prediction of plasmid realizing pairs of built-in between phenotypes cascaded via within clones. Al-Shammary verified Staph. aureus versus E. coli dependent Biofilm augmentation. An overnight Staph. aureus versus E. coli culture grown in dsTSBYE76 at 37 °C was transferred and diluted (titrated or standardized) in microtiter plate as (0.5) ml virtually log5 McFarland to five ml freshly prepared dsTSBYE76 inoculated for each well. Each isolate was tested in triplicate. Consequently, visualized wells with dsTSBYE76 unaccompanied were posted as control negative vs control positive Human primed strains. The plates were incubated for 48 h at 37 °C in order to construction authorization of visible clear biofilm adhesive poly-mucoid structures, layers and dots augmenting inside periphery or rims and in the bottom of charged wells. Furthermore, the culture was removed and plates were washed three times with sensitive phosphate-buffered saline to remove non-adherent cells and dried in an inverted position.

Adherent biofilm was fixed with 2 % sodium acetate for five minutes and was stained with double modified 20 % biofilm-crystal violet and 20 % biofilm-safranin for (15-30) minutes depending on secretory power for each clone, sensitivity, and specificity for each dye. Then, unbound stain was removed and the wells were washed three times with PBS. Plates were settled (2-3) hours for dryness then stained layers and dots of biofilm in bottom and around internal rims of wells were photographed, measured and scored according to the degree of formation, type of stain and type of isolate. Obvious result revealed after few hours to av day after complete dryness of induced biofilm. Optical density (OD) of stained adherent biofilm can be achieved by using micro-ELISA auto reader at wavelength (OD 570-600) nm or real time impedance-based cell analyzer, biosensors, fluorescent- or scanning electron microscopy. Cut-off values for biofilm production can be calculated according to verified methods (Stepanovic et al., 2007; Gutiearrez et al., 2016; Larimer et al., 2016; Hashem et al., 2017; Kirmusaoglu, 2019).

Built-in quorum sensing trusted platform module overtone via a cascaded chain of multi stress hardening genetically hidden sophisticated bionetworks augmented inside a denominator lineage, shifting an emergent trouble to end point of recalcitrant views. Antimicrobial susceptibility testing (AST 27 GP & 17 GN ID panels series) of microbial isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Until the 1950s, laboratories were lacking in the methodologies and equipment for the accurate determination of in vitro responses of organisms 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, 1966) study the sensitivity and susceptibility patterns of recovered Staph. aureus versus E. coli 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).

Staph. aureus mecA antibiotics resistance & fib biofilm gene mapping versus E. coli ESβL antibiotics resistance & fimA biofilm gene mapping. To drive the conversation around AMR's scope and burden and the urgent need for political will, feasible targets, accountability frameworks, and funding to address the problem, the One Health Trust, along with the Africa Centers for Disease Control and Prevention, the United States Centers for Disease Control and Prevention, the International Federation of Pharmaceutical Manufacturers & Associations, Global AMR R & D Hub, FIND, the AMR Action Hub, the International Centre for Antimicrobial Resistance Solutions, the Infectious Disease Society of America, and the Bill & Melinda Gates Foundation held a side event on AMR on the eve of the UNGA High-Level Meeting week (https://onehealthtrust.org/events/live-stream-ensuring-sustainable-access-to-antibiotics-from-unga-to-impact, 2024). Intercellular adhesion and biofilm production by Staph. aureus makes these bacteria resistant to antimicrobial therapy. Here, Methicillin-resistant Staph. aureus (MRSA) strains were characterized and the prevalence of genes encoding adhesion factors and biofilm formation was determined. All MRSA-XDR isolates identified by cefoxitin disc diffusion were positive for the mecA gene. SCCmec was the most frequently detected genotype. The quantitative microtiter plate assay showed that all the isolates were able to produce biofilm with levels ranging from high to moderate (Azmi et al., 2019). In this scheme, proceeds performed a cross-sectional roadmap to determine the antimicrobial resistance, adhesion and virulence factors, biofilm formation and SCCmec typing of clinical Staph. aureus isolates in Baghdad. Staph. aureus

isolates were identified using microbiological standard methods and antibiotic susceptibility tests were performed as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Inducible resistance phenotype and biofilm formation were determined using tissue culture plate assay. Multiplex-PCRs were performed to detect adhesion and virulence factors, antibiotic resistance genes, biofilm formation and SCCmec typing by specific primers (Tabandeh et al., 2022).

S. aureus isolate was grown in Louria Bertani (LB) broth at 37 °C for 24 h in an orbital shaker at 120 rpm. Chromosomal DNA from S. aureus was extracted. Briefly, the bacterial cells were lysed with 3–5 mg/mL lysozyme in the presence of 1/10 volume of 10% sodium dodecyl sulfate (SDS) at high pH and the lysate was neutralized. Subsequent deproteinization with 1:1 phenol: chloroform, chromosomal DNA was precipitated with ethanol by spinning at high speed and extracted DNA was stored at 4 °C (Gaire et al., 2021). The forward primer 5′-ACTGCTATCCACCCTCAAAC-3′ and reverse primer 5′-CTGGTGAAGTTGTAATCTGG-3′ were used for the amplification of mecA gene. The reaction mixture for the mecA gene was 25 µL and consisted of 21 µL of 1X Qiagen master mix, 0.5 µL of 10 pmole primer (forward and reverse) and 3 µL of extracted DNA template. The temperature profile for PCR included initial denaturation at 94 °C for one minute of 35 cycles, annealing at 55 °C for one minute of 35 cycles for mecA gene, extension at 72 °C for one minute of 35 cycles and final extension at 72 °C for seven minutes. The PCR amplification products were separated by electrophoresis through 2.5% agarose gel and visualized by staining with ethidium bromide. The 163 bp amplicon was detected against 1 kb DNA ladder (Gaire et al., 2021). Biofilm-encoding genes were amplified using the primer sets listed in Supplementary Table 3.5. The PCR amplification was performed using DreamTaq PCR Master Mix according to the manufacturer's recommendation (Thermo Fisher Scientific, USA) in a Veriti™ 96-Well Thermal Cycler (Applied Biosystem, USA) as follows: 96 °C for 3 min; 96 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min repeated for 35 cycles; final extension was performed at 72 °C for 7 min. The amplified products were electrophorized on 2% (w/v) agarose gel, stained using 0.5 µg/ml ethidium bromide solution, and captured using the FluroChem Q system (ProteinSimple™, USA) (Naorem et al., 2020).

The strains confirmed for E. coli via MALDI-TOF were recultured, and the total DNA was extracted from single colonies with either the GeneMATRIX Tissue & Bacterial DNA Purification Kit (E3551, EURx Ltd., Gdańsk, Poland) or the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA) or through crude lysate preparation. The lysates were made by dissolving one bacterial colony in 100 µL of lysis buffer of 0.05 M NaOH and 0.125% sodium dodecyl sulfate (final concentrations), and samples were incubated for 17 min at 90 °C. The DNA concentration and purity were determined with NanoDrop Lite (Thermo Fisher Scientific Inc., Waltham, MA, USA) (Ballen et al., 2022; Kaleva et al., 2023). Determination of ESβL and Carbapenemase Genes PCR detection and gene identification were performed for different β-lactamase gene families. In brief, DNA was extracted by boiling one colony suspended in 50 µL double-deionized water (95 °C for 10 min). After centrifugation for 1 min at 13,000 rpm (Centrifuge 5415 R, Eppendorf, Nijmegen, The Netherlands), the supernatant was used for the PCR reaction. Standard PCR protocols and conditions were modified in the following way: initial denaturation at 94 °C for 5 min; 35 cycles at 95 °C for 30 s, 52 °C for 45 s, and 72 °C for 60 s; and final incubation for 10 min at 72 °C using Taq DNA polymerase and dNTPs from QIAGEN (Hilden, Germany). Sequencing was performed with Eurofins overnight sequencing service (Eurofins, Hamburg, Germany) (Skof et al., 2024).

DNA was extracted from colonies grown on sheep blood agar medium using the InstaGene Matrix (Bio-Rad, Reinach, Switzerland) following the manufacturer's instructions. Molecular TEM and SHV ESBL detection were carried out as described elsewhere. Each sequence electropherogram was screened for the presence of double peak signals, which would indicate the presence of multiple SHV or TEM variants (Polsfuss et al., 2012). In all genomes, contigs harboring ESBL/AmpC-encoding genes were analyzed in detail to gain insights into the genetic context of the ESBL/AmpC-encoding genes. First, nucleotide BLAST at NCBI was used to determine the level of identity of the contig with publicly available sequences. Then, Open Reading Frames (ORFs) were predicted and annotated using Artemis software version 8 (Carver et al., 2012), and each predicted protein was compared against the all-protein database at NCBI using BlastP. ISfinder was used for identification of insertion sequences (IS) (Siguier et al., 2006). Comparisons of contigs harboring identical ESBL/AmpC-encoding genes were performed using CLC genomics workbench v.8. Furthermore, the Artemis comparative tool (Carver et al., 2005) was used to perform in-depth comparative genomics between ESBL-hosting contigs of our samples and their closest references from NCBI (Hounmanou et al., 2021).

Built-in primeval statistical design dependent software of Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) for scan significance variations among data frequencies (percentages fitness and goodness). According to null hypothesis, Staph. aureus mecA antibiotics resistance & fib biofilm gene mapping versus E. coli ESβL antibiotics resistance & fimA biofilm gene mapping predicted to be not encountered from cascaded chain of local dairy products sourced or supplies in accordance to predominant denominator in cases of Human origin associated with UTI positive cases and carriers (active and passive). Complete randomized experimental design with observed and expected module of Staph. aureus & E. coli lineage was enrolled and tested bio statistical in order to verify significance differences among denominators, sample brands, geographically scanned zones and in related and specified episodes

(times and periods intervals) in comparison to Staph. aureus & E. coli ecosystem at level ($p \leq 0.05$).

It deciphers the relationship between collected and calculated data in terms of independent association as null hypothesis and dependent association as alternative one. Targeted topics were premeditated by subtracting the expected frequency from the observed one cascaded by squaring it and dividing by the expected frequency for each category of data being tested. The degrees of freedom (df) for a topic denominator were equal to (r - 1) (c - 1), where r = number of rows and c = number of columns in the likelihood table being tested. Rejection of null hypothesis with acceptance of alternative concept philosophies revenue association of abnormally adapted Staph. aureus & E. coli in food chain ecosystem. When the differences between variants percentages equal to or exceeding the calculated values of $\chi 2$, then they be significant but not always it's a true parameter in bacteriology as Jay et. al. recommended (2005) i.e., Clinical significance among cross-section special topics really clear, augmented and satisfied to verify a final product or Thesis aims and objectives in food microbiology in spite of insignificant differences from biostatistics point of view.

3. RESULTS AND DISCUSSION

Emergent Microbial Pollution Violence: Unhygienic predominant pollution of dairy chain cascaded by series of frequency & distribution microbial growth epidemiological patterns of genetically modified, highly infectious prohibited predominant foci of Staph. aureus & E. coli derived from resident portals of entrance throughout cascaded bridges to be deposited within Baghdad environment (specifically among milk ice creams) with continuous stress adaptation & hardening accommodation strategies. These predominant, violent, biohazard targeted topic denominators were genetically well-equipped entities augmented to survive harsh environments with other infectious & contagious foci inside a recalcitrant barrier of electromagnetic clouds of biofilm to establish an unsafe & sophisticated struggling hygienic problem with diverse & versatile, multi stressors impendency, drift-shift antigenic transformations behaviors ending with banned sequels (AL-Yais & Al-Shammary, 2024; Jabuk et al, 2024; Machado et al, 2024; Taha, 2023 : Al-Shammary, 2019; Fadihl et al, 2019; Younis et al, 2018; Al-Shammary & Madi, 2016; Kanaan & Al-Shammary, 2013). Contamination of food chain by antimicrobial resistance genes across sophisticated interconnected mechanisms of vertical & horizontal gene transfer in which, "vertical multiplication & 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 overwhelmed with recalcitrant biofilm." (Bourdonnais et al., 2024; Okaiyeto et al., 2024; Sun et al., 2024; Trapote et al., 2024; Sagar et al., 2023; Samtiya et al., 2022; Verraes et al., 2013).

Untimely or overuse of antibiotics has led to the emergence of resistance violation. This not only jeopardizes the continued effectiveness of infection treatment policy but also seriously affects the safety and quality of animal products and even accelerates the spread of resistance genes across food chain, environment then to humans and vice versa recycled again (Sun et al., 2024; Qamar et al., 2023; Yoon et al., 2021). The ongoing evolution of resistance problems was a result of persistent resource competition among microorganisms with resistance acquisition clever stress hardening strategies (Sun et al., 2024; Al-Shammary & Mounam, 2023; An et al., 2023; Davies & Davies, 2010). The selective pressure exerted by antibiotics promotes the widespread dissemination of resistance genes among bacteria in both recycled environment and hosts settings, therefore predispose enhancing the accumulative mutation rate versus horizontal gene transfer in pathogenic and virulent microbes (Sun et al., 2024; McCorquodale-Bauer et al., 2023; Alcock et al., 2020). Alterations were predominantly manifested in food chain across translocation pollution of soil, aquatic systems, atmospheric conditions, and host-associated microbial communities. Recent studies have indicated that emerging evolutionary patterns and the accumulation of mutant genes complicate disease prevention and treatment. Therefore, a comprehensive analysis of the interplay between microbial communities in humans, animals, and the environment is essential to tackle this global health issue (Sun et al., 2024; Chow et al., 2021; Andersson et al., 2020; Graham et al., 2019; Bengtsson-Palme et al., 2018). While significant progress has been made in the management of on-farm antibiotic resistance years, the limitations of various approaches that are only applicable to their respective fields remain a concern.

Developing new integrated strategies built-in neuron processing units' chips (NPU) to combating resistance contamination in farms was a critical update. Guidelines for artificial intelligence (AI) was a powerful planned tool for detection, analysis, verification and clarification of problems. The availability of preferred datasets from diverse and versatile sources enables these futuristic techniques to identify updated resistance more efficiently and offers valuable insights across the identification of new genes, mutations, drug interactions, and conditions that facilitate transmission (Sun et al., 2024; Ali et al., 2023). Natural neuron engine language processing models of AI process has enabled demonstrate superior capabilities for reasoning, planning, decision-making, contextual learning, and zero-sample answering, in addition to broad domain adaptability, including driven shots, multimodal tasks, robotics, tool manipulation, questions versus answers, and autonomous learning (Sun et al., 2024; Naveed et al., 2023). These naturally & intentionally mutated entities to verified stressors had an ability & capacity to changes their genotypic contents from clinical phase to antigenic drift & shift food adapted contaminant phase with clever artificial intelligence behaviors in hiding & regulation virulence biomarkers. Modified mutated new progenies could be translated from generation timelines to cascaded brain like machines.

Regulation of Quorum sensing networks behaviors with sigma factors via sophisticated cleaver strategies augmented by epigenetic drifted temporary tolerant transient phases cross environmental stimuli surrounding their genetic material to becomes persisters (ALYais & Al-Shammary, 2024).

Emergence critical revival of stress adapted infrastructural targeted entities in association with an ESKAPE violent series of multidrug resistance prohibited chain (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa & Enterobacter) versus prehistoric silent enemy microbiome hiding under the ice, especially in the Siberian region, due to climate atmosphere remodeling modified changes is wreaking havoc via High-frequency Active Auroral Research Program (HAARP) & Defense Advanced Research Projects Agency (DARPA) projects constructed from futurist Nikola Tesla. Such new emergent struggling was the experience prediction of multi stress adaptation phenomenon of nosocomial human semiconservative pathogen predominant entities with phase or phenotype variation and transforming under stress adaptation from host specific phase to adaptive versatile phase resident on food chain by unknown survival and revival mechanisms (CRISPR-CAS genes sharing strategies built-in an artificial intelligence brain machines chips of quorum sensing behaviors within an enclosed recalcitrant biofilm barriers patterns) resident and deposited within cascaded Human-Dairy ecosystem in Baghdad to become a forbidden "CHIMERAS)". Prokaryotic DNA sequences designed as clustered regularly interspaced short palindromic repeats (CRISPR-CAS) (AL-Salihi & Al-Shammary, 2024; AL-Yais & Al-Shammary, 2024).

Ecomap's segregation prevalence frequency & distribution patterns of targeted special topic denominators S. aureus & E. coli from retail milk ice creams ecosystems in Baghdad within specified timelines episodes from June to September (2024) unveiled phenotypic isolation, biochemical segregation & molecular 16S rRNA PCR confirmation of thirty-four isolates (34: 28.33 %) out of proceeds processed one hundred twenty (120) traditional versus commercial ice creams from retail markets of Al-Fudhaliyah, Al-Sadrya & Abu-Ghraib within Baghdad province. Prevalence patterns of recovered S. aureus was thirty isolates (30: 25 %) out of overall cascades in which, six isolates (6: 5 %) from Al-Sadrya markets, eight isolates (8: 6.67 %) from Abu-Ghraib markets and sixteen isolates (16: 13.33 %) from Al-Fudhaliyah markets. Prevalence patterns of recovered E. coli was predominant, deposited & resident only in Al-Sadrya markets as four isolates (4: 3.33 %). E. coli was not recovered from other samples in retail markets of Al-Fudhaliyah & Abu-Ghraib. Documentary Staph. aureus cascaded pollution percentage were increased progressively to be predominant in August and, fluctuated between June and September (2024) while, E. coli pollution was cascaded predominant progressively from August to September (2024). This partly reflects thermal susceptibility of isolates according to environmental temperature and situation located.

Unacceptable pollution log levels of targeted clinical isolates were documented & recorded in which, one detected colony phenotype deciphered as colony forming units (CFU.ml-1 or g-1) in food chain considered notifiable emergency must be carefully isolated as banded region until HACCP policy guided government call for hygienic biosafe termination. Twenty-four isolates (24: 80 % & overall resistance patterns 20 %) of S. aureus out of thirty recovered ancestors versus two isolates (2: 50 % & overall resistance patterns 1.66 %) of E. coli out of four recovered ancestors where exhibit sever tolerance versus resistance (cascades from multidrug resistance to more than two antibiotics, to methicillin's versus vancomycin resistance to more than seven antibiotics as VRSA-MRSA ancestors, to extended beta lactamase resistance to more ten antibiotics as ESβL ancestors, to extensive & sever resistance to all antibiotics expressed scientifically as forbidden selective pressure clones of XDR ancestors Chimeras) to in vitro tested Vitek 2 fingerprint module cascades series of antibiotics. Local cocoa & mixed sticks milk ice creams displayed highest pollution percentages by S. aureus from retail markets in Al-Fudhaliyah. Local vanilla cones milk ice creams displayed highest pollution percentages by S. aureus from retail markets in Abu-Ghraib. Local watermelon sticks milk ice creams displayed highest pollution percentages by S. aureus & E. coli from retail markets in Al-Sadrya. Pollution contaminations levels exceed the normal residual limits versus zero tolerance values in ice creams.

Biostatistical integration was a predominant tool for deciphering and matching displayed calculated cascaded results series at confidence intervals 95 & 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 $p \leq 0.05$ & $p \leq 0.01$ via dependent analysis of variance (ANOVA) with least significant difference (LSD) cascaded by Chi square ($\chi 2$) to understanding normal distribution patterns of samples and their replicates. Significant values mean significant clinical observed and calculated trials of experimental design and & objectives. Not always non or insignificant results means they were not important clinically or scientifically in accordance to pairs of null & alternative experimental hypothesis design but this dependent primarily on virulence indices cascaded by type of evolved isolate i.e., their genetic makeup in terms of genetically modified microorganisms as priority 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 conclusions: Clinical observed trials were very important in spite of their statistical values were not significant in some situations. Downstream cascaded biostatics' (SAS, 2018) series tables (4.1. – 4.10.), integrated with photographic figures versus flowcharts (4.1. – 4.4.) deciphered these interconnected augmented situations as deciphered these interconnected augmented situations and Figure 4.5.detection of 16s rRNA PCR fingerprint alignments bands of Staphylococcus aureus & Escherichia coli PCR.

Table 4.1. Frequency and distribution epidemiological patterns of PCR primed (16) isolates of *Staph. aureus* from milk ice creams in Al-Fudhaliyah retail markets with episodes integrated mean log count (CFU.g^{-1}).

Episode	Number of Samples	Staph. aureus	Count CFU.g^{-1}	Recovery (%) (n=120)
June	10	2(20%)	1.00 ±0.00 c	1.667
July	10	4(40%)	2.477 ±0.08 b	3.333
August	10	6(60%)	4.361 ±0.17 a	5
September	10	4(40%)	3.441 ±0.12 ab	3.333
Total	40	16(40%)	2.819 ±0.17	13.333
X^2 value		3.33		2.07
P- value		0.34		0.15
L.S.D (P- value)	------	----- 0.0457	1.074** (0.00919)	1.167** (0.0087)
Means with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).				

Al-Fudhaliyah milk ice creams brands & flavors: White milk with kunafa cups, Pomegranate sticks, Cocoa sticks, Mixed sticks & Fruits sticks

Table 4.2. Frequency and distribution epidemiological patterns of PCR primed (8) isolates of *Staph. aureus* from milk ice creams in Abu-Ghraib retail markets with episodes integrated mean log count (CFU.g^{-1}).

Episode	Number of Samples	Staph. Aureus	Count CFU.g^{-1}	Recovery (%) (n=120)
June	10	1(10%)	2.00 ±0.08 c	0.833
July	10	1(10%)	2.00 ±0.08 c	0.833
August	10	2(20%)	4.845 ±0.19 b	1.667
September	10	4(40%)	6.146 ±0.35 a	3.333
Total	40	8(20%)	3.747 ±0.16	6.667
X^2 value		3.75		3.05
P- value		0.28		0.38
L.S.D (P- value)	------	----- 0.0812NS	0.994** (0.0082)	0.702* (0.0286)
Means with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).				

Abu-Ghraib milk ice creams brands & flavors: White milk cones, Vanilla cones, White milk sticks, Orange sticks & Fruits sticks.

Table 4.3. Frequency and distribution epidemiological patterns of PCR primed (6) isolates of *Staph. aureus* from milk ice creams in Al-Sadrya retail markets with episodes integrated mean log count (CFU.g^{-1}).

Episode	Number of Samples	Staph. Aureus	Count CFU.g^{-1}	Recovery (%) (n=120)
June	10	1(10%)	3.698 ±0.14	0.833
July	10	1(10%)	3.698 ±0.14	0.833
August	10	2(20%)	3.845 ±0.17	1.667

September	10	2(20%)	3.301 ±0.10	1.667
Total	40	6(15%)	3.635 ±0.14	5
X^2 value		0.78		0.67
P- value		0.85		0.87
L.S.D (P- value)	------	----- 0.881NS	0.391 NS (0.715)	0.477* (0.0367)

Means with the different letters in same column differed significantly. * (P≤0.05).

Table 4.4. Frequency and distribution epidemiological patterns of PCR primed (4) isolates of *E. coli* from milk ice creams in Al-Sadrya retail markets with episodes integrated mean log count (CFU.g^{-1}).

Al-Sadrya milk ice creams brands & flavors: Watermelon sticks, Melon sticks, Cream sandwiches, White milk cones & Cocoa cones.

Table 4.5. Frequency and distribution epidemiological patterns of PCR primed (10) isolates of *Staph. aureus* & *E. coli* from milk ice creams in Al-Sadrya retail markets with episodes integrated mean log count (CFU.g^{-1}).

Episode	Number of Samples	Staph. Aureus	Count CFU.g^{-1}	Recovery (%) (n=120)
June	10	1(10%)	2.5 ±0.10 b	0.833
July	10	1(10%)	2.5 ±0.10 b	0.833
August	10	4(40%)	10 ±0.52 a	3.333
September	10	4(40%)	10 ±0.52 a	3.333
Total	40	10(2.5%)	25	8.333
X^2 value		4.08		3.67
P- value		0.18		0.29
L.S.D (P- value)	------	----- 0.0834NS	1.951** (0.0001)	1.649* (0.0256)

Means with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).

Table 4.6. Frequency and distribution epidemiological patterns of PCR primed total isolates from milk ice creams in retail markets with integrated episodes

Episode	Number of Samples	Total isolates	Recovery (120%)
June	30	4 (13.33 %)	3.33 ±0.12 c
July	30	6 (20 %)	5.00 ±0.26 b
August	30	12 (40 %)	10 ±0.52 a
September	30	12 (40 %)	10 ±0.52 a

Total	120	34 (28.33 %)	28.33 ±1.07
L.S.D. (P-value)	---	--- 0.0398 *	1.459 ** (0.0001)
Means with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).			

Table 4.7. Frequency and distribution epidemiological patterns of dual isolates (16) recovered from Al-Fudhaliyah milk ice creams according to brands & flavors.

Brands & Flavors	*Staph. aureus*		*E. coli*		Recovery (%) (n=120)
	Local	Imported	Local	Imported	
White milk with kunafa cups	0	3	0	0	2.5
Pomegranate sticks	0	3	0	0	2.5
Cocoa sticks	4	0	0	0	3.33
Mixed sticks	4	0	0	0	3.33
Fruits sticks	2	0	0	0	1.67
X^2 value	16				0.89
P- value	0.19				0.92
L.S.D (P- value)	--	--	--	--	0.802* (0.0388)

Table 4.8. Frequency and distribution epidemiological patterns of dual isolates (8) recovered from Abu-Ghraib milk ice creams according to brands & flavors.

Brands & Flavors	*Staph. aureus*		*E. coli*		Recovery (%) (n=120)
	Local	Imported	Local	Imported	
White milk cones	1	0	0	0	0.83
Vanilla cones	4	0	0	0	3.33
White milk sticks	1	0	0	0	0.83
Orange sticks	1	0	0	0	0.83
Fruits sticks	1	0	0	0	0.83
X^2 value	0.00				4.56
P- value	1.00				0.33
L.S.D (P- value)	--	--	--	--	0.771* (0.0046)

Table 4.9. Frequency and distribution epidemiological patterns of dual isolates (10) recovered from Al-Sadrya milk ice creams according to brands & flavors.

Brands & Flavors	*Staph. aureus*		*E. coli*		Recovery (%) (n=120)
	Local	Imported	Local	Imported	
Watermelon sticks	2	0	4	0	5
Melon sticks	1	0	0	0	0.83
Cream sandwiches	1	0	0	0	0.83
White milk cones	0	1	0	0	0.83
Cocoa cones	0	1	0	0	0.83
X^2 value	13.33				8.49
P- value	0.34				0.3
L.S.D (P- value)	--	--	--	--	0.749** (0.0001)

Table 4.10. Frequency and distribution epidemiological patterns of dual isolates recovered from milk ice creams according to brands & flavors

Brands & Flavors	*Staph. aureus*		*E. coli*		Recovery (%) (n=120)
	Local	Imported	Local	Imported	
Total	22	8	4	0	28.33
L.S.D (P- value)	--	--	--	--	0.774** (0.0145)
Means with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).					

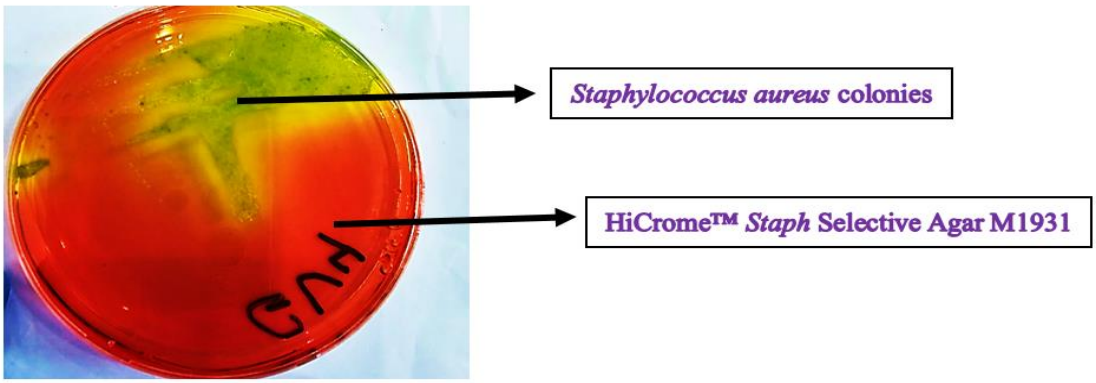


Figure 4.1. Illustrate *Staphylococcus aureus* as green diffuse scattered biofilm convex colonies phenotypes on surface of red HiCrome™ *Staph* Selective Agar M1931 after 24 hrs. incubation at 37 °C.

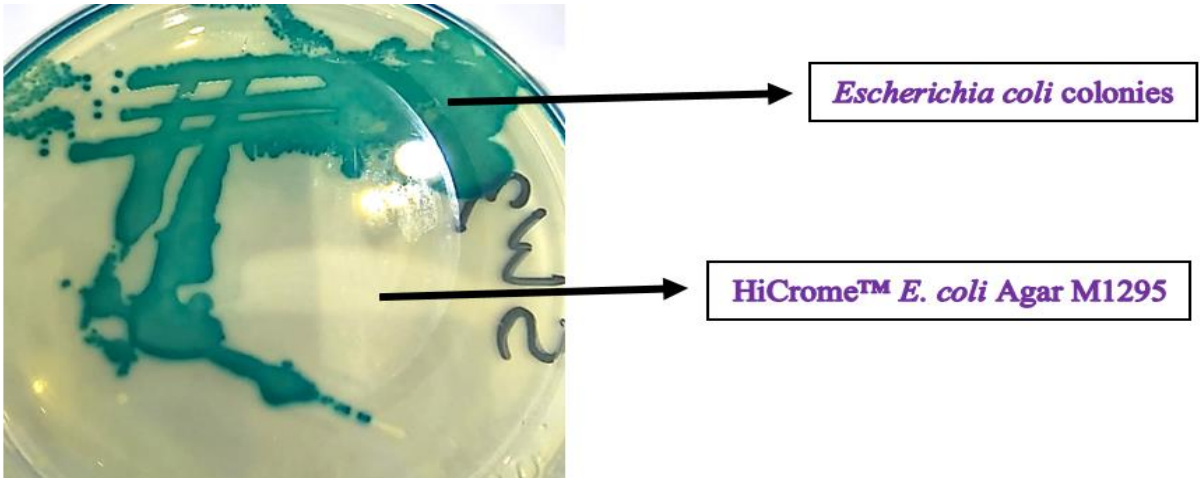


Figure 4.2. Illustrate *Escherichia coli* as green diffuse scattered biofilm convex colonies phenotypes on surface of translucent HiCrome™ *E. coli* Agar M1295 after 24 hrs. incubation at 30°C for 4 hrs. and then at 44°C for 18 hrs.

Downstream Figures (4.3. – 4.4.) (cascaded series were displayed in appendix-A) of BioMérieux's VITEK2 64 ID integrated biochemical laser compendia identification panels for documented recovered isolates of *Staphylococcus aureus* & *Escherichia coli* biotypes from milk ice creams:

bioMérieux Customer: Microbiology Chart Report Printed June 26, 2024 1:59:36 PM CST

Patient Name: Madin,FR1. Patient ID: fhjuio
Location: Physician:
Lab ID: 242 Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 3.87 hours	**Status:** Final
Selected Organism	99% Probability **Staphylococcus aureus** **Bionumber:** 010402062763231	
ID Analysis Messages		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	lLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 4.3. Biochemical ID online certificates of *Staphylococcus aureus* via VITEK2.

bioMérieux Customer: Microbiology Chart Report Printed September 24, 2024 2 PM CST

Patient Name: Madin,SW3,1 Patient ID: fgjutru
Location: Physician:
Lab ID: 258 Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	3.90 hours	**Status:**	Final
Selected Organism	99% Probability	**Escherichia coli**		
	Bionumber:	0405610550406610		
ID Analysis Messages				

Biochemical Details

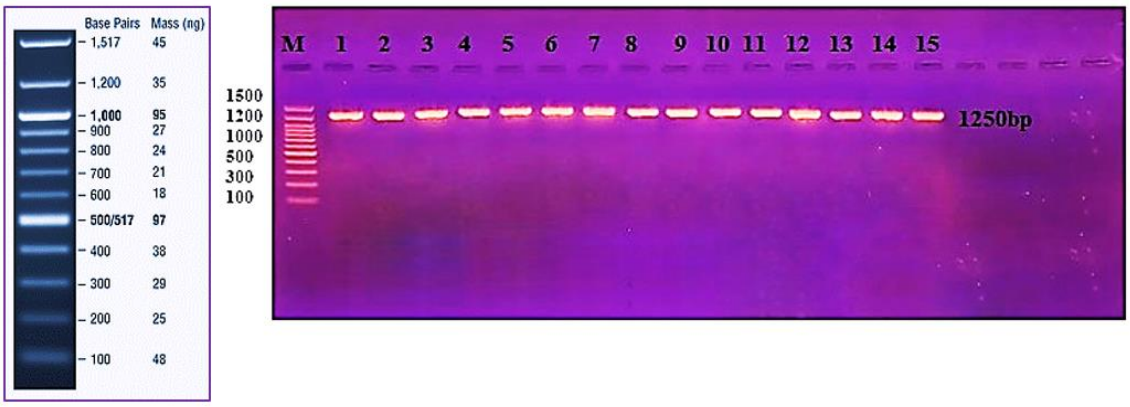
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Further cascaded series were displayed in appendix A1 (appendix-A).

Figure 4.4. Biochemical ID online certificates of *Escherichia coli* via VITEK2. Further cascaded series were displayed in appendix A2 (appendix-A).

NCBI BLASTN genetic online database software was dependent for matching and genetic identification and reading of 16s rRNA PCR genetic codes in terms of FASTA sequences patterns of phenotypically recovered *Staphylococcus aureus* & *Escherichia coli* from milk ice creams. In terms of query input and database selection, the query sequences should be pasted in the search text area to be used for a BLAST search. BLAST accepts different types of input number cascaded format automatically. Employers informs to upload a text file clarifying query in FASTA setup. Cascades resolved in the "Form" and "To" boxes powered under "Query subrange" to specify segment position. For instance, to limit matches of query sequence region 24 to 200, must enter 24 in "From" and 200 in "To" arenas. Intersection of the complete FASTA alignment in case of mismatching (NCBI, 2023). Translation, matching & deciphering of query genetic code with percent identity and purity according to online reference microbial nucleotides genomic bank database within NCBI BLAST. A BLAST exploration can be limited to the result of an Entrez query versus the database chosen. Significant expression content for database & applying the Entrez terms. Default filtering is individual translation products not to sequences database as DUST for BLASTN versus SEG for other agendas (NCBI, 2023). Customization & verification of amino acid substitution matrices to compensate for their sequence's compositions equated (Schaffer *et al*., 2001). Accurate E-values profit from mounted scores (Yu *et al*., 2003; Yu & Altschul, 2005; Altschul *et al*., 2005). Such "compositional score matrix adjustment" might be only accepted under confident statistical situations, otherwise, compositional adjustment might be summarized universally (Altschul *et al*., 2005).

Empirically, BLAST redirected to refreshing word-matches between query & database sequences. "Hot-spots" BLAST verify to initiate extensions that primed to full-blown alignments. Before an extension is initiated, an exact match of the entire word is required for nucleotide-nucleotide searches (i.e., "blastn"), consequently regulates sensitivity & speed of search by increasing or decreasing the word size. Varied amount of similarity documented. The network permits the word sizes 2, 3, & 6 setting to specifies the statistical significance threshold for reporting matches versus database sequences. Revealed



database sequences aligned to the query sequence deciphered via graphical outline. Separately alignment score is indicated by one of five diverse colors, which segregates the range of scores into five clusters. Diverse alignments segments of identical database sequence are connected by a thin grey line. Pairwise alignments databases were displayed as matches pairs between query and directed sequence in which, middle line displays letter status. Aimed at protein alignments (e.g., BLASTP/BLASTX/TBLASTN), identities current letter, conservative substitutions current a "+", and otherwise naught. For nucleotide alignments (e.g., BLASTN and mega BLAST) a "|" is exposed for matches and naught for mismatches. Mega BLAST Pattern is designed specifically for comparison of diverged sequences, especially theirs from diverse organisms, which have low degree of identity alignments. Achieved gapped extension to outcome initial offset pairs directed to verify difference of 'discontinuous word' scheme. Contingent on exact match length to twitch alignments from, it either misses a lot of statistically significant orientations, or on divergent discoveries too many short random orientations (NCBI, 2023). Deciphered FASTA codes for each recovered isolates within NCBI BLASTN authenticated with online certificates of identity and alignment illustrated in Tables (4.11. - 4.13) & Figures (4.5 - 4.9) (cascaded series were displayed in appendix-B).

Figure 4.5. 16s rRNA PCR fingerprint alignments bands of *Staphylococcus aureus* & *Escherichia coli* PCR amplification and PAGE analyzed genomic proteins bands of targeted denominator isolates. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

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