Extraction of Outer Membrane Proteins of *Proteus Mirabilis* Isolated From Urinary Tract Infections and their Immunological Effect *In Vitro*

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Abstract

300 urine samples were collected from patients visiting Fallujah city hospitals (consulting clinics of Fallujah Teaching Hospital and Women and Children Hospital) for the period from 1/9/2020 to 30/12/2020, of different ages and for both sexes. The results showed that out of 300 urine samples were 244(81.33%) A sample with a positive result for bacterial culture, and it was found there were high significant differences between the positive and negative cultures. Females and males (64.70) (35.29), respectively.

Isolates of *Proteus mirabilis* were diagnosed by observing the cultivar (colonies) and microscopic (bacterial cells) characteristics, biochemical tests and diagnostics with Vitek device. Antibiotic sensitivity were done at 12 types, Antibiotic resistance wre vary from Cefotaxime, Rifampicin, Ceftriaxon (100%), Cefixime, Ciprofloxacin (76.4%), Levofloxacin (64.7%), Nitrofurantoin (58.8%), Gentamicin (52.9%), Amikacin, Nalidixic acid (35.2%) (17.6%) Imipenem (11.7%).

Extraction and partial purification of outer membrane proteins (OMPs) from the most antibiotic-resistant isolate using lysozyme, DNase, RNase, and N-Lauroyl-Sarcosinate enzymes. associated with the ion exchanger.

To study the effect of the antigen of outer membrane proteins, some immunoassays were performed *in vitro* and the following results appeared: The use of concentrations (100, 50, 25) of OMPs antigen led to low significant of lymphocyte viability percentage (96.66, 97.66,98.0), respectively compared to With the negative control (99.66), the concentrations (100,50)% significantly reduced the percentage of survival of PMNs cells (97.66,98.33), respectively, compared with the control (99.66), while the concentration of 25 did not affect the survival of PMNs cells (99.66). The above concentrations showed a low significant in the diameter of the migration circuit of PMNs cells (8.830,10.59,12.21) compared with the negative control (15.21), while these concentrations led to a high significant in the percentage of formazone-forming PMNs cells (64.66,58.0,47.0) compared with the control (36.66).) and a high significant in the sensitivity of lymphocytes (4.0,3.39,1.87), as the absorption spectrum values for concentrations (100,50,25) reached (0.92,0.74,0.41), respectively, compared with the negative control (0.22) and the positive control (1.53). The concentrations of the used proteins increased the phagocytic index (PI) of PMN cells ,The phagocytosis coefficient was high significantly with time until it reached its maximum at 90 minutes, and from the statistical analysis it was found that the phagocytosis coefficient increases with increasing concentration of proteins.

Keywords: Proteus mirabilis, urinary tract infections

Introduction

Proteus spp. Part of the natural flora in the intestines of humans and animals and spreads in water and soil as a result of pollution [1]. They are Gram-negative bacilli and part of the family Enterobacteriaceae, It is moved by flagella and is characterized by the phenomenon of swarming. There are more than one species belonging to this genus, the most important of which is *P. mirabilis*, which is a common cause of urinary tract infections. These bacteria possess many virulence factors, fimbriae, motility, enzymes such as urease, toxins such as hemolysin, and factors of avoidance or immune evasion, and they are among the opportunistic pathogens responsible for many infections and nosocomial infections, especially in immunocompromised patients [2].

The outer membrane (OM) is found in gram-negative bacteria and is composed of an asymmetric bilayer, with phospholipids inward and lipopolysaccharide (LPS) outward. This asymmetry is important to maintain a tight permeable barrier. Almost half of the outer membrane mass is protein. It consists of integral OMPs and inwardly anchored lipoproteins [3]. Outer membrane proteins perform essential functions such as adhesion and nutrient uptake, are essential for maintaining the integrity and permeability of bacterial membranes, play an important role in bacterial virulence, and are powerful immune components. They are highly immunogenic and therefore would be an effective materials for vaccine development [4]. Activation of cellular and humoral immunity depends on the nature of antigens, and complex proteins such as bacterial outer membrane proteins can successfully activate both cellular and humoral immunity [5].

Materials and Methods

Sample collection and culture

300 urine samples were collected from clinical cases during the period from 1/9/2020 to 30/12/2020 from patients with urinary tract infection or suspected cases, according to the diagnosis of the specialist doctor from Fallujah General Hospital, Women's Hospital and private laboratories for different age groups and for both sexes. Collection of samples The first drops of urine are neglected and the mid -stream urine of it is taken in special sterile tubes, then the urine samples were transferred to the laboratory for the purpose of culture and diagnosis, t was cultured on petri dishes containing MacConkey medium as well as solid blood agar medium and incubated at 37 °C for a period of 18-24 hours. Diagnosis of bacteria as well as the using of the Vitek 2 compact device for the purpose of confirming the diagnosis of genus and species .

Antibiotic sensitivity test

The sensitivity of the isolates was tested by the disc method using twelve antibiotics including (Amikacin, Cefixim, Levofloxacin, Ciprofloxacin, Imipenem, Nitrofurantoin, Cefotaxime, Ceftriaxone, Norfloxacin, Gentamicin, Nalidixic acid, Rifampicin) according to [6].

Extraction and purification of outer membrane proteins

The method was followed by [7] in extracting outer membrane proteins (OMP), which includes the use of enzymes DNase, RNase, Lysozyme and N-Lauryl sarcosinate, and the cells were broken down using an sonicator, after which several centrifugation steps were performed, and the protein solution was concentrated Using polyethylene glycol, then membrane sorting was carried out against the same solution, then membrane filtration of the protein solution was carried out using membrane filters with holes with a diameter of 0.22 μm, and then proteins were purified by ion exchange chromatography, DEAE-Cellulose column was prepared according to

[8], the proteins that were extracted in the previous step were added to a DEAE-cellulose column that was titrated using Tris-HCl buffer 0.05M, then the column was washed with an equal volume of the same buffer, and the proteins were gradually filtered using gradual concentrations of sodium chloride(0.9-0.7-0.5-0.3-0.1) NaCl, the flow rate in the column was 4 ml/part and the absorbance of each fraction was measured at 280 nm, the protein concentration was estimated according to Bradford method [9].

Immunological experiments

The method of [10] was adopted to isolate PMNs from blood and method of [11] to isolate lymphocytes from blood, and the method of [12] was used to study the effect of outer membrane proteins on nitro blue tetrazolium reduction test (NBT test). In order to study the effect of outer membrane proteins on the viability of lymphocytes and the viability and migration of PMNs under Agarose the method of [13] and method of [10] was adopted to study the effect of outer membrane proteins onThe external phagocytosis of the heat-killed Candida yeast. The method of [14] was also based on the effect of outer membrane proteins (OMPs) on lymphocyte transformation assay.

Results and Discussion

17 *Proteus mirabilis* isolates were identified from among the 300 urine samples. The isolates were diagnosed by their culture characteristics, as they were characterized by an odor resembling the smell of rotting fish, and they grew on MacConky agar medium. Blood Agar in order to observe the diagnostic character of bacterial colony, which is the phenomenon of swarming phenomena, as diagnosed based on the results of biochemical tests. All of these isolates were positive for urease, catalase, methyl red, citrate consumption and H2S gas production, and negative for the oxidase, indole and vogas – proskaur tests as shown in Table(1)

Table (1): Results of Biochemical Tests for Diagnosing *Proteus* spp.

Biochemical tests	Results
Catalase	+
Oxidase	–
Urease	+
TSI	Acid butt/Alkaline slant H2S +
Indole	–
Methyl red	+
Vogas – Proskaur	–
Citrate utilization test	+

The Vitek2-Compact System was used to confirm the diagnosis of bacterial isolates isolated from clinical samples (urinary tract infections patients). This device performs 64 biochemical tests necessary for diagnosing bacterial isolates.

Antibiotic sensitivity test

An antibiotic sensitivity test was conducted for 17 isolates of *P. mirabilis* bacteria that were isolated from UTI patients, 12 types of antibiotics were used. The results in the in figure(1), which shows the percentage of resistance, sensitive and intermediate sensitivity by Measure the zone of inhibition around each disc used.

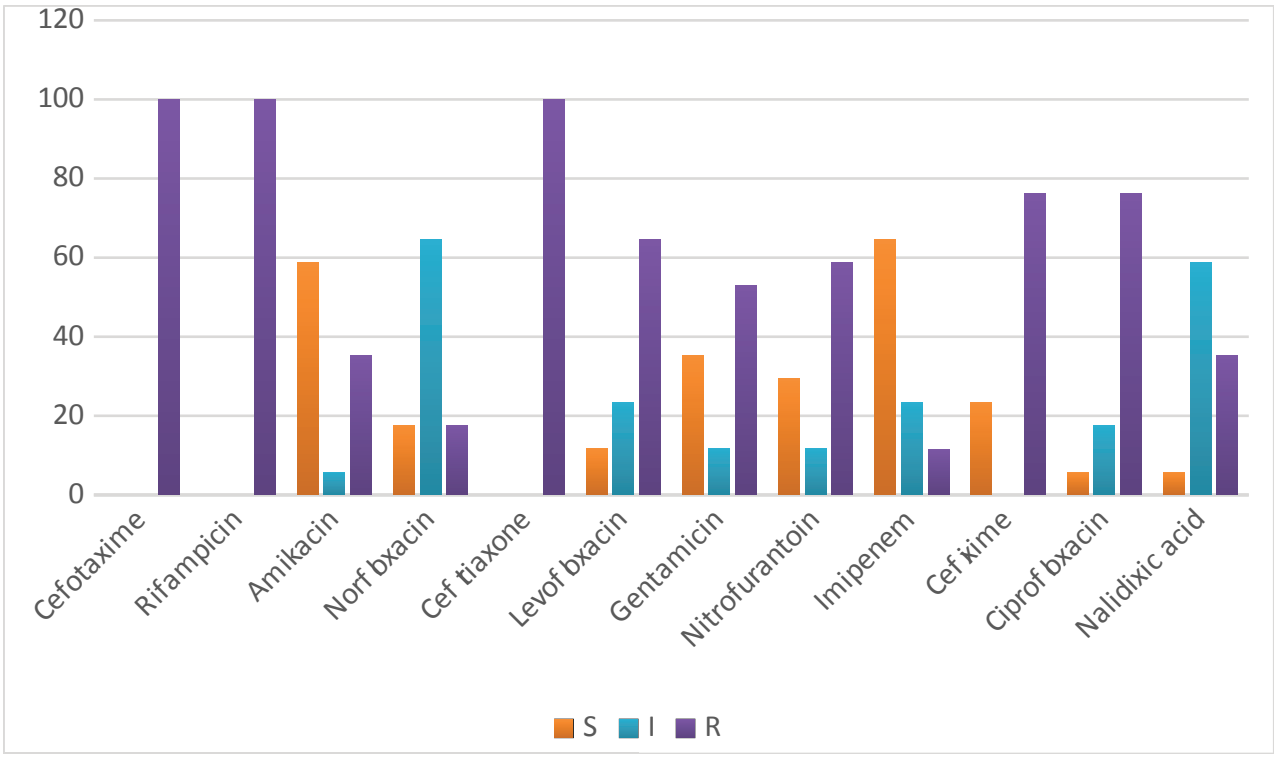

Figure (1), which shows the percentage of resistance, sensitive and intermediate sensitivity of *P. mirabilis* isolates (S : Sensitive, I : Intermediate , R: Resistant)

The results of this study showed that all isolates were resistant to the Cefotaxime and Ceftriaxone (100%), which is of the third generation of cephalosporins, while Cefixime, which is also of the third generation of cephalosporins, had a resistance rate of (76.4%) and the isolates also showed resistance (100%) For Rifampicin, as for Ciprofloxacin, the resistance was (76.4%), and the resistance to Levofloxacin reached (64.7%), , and the resistance to Nalidixic acid, , was also 35.2% while the resistance to Norfloxacin was (17.6%). As for the anti-Nitrofurantoin, the results of the current study showed a resistance (58.8%). As for the resistance to Gentamicin, a group of aminoglycosides, it was (52.9%), and the resistance to Amikacin, which It also belongs to the group of aminoglycosides (35.2%) .Imipenem, which is from the Carbapenem group Most of the isolates are sensitive to it, reached (11.7%).

Extraction of the outer membrane proteins of *Proteus mirabilis*

Outer membrane protein antigen was extracted from isolate PM8, DNase, RNase enzymes were added to reduce the viscosity of the solution by breaking DNA, RNA [15]. On it (peptidoglycan layer), where it works to cleave the murine, thus weakening the binding of peptidoglycan to the proteins of the outer membrane, and this makes the exposure greater to the detergent that was used in the extraction process. Triton X100 detergent also does not dissolve outer membrane proteins and selectively dissolves the inner-membrane protein [16].

Ion exchange chromatography

The resin used in this step is Diethylaminoethylcellulose (DEAE-cellulose). The nature of this resin allows anionic proteins to bind and cationic proteins to cross. The binding of the protein to the resin depends on the PI value of the protein and the PH of the buffer solution that has been created. Used for equilibrated resin, DEAE-cellulose resin has many advantages including ease of handling, good separation, high accuracy and the ability to be reused several times and the principle on which it is based is charge difference [17]. Use a column with dimensions (2X20) cm for ion exchange chromatography of the extract of outer membrane proteins of *Proteus mirabilis*, where washing was carried out by means of a buffer and the fractions were collected at a rate of 4 ml per part at a flow rate of 30 ml / hour, then the proteins bound to the exchanger were eluted using gradual concentrations of sodium chloride (0.9- 0.7- 0.5-0.3-0.1) NaCl, and the amounts of protein in the fractions were followed by a . reading Absorption at a wavelength of 280 nm using a UV – Visible spectrophotometer, where a peak of proteins not bonded with the ion exchanger appeared as shown in Figure (2). The protein concentration was estimated according to the Bradford method [9] drawing the standard curve between the concentrations of BSA versus the absorbance at 595 nm, and the results showed that the concentration of the extracted protein was 0.6 mg/ml.

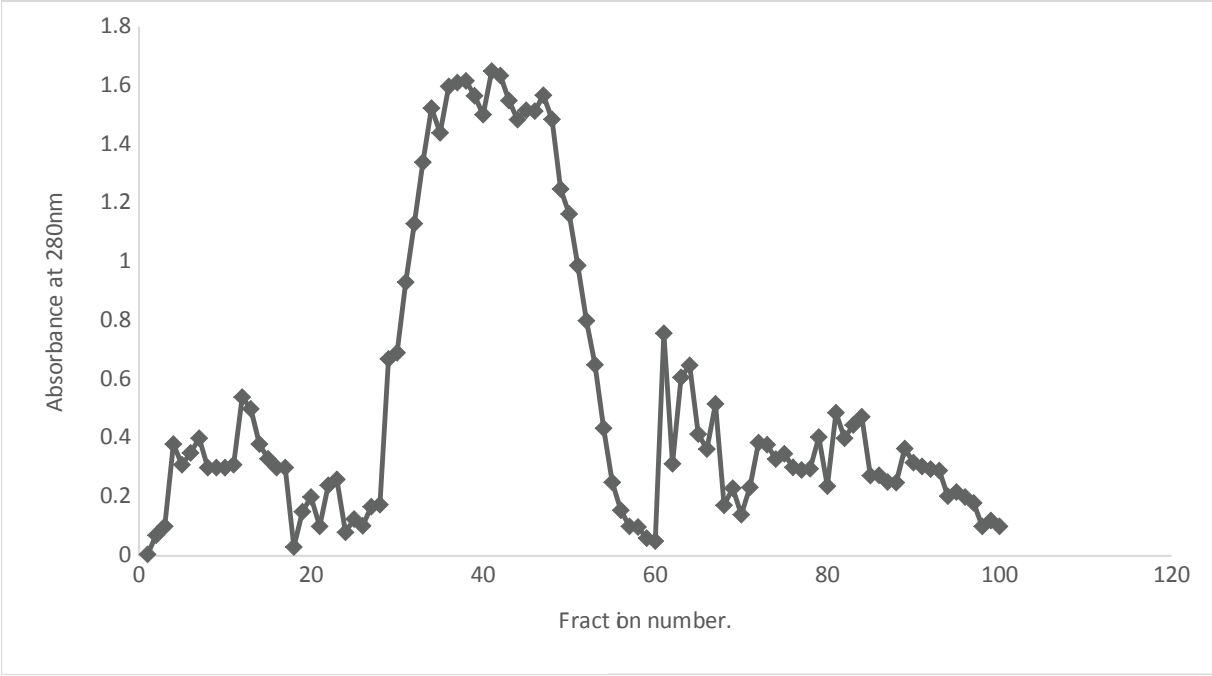

Figure (2) Ion exchange chromatography of *P. mirabilis* outer membrane protein extract by DEAE-Cellulose column equilibrated by Tris-HCl buffer 0.05M and elution by salt gradient 0.1-0.9 NaCl in a 20X2 column at 30 ml flow rate. /hour.

Effect of OMPs antigen on the viability of PMNs and lymphocytes

The results of the statistical analysis showed that the 25% concentration had no significant effect on the viability of PMNs cells. As for the concentrations (100,50), it was found that there is a low significant effect compared to the negative control, as the viability of phagocytic cells decreased from (99.667) in the treatment of Control to (97.667, 98.333) when using concentrations (100,50), respectively, as shown in Table (2).

Table (2) Effect of OMP antigen on the viability of PMNs . macrophages

Concentration of OMP (%)	Percentage of survival of PMNs (average ± standard deviation)
Zero (control)	99.667b ± 0.5774
25	99.667b ± 0.5774
50	98.333a ± 0.5774
100	97.667a ± 0.5774

* Different letters within the column indicate significant differences at (P<0.05) level.

* Similar letters within the column indicate that there are no significant differences at the level (P<0.05).

As for Lymphocyte cells, the results of the statistical analysis in Table (3) showed that all the concentrations used led to a low significant in the level of viability of lymphocytes, as rate reached (96.66, 97.66, 98) compared to the viability of the control (99.66). Al-Dahan [18] found it, showing that the OMPs antigen extracted from *Moraxella catarrhalis* led to a low significant in the survival rate of both lymphocyte and phagocytic polymorphonuclear cells (PMNs). Cells lose their selective permeability and consequently cell death [19].

Table (3) Effect of OMP antigen on the viability of lymphocytes

Concentration of OMP (%)	Percentage of survival of lymphocytes (average ± standard deviation)
Zero (control)	99.667c ± 0.5774
25	98.000b± 0.5774
50	97.667b ±0.5774
100	± 96.667 *a* 0.5774

* Different letters within the column indicate significant differences at (P<0.05) level.

* Similar letters within the column indicate that there are no significant differences at the level (P<0.05).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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