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A PARTIALLY PURIFIED LIPOPOLYSACCHARIDE EXTRACTED FROM *KLEBSIELLA PNEUMONIA* AS ANTIBACTERIAL

Ruaa SH.*, Suhad Mohammad and Esraa Sameer

Kufa University/Science College.

***Corresponding Author: Ruaa SH.**

Kufa University/Science College.

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ABSTRACT

50 isolates of *Klebsiella pneumonia* were isolated from150 urine specimens taken from recurrent –urinary tract infections (UTI) patients attended to AL-Sadar Hospital. Specimens were cultured on specific media, and then bacterial isolates were identified depending on morphological, biochemical and VITK-2. The results showed that *Klebsiella pneumonia* comprise 29 (58%), 5 (10%) and 16 (32%) from recurrent UTI, kidney stone and Catheters samples respectively. The isolate that appeared multidrug resistance(MDR) to most antibiotics used in this study were chosen to extracted lipopolysaccharide. The results of The antibacterial activity assay of LPS extract of *Klebsiella pneumonia* were done against *E. coli, Klebsiella pneumonia, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staph aureus,* and *Streptococcus pneumonia* at different concentration (200,300,400) µg /ml were evaluated by measuring the diameters of zones of growth inhibition on bacterial, and compare with the inhibition zone of amoxicillin as control, the result show all bacteria sensitive to LPS (100%) at concentration (200,300,400) µg /ml and the inhibition zone ranging from (25 to33)mm, compare with amoxicillin that all bacteria resistance to it(100%). **In Conclusion:** *LPS are promising a new medicine lead to kill gram positive and gram-negative bacteria.*

KEYWORD: Lipopolysaccharide, *Klebsiella pneumonia*, urinary tract infection, amoxicillin, Antibiotics.

INTRODUCTION

Klebsiella pneumoniae is a gram-negative, encapsulated, non-motile, facultative anaerobic rod designed bacterium originate in the normal flora of the intestines and characteristically colonizes human mucosal surfaces of the oropharynx and gastrointestinal tract. It can show high degrees of virulence and antibiotic resistance.[1]

Klebseilla pneumonia cause a nosocomial infection, and reflected the greatest public cause of hospital-acquired pneumonia. It is a second pathogen afterward *E. coli* to urinary tract infections. It is as well an opportunistic pathogen for patients by chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma.[2]

Lipopolysaccharide (LPS) is the main part of the outer membrane of Gram-negative bacteria and it covers other than 90% of the cell surface in its outer leaflet, however phospholipids that are placed in the inner leaflet. Similar other memberships of the family Enterobacteriaceae, the LPS of *Klebsiella pneumoniae* consist of three basic areas the hydrophobic lipid A, which is a main part of the outer leaflet of the Gram-negative outer membrane, while core oligosaccharide is associated to lipid A and be responsible for the attachment site for the elongated chain polysaccharide (O antigen; O chain).[3] LPS works as a physical barrier providing the bacteria defense from its surroundings and known by the immune system as a marker for the recognition of bacterial pathogen invasion, and responsible for the increase of inflammatory response.[4]

MATERIAL AND METHODS

Bacterial Characterization

A whole of 150 urine specimens were collected under aseptic condition from patients joining to Al-Sadar Medical City in AL-Najaf province suffering from recurrent UTI, kidney stone and long –used catheter were inoculated on MacConkey agar and Blood agar and incubated at 37 C° for 24 h. The morphological characteristics of the colonies counting size, shape, color, were recorded, the suspected *Klebsiella pneumonia* were relevant by biochemical test[5], lastly confirmed by using Vitek-2 Compact (Bio Mérieux, France,). Antibiotic susceptibility test were done for all isolates by using Vitek -2 system with AST-XN05 card.

Extraction of lipoopolysacharide

A- Cell Preparation

Klebsiella pneumonia isolate were cultured all nightly in flask containing 25 ml of Luria Broth (LB) (for bacterial activation) at 37ºC for 18 hours. The fresh cultures then inoculated 3.5 L of LB broth suspended in 500ml conical

flasks containing 200 ml broth. Incubated at 37ºC for 24 hours with shaky at 150 rpm. After that centrifugation at 3000 rpm for 15 minutes, the pellet have been washed twice with phosphate buffer. Cells were suspended in phosphate buffer containing 0.5% formalin (pH= 7.2) and were kept at 4ºC for 18 hours. The precipitate were centrifuged at 3000 rpm for 15 minutes furthermore washed with phosphate buffer. Finally, cells were dried using cold acetone by ten times the sample's volume.[6]

B- Lipopolysaccharide Extraction

The *Klebsiella pneumonia* LPS was removed from MDR isolate by the hot EDTA method given by.[7]

Partial Purification of LPS by Gel Filtration (Sephacryl S-300)

Sephacryl S-300 gel was primed according to the information of the manufacturer company (Sigma, Germany). It was washed and suspended in 0.025 M of phosphate buffer(PB) (pH 7.2), degassed by utilizing vacuum pump then poured with care to avoid bubbles into a column with measurement of 75×2 cm. The last volume of the column was 235.5 cm^3. Then the column was equilibrated with 0.025 M of phosphate buffer saline (PBS) (pH 7.2), and the stream rate was 75 ml/hour and the extracted sample was chemically analyzed to define the content of carbohydrate according to[8] and total protein according to.[9]

Sterility was determinate by culturing of LPS on blood and nutrient agar.

Activity of partial purify lipopolysaccharide extract as Antibacterial

The antibacterial activity of lipopolysaccharide extract were determined by agar diffusion method regarding to.[10] Petri plates containing Twenty-five ml of sterile Muller – Hinton agar inoculated along with *E. coli. Klebsiella pneumonia, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staph aureus* and *Streptococcus pneumonia* after adjusted final inoculums of these bacteria to 1.5 x10 ^8 (CFU)/ml, by comparison with the 0.5 McFarland standards tube. Cut wells have (6mm) in diameter in agar by using a sterile Pasteur pipette and removed the agar discs by a sterile forceps, after that filled wells with 0.1ml of each concentration of lipopolysaccharide extracts(200,300,400 µg/ml) then incubated in the upright position to keep the LPS extract in the wells at 37 o C for 24 hours. Measured inhibition zone diameter formed around each well assessment the antibacterial activity of LPS and match up against amoxicillin.

RESULT AND DISCUSSION

Results

1- Characterization of *Klebsiella pneumonia*

The results showed that 144 specimens (96%) out of 150 urine specimens gave positive results for bacterial growth on MacConky agar. Amongst the positive growth and even depending on features of the microscopic, morphological, biochemical tests and Vitek 2 system only 50 isolates belong to *Klebsiella pneumonia* 29 (58%), 5(10%) and 16 (32%) were restored from recurrent UTI 45(31.2%), kidney stone 55(38.1%) and Catheters 44(30.5%) samples, respectively. The remaining isolates 94(65.6%) showed growth of *P. mirabilis* 25(17.3%), *Pseudomonas* 17(11.8%), *E. coli* 52(36.1%).

2-Extraction of Lipopolysaccharide from chosen isolate

The isolate that offered high resistance to antibiotics in VITEK-2 Compact system used to extract LPS, which was isolated from recurrent UTI patient.

The results of extracted LPS looked that the existent employed method produced a bacterial mass of 20 gm dry weight bacteria and obtained 170 mg of crude lipopolysaccharide.

3-Partial Purification

The results of chemical analysis of LPS in 1 ml of crud and partial purified LPS showed that Carbohydrate and protein was (98.8% and 1.2%) respectively in crud extraction and (93.6% and %6.4) in partial purified LPS respectively.

In–vitro Activity of Partial Purification LPS as Antibacterial

The antibacterial activity assay of LPS extract of *Klebsiella pneumonia* were done against *E. coli, Klebsiella pneumonia*, *Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staph aureus, Streptococcus pneumonia* at different concentration (200,300,400) µg /ml were estimated by determining the diameters of zones of growth inhibition on bacterial, and match with the inhibition zone of amoxicillin as control, the result show all bacteria sensitive to LPS (100%) at concentration (200,300,400) µg /ml and the inhibition zone ranging from 25 to33mm, compare with amoxicillin that all bacteria resistance to it (100%) according to[11] and the results are presented as shown in table (1) and figures (1).

Table (1): Concentration of LPS Ug/ml, Size of inhibition zone diameter for three replicated (mm).

concentration	*E.coli*	*Salmonella typhi*	*Proteus mirabilis*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Staph aureus*	*Bacillus subtilis*	*Streptococcus pneumonia*
200	27	28.5	25	26	25	26	27	27
	28	29	26	27	26	25	27	26
	29	29	25	28	26	27	28	25
mean		64.6	25.3	27	25.6	26	27.3	26
300	31	30	28	28	28	28	29	29
	31	31	29	29	27	29	30	28
	32	31	28	29	29	28	30	29
mean	31	30.6	28.3	28.6	28	28.3	29.6	28.6
400	30	30	30	31	30	30	30	30
	32	32	29	31	32	31	32	30
	32	31	31	31	32	31	32	31
mean	31.3	31	30	31	31.3	30.6	31.2	30.3
Amoxicillin (control)	0	0.4	0	0.3	0	0	0	0.2
	0	0.1	0	0	0.2	0	0.2	0.3
	0.3	0.4	0	0.1	0	0	0.1	0
mean	0.1	0.3	0	0.13	0.06	0	0.1	0.3

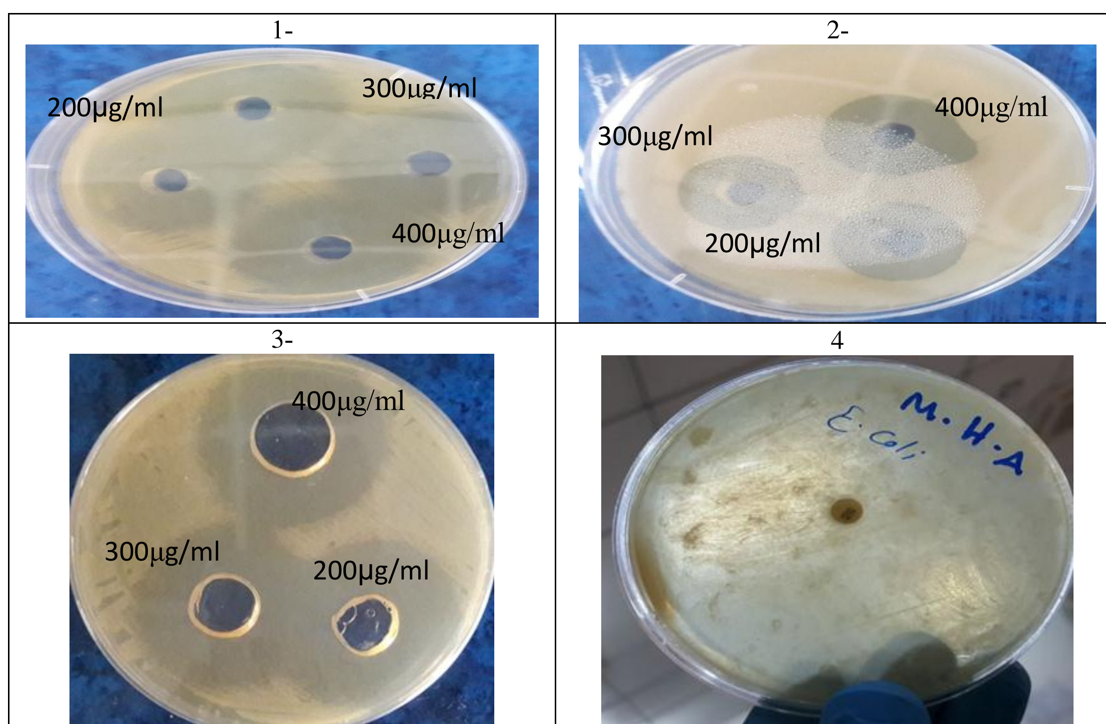

Fig. (1): The inhibition zone diameter for different concentration of partial purification LPS aganist1- *Staphylococcus aureus* 2- *Sallmonella typhi* 3- *Proteus mirabillis* compare with 4- amoxicillin.

DISCUSSION

In this study, the isolate of *Klebsiella pneumonia* that gave high resistance to antibiotics in VITEK-2 Compact system was used, which isolated from recurrent UTI patient. The resistance of bacteria were connected to alterations within the lipopolysaccharide is thought to act as a permeability barrier, making the outer membrane relatively impermeable to antibiotics and detergents.[12]

Extraction of LPS from bacteria more antibiotics resistance and used it to eradicate hazardous pathogen. The results of extracted LPS appeared that the present engaged method yielded a bacterial mass of 20 gm dry weight bacteria and obtained 170 mg of crude lipopolysaccharide. This result Exposed that Carbohydrate and protein in crude LPS was (98.8% and 1.2%) respectively and (93.6% and % 6.4) in partial purified LPS respectively. However, (13) founded that 1.4 g LPS was recovered from 10.5g of dried cell. Carbohydrate and protein was (2.3% and 0.9%) respectively in crud extraction and (9.5% and % 0.06) in partial purified LPS. also[14] observed that partial purification of LPS extracted from pathogenic and standard P .mirabilis revealed the first peak contained the active LPS more than the second peak in pathogenic. While the standard bacteria in the second peak contained

the active LPS more than the first one. The increase in the carbohydrate amount in the purified sample could be due to the removal of some impurities.[15]

The[16] found that LPS in different concentration (700,800,900,1000,1100, 1200 Ug/ml) gave different inhibition zone to *E coli* range from(27-37mm), and[17] found inhibition zone of polysaccharide, ranging between (2.7-11.5 mm) against *E. coli* and *P. aeruginosa.*

The unique structure of LPS become active against different bacterial strains (gram positive and gram.[18] The susceptibility of Gram-negative cells to LPS is associated with factors that facilitate the transport of the LPS across the outer membrane, also the variations in the chemical structure of LPS, e.g., in the composition of the sugar head group and to the highly charged O-specific sugar side chains as the causes for the bacteria sensitive towards LPS.[19] The O-antigen might be a reliable indicator of virulence potential and these important features often differ within the same bacterial strain, this will help in the designing of novel antibacterial as future therapeutics.[20]

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