JNROnline Journal
ISSN: 2320-3358 (e)
ISSN: 0972-5547(p)

Journal of Natural Remedies
Vol. 21, No. 5(S2),2020

EXTRACTION AND PURIFICATION OF UROPATHOGENIC *E. COLI* OUTER MEMBRANE VESICLES (OMVS) ISOLATED FROM URINE SPECIMEN PATIENT SUFFERING URINARY TRACT INFECTION (UTI)

PhD Student Mahmood Abed Hamzah[*1]; Prof.Dr. Suzan Saadi Hussain[*2]; Prof. Dr. Ali Hussein Alwan[*3]

[*1,2,3] Department of Biology, College of Science, Al-Mustanisiriyah University, Iraq.

ABSTRACT

Twenty-five samples of urine were collected from patients suffering Urinary Tract Infection (UTI) from hospital in Baghdad, bacterial isolates have been submitted to the cultural, microscopical and Vitek2 system. From our result showed the *E.coli* number 5 is the best bacterial virulence factor for producing OMV and showed by SEM that outer membrane vesicles *E.coli* is spherical particles of 61.08 to 103.2 nm size also we purification the OMV by gel filtration chromatography used of Sephadex G-200 column 1.5×35cm equilibrated by 0.1 M Tris-HCL buffer pH8 and the our result showed only single peak of *E.coli*drived protein, after that we determined the purity degree of *E.coli* outer membrane vesicles protein molecular weight by used electrophoresis with a polyacrylamide gel, and our result showed single band after gel filtration stage was determined that the molecular weight of the distilled *E.coli* membrane protein is recorded 50 kDa.**Conclusion:** Extraction of *E. coli* OMVs by used cold absolute ethanol at 4oC this resulted in rough precipitation isolation. Purified OMV protein by Gel chromatography, the molecular weight of OMVs protein from *E. coli* was 50kDa. So, the concentration of the purified OMVs protein was measured using the Lowry method and found to be equal 2.757 mg/ml while for crude protein equal 7.510 mg /ml.

Keywords: Extraction; purification; Uropathogenic*E. coli*; Outer Membrane Vesicles (OMV); Urinary Tract Infection (UTI).

INTRODUCTION:

Outer membrane vesicles (50 – 250 nm in diameter) are formed by both pathogenic and non-pathogenic bacteria, involved in various functions including intercellular bacterial contact mediation, virulence promotion, uninjured compound removal, and immune response modulation[(1)].

Several bacterial species such as *Escherichia coli*, *Pseudomonas aeruginosa* have been reported to produce OMVs, among other species[(2)].They have lipopolysaccharide (LPS), phospholipid, peptidoglycan, outer membrane protein (OMP), cell walls, protein, nucleic acids (DNA, RNA), ion metabolite, and signaling molecules as a cargo[(3)]. OMVs can to transfer biological molecules to host cells and their development among gram-negative bacteria is intended. Apart from their role in the communication of bacteria, virulence factors are transferred to the host for cargoes to OMVs to boost bacterial survival[(4)]

OMV membrane contains inside phospholipholipids (PL) and outside LPS and PL mixed with membrane proteins in different positions which represent, in large part, the structure of the external membrane. Vesicle lumens may contain a range of periplasm or cytoplasmic compounds such as proteins, RNA / DNA, and peptide glycol (PG) [(5)].

Outer membranes vesicles have properties which permit the transmission of DNA fragments, autolysins, cytotoxins, virulence factors, and various other biomolecules to be mediated by them and their secretion allows bacteria to interact with and within the species and also strengthens their contact with the host. OMVs were recognized for their role in nutrient procurement, stress reactions and toxin transmission, adherence to and virulence factors in avoiding host defense systems among the prominent roles in various pathological and physiological functions [(6)]

MATERIAL AND METHODS:

Samples Collection

Twenty- five (25) urine samples were collected in UTI patients with certain clinical symptoms. These samples have been collected in many Baghdad hospitals, especially in Al-Karama Hospital with two different sexes and ages.andinoculated by a loop to include a repeat of the urine specimens on MacConkey agar and blood agar plates. Bacterial development was examined following inoculation during the night at 37 ° C. [(7)]

Diagnostic laboratory:

Identification of *E. coli* via VITEK2 compact system

To classify the insulated bacteria, the compact device VITEK2 was also used. In MacConkey agar plates, the bacterial isolates were undergrown. Bacterial suspensions were used in 0.45% sterilized NaCl solution identically to the 0.5 MacFarland standard. A densitometer has been used to modify the bacterial suspension turbidity. VITEK 2 ID-GN cards and AST-No cards. The VITEK 2 compact system was loaded by hand with 12 cards and bacterial suspension. A bacterial suspension was sealed and incubated for 6 hours mechanically to fill each test card. The cards were recited every 15 minutes through the kinetic fluorescence measurement. The compact system software VITEK 2 initially analyzed the data and then mechanically reported its impact [(8)].

Method for preparation of outer membrane vesicles (OMV)

Overnight on lysogeny broth, bacterial cells were cultivated with shaking at 37 ° C (150rp.m.) until the OD600 reached 1.5 (1 percent tryptone, 0 percent yeast extract, 1 percent NaCl, 7.0 pH). At 5000rpm twice for 20min, at 4°C, the cultivated cells were pelleted. The supernatant was filtered with a 0.4 5 µm pore filter and then applied supernatant to cold absolute ethanol at 4°C (twice the volume of supernatant), which lasted 24 hours. This resulted in rough precipitation isolation. The precipitation was dissolved in deionized water and dialyses using a 14000D tubular dialysis membrane for 24-48 hours with Spectra / Por molecular pore.At 15000rpm twice for 20min, at 4°C, the precept cells were pelleted by using high speed centrifuge. A filter with a pore size of 0,22µm and the OMV pellet were filtered again into water and held at −80 ° C for further experiments.

gel chromatography purification

The Pharmacia Fine Chemicals Company has prepared Sephadex G-200 as recommended. A quantity of Sephadex G-200 has been suspended, degassed, and wrapped in 0.1 M Tris-HCl buffer pH8 (1.5x80 cm), the gel has been preserved for 72 hours. Then balanced with the same buffer at room temperatures for swelling. Elution was done at a flow rate of 30 ml /hr and the same control buffer was used. At 280 nm, absorbance was measured for each fraction.

Characterization of protein Purified OMV

Molecular weight estimate for OMV protein

Molecular OMVs weight has been measured at sodium decyl sulfate (SDS) in polyacrylamide gel electrophoresis (SDS PAGE), using a solution in (2,2.4.4.1) under [(9&10)].

- 10% gel was prepared by a mixture of 10ml acrylamide-bisacrylamide solution and 7.5ml gel buffer solution with 12.5ml D.W.
- 100µl of ammonium persulfate (Aps) and 20µl (TEMED) were added to the separate gel, gently mixed and poured into the chamber.

- Mix 1.3ml of acrylamide-bisacrylamide solution with 2.5ml of gel-buffer solution and 6.1ml D.W
- Stacking gel was made by mixing the solution.
- The holes were filled and then mixed in 50µl (Aps) and 10µl (TEMED) and filled up easily.
- One ml of sample enzyme with 1 ml of a solution of sample buffer was combined.
- In 1 ml of sample buffer solution, 2 mg of each standard protein was dissolved.
- Samples were heated at 100 ° C for 5min (enzyme solution, uniform protein solution).
- Proteins in a gel is fastened and stained for 4 hours at the same time.
- Excess dye in the gel can be removed with the solution detained
- Proteins on a clear background were identified as blue bands.
- In order to retain the solution, the gel was retained.
- Relative protein band mobility (RM) has been measured. (RM) refers to the movement of a polypeptide form in relation to other protein bands through a gel:

$$RM = \frac{\text{migration distance of protein}}{\text{migration distance of dye front(Bromophenol blue)}}$$

Relatives mobility of a polypeptide band is related to its molecular weight, and a standard curve of mass versus relative mobility can be created using a set of protein standards of known molecular weight from which estimates of apparent weight from unknown protein can be made.

Statistical Analysis

Statistical analyses were performed by the use of IBM SPSS computer program version 21. Differences between the groups were statistically analyzed by ANOVA table. Data are expressed as mean ± standard error (SE). A p value of ≥ 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION:

Isolation and Identification of *Escherichia coli*

Twenty-five urine samples have been taken from several Baghdad hospitals of different two genders and different ages of urinary tract infections (UTI) in patients. Samples of urine have been inoculated by direct streaking methods on MacConkey agar and blood agar plate[7].The isolation of bacteria as a pure colony in MacConkey-agar and the use of gram stain technique and cultural recognition for biochemical tests (Oxidase, catalase, IMVIC) and Vitek2 method was performed on microscopic isolation of Eosin methylene-blue agar. The selective media cultivated bacterial isolates have been identified as such[11].

Proof of identity Using Vitek2 System

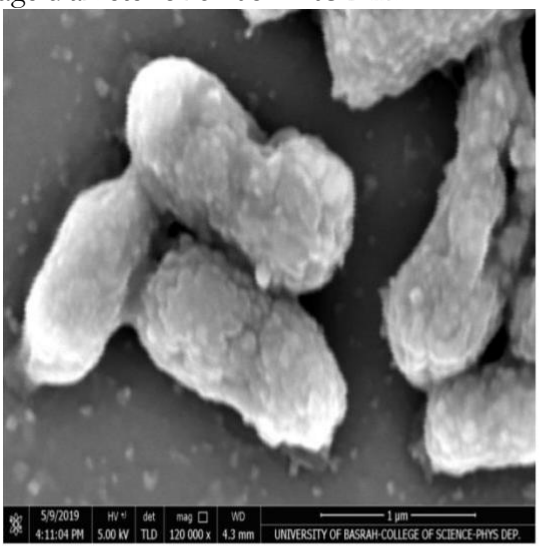
Vitek2 is a promising, highly automated, new method for rapid gram-negative bacteria detection for their species. The findings showed that our 22 isolated out of 20 of them, 90.90% of isolated*E. coli* were the most prevalent in UTI.

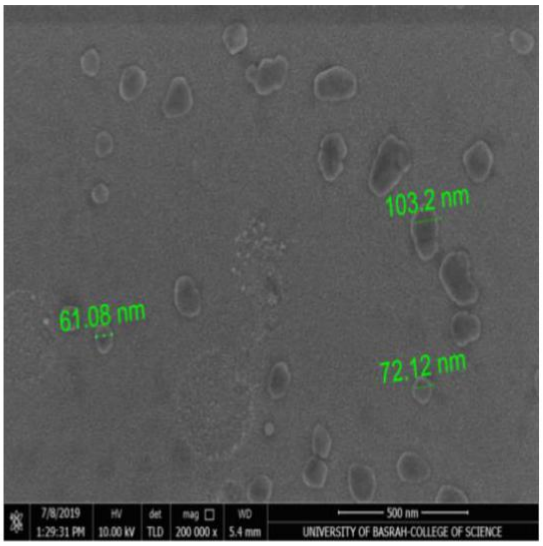
Isolation of outer membrane vesicles) OMVs(

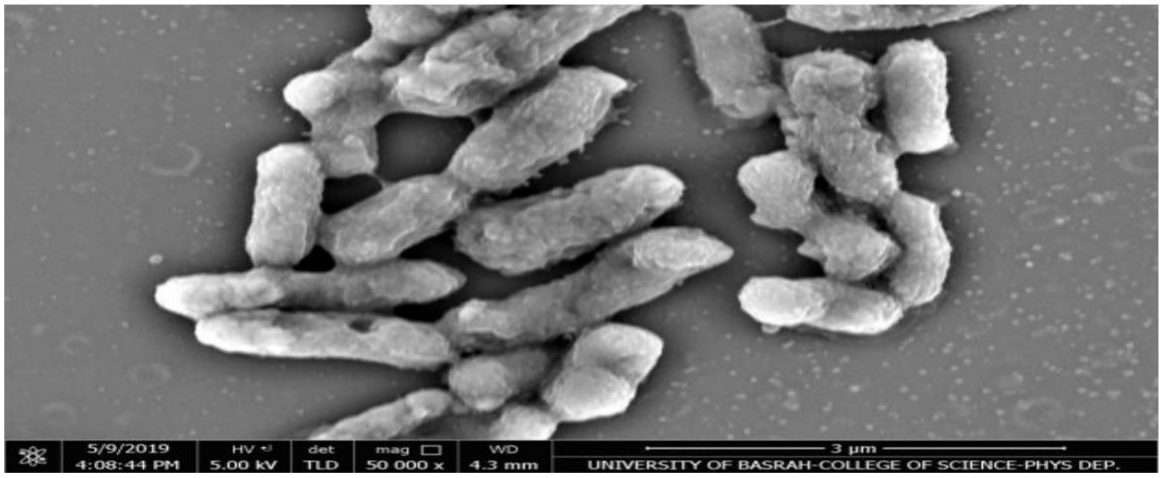
The results of screening of the highest and best bacterial virulence factors isolates for producing OMV showed that the isolate number five 5 is the best for the ability to produce OMV Figure (1 and 2) with an average diameter of 61.08 – 103.2 nm.

Isolation OMV

The results of screening the highest and best bacterial virulence factors isolate for producing OMV showed that isolate number five 5 is the best for the ability to produce OMV Figure (1&2) with an average diameter of 61.08 – 103.2 nm.







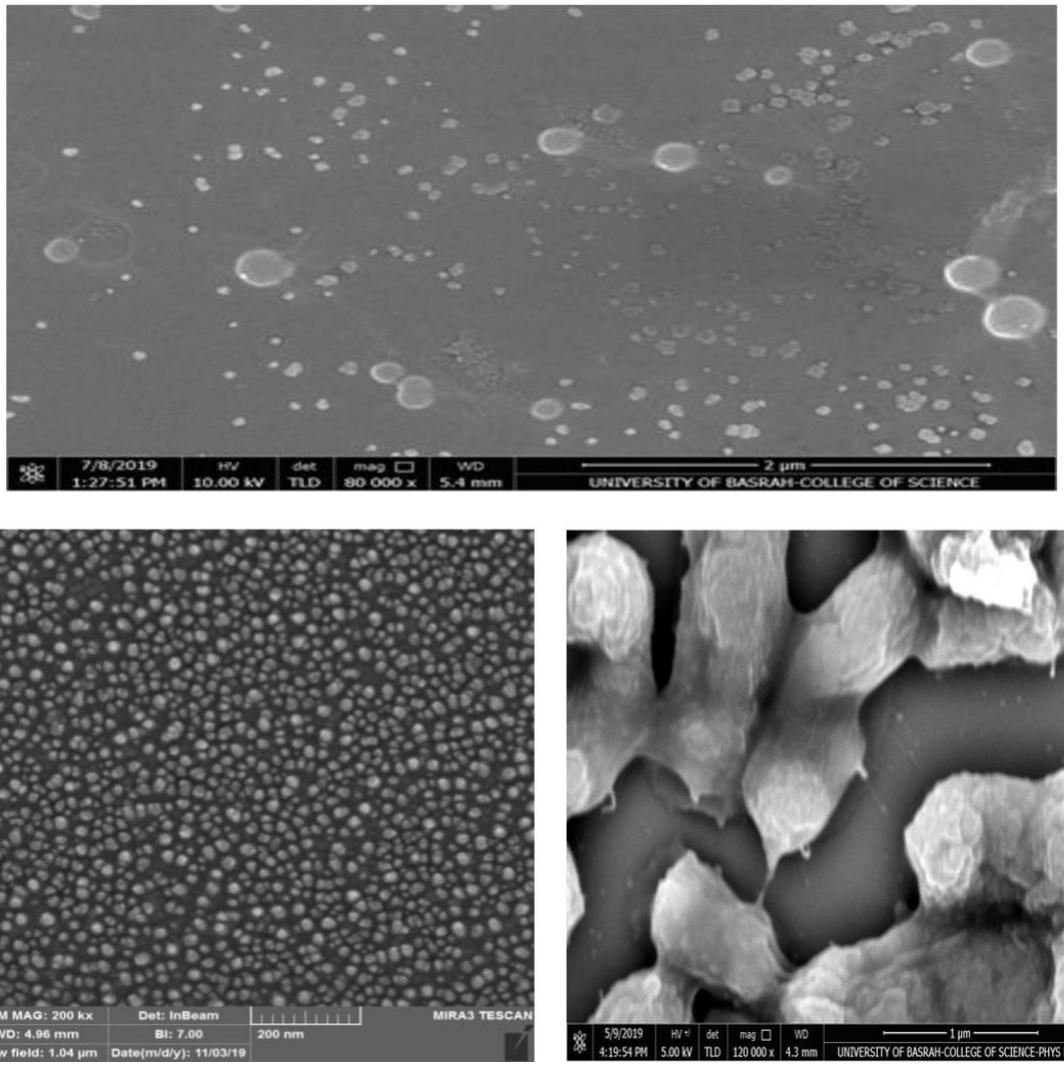


Figure (1): SEM shows the ability of isolate no. 5 to produce outer membrane vesicles (OMV)

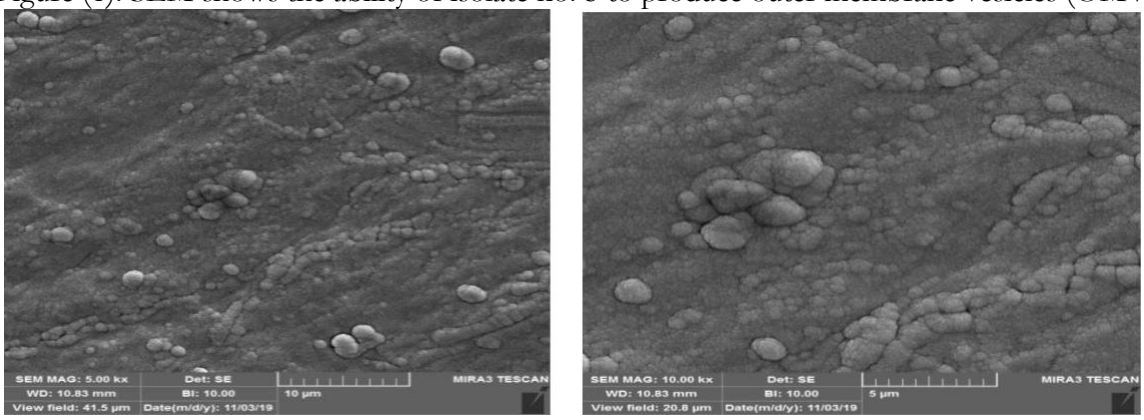


Figure (2): SEM shows the ability of isolate no. 5 to produce outer membrane vesicles(OMVs) by gel filtration chromatography method

Kim*et al* . (2017) [12]have shown both *E. coli* wild type and mutant bacteria-derived OMVs have nano-dimensional lipid-bilayer vesicular structures, both *E.coli* of which showed with an average diameter

of 38.6±3.6 and 38.7±4.2 nm along with the wild-type *E. coli*, OMVs had a higher range of production. OMV *E.coli*, giving an extra advantage of OMVs, as naturally generated OMVs have low productivity issues.

Lee*et al.*,2018[13] have shown that they are isolated *E. coli* OMVs from cell culture supernants by used ultrafiltration and ultracentrifugation methods mix and characterized isolated *E. coli* OMVs with TEM microscopic and dynamic light dispersion electron transmission SDS – PAGE, analysis showed that *E. coli* OMVs contain distinct proteins in comparison to whole cell lysates, and spherically bilayer vesicular structures of diameters ranging from 20 to 100 nm. OMVs have been supplemented with external membrane proteins like OmpA. From the cell culture supernatants, we isolated E. coli OMVs by the combination of ultrafiltration and ultracentrifugation as previously reported [14].

Behrouzi*et al.,* (2018) [15]showed that the extracted OMV was 20–75 nm from the disease strain, whereas the OMV was 45–270 nm from the non-pathogenic strain. Anand and Chaudhuri., (2016) [16]have shown that both pathogenic and nonpathogenic bacteria are made of outer membrane vesicles (OMVs) (50-250 nm in diameter) as the canonical result of secretion.

Purification by gel filtration chromatography

The column of the Sephadex G-200 is split from (5000-600,000) Dalton. It allowed more effective separation capability with a high purification degree. Moreover, a high running, simple preparation, long recovery, and stability, which could be used in protein segments for several times, characterized it by the fact that only one single peak of *E. coli*- derivate protein external membrane vesicles was found following elusion by the Tris-HCl buffer (figure 3).

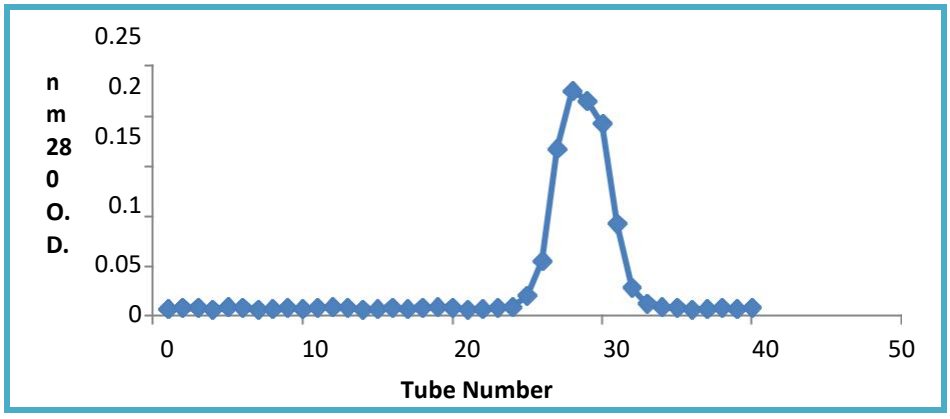


Figure (3):Shows Gel filtration chromatography for isolate no. 5 outer membrane vesicles protein using Sephadex G-200 column (1.5x35cm) equilibrated by 0.1 M Tris-HCL buffer pH8

Determination the Purity Degrees of *E. coli* outer membrane vesicles protein Molecular Weight:

Electrophoresis with a polyacrylamide gel determined the degree of purity of *E. coli* external membrane protein. Protein profile analysis for purified *E.coli* external membrane protein has given the OMV protein purity one band after the gel filtration stage (Fig.4). It was determined that the molecular weight of the distilled *E. coli* membrane protein is 50 kDa.

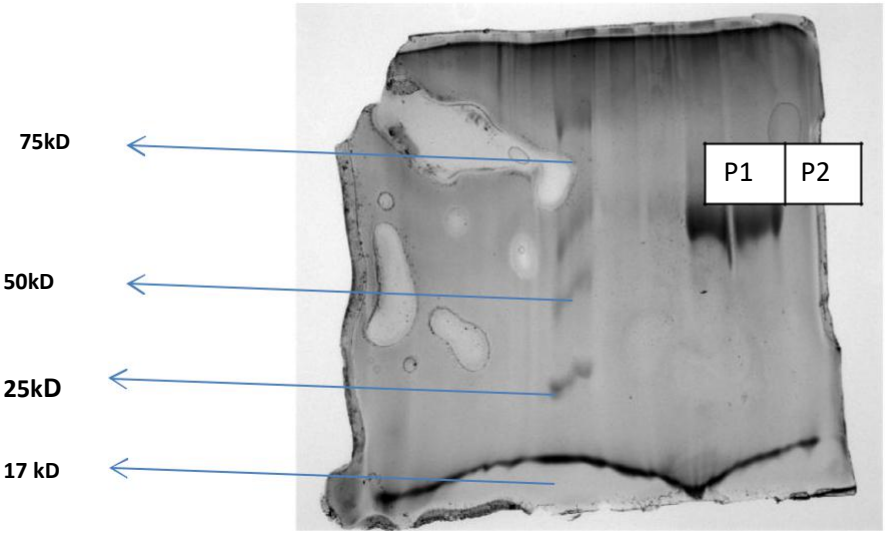


Figure (4): Shows SDS Polyacrylamide gel electrophoresis of purified isolate no. 5outer membrane protein (1): Proteins marker. (2): Protein of isolate no. 5OMVs band after gel filtration step

Premjani*et al*., 2014 [17]showed that an apparent molecular weight was found in OmpT protein. The over-expressing strain of ompT 35 kDa for cell lysates and wild-type OMVs EHEC. The profiles of OMVs type *E. coli* have been shown by [18] a comparison of protein patterns of whole-cell extract and inner membrane and outer membrane proteins with OMV protein patterns and outer membrane protein molecular weight of equal to 35,5 kDa, and which found that 316 proteins have been classified with high confidentiality using *E.coli* Protein sequence database of coli MG1655 the findings indicate that the selective sorting of protein in OMV is a highly successful one.

CONCLUSION:

Escherichia coli is the most common uropathogenic in urinary tract infection in this study. Extraction of *E. coli* OMVs by used cold absolute ethanol at 4oC this resulted in rough precipitation isolation. Purified OMV protein by Gel chromatography, the molecular weight of OMVs protein from *E. coli* was 50kDa. The concentration of the purified OMVs protein was measured using the Lowry method and found to be equal 2.757 mg/ml while for crude protein equal 7.510 mg /ml.

Conflict of interest: none

Source of findings: self-findings.

Ethical clearance: none

REFERENCES

[1] Schwechheimer, C., & Kuehn, M. J. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. Nature reviews microbiology, 2015.13(10), 605.

[2] Elhenawy, W., Bording-Jorgensen, M., Valguarnera, E., Haurat, M. F., Wine, E., & Feldman, M. F. LPS remodeling triggers formation of outer membrane vesicles in Salmonella. MBio, ,2016. 7(4), e00940-16.

[3] Koeppen, K., Hampton, T. H., Jarek, M., Scharfe, M., Gerber, S. A., Mielcarz, D. W. & Stanton, B. A. A novel mechanism of host-pathogen interaction through sRNA in bacterial outer membrane vesicles. PLoS pathogens,2016. 12(6).

[4] Chattopadhyay, M. K., &Jaganandham, M. V. (2015). Vesicles-mediated resistance to antibiotics in bacteria. Front. Microbiol. 6:758.

[5] Bonnington, K. E., & Kuehn, M. J. Protein selection and export via outer membrane vesicles. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research,2014.1843(8), 1612-1619.

[6] Biller, S. J., Schubotz, F., Roggensack, S. E., Thompson, A. W., Summons, R. E., & Chisholm, S. W.Bacterial vesicles in marine ecosystems. science, 2014.343(6167), 183-186.

[7] Forbes , B. A. ; Sahm, D. F. &Waissfled, A. S. Baily and Scotts Diagnostic Microbiology,12th ed. ,2002.Mosby Company , Baltimorc, USA.

[8] Simgamsetty, S., Yarlagadda, P., Yenigalla, B. M., & Myneni, R. B. Ease with VITEK 2 systems, biomerieux in identification of non-lactose fermenting bacteria including their antibiotic drug susceptibility: our experience. Inter. J. Research in Med. Sciences,2016. 4(3), 813-817.

[9] Laemmli, U. K. Cleavage of Structural proteins during the assembly of the head of bacteriophage T4. Nature (London).1970,227:680-685.

[10] Piljac, V., 10Piljac, G., &Bozine, N.Horizontal electrophoresis in genetic engineering centrifugation and electrophoresis Yugoslavia the Tiz. Zriaski, Cakovee.1986.

[11] Forbes , B. A. ; Daniel, F.S; & Alice, S.W. Baily and Scotts Diagnostic Microbiology,11th ed.,2007.Mosby Elsevier Company , USA.

[12] Kim, S., Choi, J. E., Choi, J., Chung, K. H., Park, K., Yi, J., & Ryu, D. Y. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in vitro, 2009. 23(6), 1076-1084.

[13] Lee, J., Yoon, Y. J., 12Kim, J. H., Dinh, N. T. H., Go, G., Tae, S., ... & Di Vizio, D. Outer membrane vesicles derived from Escherichia coli regulate neutrophil migration by induction of endothelial IL-8. Frontiers in microbiology, 2018.9, 2268.

[14] Kim, O. Y., Park, H. T., Dinh, N. T. H., Choi, S. J., Lee, J., Kim, J. H., ... &Gho, Y. S. Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response. Nature communications, ,2017. 8(1), 1-9.

[15] Behrouzi, A., Vaziri, F., Rad, F. R., Amanzadeh, A., Fateh, A., Moshiri, A., &Siadat, S. D. Comparative study of pathogenic and non-pathogenic Escherichia coli outer membrane vesicles and prediction of host-interactions with TLR signaling pathways. *BMC research notes*, 2018.*11*(1), 539.

[16] Anand, D., & Chaudhuri, A. Bacterial outer membrane vesicles: New insights and applications. *Molecular membrane biology*, 2016.*33*(6-8), 125-137.

[17] Premjani, V., Tilley, D., Gruenheid, S., Le Moual, H., &Samis, J. A. Enterohemorrhagic Escherichia coli OmpT regulates outer membrane vesicle biogenesis. FEMS Microbiology Letters, 2014.355(2), 185-192.

[18] Kulkarni, H. M., Nagaraj, R., &Jagannadham, M. V. Protective role of E. coli outer membrane vesicles against antibiotics. *Microbiological research*, 2015.*181*, 1-7.