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Comparing inhibitory effect some of probiotics and antibiotics against pathogenic bacteria isolated from injuries of military operations

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Abstract

This study aimed to isolate the antibiotics-resistant bacteria causing burns and wounds infections in military operation patients, and to compare the inhibitory efficacy of probiotics with the most common effective antibiotics. For this purpose, collected a total of 52 male patients of ages from 18 to 55 years suffering of wounds and burns infection from Military Muthanna Hospital form the period September 2019 to January 2020. Distributed between 34 of the samples were collected from wounds and 18 from burns. 27 (51.92%) from the wounds and 14 (26.92%) from the burns gave positive results for bacterial growth. A total of 61 bacterial isolates, 40 from wounds and 21 from burns, were obtained after culturing on MacConkey agar and blood agar and incubating at 37ºC for 24h. The isolates were identified by cultural, microscopic and biochemical tests and found to belong to both Gram positive and Gram-negative bacteria .The susceptibility test was performed by subjected each of the wound and burn isolates to four most common antibiotics to each isolate bacterium. Results showed that majority of the isolates were highly sensitive to Ciprofloxacin and Gentamicin. 8 of the highly antibiotic-resistant isolates were selected for treatment by two bacterial probiotics; *Lactobacillus casei and L. paracasei* Cultivated independently, in MRS broth (de Man, Rogosa and Sharpe broth) medium. The fermentation products were then concentrated (50, 25, and12.5) ml and the inhibitory activity of the eight most antibiotic-resistant isolates used was tested. Experiment was repeated three times and analysed with SPSS software. ANOVA Table with Tukey's Multiple Comparison Test. Results showed that despite the three-fold concentrated filtrates of both probiotics exerted good inhibitory activity against the pathogenic isolates, but *L. casei* filtrate was highly effective than that of *L. paracasei*. Moreover, *L. casei* filtrate was even competitor to Ciprofloxacin and Gentamicin.

Introduction:

The skin largest organ and first barrier in the body, the skin has multiple important functions, such as preventing pathogens and dehydration, regulating body temperature, and supplying sensation. The skin is also an active immune organ, hosting cellular elements of the innate and adaptive immune systems [1]. Serious and widespread skin damage, such as burn injury, threatens the entire organism and impairs the capacity for skin regeneration.

Moreover, with the increased prevalence of such diseases as diabetes, vascular disease, and obesity, chronic wounds are becoming a major global issue with limited treatment strategies, unsatisfactory therapeutic effects, and significant medical costs [2]. More than 200 different types of bacteria live naturally on the skin. Open wounds provide a moist, warm and ideal environment for microbial colonization and spread [3]. Urgent responding to medical needs for military operations drove for decades the pace of improvements in treating wounds, burns, infections as well as orthopaedic injuries [4]. Most specific antibiotic resistant bacterial strains are composed of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii* [5].

Probiotics can restore the normal intestinal flora and prevent the growth of harmful bacteria. They may be used to compensate and reduce complications caused by antibiotics [6]. Probiotics possess many mechanisms to exert their beneficial effects such as inhibiting cell colonization adhesion, pathogen invasion having antimicrobial activity and modulate immune response of the host [7]. Moreover, Hadid [8] found among all, *P. aeruginosa*1 was the most affected isolate with highest recorded inhibition zone of 18 mm. Adversely, the least effective inhibitory effect was recorded against *Acinetobacter baumannii* with an inhibition zone 7 mm. Aim of this study isolate the antibiotics-resistant bacteria causing burns and wounds infections in military operation patients, and to compare the inhibitory efficacy of probiotics with the most common effective antibiotics.

New approaches and ways are needed and considered to be important in controlling wound and burn infections. Probiotics are one of the promising means in enhancing the effects of antibiotics and reducing the resistance of pathogenic bacteria to antibiotics.

Materials and Methods

Collection and Isolation of Pathogens

We were collected 52 sample from male patients of ages between 18 to 55 years suffering from burns and wounds infection of Military Muthanna Hospital form the period September 2019 to January 2020. Samples were taken by sterile disposable cotton swabs before returning to the transport medium. They were, then, cultured onto (MacConkey agar, Blood agar and Mannitol Salt Agar/Himedia) plates before incubating at 37ºC for 24h. After incubation, identified based on colony morphology, microscopic Gram stain investigation, and standard biochemical tests [9].

Antibiotic susceptibility test

The Antibiotic susceptibility of samples was determined by disk diffusion method using (Mueller-Hinton agar/Himedia) according to the clinical and laboratory standards institute (CLSI, 2019) recommendations [10]. In the current study, the following 4 antibiotic disks (Conda/Spain) were used: Amikacin(30µg), imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (30 µg), Vancomycin (30µg) and Erythromycin (15µg).

In the bacterial suspension (0.5 McFarland) were cultured on Mueller-Hinton agar plates and then antibiotic discs were placed on the medium and incubated at 37°C for 16 -24 hours [11].

Activation of probiotics

In the current study, 2 probiotic strains were taken by the laboratories of the Department of Biotechnology / University of Baghdad. The local *Lactobacillus* strains included *L. caesi and L. paracaesi*. Probiotics were cultured in MRS broth (de Man, Rogosa and Sharpe broth/Himedia) , a selective medium for profuse growth of lactic acid bacteria, and incubated under anaerobic conditions at 37°C for 48 - 72 hours [12].

Then, they were transferred to MRS agar (de Man, Rogosa and Sharpe agar/Himedia) Experiment was repeated three times and analyzed with SPSS software. ANOVA Table with Tukey's Multiple Comparison Test Finally, probiotics were aseptically -4°C for 2 weeks.

Preparation of Probiotic Filtrates:

A volume of 100 ml of the fermented product (considered as unconcentrated filtrate) was concentrated to 50 ml by putting in the Rotary evaporator at (40-45)°C to make the one-fold concentrated filtrate. The experiment was repeated on the one-fold concentrated filtrate to obtain the two-fold concentrated filtrate (25 ml), and same thing was done for the two-fold concentrated filtrate to obtain the three-fold concentrated filtrate (12.5 ml).

Determination of Probiotics Activity against Pathogenic Bacteria:

Agar well bioassay Aslim and Kilic [13] was applied for testing the antibacterial activity of probiotics against pathogenic bacterial isolates. The probiotic bacterial isolates were prepared by inoculating 2% of the inoculum of *L. casei*, *L. para casei* (6×10^8) or the mixture of them (1×10^9 for each) in MRS broth of pH 6. Then it was incubated anaerobically in a candle jar at 37°C for 48h (this process was repeated three times to increase the intensity of bacterial cells). Then placed in a centrifuge at 6000 rpm for 20 minutes, and the filter was sterilized using a Millipore filter (0.45) and the precipitate was neglected.

Pathogenic bacteria cultured in Brain-heart infusion broth were prepared and activated in a Nutrient broth medium, and then incubated in aerobic conditions at 37°C for 24h. Take (0.1) ml of bacteria stuck at concentration ($1.5 * 10^8$ bacterial cells / ml) (compared with the turbidity of the McFarland standard solution).

Then spread it on surface of Muller-Hinton with Cotton Swab in three directions with the plate rotated. At an angle of 60 in all directions, then the dishes are allowed to dry at room temperature for 15 minutes, 5 mm diameter wells were made by sterile cork borer in the center of Muller-Hinton agar separately cultivated pathogenic bacteria. Each well was filled with 0.1 mL of concentrated filter with three folds (50, 25, and 12.5) mL, and then incubated at 37 ° C for 24 hours. Antibacterial activity was estimated by measuring the diameters of the inhibition zone (in inches) around the well by a ruler

Results and Discussion

The 52 Samples were collected from patient males of the Military Muthanna Hospital which composed of 34 wound infections and 18 of burn infections swabs. Figure 1 shows that highest occurrence 11 cases 32.35% among wound patients was recorded in the age group of 30-40 year, while the lowest 3 cases 8.82% were in the age group of over 50 year. Regarding burn infection patients, highest occurrence7 cases 38.8% was recorded in the 20-30 year age group and the lowest1case 5.5% was in 40-50 year age group. Results showed the most injured group with wounds and burns less than 20 and 20-30 years. The reason is due to the most age groups present on the front lines of military operations, in addition to the lack of military and security experience.

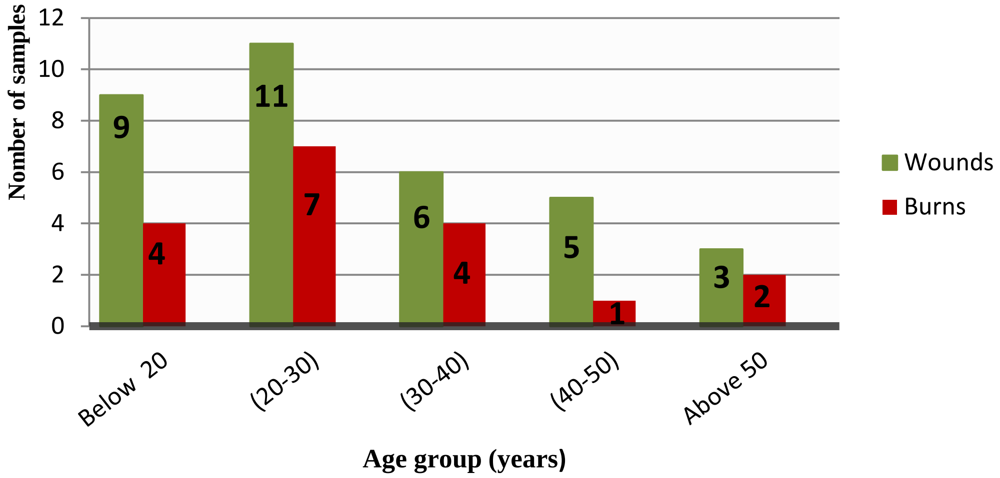

Fig1: Distribution of infections according to their sources and group of patients

Isolation of bacteria:

After propagating infection samples on differential media (MacConkey agar and blood agar), 27 of the 34 wounds samples 79.41 % gave positive result for the occurrence of bacterial growth, compared to 14 (77.77%) of the 18 burns samples. Adversely, the rest 7 samples (20.58 %) of wounds and 4(22.22%) of burns were negative for bacterial growth. As a net result, from the total of 62 patients who suffered from wound and burn injuries, 41were found to be infected with bacterial growth while 11 patients were free of this growth.

As shown in figure 2 , Gram-positive bacteria were predominant in wound infections with 24 isolates 60 % compared to 16 (40%) isolates of Gram-negative bacteria. Adversely, Gram-negative bacteria were predominant in burn infections with 17 (80.95%) isolates with only 4 isolates 19.04% as Gram-positive bacteria.

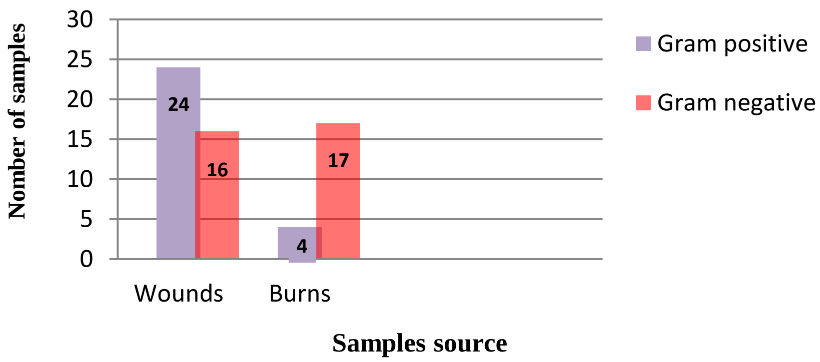

Fig 2: Distribution of wound and burn infection samples according to Gram reaction of bacteria.

Distribution of identified bacterial isolates on sources of infections

Table 1 shows the numbers and percentages of bacterial species identified in patients suffering of wound and burn infections. Six species were detected in patient samples of wounds infection with various numbers of isolates for each. *Staphylococcus aureus* was the most prevalent species detected in wounds infection with a number of 18 isolates and a percentage of 45 %, followed by 9 isolates for *Escherichia coli* in a percentage of 22.5%, then 6 isolates 15% for *S. epidermis.*

In this regard, Isibor *et al*., [14] found in their study that *S. aureus* was the main cause of wounds infection with a percentage of 35% . A percentage of 10 % was recorded by 4 isolates of *Pseudomonas aeruginosa* in wounds infection, which is a little lower percentage than that reported by Yeoman, and Edwards [15] who found it 16.00% and highly below than 27.8% of (year) in their studies performed also on wound infections. Adversely, *Pseudomonas aeruginosa* was the most dominant bacteria in samples of burn infections when it is accounted for 8 isolates (a percentage of 38.09% of the 21 isolates of this source.

This result came in accordance with the study performed in RML Delhi Hospitable by Sharma and Hans [16] who found *Ps. aeruginosa* as the most common bacteria in burn infections. Occurrence of *Staphylococcus aureus* in burn infections came in the second place with a number of 4 isolates in a percentage of 19.04%.

Oncul *et al*.,[17] who recorded in the Teaching Hospital in Istanbul as 25% in their studies on burn infections. *Escherichia coli* occurred in a percentage of 14.28% by 4 isolates. Each of *Klebsiella pneumoniae* and *Proteus putida* presented in burn infections by 3 isolates 9.5% which came in accordance with what was found 7-10% by Mehta *et al*., [18].

It could be concluded regarding occurrence of bacteria in wound and burn infections that all species listed in table 1, except *Staphylococcus epidermis* were detected in burn infections, while both *Enterobacter cloacae* and *Acinetobacter baumannii* were not present in wound infections.

Table 1: Bacterial species obtained from wounds and burns infections

Source of isolates / Species of bacteria	Wounds		Burns	
	No.	%	No.	%
Staphylococcus aureus	18	45	4	19.04
Escherichia coli	9	22.5	3	14.28
Staphylococcus epidermis	6	15.	0	0
Pseudomonas aeruginosa	4	10	8	38.09
Klebsiella pneumoniae	2	5	2	9.52
Proteus putida	1	2.5	2	9.52
Enterobacter cloacae	0	0	1	4.76
Acinetobacter baumannii	0	0	1	4.76
Total	40	100	21	100

Antibiotic susceptibility of pathogenic bacteria

By using the disk diffusion method to test susceptibility of the bacteria isolated from wounds and burns toward six different types of antibiotics [12], the following results were obtained:

Antibiotics susceptibility of wounds bacteria:

As shown in table 2, majority of the *Staphylococcus aureus* 18 isolates were resistant to two of the four antibiotics used; namely Vancomycin by 13 isolates 72.22% and Erythromycin by 12 isolates 66.66%. A closed result was reported by Hussain [19] who found that the percentage of Erythromycin resistance to *Staphylococcus aureus* was (52%). Adversely, majority of the 18 *S. aureus* isolates were sensitive to the other two antibiotics, Ciprofloxacin 13 isolates 72.22% and Gentamicin 11 isolates 61.11%.

This result is close to that reported by Al-Jumaily [20] who found that 80% of the *S.aureus* isolates were sensitive to Ciprofloxacin. On the other hand, Rafiq [21] reported different results when found that sensitive to 100%. Regarding *Escherichia coli*, majority of its 9 isolates were sensitive to all four antibiotics used; 7 (77.7%) to Imipenem, 6 (66.67%) to each of Amikacin and Gentamicin, and 5 (55.56%) to Ciprofloxacin.

Results of a study by Desai *et al.,* [22] showed that 23.60% of the *E. coli* isolates were sensitive to Ciprofloxacin and 68.55% to Gentamicin. The 6 isolates of *S. epidermidis* were sensitive to all four above antibiotics, which came in agreement with a study conducted. All 4 isolates of *Pseudomonas aeruginosa* were resistant to Ciprofloxacin, while 3 (75 %) of them were sensitive each of Amikacin and Gentamicin as shown in Figure 3. Hamad [23] found that 70% of isolates of this were sensitive to Gentamicin and Ciprofloxacin

Table 2: Susceptibility of bacterial isolates of wound samples toward the most common antibiotics

Bacterial isolate	S\R	Antibiotic					
		Amikacin	Erythromycin	Ciprofloxacin	Gentamycin	Imipenem	Vancomycin
Staphylococcus aureus	S	-	6	13	11	-	5
(n=18)	R	-	12	5	7	-	13
Escherichia coli	S	6	-	5	6	7	-
(n=9)	R	3	-	4	3	2	-
Staphylococcus epidermis	S	-	6	6	6	-	6
(n=6)	R	-	0	0	0	-	0
Pseudomonas aeruginosa	S	3	-	0	3	2	-
(n=4)	R	1	-	4	1	2	-
Klebsiella pneumoniae	S	2	-	1	2	2	-
(n=2)	R	0	-	1	0	0	-
Proteus putida	S	1	-	1	1	1	-
(n=1)	R	0	-	0	0	0	-

n: Number of isolated bacteria; S: Sensitivity; R: Resistance,: No test

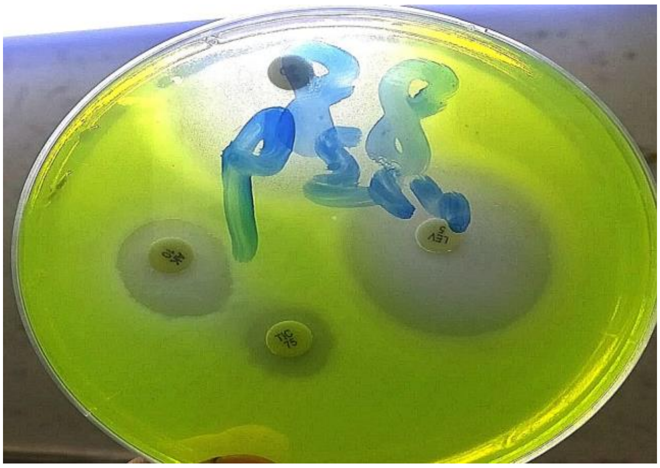

Fig 3: Inhibition zone given by Antibiotic against Pseudomonas aeruginosa isolated from burn wound infections.

Antibiotics susceptibility of burns bacteria:

As shown in table 3, highest number of isolates obtained from burn infections were belonged to *Pseudomonas aeruginosa* with 8 of the 21 total isolates. At the time that 6 (75 %) these bacterial isolates were resistant to antibiotic Amikacin, 6 of them were sensitivity to Imipenem. In this regard, Hamad [23] reported 70.00% resistance of *P. aeruginosa* isolates to the Amikacin. Variable susceptibility results were recorded by the 4 isolates of *Staphylococcus aureus* toward the antibiotics used. While all 4 (100%) isolates were resistant to Gentamicin, 3 (75 %) were resistant to Ciprofloxacin. Davoudi *et al.,* [24] reported that (71.42%) of *S. aureus* isolates were resistant to Gentamycin. Two of the 3 (66.66%) *E. coli* isolates were sensitive to all four antibiotics used. Moş *et al.,* [25] reported high sensitivity of *E. coli* toward antibiotics Imipenem and Amikacin. One of the two isolates of *Klebsiella pneumoniae* was sensitive to all four antibiotics used while the other was resistant. The only one isolate of each of *Acinetobacter baumannii* and *Enterobacter cloacae* was sensitive to three of the four antibiotics used and resistant to only one; Imipenem and respectively. Sharmin *et al.,* [26] found that *Enterobacter* spp. and *Klebsiella spp*. were resistant to Erythromycin 100% For both of them. Espinal *et al.,* [27] related sensitivity of *A. baumannii* towards antibiotics to its ability for forming biofilms which enables it to survive.

Table 3: Susceptibility of bacterial isolates of Burn samples toward the most common antibiotics

Bacterial Isolate	S\R	Antibiotic					
		Amikacin	Erythromycin	Ciprofloxacin	Gentamycin	Imipenem	Vancomycin
Pseudomonas aeruginosa	S	2	-	3	4	6	-
(n=8)	R	6	-	5	4	2	-
Staphylococcus aureus	S	-	2	1	0	-	2
(n=4)	R	-	2	3	4	-	2
Escherichia coli	S	2	-	2	2	2	-
(n=3)	R	1	-	1	1	1	-
Klebsiella pneumoniae	S	1	-	1	1	1	-
(n=2)	R	1	-	1	1	1	-
Proteus putida	S	2	-	0	1	2	-
(n=2)	R	0	-	2	1	0	-
Acinetobacter baumannii	S	1	-	1	1	0	-
(n=1)	R	0	-	0	0	1	-
Enterobacter cloacae	S	1	0	1	-	1	-
(n=1)	R	0	1	0	-	0	-

n: Number of bacterial isolates S: Sensitive; R: Resistant; -: No test

Inhibition zones of highly antibiotics-resistant isolates

Two (BPSa39 and WPSa52) of the three *P. aeruginosa* isolates gave highest inhibition zones (14 and 13 mm, respectively) against Imipenem, while the third one (BPSa21). It is not affected by inhibition; instead it gave highest inhibition zone (16 mm) against Ciprofloxacin. At the time that *S. aureus* WSa22 isolate gave inhibition zones against all four antibiotics used, its highest zone (16 mm) was recorded against Vancomycin. On the other hand, isolate *S. aureus* WSa56 of this species produced inhibition zones of 11 mm and 9 mm against Vancomycin and Ciprofloxacin, respectively, and no any zone against Erythromycin and Gentamycin. Regarding the two isolates of *E. coli*, inhibition zones of 17mm and 15 mm were recorded by WEc111 isolate against Gentamycin and Imipenem, respectively, but no zones against each of Amikacin and Ciprofloxacin. Despite that the second isolate of *E. coli* BEc153 produced no any zone against Imipenem, it gave 13, 11- and 7-mm zones against Ciprofloxacin, Gentamycin and Amikacin, respectively.

The last of the 8 isolates *Klebsiella pneumoniae* BKp41 produced its highest inhibition zone (11 mm) against Imipenem, then Amikacin (8 mm), but no any zone against both Ciprofloxacin and Gentamycin. Most resistance of the pathogen to the drug used may be due to the overuse of antibiotics in treatment, which leads to greater pathogen resistance to the antibiotics [28]. Paterson [29], mentioned that taking an antibiotic much longer in how it is taken orally also affects the rate at which it is absorbed into the bloodstream.

Antibacterial activity of probiotic filtrate pathogenic bacteria

Results in tables 4 and 5 showed that the unconcentrated, one-fold and two-fold concentrated filtrates of both probiotic bacteria *Lactobacillus casei, Lactobacillus paracasei* had no inhibitory effect against any of the eight wounds and burns bacterial isolates. Adversely, the three-fold concentrated filtrates of both probiotics excreted seriously inhibitory activity against majority of the isolates. Generally, the three-fold filtrate of *L. casei* was more effective on the pathogenic isolates than *L. paracasei.* as shown in Figure 4.

Table 4: Inhibitory effect of unconcentrated and concentrated filtrates of probiotic bacteria *Lactobacillus casei* on pathogenic bacterial isolates causing wound and burn infections.

Bacterial isolate	Inhibition zone (mm)			
	Unconcentrated filtrate	One-fold concentrate d filtrate	Two-fold concentrate d filtrate	Three-fold concentrate d filtrate
P. aeruginosa WPSa52	0	0	0	8.5
P. aeruginosa BPSa39	0	0	0	5.5
P. aeruginosa BPSa21	0	0	0	10.3
S. aureus WSa56	0	0	0	14.0
S. aureus WSa22	0	0	0	10.3
E. coli WEc111	0	0	0	11.3
E. coli BEc153	0	0	0	12.6
K. pneumonia BKp41	0	0	0	14.6

W:Wound , B: Burn, N:Namber of isolate bacteria , Psa: *P.aeruginosa*, Sa : *S.aureus*, EC: *E.coli*, Kp *: K. pneumonia.*

Table 5: Inhibitory effect of unconcentrated and concentrated filtrates of probiotic bacteria *Lactobacillus paracasei* on pathogenic bacterial isolates causing wound and burn infections.

Isolate bacteria	Inhibition zone (mm)			
	Unconcentrated fermentation	One-fold) Concentrated	Two-fold) Concentrated	Three-fold Concentrated

P. aeruginosa WPSa52	0	0	0	6.6
P. aeruginosa BPSa39	0	0	0	0
P. aeruginosa BPSa21	0	0	0	10.0
S. aureus WSa56	0	0	0	0
S. aureus WSa22	0	0	0	0
E. coli WEc111	0	0	0	9.6
E. coli BEc153	0	0	0	0
K. pneumonia BKp41.	0	0	0	14.3

W:Wound , B: Burn, , Psa: *P.aeruginosa*, Sa : *S.aureus*, EC: *E.coli*, Kp *: K. pneumoniae,* n:Namber of isolate bacteria

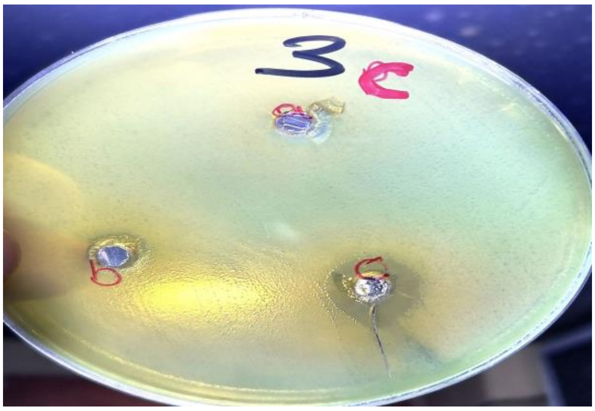

Fig 4: Inhibition zone given by *Lactobacillus casei* (a) one-fold concentrated fermentation product (b) Tow-fold concentrated (c) Three-fold concentrated fermentation product against (3c) *S. aureus* isolated from burn wound infections.

Comparing antibacterial activity of probiotic filtrate and antibiotics:

The efficacy in inhibiting growth of the antibiotics resistant bacterial isolates was compared between two the probiotics (*Lactobacillus casei, L. paracasei*) and two antibiotics (Gentamicin, Ciprofloxacin). Results in table 6 illustrate that *Lb. casei* three-fold concentrated filtrate excreted high inhibitory effect against all bacterial isolates despite varying inhibition zone diameters, followed by antibiotics Gentamicin Ciprofloxacin**.**

Chuayana *et al.,* [30] reported that most of the antimicrobial activities demonstrated by probiotics were bactericidal in nature, with the exception of *L. casei* which was antibacterial against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This corresponds to a previous investigation which showed that the bacteria isolated from milk which identified as *L. casei* was bacteriostatic against *Staphylococcus aureus, Pseudomonas aeruginosa* sensitive to methicillin, and some other bacteria**.**

Dasari *et al.,* [31] observed through the study that the areas of inhibition of probiotics are greater than those of antibiotics. When this study was conducted on *P. aeruginosa,* results showed that in about 72% of cases, areas inhibiting probiotics were greater than those of antibiotics**.**

Table 6: Inhibition zones formed by antibiotics (Ciprofloxacin, Gentamycin) and three-fold concentrated filtrates of probiotics (*Lactobacillus casei, L. paracacsei*) against wounds and burns pathogenic bacteria.

Isolate bacteria	Inhibition zone (mm)								
	Lb. casie	*Lb. paracasie*	Amikacin	Erythromycin	Ciprofloxacin	Gentamycin	Imipenem	Vancomycin	*p* value[a]
P. aeruginosa WPSa52	8.7	7.3	0	-	0	0	13	-	0.01**
P. aeruginosa BPSa39	3.7	0.0	10	-	9	0	14	-	0.02*
P. aeruginosa BPSa21	10.3	10.0	9	-	16	12	0	-	0.09[NS]
S. aureus WSa56	14.0	0.0	-	0	9	0	-	11	0.02*
S. aureus WSa22	11.0	0.0	-	11	14	10	-	16	0.17[NS]
E. coli WEc111	11.3	9.7	0	-	0	17	15	-	0.14[NS]
E. coli BEc153	12.7	0.0	7	-	13	11	0	-	0.04*
K. pneumonia BKp41.	14.7	14.3	8	-	0	0	11	-	0.08[NS]
p value[b]	-	0.03*	0.14[NS]	0.5[NS]	0.3[NS]	0.13[NS]	0.7[NS]	0.8[NS]	
p value[c]	0.03*	-	0.73[NS]	0.5[NS]	0.6[NS]	0.7[NS]	0.6[NS]	0.1[NS]	

[a] Data presented as T test.
[b] Data presented as T test, antibiotic with *Lb. casie*.
[c] Data presented as T test, antibiotic with *Lb. paracasie***.**
NS: Non-significant. * The correlation is significant at the $P < 0.05$ level (Significant). ** The correlation is significant at the $P < 0.01$ level (Highly Significant). – no test.

Conclusions

Occurrence of wound injuries in military operations is more common than those of burns.The multibacterial pattern in wound burn infections was most common than that of monobacterial. In wound infections, Gram positive bacteria, especially *Staphylococcus aureus*, were the predominant pathogens, while in burn infections, Gram negative bacteria especially, *Pseudomonas aeruginosa* were the most common**.** All bacterial isolates were sensitive in different percentages to the antibiotics Ciprofloxacin and Gentamicin. As probiotics, *Lactobacillus casei* and *L. paracasei* exhibited good antibacterial activity against pathogenic bacterial isolates.

The three-fold concentrated filtrates of *L. casei* and *L. paracasei* were effective in inhibiting growth of bacterial pathogens. In fact, *L. casei* filtrate gave remarkable inhibitory effect compared to that of *L. paracasei.*

References

1. Ehrhardt, P., Brandner Johanna, M., & Jens-Michael, J. (2008). The skin: an indispensable barrier. Exp Dermatol, 17, 1063-72.

2. Sorg, H., Tilkorn, D. J., Hager, S., Hauser, J., & Mirastschijski, U. (2017). Skin wound healing: an update on the current knowledge and concepts. European Surgical Research, 58(1-2), 81-94.
3. Young, L. (2012). Identifying infection in chronic wounds. *Wound Pract. Res. J. Aust. Wound Manag. Assoc.* 20, 38 .
4. Dougherty, P. J., Carter, P. R., Seligson, D., Benson, D. R. & Purvis, J. M. (2004).Orthopaedic surgery advances resulting from World War II. *JBJS* 86, 176–181.
5. Murray, C. K., Roop, S. A., Hospenthal, D. R., Dooley, D. P., Wenner, K., Hammock, J., ... & Gourdine, E. (2006). Bacteriology of war wounds at the time of injury. *Military medicine*, 171(9), 826-829.
6. Sharma, J. & Chauhan, D. (2014).Inhibition of *Pseudomonas aeruginosa* by antibiotics and probiotics combinations-In vitro study. *Eur. J. Exp. Biol.* 4, 10–14.
7. Doron, S. & Gorbach, S. L. (2006).Probiotics: their role in the treatment and prevention of disease. *Expert Rev. Anti. Infect. Ther.* 4, 261–275.
8. HADID, M. A. (2015).Evaluating the Effects of Probiotics and Vinegars on Bacteria Isolated from Burns and Wounds Infections.
9. James, C., & Natalie, S. (2014). Microbiology. A laboratory manual. Pearson Education.
10. Kahlmeter, G., Giske, C. G., Kirn, T. J., & Sharp, S. E. (2019). Point-Counterpoint: Differences between the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute Recommendations for Reporting Antimicrobial Susceptibility ResultsPoint-Counterpoint: Differences between the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute Recommendations for Reporting Antimicrobial Susceptibility ResultsPoint-Counterpoint: Differences between the European Committee on .... *Journal of* clinical *microbiology*, 57(9), e01129-19
11. Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol.
12. Aslim, B. and Kilic, E. (2006) 'Some probiotic properties of vaginal lactobacilli isolated from healthy women', *Japanese journal of infectious diseases*, 59(4), p. 249.
13. Lewus, C. B., Kaiser, A. & Montville, T. J. (1991).Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl. Environ. Microbiol.* 57, 1683–1688.
14. Isibor, J. O., Oseni, A., Eyaufe, A. & Turay, A. (2008). Incidence of aerobic bacteria and *Candida albicans* in post-operative wound infections. *African J. Microbiol. Res.* 2, 288–291.
15. Yeoman, K. H. & Edwards, C. (1997).Purification and characterization of the protease enzymes of Streptomyces thermovulgaris grown in rapemeal-derived media. *J. Appl. Microbiol.* 82, 149–156.
16. Sharma, S. & Hans, C. (1996).Bacterial infections in burns patients: a three years study at RML Hospital, Delhi. *J. Commun. Dis.* 28, 101–106.
17. Oncul, O., Yüksel, F., Altunay, H., Açikel, C., Çeliköz, B., & Çavuşlu, Ş. (2002). The evaluation of nosocomial infection during 1-year-period in the burn unit of a training hospital in Istanbul, Turkey. *Burns*, *28*(8), 738-744.
18. Mehta, M., Dutta, P. & Gupta, V. (2007).Bacterial isolates from burn wound infections and their antibiograms: A eight-year study.
19. Hussain, S.S.,Obied, K.A.(2017). Study of the effect of some antibiotics on Pseudomonas aeruginosa and Staphylococcus aureus isolated from burn and wound infections. *Babylon University Journal*, 25 (1), 69-80.
20. Al-Jumaily, E. F., Al-obali, Y. A. J. & Al-nasser, N. E. (2011).Extraction of caffeine from green tea plants (Camellia sinensis). *J. Biotechnol. Res. Cent.* 5, 9–15.
21. Rafiq, R. M. H. S. S. N. & HAMAD, P. A. (2017). Vancomycin Resistance among Methicillin Resistant *Staphylococcus aureus* isolated from Clinical Samples in Erbil City, Iraq. *kirkuk*

Univ. J. Sci. Stud. 12, 108–120 .

22. Desai, K. J., Malek, S. S. & Parikh, A. (2010).Neonatal septicemia: bacterial isolates & their antibiotics susceptibility patterns. *NJIRM* 1, 12–15 .
23. Hamad, L. R. (2014).Study of some plant extracts' effect on the growth of *pseudomonoas aeruginosa* bacteria (pathogenic and wild) and its production of pyocyanin pigment. *Al-Anbar J. Vet. Sci.* 7, 26–32.
24. Davoudi, A., Najafi, N., Alian, S., Tayebi, A., Ahangarkani, F., Rouhi, S., & Heydari, A. (2016). Resistance pattern of antibiotics in patient underwent open heart surgery with nosocomial infection in North of Iran. *Global journal of health science*, 8(2), 288.
25. Moş, I., Micle, O., Zdrâncă, M., Mureşan, M. & Vicaş, L. (2010).Antibiotic sensitivity of the *Escherichia coli* strains isolated from infected skin wounds. *Farmacia* 58, 637–645.
26. Sharmin, S., Alamgir, F., Fahmida, M. & Saleh, A. A. (2009).Antimicrobial sensitivity pattern of uropathogens in children. *Bangladesh J. Med. Microbiol.* 3, 18–22 .
27. Espinal, P., Marti, S. & Vila, J. (2012).Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *J. Hosp. Infect.* 80, 56–60.
28. Rice, L. B., Willey, S. H., Papanicolaou, G. A., Medeiros, A. A., Eliopoulos, G. M., Moellering, R. C., & Jacoby, G. A. (1990). Outbreak of ceftazidime resistance caused by extended-spectrum beta-lactamases at a Massachusetts chronic-care facility. *Antimicrobial agents and chemotherapy*, *34*(11), 2193-2199.
29. Paterson, D. L. (2002).Looking for risk factors for the acquisition of antibiotic resistance: a 21st-century approach.
30. Chuayana, E. L., Ponce, C. V, Rivera, M. R. B. & Cabrera, E. C. (2003) Antimicrobial activity of probiotics from milk products. *Phil J. Microbiol. Infect. Dis* 32, 71–74.
31. Dasari, S., Shouri, R. N. D. (2014).Wudayagiri, R. & Valluru, L. Antimicrobial activity of *Lactobacillus* against microbial flora of cervicovaginal infections. *Asian Pacific J. Trop. Dis.* 4, 18–24.

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مقارنة الفعالية التثبيطية للمعززات الحيوية والمضادات الحيوية ضد البكتريا المرضية المعزولة من المصابين بالعمليات العسكرية

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الخلاصة:

هدفت هذه الدراسة إلى عزل المسبب البكتيري الذي يسبب التهابات الجروح والحروق ومقارنة الفعالية المثبطة للمعززات الحيوية والمضادات الحيوية الأكثر شيوعًا. لهذا الغرض تم جمع 52 مسحة من الذكور تتراوح أعمارهم من 18 إلى 55 عاما يعانون من التهابات الجروح والحروق من مستشفى المثنى العسكري خلال الفترة من ايلول 2019 إلى كانون الثاني 2020. موزعة 34 عينه من الجروح و 18 عينة من الحروق. تمت زراعة العينات على أجار مانكونكي وأجار الدم كخطوة أولى في الزراعة البكتيرية. اعطت 27(51.92٪) من العينات الجروح و 14(26.92%) من الحروق موجبة لإعطاء نمو جرثومي من كلا الحالتين. وأظهرت النتائج وجود 61 عزلة بكتيرية موزعة بين (40) عزلة من الجروح و (21) عزلة من الحروق و بعد التشخيص بواسطة الزرع والفحص المجهري والفحوصات البايوكيميائية ووجد أنها تنتمي إلى موجبة الجرام وسالبة الجرام بعد أن خضعت هذه العزلات لاختبارات الحساسية ، تم إعطاء ستة من المضادات الحيوية الأكثر استخدامًا للمرضى في المستشفى ، وتم الإعلان عن النتائج ووجد ان Gentamycin وCiprofloxacin هما أكثر المضادات الحيوية فعالية ضد كل من البكتيريا موجبة الكرام وسالبة الكرام. كمعززات حيوية، تم استخدام نوعين من *Lactobacillus casei* و *Lactobacillus paracasei* ، زرعت بشكل مستقل، في وسط مرق (de Man, Rogosa and Sharpe broth MRS). بعد ذلك تم تركيز منتجات التخمير (12.5،25،50) مل واختبار الفعالية المثبطة للعزلات الثمانية الأكثر مقاومة للمضادات الحيوية المستخدمة. تم تكرار التجربة ثلاث مرات وتم تحليلها باستخدام برنامج SPSS (ANOVA) مع اختبار Tukey للمقارنة المتعددة ، أظهرت النتائج أن *Lb. casei* سجل منتج بتركيز ثلاثي (12.5 مل) أعلى فعالية مثبطة ضد العزلات البكتيرية المرضية مقاومة المضادات الحيوية. يليها منتج بتركيز ثلاثي *Lb. paracasei*
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