RESEARCH ARTICLE

Evaluation of the Antibacterial Activity and Immunomodulatory Effect of Purified Exopolysaccharides (EPSs) Produced from Vancomycin Resistant *Enterococcus faecalis*

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ABSTRACT

This study was carried out evaluation the antibacterial activity of purified Effect of Purified Exopolysaccharides (EPSs) from vancomycin-resistant *Enterococcus faecalis* against gram-positive bacteria *Staphylococcus aureus*, and gram-negative bacteria *Proteus mirabilis*, *Pseudomonas aeruginosa* as well as evaluation the immunomodulatory effect on the immunity system, a total 100 the urinary tract infection sample was contained 1% vancomycin-resistant *E. faecalis*. Moreover, the total carbohydrates contents of purified EPSs was 88% expressed as a percentage of EPS dry weight (w/w%), the results of antibacterial activity displayed the EPSs has activity against studied isolates depending on inhibition zone diameter at 0.5 mg/mL and 1 mg/mL concentration, but fewer inhibition zones in both concentrations were recorded in *S. aureus*, at 0.5 mg/mL was (7 mm), and 1 mg/mL was (8 mm). Furthermore, the highest inhibition zone was recorded in *P. mirabilis* at 0.5 mg/mL was (17 mm), 1 mg/mL was (20 mm); on the other side, the in vitro experiments revealed EPSs acted positively on the immunity system in the group that was injected with EPS combination with *S. aureus* and the highest value was recorded in the group received 1 mg/ml concentration combination with *S. aureus* in all assays as following TLR-2 level was (8.733 ± 2.136) with significant differences at ($p \leq 0.05$) comparison with other groups, while phagocytic activity was (24.2%) as well as IL-10 level was (78.12 ± 3.16),significant different at ($p \leq 0.05$), whoever Arthus reaction recorded highest results at 1mg/mL (4.26 ± 0.05) Furthermore delayed hypersensitivity test was reported (4.14 ± 0.00 , 4.24 ± 0.00, 3.50 ± 0.50) after 24 hr, 48 hr, and 72 hours respectively in the same group. Depending on obtained results in this present study, the (EPSs) produced by Vancomycin-Resistant *E. faecalis* appeared an antibacterial activity against gram-positive and negative bacteria in vitro, also the in vivo experiments showed EPSs worked positively on the immunity system.

Keywords: Exopolysaccharides (EPSs), Vancomycin Resistant *E. faecalis,* Antibacterial Activity, Immunomodulatory, innate immunity, adaptive immunity.

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INTRODUCTION

E. faecalis is a gram-positive bacterium caused various nosocomial infections, but urinary tract infections was the most common, *E. faecalis* can be found anywhere in environments such as water, soil, foods, and even in plants, also be found in human and animal gastrointestinal tracts[1], in same time this bacteria used a probiotics in food biopreservation attributed to the development of antimicrobial called bacteriocins[2] which are live cells with numerous beneficial human body properties, such as immune enhancement (anti-inflammatory activity), hypocholesterolemic effects[3], and disease prevention/treatment, using this bacteria as probiotic was triggered important debates due to their opportunistic pathogenicity involved in nosocomial infections as well as their antibiotic resistance to their virulence factors.[4] However, as a possible strategy to modulate the internal microbial environment, probiotics may be used to cause beneficial effects without causing harm,[5] for example, applications of probiotic bacteria, could be demonstrated in support of healthy microbiota maintenance in the intestines6. Lactic acid bacteria LAB as *E. faecalis* have been found in different types of fermented foods. They are also enteric microbiota that is often in close contact with the intestinal immune system,7 chronic inflammation relief, blood pressure reduction and cholesterol, and anti-carcinogenic. Immune-modulating effects were the most important benefits to the intestinal tract in this study[8]—primarily focused on

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related studies documenting the advantageous effects of EPSs of *E. faecalis* on the immune system.[9,10] this study aimed to evaluate the antibacterial activity of purified EPSs from E. faecalis against gram-positive and gram-negative isolates and evaluate the immunomodulatory effects.

MATERIAL AND METHODS

The study was included two aspects which are in vitro aspect contained isolation and identification *E. faecalis*, extraction, and purification EPSs. While the second aspect was the determination of the Immunomodulatory Effect of EPSs on the immunity system in albino male mice (Balb-c).

Isolation and Identification from UTI

A total of 100 samples of urinary tract infections was collected from Al-Hussein Teaching Hospital in Al-Muthanna provinc. Samples were activated by nutrient broth for 24 hours at 37 C, then subcultured on esculin azide agar and blood agar after diagnosis the *E. faecalis* isolates depending on the morphological characterization and biochemical test, the isolates were confirmed by Vitek 2 system,

Extraction of and Purification of EPSs

Exopolysaccharides were extracted according to procedure[11] with some modifications. Firstly *E. faecalis* was cultured in (MRS) De Man, Rogosa and Sharpe broth at 37 ° C for 24 hours, Trichloroacetic acid 14% was added at a final concentration to denature all proteins, then the mixture put in a shaker incubator at 90 rpm for 30 minutes. The next step the mixture by cooling centrifuge at 8000 x g for 20 min at 4°C, the supernatant was collected. Absolute cold ethanol (2:1) was added to the supernatant at 4°C for 24 hours for precipitation of the exopolysaccharide followed by centrifugation 8000 x g for 15 minutes 4°C; precipitated exopolysaccharides was dissolved in an equal volume of deionized water against sugar. The final step, the purification of exopolysaccharides, was done with dialysis bags (12,000 of 14,000 Daltons) for 24 ~ 48 hours. The purified EPSs were dried to remove ethanol incubation at 37 for 47 hours.

Determination Carbohydrate Contents

The concentration of carbohydrates in the EPSs was estimated according to method phenol-sulfuric-acid-method[12] relying on the standard curve of glucose, as shown in Figure 1. Absorbance corresponds to 0.1 mL of the test = ‘x’ mg of glucose. A total of 100mL of the sample solution contains = (‘x’ ÷ 0.1) × 100mg of glucose = % of total carbohydrate.

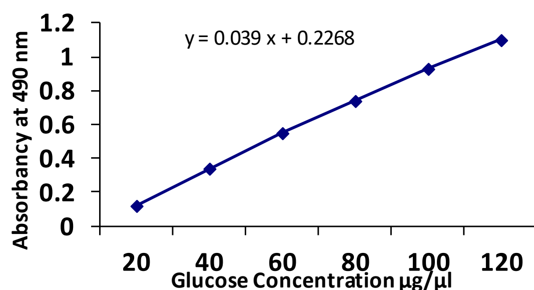

Figure 1: Standard curve of total carbohydrate using glucose

Determination of the Antibacterial Activity.

The isolates that used in this assay were *S. aureus*, *P. mirabilis*, *P. aeruginosa* obtained from the microbiology laboratory/ college of science/Al-Mustansiria University, confirmed by Vitek 2 system, later different antibiotics were applied toward those isolates to select the antibiotic with high inhibition zones to use as a control to comparison with different concentrations of EPSs.

Agar Diffusion Method

Two concentrations were prepared fromEPSs 0.5 mg/mL and 1 mg/ml used to determine the antibacterial activity against *S.aureus*, *P. mirabilis*, *P.aeruginosa*, isolates were adjusted to 1.5 x 108 CFU/mL, cultured on Muller-Hinton agar containing wells (6 mm in diameter). The wells were filled with 100 mL of prepared EPSs concentrations then incubated at 37 C for 24 hours, the diameter of the inhibition zones were measured.[13]

The Laboratory Animals

The research was achieved on male albino mice (Balb-c), their age ranged between (6-8) weeks were obtained from Research and Cancer Center in Baghdad, all experiments were done in the laboratories of the college of science in Al-Mustansiriyah University.

The Studied Groups

This aspect of this study was included groups and used *S. aureus* depending on the inhibition zone against EPSs the groups were treated as follows:

Group: the mice without any treatment,
Group: injected Intraperitoneally with normal saline daily,
Group: injected Intraperitoneally with 100 mL of 1.5 x 108 CFU/ml of *S. aureus*
Group: Injected Intraperitoneally with 100 mL of EPS (0.5 *mg/mL)* daily.
Group: Injected Intraperitoneally with 100 mL of 1.5 x 108 CFU / mL of *S. aureus* and after 4 hours injected daily with 100 mL of 0.5mg/mL of EPSs.
Group: Injected Intraperitoneally with 100 mL of EPSs (1 mg/mL) daily.
Group: Injected Intraperitoneally with 100 mL of 1.5 x 108 CFU/mL of *S. aureus* and after 4 hours injected daily with 100 mL of 1 Mg/mL of EPSs.

After 14 days of treatment, the blood was collected from the mice and divided; serum was obtained and stored at – 4 C for the next assays.

Immunological Assays

Phagocytic Activity

Based on the method provided by[14] .100 ul Blood, the procedure was performed, with 175 uL MEM media added to each well of the microtiter plate followed by the addition of 25 uL NBT and incubated at 28°C for two hours. The supernatant was cautiously extracted. Cells were fixed for 5 minutes with 100%

methanol (v/ v) washed twice with 70% 125 mL methanol. Finally, 125 mL of potassium hydroxide (2N) and 150 mL of DMSO were applied to dissolve NBT, read by Elisa at 650-wave length, and measure the percentage using the equivalent below.

Phagocytic activity percentage (%) = [(A – B)/B]×100

A = (Average) Treated groups; B = (Average) Negative control group.

Arthus Reaction and Delayed Hypersensitivity Test

Every animal in the studied groups was injected with 50 uL of S.aureus on day 5 in the right footpad and normal saline in the left footpad (except positive control injected by normal saline) for Arthus reactions, swelling in the footpad after 4 hours was determined while delayed form hypersensitivity was determined by a digital Vernia after 24, 48, and 72 hours and given in a unit of a millimeter, as suggested by.[15]

Measurements of TLR-2 and IL-10 Level

All the procedures were performed according to the manufacturer's instructions of Elabscience.

Statically Analytics

The values of the parameters were given in terms of mean ± standard deviations (S.D), using the computer programmer SPSS version 7.5 by performing variance analysis (ANOVA), the least significant difference (LSD).

RESULTS AND DISCUSSION

The results of the isolation and identification of *E. faecalis* from UTI reported only 4% of the samples contained *E. faecalis* (five isolates) and only one isolate was confirmed as Vancomycin-resistant *E. faecalis*, (1% of the total sample) which will be used to the extraction of EPSs as Figure 2.

This agreed with the obtained results by Li M, *et al.*,[16] isolated and diagnosed vancomycin-resistant *E. faecalis* from urine samples for urinary tract infections based on the next-generation sequencing, *E. faecalis* considered to be one of the causes of urinary tract infection and quickly became a major cause of urinary tract infections Healthcare-associated (urinary tract infection). Enterococci spp is considered 15% of all urinary tract-related urinary tract infections; catheters are more common in men and are usually associated with frequent urinary tract infections.[17] On the other hand, the results carbohydrates rate in purified EPSs revealed the carbohydrate content was 88% expressed as a percentage of EPS dry weight (w/w%) of by using method phenol-sulfuric-acid-method,[12] while the 12% other components such as uronic acids, Hexominase and acetyl group and Ketal Linked pyruvate group[18]. However TCA was used to denature the protein during the extraction[11], This came in line with[19] reported that the EPSs extraction was long-chain molecules consisting of branched units of sugars or sugar derivatives. Glucose, galactose, and rhamnose appear mainly in different proportions in these sugar units[20], *E. faecalis* is one of the strains that produced heterogeneous exopolysaccharides characterized as consisting of recurrent linear or branched units[21], EPSs displayed Carbohydrates contents higher than other components.

Besides the susceptibility test of isolates was revealed *S. aureus* was sensitive toward tetracycline while *P. mirabilis*, *P. aeruginosa* were sensitive toward Ciprofloxacin. However the current study was revealed the antibacterial activity assay of EPSs toward gram-positive isolates as S. *aureus* and gram-negative as *P. mirabilis*, *P. aeruginosa* showed various responses against those isolates depending on the inhibition zones since *S. aureus* displayed resistance to EPSs in different concentrations which the inhibition zone diameter of concentration in 0.5 mg/mL was (7 mm) and 1 mg/mL was (8mm). Furthermore, the highest inhibition zone was recorded in *P. mirabilis* as in 0.5 mg/mL was (17 mm) and 1 mg/mL was (20 mm), while the antibacterial activity against *P. aeruginosa* revealed the inhibition zone diameter in 0.5 mg/mL was (9 mm) and (12 mm) in 1mg/ml concentration as shown in Figure 3.

These findings were accepted with[22], which revealed the operation of the aqueous EPS extract against *S. epidermi* and *S. typhimurium*. Numerous theories set due to the antibacterial activity mechanism of EPS, the EPS worked to weaken, decrease cell division, even disrupt cell wall and cytoplasmic membranes, and DNA analysis[23]. Moreover, the other aspect (in vivio) of the present study was evaluation of the influence of EPS particularly on the immune system (innate immunity and adaptive immunity). the EPSs showed positive effects on the immune response generally (innate immunity and adaptive immunity), the assays of determination of influence EPSs on innate immunity were included measurement TLR-2 and phagocytic activity index. The results of the effect EPSs on

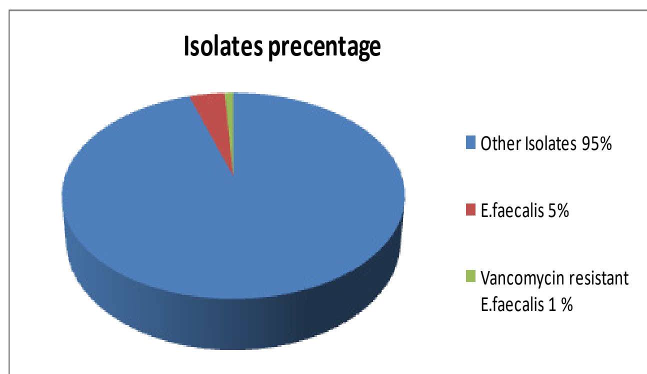

Figure 2: *E. faecalis* percentage in UTI samples

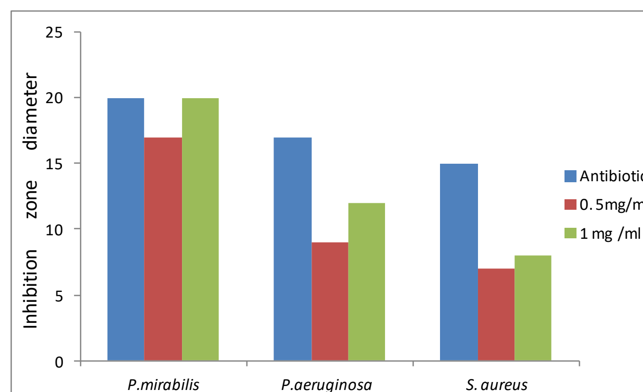

Figure 3: Inhibition zones diameter (mm) of Antibacterial Activity of EPSs

TLR-2 level showed the groups that injected in EPSs (different concentration) combination with *S. aureus* was higher than other groups but the highest value in the group that injected with 1 mg/mL (8.733 ± 2.13) while the group that injection with 0.5 mg/mL (6.824 ± 0.88) with a significant difference at ($p \leq 0.05$) while the other group showed various values as shown in Table 1.

These findings were in line with several studies using EPS from various probiotic strains,[24] which identified the EPSP from *L.Casei* in their study induced higher expression of TLR-1 and TLR-2 and modulated the pro-inflammatory and anti-inflammatory cytokine expression profile in the liver fish cells. The common pathway of innate immune cells triggered by *S.aureus* was identified by TLR-2, which identifies. Lipoproteins derived from *S. aureus*[25]. EPS was increased TLR-2 expression on the cells attributed to the living probiotics and their products, such as EPSs were promoted TLR-2 mRNA transcription, while some studies showed the heat-killed probiotics were enhanced transcription of TLR-2, TLR-3, TLR-4, and TLR-9 in the murine macrophage.[26] TLR-2 have roles in attractive the phagocytic cell to the infection sites signaling[27]and this was noticed in results of the phagocytic activity index were showed the mice of the group had a combination injection by S.*aureus* and EPSs recorded high values with significant differences at ($p \leq 0.05$) comparison with another studied group in both concentration, but the highest value recorded in the group that received combination injection of 1 mg/mL of EPS and *S. aureus* (24.2%) followed by the group that injected only with 1 mg/mL EPSs (10.4%), while in 0.5 mg/mL concentration, the results also reported the group that received combination injection of 0.5 mg/mL EPSs and *S. aureus* showed higher (22.4%) than the group that received only 0.5mg/mL (8.2%) and *S. aureus* only (4.4%) with significant difference ($p \leq 0.05$) between the groups in both concentrations as in Table 2.

These findings were accepted with data obtained by lei Xiu, *et al.*,[28] using the *L. casei* strain EPSs as an adjuvant and found that proliferation and phagocytic activity were enhanced as well as the development of NO, TNF-α, IL-1β, and IL-6 in macrophages as well as agreed with[29], EPS and selenium EPS increased phagocytosis and increased secretion of nitric oxide, IL-1, IFNγ, IL-6, and IL-12 in peritoneal macrophages, but this action itself allowed macrophages to switch from a pro-inflammatory to an anti-inflammatory phenotype, also referred to as M2 or "alternatively activated" macrophages.[30] However, EPS may play a role in regulating the mRNA level of iNOS macrophages through NF-_B activation and induction TNF, IL-1and 2 IL-6[31].

Moreover the results of measurement IL -10 level in the studied group of this study noticed the groups that injected in both EPSs(different concentrations) and *S. aureus* were given high value than other groups but the highest value in the group that injected with 1 mg/ml and *S. aureus* (78.62 ± 3.16) while the group that injection with 0.5 mg/mL and *S. aureus* (69.94 ± 3.99) with a significant difference at ($p \leq 0.05$). In contrast, the other groups showed various values as shown in Table 3.

This increasing in the IL-10 level was consistent with Mallory L, *et al.*,[32] reported the EPS from *B. subtitles* increased the level of M2 macrophage and the level of IL-10 in mice. Dinić in 33 EPS from *L. paraplantarum* increased IL-10 level by reducing inflammatory mediators, preventing inflammatory cells (macrophages) and enhancement the tissue repair anti-inflammatory molecules such as IL-1 receptor antagonists and IL-10[34], the transition from a pro-inflammatory macrophage-dominant wound to an anti-inflammatory macrophage-dominant environment is essential t to overcome the inflammations

Table 1: TLR-2 level (pg/mL) in studied groups in different concentrations

	Different concentration of EPS (Mean ± S.D)	
Groups	*0.5 mg/mL*	*1 mg/mL*
Control positive	1.62 ± 0.53 c	1.62 ± 0.53 c
Control Negative	1.31 ± 0.70 c	1.31 ± 0.70 c
S. aureus	3.50 ± 0.14 b	3.50 ± 0.14 b
EPS	4.86 ± 0.31 a	5.58 ± 0.92 b
EPS+ *S. aureus*	6.82 ± 0.88 a	8.73 ± 2.13 a

*The different letters denoted that significant differences among the groups at $p \leq 0.05$
*The similar letters denoted that non-significant differences among the groups at $p \leq 0.05$

Table 2: Phagocytic Activity in 0.5 mg/mL and 1mg/mL concentration

	0.5 mg/mL, 1 mg/mL concentration of EPS (Mean ± S.D)			
Groups	*0. 5 mg/mL*	*Phagocytic activity %*	*1 mg/mL*	*Phagocytic activity %*
Positive control	0.47 ± 0.00 c	0 %	0.47 ± 0.00 c	0 %
Negative control	0.45 ± 0.02 c	0 %	0.45 ± 0.02 c	0 %
S.aureus	0.64 ± 0.05 b	4.4 %	0.64 ± 0.05 b	4.4 %
EPS only	0.82 ± 0.06 **b**	8.2%	0.92 ± 0.00 **b**	10.4 %
EPS + *S.aureus*	1.46 ± 0.15 **a**	22.4 %	1.54 ± 0.497 **a**	24.2%

*The different letters denoted that significant differences among the groups at $p \leq 0.05$
*The similar letters denoted that non-significant differences among the groups at $p \leq 0.05$

Table 3: IL-10 level (pg/ml) in studied groups in different concentrations

	Different concentrations of EPS(Mean± S.D)	
Groups	*0.5 mg/mL*	*1 mg/mL*
Control positive	8.80 ±36.85^c	8.80 ± 36.85^c
Control Negative	1.33 ±33.41c	1.33 ± 33.41c
S. aureus	2.43 ± 52.210b	2.43 ± 52.210b
EPS	63.99a ± 0.18	67.25b ± 4.09
EPS + *S. aureus*	69.94a ± 3.99	78.12a ± 3.16

*The different letters denoted that significant differences among the groups at $p \leq 0.05$
*The similar letters denoted that no significant differences among the groups at $p \leq 0.05$

Table 4: Results of Arthus reaction of studied concentrations (Means ±S.D, mm)

Groups	*(0.5 mg/mL)*	*(1 mg/mL)*
Positive control	3.00 ± 0.00 ^c	3.00 ± 0.00 ^c
Negative control	3.00 ± 0.00 ^c	3.00 ± 0.00 ^c
S. aureus only	4.15 ± 0.00 ^a	4.15 ± 0.00 ^a
EPS only	3.25 ± 0.25 ^b	3.5 ± 0. 00 ^b
EPS and *S. aureus*	4.20 ± 0.00 ^a	4.26 ± 0.05^a

*The different letters denoted that significant differences among the groups at $p \leq 0.05$
*The similar letters denoted that no significant differences among the groups at $p \leq 0.05$

Table 5: Results of DHT in 0.5 mg/mL concentration after 24, 48, 72 hours (Mean ± S.D, mm) studied groups.

Groups	*24 hr*	*48 hr*	*72 hr*
Positive control	3.00 ± 0.00 ^c	3.00 ± 0.00 ^c	3.00 ± 0.00 ^c
Negative control	3.00 ± 0.00 ^c	3.00 ± 0.00 ^c	3.00 ± 0.00 ^c
S. aureus only	4.24 ± 0.00 ^a	3.56 ± 0.50 ^b	3.53 ± 0.50 ^b
EPS only	3.50 ± 0.50 ^b	3.52 ± 0.50 ^b	3.55 ± 0.50 ^b
EPS and *S. aureus*	3.50 ± 0.50^b	4.24 ± 0.00 ^a	4.14± 0.00 ^a

*The different letters denoted that significant differences among the groups at $p \leq 0.05$
*The similar letters denoted that no significant differences among the groups at $p \leq 0.05$

and prepare the wound for successful healing.[35] EPS has been influenced to induce macrophages to switch from a pro-inflammatory phenotype to an anti-inflammatory phenotype.[29]

Furthermore, the effect of EPSs toward adaptive immunity in the current study was represented by Humoral immunity (Arthus reaction) and by for cell-mediated immunity (DHT). Anyway, the results of Arthus reaction and DHT displayed the same results; the group with a combination injection by EPS and *S. aureus* showed higher value than the other group, while in the Arthus test both concentrations recorded same value in the group injected with EPSs and *S. aureus* which was (4.26 ± 0.05) 1 mg/mL and (44.20 ± 0.00) at 0.5 mg/mL, as shown in Table 4.

The beneficial effect of EPS on humoral immunity was agreed with28 the subcutaneous administration of EPS significantly promoted both humoral and cellular immune responses by enhancing serum antibodies and T cell proliferation, enhancing cytokine expression, and enhancing DC maturation. EPS could significantly increase the titers of antibodies and significantly enhanced T cell proliferation. Mayyada[36] was revealed EPSs could increase the expression of both IL-4 and INF-π in CD4+T cells affecting TH1 and TH 2 cells promoting both humoral immunity and cell-mediated immunity from infection.

These results were confirmed by the DHT, the group that had a combination injection with EPS and *S. aureus* expressed higher value than the other group, but the highest value recorded in 0.5 mg/mL concentration with significant differences after 24, 48 was (3.50 ± 0.50, 4.24 ± 0.50) respectively as in Table 5, while at 1 mg/mL after 24 hours was (4.00 ± 0.00) after 48 hours was (4.10 ± 0.00) and less value was after 72 hours (3.50 ± 0.50) $p \leq 0.05$ as shown in Table 6.

The obtained results agreed with Rubab I, *et al*.,[37] by using extracted EPS from *C. freundii* to induce an immune response against bacteria *(C.freundii*) explained enhancement of cellular immunity, which characters may be swelling, redness due to the infiltration of macrophage, neutrophil and lymphocyte at the site of inflammation that recognizes antigen and secreteing IL-1 that enhanced proliferation and differentiation of other T-cell into Th cells. In turn ,it will produce IL-2 to attract macrophages and INF-γ led enhancing the cytolytic activity of accumulated macrophages leading to skin thickness. The role of EPS in DHT was suggested EPSs may increasing expression both of I-4, INF-γ in CD4+T cells that effect on TH1 and TH2 cells that support both of humoral immunity and cell-mediated immunity.[36] The reduction in thickness of the footpad after 72 hours in the group that received 1 mg/ml EPSs and *S. aureus* have attributed the EPS helped the macrophages to transition

Table 6: Results of DHT in 1 mg/ml concentration after 24hr, 48hr, 72 hr (Mean ± S.D, mm) in studied groups.

Groups	*24hr*	*48hr*	*72 hr*
Positive control	3.00 ± 0.00 [a]	3.00 ± 0.00 [a]	3.00 ± 0.00 [c]
Negative control	3.00 ± 0.00 [a]	3.00 ± 0.00 [a]	3.00 ± 0.00 [c]
S. aureus only	4.24 ± 0.00 [a]	3.50 ± 0.50 [b]	3.50 ± 0.50 [b]
EPS only	4.20 ± 0.00 [a]	3.00 ± 0.00 [b]	3.14 ± 0.00 [c]
EPS and *S. aureus*	4.00 ± 0.00 [a]	4.10 ± 0.00 [a]	3.50 ± 0.50 [b]

*The different letters denoted that significant differences among the groups at $p \leq 0.05$
*The similar letters denoted that no significant differences among the groups at $p \leq 0.05$

from a pro-inflammatory phenotype to an anti-inflammatory phenotype, which in turn will reduce the inflammation and produce anti-inflammatory mediators as IL-1033, the EPS in this study helped to regulate the inflammatory response.

CONCLUSION

Depending on obtained results in this present study, the exopolysaccharides (EPSs) produced by Vancomycin-Resistant *E.faecalis* were showed antibacterial activity against gram-positive and negative bacteria in vitro. While in vivo experiments EPSs worked positively on the immunity system both innate and adaptive, EPSs were helped to regulate the inflammatory response by increasing IL 10 level and decreasing in DTH test after 72 hours.

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REFERENCES

1. Perricone M, Arace E, Calò G, Sinigaglia M. Starter Cultures in Food Production, , *Ethnic fermented foods*. 2017; 384–406.
2. Olfa Ben Brek and Slim Smaoui.Enterococci: Between Emerging Pathogens and Potential Probiotics , *BioMed Research International*. 2019:1-13.
3. Huycke M, Spiegel CA, Gilmore M S. Bacteremia caused by hemolytic, high-level gentamicin-resistant Enterococcus faecalis *, Antimicrobial Agents and Chemotherapy.* 1991; 35(8):1626–1634.
4. Gilmore MS, Lebreton F, van Schaik W, Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. *,Curr. Opin. Microbiol*. 2013 ;16:10–16.
5. Lee NK, Lee S K, Paik HD. Multifunctional effect of probiotic Lactococcus lactis KC24 isolated from kimchi, LWT-Food Sci. Technol. 2015 ; 64: 1036-1041.
6. Amy E, Foxx-Orenstein and William Chey. Manipulation of the Gut Microbiota as a Novel Treatment Strategy for Gastrointestinal Disorders *,Am J Gastroenterol Suppl.* 2012;1:41– 46;
7. Huang C H, Shen C C, Liang Y C, & Jan, T. R. The probiotic activity of Lactobacillus murinus against food allergy. *Journal of Functional Foods*. 2016; *25:*231-241.
8. Han K J, Lee NK, Park H, Paik HD. Anticancer and Anti-Inflammatory Activity of Probiotic Lactococcus lactis NK34. *J Microbiol Biotechnol* . 2015 ;25(10): 1697-1701.
9. Shimada T, Cheng L, Shi HB. Effect of lysed Enterococcus faecalis FK-23 on allergen-induced immune responses and intestinal microflora in antibiotic-treated weaning mice. *J Investig Allergol Clin Immunol* . 2007;(17):70–6.
10. W Li, X. Xia, W. Tang. Structural Characterization and Anticancer Activity of Cell-Bound Exopolysaccharide from Lactobacillus helveticus MB2-1. *Journal of Agricultural and Food Chemistry* . 2015 ; 63 (13): 3454–3463.
11. Bajpai V K, R Majumder, Rather, K Kim. Extraction, Isolation and Purification of Exopolysaccharide from Lactic Acid Bacteria Using Ethanol Precipitation Method". *Bangladesh Journal of Pharmacology*. 2016 ;11:(3): 573-6.
12. Dubois M, Gilles K.A, Hamilton J.K, Rebers P.A, Smith F. Colorimetric method for determination of sugars and related substances.Anal Chem. 1956 ;28: 350-356.
13. Tetz G V, Artemenko N K., Tetz, V V. Effect of DNase and antibiotics on biofilmcharacteristics. *Antimicrob. Agents Chemother* . 2009 ;53(3) :1204–1209.
14. Zakaria,Z. A. ofiee,M. S. Teh L. K. Salleh, M. Z., Sulaiman, M. R. Somchi, M. N. *Bauhinia purpurea* leaves' extracts exhibited *in vitro* antiproliferative and antioxidant activities . *African Journal of Biotechnology* . 2011; 10(1) :65-74.
15. Tansho S A, Mizutani S, Yasuo S, Kazutoh O T and Hideyo Y. Protection of mice from lethal endogenous Candida albicans infection by immunization with Candida membrane antigen, Microbiol. mmunol . 2002; 46: 307-311.
16. Li M, Yang F, Lu Y.Identification of *Enterococcus faecalis* in a patient with urinary-tract infection based on metagenomic next-generation sequencing: a case report. BMC Infect Dis . 2020 ;20: 467.
17. Arias CA, Contreras GA, Murray BE. Management of multidrug-resistant enterococcal infections. *Clin Microbiol Infect*. 2010; 16(6) :555–562.
18. Hanan M, Mubarak , Saida M. Amer .Purification And Characterization Of Exopolysaccharides (Eps) Extracted From Saccharomyces Cerevisiae Egypt. J. Exp. Biol. 2013;9(2): 249–258.
19. Dilna SV, Surya H, Aswathy RG, Varsha KK, Sakthikumar DN. Characterization of an exopolysaccharide with potential healthbenefitproperties from a probiotic Lactobacillus plantarum RJF4. *LWTFood Sci Technol*. 2015 ;64:1179-1186.
20. Welman AD, Maddox IS. Fermantation performance of an exopolysaccharide-producing strain of Lactobacillus delbruckii subsp. Bulgaricus. *J Ind Microbiol Biotechnol*. (2003;30:661-668.
21. P. Ruas-Madiedo J, Hugenholtz, P. Zoon. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Dairy Journal*. 2002;12:163–171.
22. Majolagbe O N, Oloke J K., Adebayo E A., Adewoyin A G, Ayandele A, Bamigboye C O. Study on the Antibacterial Activity of Exopolysaccharides of Lentinus subnudus Using Swiss Albino Rats as Animal Model.American-Eurasian .*Journal of Scientific Research* . 2013 ;8 (1): 47-52.
23. Wu M H, Pan Y, Wu S, Chang M, Chang C. Exopolysaccharide activities from probiotic bifidobacterium: Im-munomodulatory

effects (on J774A.1 macrophages) and antimi-crobial properties. *Int. J. Food Microbiol*. 2010;(144): 104–110.

24. Qin C, Zhang Z, Wang Y, et al. EPSP of *L. casei* BL23 Protected against the Infection Caused by *Aeromonas veronii* via Enhancement of Immune Response in Zebrafish. *Front Microbiol*. 2017; 8:2406.
25. Hashimoto M, Tawaratsumida K, Kariya H, Aoyama K, Tamura T, Suda Y. Lipoprotein is a predominant Toll-like receptor 2 ligand in *Staphylococcus aureus* cell wall components. *Int Immunol* . 2006 ;1(18) :355–362.
26. Wang, Y., Xie, J., Wang, N., Li, Y., Sun, X., Zhang, Y. and Zhang, H. *Lactobacillus casei* Zhang modulate cytokine and Toll-like receptor expression and beneficially regulate poly I:C-induced immune responses in RAW264.7 macrophages. *Microbiol Immunol* .2013; 57: 54– 62.
27. Kaisho T, Akira S. Pleiotropic function of Toll-like receptors. *Microbes Infect*. (2004); 2 (6) :1388–1394.
28. lei Xiu, Haochi Zhang, Zhongpeng Hu, Yanchen Liang, Shuai Guo, Ming Yang, Ruiping Du & Xiao Wang .Immunostimulatory activity of exopolysaccharides from probiotic *Lactobacillus casei* WXD030 strain as a novel adjuvant *in vitro* and *in vivo*. *Food and Agricultural Immunology.* 2018 ;29:110;86-1105.
29. Daodong Pan, Jia Liu, Xiaoqun Zeng, Lu Liu, Hua Li & Yuxing Guo. Immunomodulatory activity of selenium exopolysaccharide produced by *Lactococcus lactis* subsp. *Lactis*. *Food and Agricultural Immunology*. 2015; 26(2) :248-259.
30. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest. 1998; 101(4):890–8.
31. Laiño J, Villena J, Kanmani P, Kitazawa H. Immunoregulatory Effects Triggered by Lactic Acid Bacteria Exopolysaccharides: New Insights into Molecular Interactions with Host Cells. Microorganisms. 2016; 4(3): 27.
32. Mallory L. Paynich, Sara E. Jones-Burrage and Katherine L. KnightExopolysaccharide from Bacillus subtilis Induces Anti-Inflammatory M2 Macrophages That Prevent T Cell–Mediated Disease. J Immunol. 2017 ;198 (7): 2689-2698.
33. Dinić M, Pecikoza U, Djokić J, Stepanović-Petrović R, Milenković M, Stevanović M, Filipović N, Begović J, Golić N, Lukić J. Exopolysaccharide Produced by Probiotic Strain *Lactobacillus paraplantarum* BGCG11 Reduces Inflammatory Hyperalgesia in Rats . *Front Pharmacol*. 2018; 9:1.
34. Brancato SK, Albina JE. Wound macrophages as key regulators ofrepair: origin, phenotype, and function. Am J Pathol. 2011;178(1) :19–25.
35. Mosser DM, Edwards JP. Exploring the full spectrum of macrophageactivation. Nat Rev Immunol. 2008; 8(12): 958–69.
36. Darweesh ,M,Exopolysaccharide vaccine extracted from C.freundii induce immunity .*Al-Kufa University Journal for Biology*. 2017 ;9 (2): 150-160.
37. Rubab I, Ali S. Dried fruit extract of Terminalia chebula modulates the immune response in mice. *Food and Agricultural Immunology*. (2016);27(1):1–22.