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CHECKERBOARD PATTERN OF GREEN TEA AND ROSEMARY EXTRACTS ON MULTI DRUGS RESISTANT *BACILLUS CEREUS* RECOVERED FROM SOFT CHEESE IN KIRKUK, IRAQ

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Abstract

Genetically altered and food-borne microorganisms that are multidrug resistant contain harmful pathogens that contaminate our environment. Recovery multidrugs resistant *Bacillus cereus* recovered from soft cheese in Kirkuk areas. Which show 67 positve isolate from 100 samples in different areas and Forty isolates out of sixty-seven (59.701 %) were resistant to penicillin, amoxicillin, methicillin and vancomycin but mostly sensitive to ciprofloxacin; versus thirty-seven (40.298 %) were intermediate to sensitive clones. Most experimentally processed forty multidrug resistant clones verified as intermediate to resistant to all extracts up-to twenty percent concentration versus other thirty-seven clones verified as intermediate to sensitive to all extracts below twenty percent concentration.

Key words: *B. cereus*, antibiotics, green tea, rosemary and soft cheese.

Introduction

Food plays an essential role in our daily lifetime. Globally, many billions of people eat milk and dairy products daily. Milk and dairy products contamination could occur at any stage in the course from food production to consumption ("farm to fork") (FDA, 2019 & CDCs, 2019).

Nosocomial and community associated multidrug resistant and super strain Diverse *Bacillus cereus* (MDRO-BC) owing to biofilm and capsule architecture, is a universally new emerging and growing threats in man and animals (Medscape, 2019 & CDCs, 2019). Those with compromised immune systems or who are weakened by other infections are frequently affected by Opportunistic MDRO-BC strain (Hamzah and Hasso, 2019). It is an eminent "a bridge" for transferring a multidrug resistance to another pathogenic foodborne microorganisms *in vivo* (Owusu-Kwarteng *et al*., 2017).

Bacillus cereus has a certain interest in food safety and public health because of its ability to cause food spoilage and infection by producing numerous toxins. *B. cereus* was found to have significant influence on human health, food processing and farming. *B. cereus* usually causes food products to decompose. It is also an opportunistic pathogen, causing two forms of food poisoning in humans, characterized either by vomiting and nausea or by stomach cramps and diarrhea (Owusu-Kwarteng *et al.,* 2017).

One of the most widely consumed beverages worldwide is Green tea. The potential health benefits were widely studied, developed mainly from the leaves of the *Camellia sinensis* plant in Asian countries. Green tea has antimicrobial, anti-carcinogenic, anti-inflammatory and antioxidant properties. Epig allocate chin gallate (EGCG), the powerful green tea bioactive flavonoids can fight biofilm producing and multidrug resistant clinical or foodborne microorganisms (Reygaert, 2014-2018 & Parvez *et al.*, 2019).

Several spice extracts have displayed their properties to inhibit the autoxidation of unsaturated triacylglycerols. Specially, the natural extract from the Lamiaceae family (*thyme, sage and rosemary*) has been stated in several studies for its antioxidative activity (Twegh *et al*., 2020). Thus, rosemary extract possibly will be useful for switching or even diminishing synthetic antioxidants in foods (Nieto *et al*., 2018; Stojiljkovic *et al*., 2018).

Materials and Methods

Collection and Processing of Samples: A one-hundred pooled (100) samples collected carefully under

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supervisor authority guidelines planning techniques and experimental work scheme design in environmental ecological niches from Kirkuk regions. Choices regions and samples were dependent on presence of cattles and availability of other dairy products under supervisor experience works in which they divided according to the distribution and frequency of selected ecotope (animals & samples) with environmental temperature and geographical nature for each selected region. They collected aseptically in clean, non-permeable & non-durable plastic bags (0.5 -1) L and transported as soon as possible within two hours to a milk hygiene laboratory in icebox. Then cooled for (2-3) days at 4 °C, then warmed and homogenized at laboratory temperature, then processed as two separate units (directly and indirectly) according to updated and approved procedures.

Samples Units & Regions: Locally produced fresh-sweet (home-made from raw unheated milk) and brined soft cheese with their risky whey, units replicates collected randomly from regions of Kirkuk, Alhjaweja, Laylan and Dakok in which 25 samples from each region through 4 months. In this case, a half-percent dependent and independent statistical hypothesis of prevalence authority examined against null food safety reality of absence of foodborne multidrug resistant and super strain Diverse *Bacillus cereus* (MDRO-BC) from selected regions environments.

Processing of Proceeds Replicates: Modified regimes protocols of referenced globally authorities guidelines of BAM, ISO, FDA and FSIS (2019) in food microbiology applied to analyze and recovery of foodborne multidrug resistant and super strain Diverse *Bacillus cereus* (MDRO-BC). Samples undergo critical step of refrigeration before direct and indirect intelligent culturing schemes for encouraging recovery network under cooled psychrotrophic environment inside a 4 °C refrigerator cabinet.

Collection and Processing of Soft Cheese – Whey: A one-hundred pooled samples (five-fresh soft cheese from each region per month). Collected (size volume = 250 g) and processed refrigerated soft cheese samples with their risky whey's undergoes modified direct and indirect culturing cycle. Modified direct without 2% emulsifier buffered sodium citrate and indirect with sodium citrate culturing techniques used for this level. Processing samples after refrigeration for (48-72) hours by macerating and integrating well inside collecting bags with their whey. Then taken representative sample approximately ten (10)g. Directly Inoculating and incubating pooled processed sample with ninety (90) ml doubled strengthen powered TSB – YE broth at 37 °C for 48 hours (modified one part processed sample : ten part enriched - enrichment broth). Inoculated samples mixed well again for transferring by standard HiMedia loop about fiver droplets contents (each droplet equal mathematically to 0.02 micron or ml and so on, totally inoculated processed part equal to approximately one ml). Streaking by dilution technique on tryptone soya yeast extract (TSA-YE), then incubated at 37 °C for (24 - 72) hours. Indirectly, refrigerated parts were inoculated by modified dual - drive buffered sodium citrate - doubled strengthen powered TSB - YE broth at 37 °C for 48 hours for dual emulsifying cheese globules hide pathogenic *multidrug resistant and super strain Diverse Bacillus cereus (MDRO-BC)* for better recovery cycle and the process proceeds as same as above.

Extracts (green tea and rosemary)

Boiling 0.5 liter of water then added the herb (green tea and rosemary each alone) 105 grams lets to boiling for half hour then filtrated with tea strainer.

Enumeration ecosystem: Numerous procedures in microbiology and food microbiology require that cells be counted. Counting mass of visible viable colonies on nearly all occasions actually represents the concentration of the cells per ml or g of food or clinical sample. If the cells are properly distributed on the plate, it can generally be assumed that in a sample and counting techniques each cell or colony-forming unit will produce a single colony, depending on the true density of microorganisms. The mean log count of recovery of *multidrug resistant and super strain Diverse Bacillus cereus (MDRO-BC)* was dependent on colonial phenotypes variants prisms like structures and discoloration phenomenon with haemolytic pattern of isolates. The *multidrug resistant and super strain Diverse Bacillus cereus (MDRO-BC)* load log recovery titers calculated via mean number of colonies on cultured plate x a reciprocal of dilution factor × 50 cfu / ml (Ali Al-Shammary, 2009).

Staining features and biochemical identification system: Purification and storage of executed isolates. Picked up and rebounded for 24 hours on double strength TSB-YE at 37 °C. Then transferred for (24-48) hours to double force control TSA-YE at 37 °C. Inoculated universal slanting bottles preserved as pure seeds or nucleus inside a refrigerator for other identification procedures. According to instructions of Quinn *et al*., (2004) and MacFaddin (2000) a Gram stain kit and Capsule staining technique used for demonstration of *Bacillus cereus* shapes and colors with very important feature of present polysaccharide capsule. In the former the fixed smears were placed over a sink on a staining

rack. Over the whole smear the staining solutions are flooded and left on the slide for the correct time. The smear washed beneath a gently running tap between each staining reagent, excess water tipped off and the next reagent added. The stained smear is finally washed, and air is dried. *Bacillus cereus* retains the complex of violet-iodine crystals and violet-blue stains.

In later, India ink showed capsule as a transparent hue surrounding *Bacillus cereus* gram-positive purple bacilli, in which a single drop of India ink was mounted on a clean microscope slide. Place the end of alternative clean microscope slide at an angle to the end of the *Bacillus cereus* containing slide. Spread the drop out over the smear into a film. Let the film air dry. Saturate the slide for one minute with crystal-violet. Rinse gently with water on the slide. Allow air to dry on the slide. Watch the slide under the microscope, using the right microscope technique.

Formation of faded turbidity on TSB-YE after 48 hours and after 96 hours, a thick and sticky slimy precipitates inside the universals bottoms formed with a characteristic feature of crock-screw motility during agitation, which may indicate a biofilm formation phenomenon in *Bacillus cereus* isolates. This phenomenon also founds in preserved slant seeds after a week. Catalase test preformed to detects the enzymatic activity of *Bacillus cereus* that converts hydrogen peroxide to water and oxygen. The reagent, 3% H_2O_2, should be stored at 4 ©C in dark bottle. Extra care must be taken if the isolate has been grown on blood agar, because the presence of red blood cells can lead to a false-positive reaction. The test done by two ways: first for demonstration of cell-bound catalase by taken a loopful of pure isolates on a TSA-YE placed on a clean microscopic slide and a drop of 3% H_2O_2 added. An effervescence of oxygen gas within a few seconds indicates a positive reaction. Second for demonstration of secreted cell-free cytoplasmic catalase by adding some drops of 3% H_2O_2 to overnight broth of isolate and through a seconds we noticed an effervescence or gaseous foam formation as an indication of positive reaction (Quinn *et al.*, 2004).

Phenotypic growth of *Bacillus cereus*

The majority of *Bacillus* species apparently have pathogenic potential and are rarely associated with disease in humans or in lower animals. The main exception seems to be anthrax agent *Bacillus anthracis* and *Bacillus cereus* gastroenteritis (Murray *et al.,* 2003). *Bacillus cereus* as well causes food poisoning resulting from the consumption of contaminated rice (Bouza *et al.,* 1979), other starchy foods, including such potatoes, pasta and cheese, eye infections and a broad range of other clinical conditions, including such abscess formation, meningitis, septicemia and wound infection. HiCromeTM Bacillus Agar is dependent upon this formulated MYP Agar (Mortimer and Mc Cann., 1974) used it to enumerate *Bacillus cereus* as well as *Bacillus thuringiensis* when found in abundant numbers in only some foodstuffs. The medium contains peptone and HM extract, that provide growth-enhancing nitrogenic and carbonaceous compounds, long chain amino acids, vitamins, and other nutrients. Mannitol needs to act as its fermentable carbohydrate, which can then be detected for fermentation by phenol red. Mannitol that ferments organisms such as *B. megaterium* yields yellow colonies. The chromogenic mixture observed in the medium is metabolized by the b-glucosidase enzyme observed in *B. cereus* which leads to the formation of blue colonies. While *B. cereus* and *B. thuringiensis* are biochemically equivalent to blue / green colonies, *B. thuringiensis* almost always grows with this medium. Where aseptically selective isolation of *B. cereus* or *B. thuringiensis* is necessary, add Bacillus Selective Supplement (FD324).

Biofilm Formation Assay: Congo Red Agar method (CRA)

Hassan *et al.,* (2011) described an alternate method of screening biofilm formation; demanding the use of such a specifically prepared solid media. A modification was made by replacing BHI agar with double-strengthened TSA-YE (8g Tryptone Soya Agar + 1g Yeast Extract\100 ml d.w.) supplemented with 5 percent sucrose (5gm\100 ml) and Congo red (10gm\L) for better results. Congo red applied as a concentrated aqueous solution directly or prepared with media and autoclaved at 121 ©C for 15 minutes, separately from other medium constituents, then applied when the agar had cooled to 55 ©C. Don't just boil on autoclave media (critical step). Plate was inoculated and incubated at 37 ©C for 24-48 hr. Black colonies with a dry, crystalline consistency showed good results. Weak slime producers generally remained white, although occasional darkening was observed at the colonial centers. An intermediate result was an obscuring of the colonies with the absence of a dry crystalline colonial morphology. The triplicate experiment was performed, and repeated three times.

Antibiogram-Antibiotics Susceptibility-Sensitivity Assay

Proper selection of antibiotic for treatment depends on right diagnostic gesture in which, antibiotics susceptibility test (AST) confirm these guidelines.

Increased resistance problems and emergence of new multidrug pathogens that transferred to food chain especially in Iraqi environment play a critical tool for hygienic community and economic strategy for production and trading of safe food and customer satisfaction (Kanaan, 2013). In clinical practice prescribed antibiotics most commonly are based on the general recommendations and susceptibility awareness. Although susceptibility can differ even within the same species (where certain strains will be more resistant than others, antibiotic susceptibility testing (AST) is typically performed in order to determine which antibiotic would be much more effective in the treatment of it being in vivo bacterial infection. A semi-quantitative approach based on diffusion (Kirby-Bauer process); small disks containing different antibiotics or impregnated paper disks are dropped on an agar plate, which is a nutrient-rich environment in which bacteria can develop, in various zones of the culture. The antibiotic will spread throughout the area surrounding each tablet, and a bacterial lysis disc will become visible. Although the antibiotic concentration has been the highest there at center and the lowest just at edge of this zone, the diameter is indicative for MIC or Minimum Inhibitory Concentration (conversion of the diameter into MIC in µg/ml is dependent on established linear regression curves).

Statistical analysis

The data were analyzed using statistical software, the Social Sciences Statistical Package (SPSS, version 25, 2019), including t-test, ANOVA and Chi-square to check the variations in significance among the revealed data.

Results and Discussion

Total Recovery Ecomap

Checkerboard calculated data illustrate recovery of sixty-seven isolates of *B. cereus* out off one-hundred pooled samples of soft cheese ecosystem (67 from 100: 67 %) in selected and scanned tetra-regions in Kirkuk during November (2019) until March (2020). Ecomap distribution chain revealed isolation and identification of twenty-seven isolates (34.328 %) from Alhaweja, seventeen isolates (25.373 %) from Dakok, thirteen isolates (19.403 %) from Laylan and ten isolates (14.925 %) from Kirkuk territories. Estimated data described according to focused region and month as illustrated in table 1 and 2.

Revised Antibiotics Susceptibility

Depending on guidelines of disc diffusion method and instructions of CLSI, including standardized reference table of susceptibility of *B. cereus* to selected antibiotics; the recovered clones were categorized into versatile groups: Susceptible to Resistant phenotypes. Forty

Table 1: Recovery pattern of *B. cereus* from soft-cheese ecosystem according to selected region.

Month	Number of Samples	Recovered *B. cereus*	Recovery ratio	
			25 %	100 %
Alhaweja	25	23 (34.328 %) A*	92	23
Dakok	25	17 (25.373 %) B	68	17
Laylan	25	13 (19.403 %) C	52	13
Kirkuk	25	10 (14.925 %) D	40	10
Total	100	67		67

*: Indicate highest isolation ratio of *B. cereus* from Alhaweja.
A, B, C, D: Show the vertically important clinical differences between regions at level (P≤0.05).

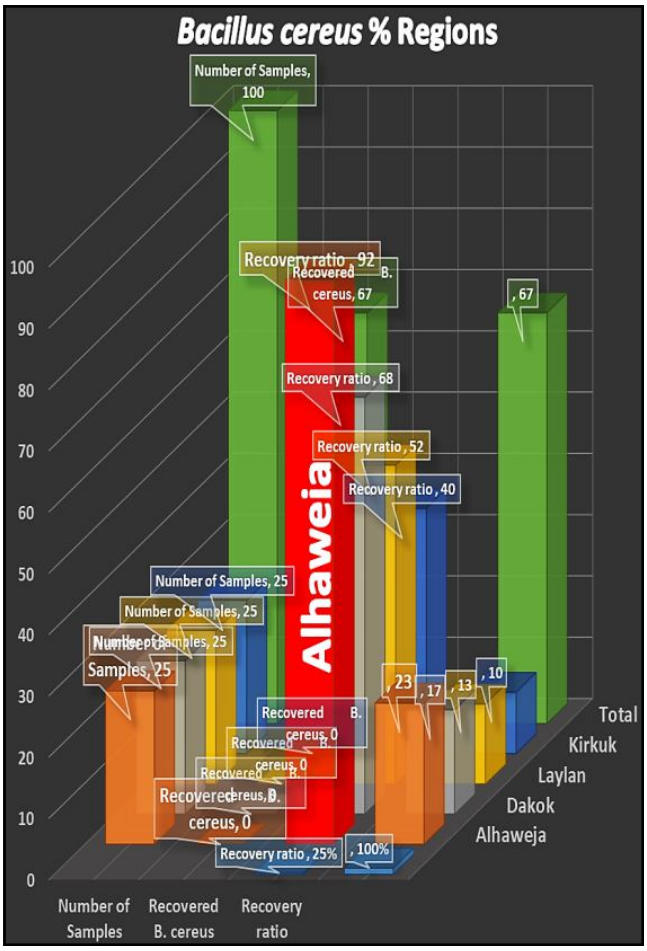

Fig. 1: Illustrate recovery percentage Regions.

Table 2: Recovery pattern of *B. cereus* from soft-cheese ecosystem according to selected month.

Month		Number of Samples	Recovered *B. cereus*	Recovery ratio	
				25 %	100 %
2019	November	20	8 (11.940 %) D	40	8
	December	20	13 (19.403 %) C	65	13
2020	January	20	19 (28.358 %) A*	95	19
	February	20	17 (25.373 %) B	85	17
	March	20	10 (14.925 %) D	50	10
Total		100	67		67

Table 3: Recovery mean log count of *B. cereus* (CFU.ml^{-1}) from soft-cheese ecosystem.

Region	Number of Samples	Mean Log Count (CFU.ml^{-1})
Alhaweja	25	6.190 ^{A*}
Dakok	25	6.039 ^{A}
Laylan	25	5.880 ^{B}
Kirkuk	25	5.867 ^{B}
Total	100	5.994

isolates out of sixty-seven (59.701 %) were resistant to penicillin, amoxicillin, methicillin and vancomycin but mostly sensitive to ciprofloxacin; versus thirty-seven (40.298 %) were intermediate to sensitive clones. Sixteen isolates (23.88 %) were resistant to selected antibiotics versus three excluded (4.477 %) were sensitive to ciprofloxacin in Alhaweja, ten isolates (14.925 %) were resistant to selected antibiotics and sensitive to ciprofloxacin from Dakok, seven isolates (10.447 %) were resistant to selected antibiotics and sensitive to ciprofloxacin from Laylan and same statistical from Kirkuk. These revised statistics disclose development of resistance profile inside Kirkuk ecosystem that give us *in vitro* clue for the right choice of antibiotics but must be proposed that not all recovered resistance *in vitro* were true in sensing behavior because selected antibiotics might work *in vivo* especially in clinical phase on *B. cereus* in combination with other innate and adaptive immune defense barriers. Powered shields of capsule-biofilm architecture might encounter resistance profile index. Table 5 and 6 with Fig. 7 illustrate these behaviors.

Green tea & Rosemary Susceptibility Pattern

Most experimentally processed forty multidrug resistant clones verified as intermediate to resistant to all extracts up-to twenty percent concentration versus other thirty-seven clones verified as intermediate to sensitive to all extracts below twenty percent concentration. Green tea was better than other formulas followed by mixed

Fig. 2: Blue colonies of B. cereus on HiChrome Bacillus Brilliant Agar.

Table 5: Resistant (R), Intermediate (I) and Susceptible (S) antibiotics segregation chain.

Regions	Recovered *B. cereus* Strains	Selected Antibiotics														
		Penicillin (P 10µ)			Amoxicillin (AM 10µ)			Methicillin (ME 5µ)			Vancomycin (VA 30µ)			Ciprofloxacin (CIP 5µ)		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Alhaweja	27	16	10	1	16	10	1	27	0	0	27	0	0	0	1	26
Dakok	17	10	5	2	10	5	2	17	0	0	17	0	0	0	0	17
Laylan	13	7	3	3	7	3	3	13	0	0	13	0	0	0	0	13
Kirkuk	10	7	3	3	7	3	3	10	0	0	10	0	0	0	0	10
Total	67	40	21	9	40	21	9	67	0	0	67	0	0	0	1	66

Table 7: Efficacy interphase threshold breakpoint of extracts (Standard Control Penicillin (CLSI).

Regions	Selected MDRO Strains	Threshold Concentration (20 %)					
		Green tea		Rosemary		Mixed	
		R	S	R	S	R	S
Alhaweja	16	16	0	16	0	16	0
Dakok	10	9	1	10	0	9	1
Laylan	7	6	1	7	0	6	1
Kirkuk	7	6	1	7	0	6	1
Total	40	37 (92.5)^{A}	3 (7.5)^{B}	40 (100)^{A}	0^{C}	37 (92.5)^{A}	3 (7.5)^{B}

A,B,C: Indicate bio statistically significant differences horizontally at level ($p \leq 0.05$).

then rosemary, with MIC breakpoints from twenty percent interphase and MBC breakpoints up-to twenty percent in most examined strains. These features unfortunately reflect the development of recalcitrant biofilm clones that unaffected by these natural antimicrobial preparations *in vitro* model but, might integrated with antibiotics or inside the host with other natural defense barriers or factors as a potentiation pattern to inhibit or kill the targeted foodborne *B. cereus*. Powerful and highly dangerous multidrug and multi-extract clone was determined from soft-cheese ecosystem from Alhaweja. HACCP guidelines must be verified and supported by Hurdle technology using integration module of pasteurization followed by preservation and flavoring with powerful antioxidant scavengers (green tea and rosemary) might be the right untested choice for better hygienic profile index. Table 7 and 8 with Fig. 8 illustrate the tested null hypnosis with alternative pair (Coherent study).

Checkpoints Box: From tables above estimated and calculated data reveled insignificant differences in my results were 67% and may friends found 63.33 isolates of *B. cereus* (Abd and Ali., 2015) and Ahmed *et al.,* (2010) were isolate *B. cereus* as bacterial load of contaminants in fresh meat. According to standard microbial log count formula of Jay (2005-2019) in food microbiology in which, each decreasing or increasing 0.5 log count of microbe in food indicate clinically significant differences affecting hygienic status and this might not in necessary indicate statistical differences. Such variations in numbers were due either to the effects of the sanitation system implemented at these sites or to the animal management policy, which may include grooming, the type of food and water provided to these animals and the climate around these sites (AL-Allaf., 2011).

Hence, it is essential to understand the importance of biofilms and other virulence factors which contribute with most pathogens' ability to colonize & establish infections (Gomes *et al.,* 2016). Biofilms sometimes account for recurring infections. Biofilm formation is caused by major genetic and resulting physiological changes in the microorganisms leading to a loss of immunity to approximately all antibiotic groups (Gomes *et al.,* 2016).

While testing data on susceptibility we captured variable associated accessories, different growth phenotypes and the existence of tolerant clones develop within the inhibition zone categorized as persistent. Resistance behavior which is either natural or acquired through strategies for sharing genes We found abnormal growth of some subclones within the inhibition zone from the same recovered isolate. This may indicate the challenge of certain colonies formed by the same isolation to natural tolerance of selected and tested antibiotics as persistent due to genetic individual variation within the same *B. cereus* clones during reproduction (generation time: log to growth stationery phases) either ended up resisting antibiotics manifested by growth on or sensitive to Muller-Hinton agar manifested by inhibition. Uncontrolled import of contaminated feeds-foods recycled contaminated Iraqi environment after 2003 with foreign clones carrying strong & intelligent defense strategies called the immune system CRISPR-CAS. All of these scenarios with other obscure causes lead to these resistant biofilm clones emerging.

Most experimentally processed forty multidrug

Table 8: Minimum & Maximum Inhibitory Concentration of Extracts (Titer or Cut Off Values).

Regions	Selected MDRO Strains	Threshold Concentration (20 %)					
		Green tea		Rosemary		Mixed	
		MIC	MBC	MIC	MBC	MIC	MBC
Alhaweja	16	T0 (20 %)	T0 (≥ 20 %)	T0 (≥ 20 %)	T0 (≥ 20 %)	T0 (20 %)	T0 (≥ 20 %)
		0.25 Log	3 cfu.ml^{-1}	0.5 Log	5 cfu.ml^{-1}	0.5 Log	3 cfu.ml^{-1}
Dakok	10	T1 (20 %)	T1 (≥ 20 %)	T0 (≥ 20 %)	T0 (≥ 20 %)	T1 (20 %)	T1 (≥ 20 %)
		0.5 Log	2 cfu.ml^{-1}	0.75 Log	3 cfu.ml^{-1}	0.75 Log	2 cfu.ml^{-1}
Laylan	7	T2 (20 %)	T2 (≥ 20 %)	T1 (≥ 20 %)	T1 (≥ 20 %)	T1 (20 %)	T1 (≥ 20 %)
		0.75 Log	1 cfu.ml^{-1}	1 Log	2 cfu.ml^{-1}	1 Log	1 cfu.ml^{-1}
Kirkuk	7	T2 (20 %)	T2 (≥ 20 %)	T1 (≥ 20 %)	T1 (≥ 20 %)	T1 (20 %)	T1 (≥ 20 %)
		0.75 Log	1 cfu.ml^{-1}	1 Log	2 cfu.ml^{-1}	1 Log	1 cfu.ml^{-1}

resistant clones verified as intermediate to resistant to all extracts up-to twenty percent concentration versus other thirty-seven clones verified as intermediate to sensitive to all extracts below twenty percent concentration. Green tea was better than other formulas followed by mixed then rosemary, with MIC breakpoints from twenty percent interphase and MBC breakpoints up-to twenty percent in most examined strains. These features unfortunately reflect the development of recalcitrant biofilm clones that unaffected by these natural antimicrobial preparations *in vitro* model but, might integrated with antibiotics or inside the host with other natural defense barriers or factors as a potentiation pattern to inhibit or kill the targeted foodborne *B. cereus*. Powerful and highly dangerous multidrug and multi-extract clone was determined from soft-cheese ecosystem from Alhaweja.

Even among functional properties of green tea, that was first demonstrated antibacterial activity in Mc Naught (1906), that also had shown that tea destroyed the microorganisms taking responsibility for typhoid fever and brucellosis. In another study they had also said, it is remarkable that methicillin-resistant *S. aureus* (MRSA) is inhibited by tea extract at concentrations normally found in tea drinks (Cho., 2008).

The essential oil of Rosemary has indeed been tested against eight strains of bacteria: *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Bacillus pumilis, Pseudomonas aeruginosa, Salmonella poona, Escherichia coli* and ampicillin-resistant *Escherichia coli*. Results of this test for disc diffusion assay accompanied by modified resazurin indicated that the essential oil tested showed greater antibacterial activity against Gram-positive bacteria (IZ 18.0- 24.2; MIC 0.20-0.48 mg.mL^{-1}) particularly in comparison with Gram-negative bacteria (IZ 12.8-17.5; MIC 1.16-1.72 mg mL^{-1}) (Hussain *et al.,* 2010).

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