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Effect of partially purified capsular polysaccharide of *Escherichia coli* on phagocytosis and IgG levels

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ABSTRACT

Escherichia coli is considered as a source for various invasive and noninvasive diseases especially in the children of age groups two to eight. *E coli* pathogenicity is related to the presence of capsular polysaccharide. The large structure of polysaccharide is covered bacterial cell surface and act as protective barrier, facilitates adhesion, and increase bacterial virulence by inhibiting specific and non-specific host immune responses. In this paper we evaluated the effect of partial purified polysaccharide layer which has been purified from *Escherichia coli* strain on phagocyte and IgG levels and use it as adjuvant. Five strains of *E. coli* were collected from different clinical sources (urine, wound and burn). Then, the antibacterial activity of several common antibiotics toward all isolates was detected by Kirby-Bauer method. For further in vivo evaluation, treated mice were immunized by injection subcutaneously (S/C) with 50 µg/ml of partial purified capsule while control group were injected with normal saline. The results of antibiotic susceptability test showed that all isolates resistant in 100% to Ampicillin, Ceftriaxone, Amikacin, Cefepime whereas showed variable resistance to Imipenem. The results of immunization showed increased in the number of phagocyte (29), lymphocyte (21), neutrophil (10), eosinophil (1), monocyte (1), while basophil cells showed no change in their level in comparison with other cells, In addition, the concentration of IgG (Ig) antibody in the serum of treated mice was (15.1) mg/ml, while the concentration in the control group was (4.1) mg/ml. Finally, the results of challenge were shown all treated mice were still live while untreated mice (control) were dead.

Keywords: *Escherichia coli*, adjuvant, phagocytosis, antibody.

1. INTRODUCTION

The ability of bacteria to overcome bactericidal action of serum and passing the innate defenses mechanisms is related to produce of protective extracellular polysaccharide capsule [1]. Extra intestinal pathogenic *Escherichia coli* (ExPEC) strains are clinically considered as a significant group of pathogens which cause many infections in human including urinary tract infections, abdominal infections, nosocomial pneumonia, neonatal meningitis and sepsis. These strains are covered with a layer of polysaccharides that can be distinguished serologically as capsular Kl antigen [2].

Capsular polysaccharide antigens are varied in structure and many studies documented about 80 different serotypes which play a special role in enhancing the virulence of a particular gram-negative bacteria [3]. Moreover, the cell surface K1antigen, which identical in *E coli* and group B meningococci, is a linear homopolymer of α-2-8-linked N-acetylneuraminicacid (NeuNAc) and considered poorly immunogenic because NeuNAc residues are found in gangliosides and cell membrane glycoproteins [4].

K1 capsule of *E coli* bacteria is considered as a virulence factor which inhibit the ability of the host to phagocytose K1-positive *E. coli* because the capsular layer is covered opsonins -binding sites that lead to diminish encounters with neutrophils thereby maximizing its chances for survival [5]. Hence, In this paper we evaluated the effect of partial purified

capsular polysaccharide from *Escherichia coli* isolate on phagocytes and IgG levels and use it as adjuvant.

2. MATERIALS AND METHODS

2.1 Bacterial strains and antimicrobial susceptibility test

E. coli strains (n= 5) were isolated from different clinical specimens(urine, wound and burn), supplied locally by University of Baghdad/ College of Science/Biotechnology Department ,and the identification of these isolates were confirmed by cultural, microscopic and biochemical tests. Then, antibiotic susceptibility test were performed by Kirby Bauer disc diffusion method with the following antibiotics discs:(Cefepime (30µg), Amikacin (30µg), Imipenem (10µg), Ampicillin (30µg), Ceftriaxone (30µg)) .The results of susceptibility test were interpreted according to the Clinical and Laboratory Standards Institute guideline[6].

2.2 Extraction and partial purification of capsular polysaccharides

CPS was extracted from *E. coli* strain according to the modified method described by [2].In brief, a highly resistant isolate to most antimicrobial agent was selected and activated on brain heart infusion broth, then transferred to petri dishes containing modified Trypticase Soya agar, and incubated at 37ºC for 18 to 24 hr. Growth was harvested by adding 3ml of sterile phosphate buffer saline (0.01M)(PH=7.2) and washed 2 to 3 times with PBS, then the cells were separated by centrifugation for 15 min at 10,000 rpm. After that, the pellet was suspended with 5 ml of distilled water and mixed well by magnetic stirrer, then cells were lysed with cetavlon (0.125 gm)with stirring for 30 min at room temperature. The mixture was centrifuged for 30 min at 10,000 rpm. Thereafter, the materials were dissolved in CaCl2 (1M) and ethanol was added to a concentration of 25%. The mixture was then mixed with magnetic stirrer for 60 min at room temperature. These materials were centrifuged for 30mins at 10,000 rpm and the supernatant was collected. Ethanol (80%) at four times the volume of the supernatant was added and the capsule was precipitated by centrifugation at 10,000 rpm for 30 min. The capsule precipitate was dissolved in (2ml) distilled water. Chloroform: butanol (5:1) was added with the same amount of distilled water, the previous step was repeated three time. Finally the water phase containing CPS was collected. The partial purification of Lipopolysaccharide and protein in CPS preparations were detoxified according to the method by [7] by adding 2ml of a (0.1N) NaOH solution with 95% ethanol for 30 min at 37ºC.The solution was neutralized by addition of (2N) acetic acid and the supernatant was neglected. The pellet was represented as CPS dissolved in distilled water. The mixture was dialyzed for 24 hrs. against distilled water, then concentrated against sucrose at 4°C, the final volume was reduced to 5 ml. finally ,the concentration of sugar, lipid, protein and Deoxyribonuclease (DNA) were assayed by standard methods.

2.3 Immunization of mice

In this experiment, six healthy white mice (1 kg) were used and divided into two groups, each group contains three mice.

Group 1: control group injected with normal saline.

Group 2: injected with a 50µg/kg of partial purified of CPS (extracted from *E.coli*). Immunization was carried out subcutaneously (S/C) according to the method mentioned by [8] Mice were immunized with a 50µg/kg of adjuvant for seven days. After that, blood samples were collected from mice at one week later (day 7) by cardiac puncture and then transferred into EDTA tubes for determine phagocytosis index . On the other hand, serum was separated from another blood samples by centrifugation at 5000 rpm for 10 mins and stored at -20°C for serological examination by using ELISA test to detect the anti-capsular polysaccharide antibody (IgG),.

2.4 Phagocytosis index

E .coli isolate was reactivated by culturing in nutrient agar and incubated at 37ºC for 24hr. Bacterial colonies were suspended in normal saline and adjusted to1.5X 10^8 CFU/ml (0.5McFarland standards). From immunized mice, 1 ml of blood was pooled from the heart and transmitted to tubes contain EDTA as an anticoagulant, with gently mixed. An aliquot of 20 µl of mice blood were mixed gently with the bacterial suspension of *E. coli* (10^8/ml) in sterile test tube. Then, the mixture was incubated at 37ºC for 1.5 hr. Smear was prepared by taking a drop of the mixture on the cleaned slide; Triplicate slides were made for each tube, slides were air dried, stained with Giemsa stain for 10 min. and then washed by D.W. The slides were examined microscopically to calculate the number of phagocytes engulfed microorganisms [9].

2.5 Differential leukocyte count

The leukocyte differential count is determined the number of each type of white blood cell in the blood samples, according to the following protocol [4].A drop of each tested animal blood is placed on the clean slide to produce a smear which should be dried rapidly. Giemsa stain was poured over the smear for 8-10 minutes then wash off with water and dried. The dry and stained film was examined under (40X). For differential leukocyte counts, an area with the morphology of the cells is clearly visible was chosen.

2.6 Experimental Challenge

After inoculation for 14 days, the mice were scratched dorsal skin with virulent *E. coli* strain at the dose of 0.1 mL, 1.5×10^8 CFU/mL , and then observed for symptoms. Two weeks post-challenge, all the survived mice were anatomized to check the pathological lesions of skin caused by the infection with virulent E. coli [10].

3. RESULTS AND DISCUSSION

The results of antibiotic susceptability test were showed that all strains of *E. coli* were resistant to most antibiotics types except Imipenem. In addition, the results of capsule extraction and purification demonstrated that the concentration of

polysaccharides, protein, lipid and DNA before purification were 44(68.6%), 4.6 (7%), 15(23.4%), 0.5(0.7%) µg/ml respectively, while the concentration after purification were 9(86%), 2.4 (2.2%), 12(11.4%), 0.2(0.19%) µg/ml respectively (Table1).

Table 1: Extraction and partial purification of CPS

	Polysaccharides Con.µg/ml(%)	Proteins µg/ml(%)	Lipids µg/ml(%)	DNA µg/ml(%)
Before purification	44(68.6%)	4.6 (7%)	15(23.4%)	0.5(0.7%)
After purification	90(86%)	2.4(2.2%)	12(11.4%)	0.2(0.19%)

In addition, the effect of partial purified CPS extract on the differential leukocyte count and phagocytosis index was tested, The results were shown an increase in the number of phagocyte (29),lymphocyte(21), neutrophil(10), eosinophil(1), monocyte (1), basophil(0) cells in the treated mice group in comparison with none treated mice (control) which were 5,14,4,0,0,1 respectively. Furthermore, the efficiency of capsule as adjuvant was (47%) for treated mice while for untreated mice (control) was (21%) (Table 2). Moreover, the result of IgG antibody concentration in serum of treated mice group was (15.1) mg/ml, while in control group was (4.1) mg/ml. Finally, the result of challenge revealed that all treated mice were still life while untreated mice (control) were dead.

Table 2: leukocyte count and PI (Efficiency)

Cells	Control cell/field	Immunized cell/field
Phagocytes	5	29
Lymphocyte	14	21
Neutrophil	4	10
Eosinophil	0	1
Monocyte	1	1
Basophil	0	0
PI(efficiency)	21%	47%

The resistance of *E coli* is related to the presence of Extend spectrum β-lactamase ESBLs genes that responsible for confer resistance against β-lactams antibiotic, these genes are located on mobile genetic elements. In addition, some bacterial strains of *E. coli* also contain AmpC gene which confer broad-spectrum resistance to cephalosporin.

However, ESBL-producing Enterobacteriaceae are often resistant to a number of other antibiotic classes like aminoglycoside by several mechanisms that responsible for developing resistance which including (1) changes of outer membrane permeability which is usually cause reduction of the intracellular concentration of aminoglycosides that is considered as non-specific resistance mechanism, (2) the action of aminoglycoside-modifying enzymes which effect on specific sites of the aminoglycosides group that lead to deactivation of antibiotic [11](3) active efflux or drug trapping, (4) Make changes on the 30S ribosomal subunit by mutation, and finally (5) Induce changes on aminoglycoside binding site by methylation[12].

In our study, the extraction and purification of capsular polysaccharides from *E. coli* was achieved by using a simple and efficacy method in which the preparation of adjuvant becomes less tedious and cost effective with high content polysaccharides levels and a very little amount of nucleic acid and protein content in the samples . The findings of the current study are consistent with those obtained by[13] who mentioned that the amount of protein and nucleic acid concentration were much lower than polysaccharides concentration in the final capsular extract of *E. coli*.

Moreover, the CPS antigen extracted from *E. coli* has a positive effect on phagocytosis by increasing the phagocytic index (PI), this result confirmed the previous findings of [14]who claimed that the role of *E. coli* CPS contributes to enhance the phagocytosis by alveolar macrophages in spite of the main role of capsule which is antiphagocytic activity to protect the bacterium from destruction and elimination[15] However, the findings of the current study do not support the previous research as the results showed increasing in number of phagocyte cells, this result may be due to remove some components of capsule during the extraction and purification methods as result of using solvents which have highly effect on capsular structure [16]. However, phagocytosis activity increased due to the elevation in sugar moiety because phagocytes have a receptor to bind with mannose . In addition, the ability of the pathogen to attach to the mannose receptors (MR) on phagocytic cells can lead to increase the production of antibodies [17].

On the other hand, present study showed increasing in IgG levels in treated mice as a result to increase the production of lymphocytes, our above results are consistent with [18] who mentioned that the molecular mechanisms by immunogens, bacterial polysaccharides, can stimulate production of antibody. In addition, different factors effect on the IgG serum levels, such as genetic makeup, immune state and environmental factors.

After many researches that documented the role of antibodies against CPSs in protection against infection, Many attempts were performed to use polysaccharides as immunogens (adjuvant), and in the late 1940s., the first attempts were done [10].

4. CONCLUSION

This study has shown that capsular polysaccharide is responsible for bacterial persistence in the host by overcoming of the complement cascade and increased in resistance of serum sensitive. Moreover, CPS have role in increasing IgG antibody and white blood cells significantly.

5. REFERENCES

1. Moxon, E.R. and Kroll, J.S. (1990). The role of bacterial polysaccharide capsules as virulence factors. Curr Top Microbiol. Immunol. 150:65–85.
2. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. and Smith, F.(1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28(3):350-355.
3. Whitfield, C. and Roberts, I.S. (1999). Structure, assembly and regulation of expression of capsules in Escherichia coli. Mol. Microbiol.;31:1307–1319.
4. Ceelie,H.; Dinkelaar,R. B. and Gelder, W.(2007). Examination of peripheral blood films using automated microscopy; evaluation of Diffmaster Octavia and Cellavision DM96. J. Clin. Pathol. 60(1): 72–79.
5. Roberts, I. S. 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu. Rev. Microbiol. 50:285–315.
6. CLSI (2011). Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement; CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
7. Crouse, J. and Amorese, D.(1987). Ethanol precipitation: Ammonium acetate as an alternative to sodium acetate . Focus.9 (2):3-5.
8. Sikarwar, A. S. and Batra, H.V. (2011).Identification of Klebsiella pneumoniae by capsular polysaccharide polyclonal antibodies. I nter. J. Chem. Eng. and Applic. 2(2):130-134.
9. Furth, R. V.; Theda, L. and Liejilt, P. C.(1985). In vitro determination of phagocytosis and intracellular killing by polymer photonuclear phagocytosis. Hand Book of experimental immunology.. Black well scientific publication, Oxford,:125.
10. Mcleod, C.M.; Hodges, RS.; Heidelberger, M. and Bernhardt, WG. (1945). Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. J Exp Med 82: 445-465.
11. Liu, X.; Hao, Y.; Wang, S.; Zhang, A.; Wang, Y. and Guo, Y. (2013). Assessment of immunogenicity of CP8, CLFA-FnBPB and CP8-CLFA-FnBPBantigens. Am. J. Anim. Vet. Sci., 8: 134-141.
12. Llano-Sotelo, B.; Azucena, EF. Jr.; Kotra ,L.P., Mobashery, S. and Chow, C.S. (2002). Aminoglycosides modified by resistance enzymes display diminished binding to the bacterial ribosomal aminoacyl-tRNA site. ChemBiol 9: 455–463.
13. Mallick, J.(2009).Extraction and purification of capsular polysaccharide from streptococcus pneumoniae and Escherichia coli for conjugate vaccines preparation. Thesis
14. March, C.; Cano, V.; Moranta, D.; Llobet, E.; Pe´rez-Gutie´rrez, C.; Toma, J. M. ; Sua´rez, T.S.; Garmendia, J. and Bengoechea, J.A.(2013). Role of bacterial surface structures on the interaction of Klebsiella pneumoniae with phagocytes. PLoS ONE, 8(2): 1-16
15. Domenico, P.; Salo, R. J.; Cross, A. S. and Cunha, B. A.(1994). Polysaccharide capsule-mediated resistance to opsonophagocytosis in Klebsiella pneumoniae. Infect. Immun. 62 (10): 4495-4499.
16. Shakil, S.; Khan, R.; Zarrilli , R. and Khan, A.U. (2008). Aminoglycosides versus bacteria–a description of the action, resistance mechanism, and nosocomial battleground. J Biomed Sci.;15:5–14.
17. Aderem, A. and Underhill, D. M. (1999). Mechanism of phagocytosis in macrophages. Annu. Rev. Immunol. 17:593-628
18. Snapper, C.M., Rosas, F.R. Kehry, M.R. Mond, J.J and Wetzler, L.M.(1997). Neisserial porins may provide critical second signals to polysaccharide-activated murine B cells for induction of immunoglobulin secretion. Infect Immun 65: 3203-3208.

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