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Correlation Between Gel Electrophoretic Profile of Bacterial Lipopolysaccharide and its Toxicity

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ABSTRACT. Gel electrophoretic pattern of lipopolysaccharide (LPS), prepared from *Pseudomonas aerüginosa* isolates, revealed variable banding, assuming quantitative and qualitative differences leading to three main distinctive profiles. The first is a ladder-like banding arrangement of molecules, with various oligosaccharide repeating units and variable affinity to the silver stain (lightly and deeply stained bands). The second banding pattern consists of profiles which are characterized by aggregates of molecules with homogenous oligosaccharide repeating units and forming large deeply stained bands. The third banding patterns are those having limited number of bands running to the far end of the lanes and staining differently. The toxicity of the studied LPS is closely related to the number of deeply stained bands.

The lipopolysaccharide of *Pseudomonas aeruginosa* displays the biological activities of classic endotoxin (Barasanin *et al.* 1978, Cho *et al.* 1979). Although the role of this compound in the pathogenesis of the organisms is indisputable, Jarrell and Kropinski (1977) reported that LPS of *P. aeruginosa* is not toxic, while Cryz *et al.* (1984) noticed that pathogenicity of this organism is inversely correlated to the percentage of LPS

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which partitions into phenol-water. In contrast LD_{50s} of 17, 20, 42 and 125 mg.kg have been reported by Barasanin *et al.* (1978), Homma (1971) and Mlynarcik and Muszynski (1974). The recent advance in the extraction and analysis of LPS using gel electrophoresis and silver stain provided tools for investigating this compound from several genera and species of gram negative bacteria. The fact that LPS heterogeneity is applicable for both electrophoretic partition and toxicity suggests a possible relation between the two characters. Therefore, this relation has been investigated in the present study.

Materials and Methods

Collection, Selection and Maintenance of Organsims

Organisms were collected from Dammam General Hospital (Eastern Region of Saudi Arabia) over a period of eight months in batches of 50 isolates. Replica isolate from the same patients were excluded. Thus collection consisted of individual patient isolates. Selection of isolates was based on the criterion that isolates should be ofdifferent pyocin and antibiogram types on repeated tests.

Pyocin Typing

Pyocin typing was performed with both the old and the revised method (Gillies and Govan 1966, Fyre *et al.* 1984) using the eight standard indicators supplied by Govan (the University of Edinburgh, Scotland).

Antibiotic Sensitivity Test

Minimum inhibitory concentrations were determined by the agar dilution method on diagnostic sensitivity agar (Oxoid CM 261) with an inoculum of 10^5 organisms (King *et al.* 1980).

Growth and Extraction of LPS

Organism were grown from 10 ml inocula containing 10^4/ml cfu in 500 ml batch cultures of Luria Bertni medium supplemented with glucose (LBG). Cells were collected from late exponential phase by centrifugation after an incubation period of 16 h + 1. The optical density of the resulting cultures were 1.2 at 650 nm (Spectronic 20 L and B).

Collected pallets were washed in buffered saline (pH 7.2), then LPS was extracted with the aqueous phenol method. Layers containing LPS were lyophilized and the dried white powder (representing LPS) was stored in glass vials at 4°C till further use.

Polyacrylamide Gel Electrophoresis

Lipopolysaccharide (2.5 mg) was solubilized at 70°C for 30 min in 5 ml of lysing buffer containing 2% SDS, 4% mercaptoethanol, 10% glycorst,1M Tris pH (6.8) and

bromophenol blue. The solubilized materials (10, 20 and 30 µg of each preparation) were subjected to SDS-PAGE with the Laemmli buffer system (Laemmli 1970). The 4% staking and 12% separating gel did not contain SDS. The electrophoresis was done at 35 mA (constant current) with Tris pH 8.3 for 3 h.

Staining (Ag-LPS Staining)

The procedure of Hitchcock and Brown (1983) was used which consists of four essential steps: (1) fixation in propanol and acetic acid; (2) oxidation in periodic acid; (3) staining with silver nitrate, (gels should be clear after this step as gray gels are not suitable for further processing and should be neglected); (4) developing in citric acid and formaldehyde. The modified Ag-LPS staining (Formsgaard *et al.* 1990) was also used to provide sharper stained patterns and to avoid faint staining produced by deletion of fatty acids during fixation.

Lethality Bioassay of LPS

The LD_{50} of LPS preparations from the studied isolates were determined using embryonated eggs aging 11-12 days. Serial double dilution from 1.6 mg/ml to 2µg/ml were made in phosphate buffer saline at pH 7. Six eggs were injected intravenously with 0.1 ml of each dilution. Eggs were incubated at 37°C in an egg incubator and mortality was read after 24 h. The LD_{50s} were computed with the method of Reed and Muench (1935).

Results

Out of 300 clinical isolates obtained from the hospital, 20 isolates met the criteria of being different in their pyocin and antibiogram types on three consecutive typings (Table 1). The silver stained electrophoretic profiles of phenol-water partitioned LPS (10 µg) showed morphological heterogeneity, larger quantities of LPS 20 and 30 µg resulted in lack of distinctive banding. Thus, the ladder-like banding patterns obtained with the silver stain procedure (conventional and modified silver stain) revealed three groups of morphologically heterogenous LPS. A group of LPS molecules with various oligosaccharide repeating units distributed through the lanes in adjacent arrangement. The banding patterns within this group showed different affinity to the silver stain leading to deeply stained (brown) bands of isolates 13 and 15, and lightly stained (yellow) bands of isolates 3, 5, and 10 (Fig. 1). Although, isolates 9, 12 and 14 belonged to the previous group, they show fast traveling, deeply stained bands occupying the far end of the lanes. A second group of LPS molecules with almost homogenous repeating units of oligosaccharide and characterized as deeply stained bands of intermediate migrating distance isolates 6, 21, 22 and 24 (Fig. 1). The third group of LPS consist of fast migrating lightly stained molecules occupying the far end of the gel isolates 2, 16, 19, 20, 23, 25 and 26 (Fig. 1). In term of toxicity, as estimated with the chichembryoassay,

Table 1. The antibiogram and pyocin types of the studied isolates of *P. aerüginosa*

Isolate No.	Pyocin Type	MIC Antibiotics in µg/ml							Isolates No.	Pyocin Type	MIC Antibiotics in µg/ml						
		TM	GM	AN	TIC	CB	RO	RIP			TM	GM	AN	TIC	CB	RO	RIP
2	3	128	500>	4	256	500>	500>	128	16	13	128	500>	4	128	128	64	500>
3	48	32	4	4	32	128	500>	128	17	30	32	4	4	32	32	28	32
5	1	128	500>	64>	500>	500>	500>	500>	19	52	4	2	2	64	64	64	64
6	24	32	4	4	16	32	500>	256	20	36	32	4	4	16	128	500>	256
9	57	32	4	4	32	16	64	64	21	21	32	64	4	64	500>	64	500>
10	10	128	500>	4	500>	500>	16	500>	22	UT	64	500>	32	500>	500>	128	500>
12	27	32	4	4	32	16	500>	64	23	14	32	4	2	128	64	64	64
13	5	128	500>	4	250	500>	500>	500>	24	17	4	500>	4	128	500>	32	500>
14	9	32	4	4	16	32	64	256	25	2	32	4	2	128	128	64	500>
15	uc	32	500>	4	500>	500>	64	500>	26	68	8	2	2	16	16	16	16

TM; Tobramycin, GM; Gentamicin, AN; Amikcin,
PIP; Piperacillin, TIC; Ticarcillin, CB; Carbenicillin,
RO; Roceiphin. UC; Unclassified type UT; Untypable

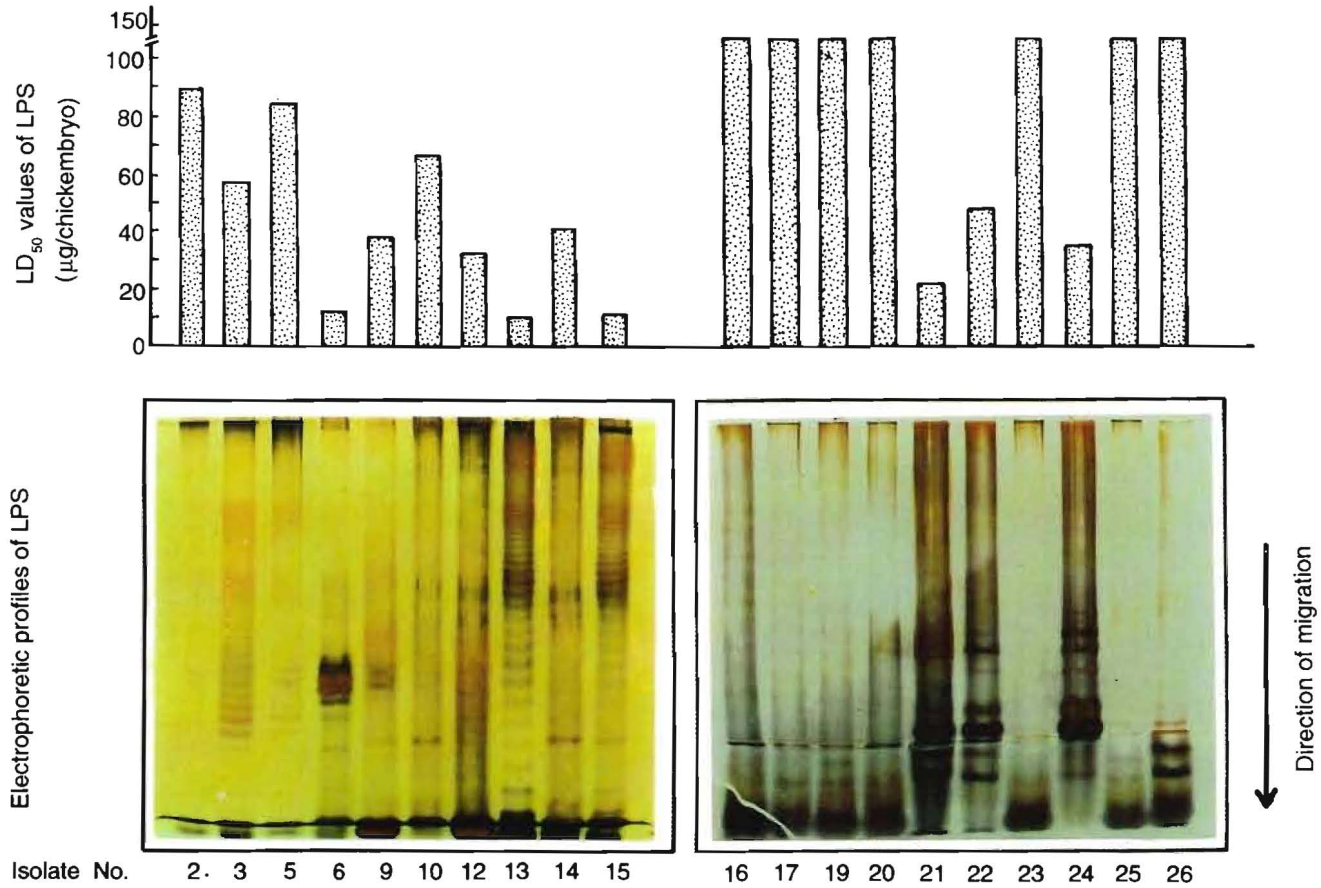

Fig. 1. The relation between LD_{50} values and polyacrylamide gel electrophoresis profiles of LPS prepared from *P. aeruginosa* isolates. Each LPS drived from one isolate has its LD_{50} value above and electrophoratic profile (below).

the LPS molecules show deeply stained banding patterns and various oligosaccharide repeating units were found to be more toxic than the same lightly stained banding patterns. An approximate level of toxicity to the deeply stained LPS molecules, with various oligosaccharide units, are those characterized by homogenous oligosaccharide repeating units, intermediate migration and forming bands patterns of aggregate molecules. Patterns showing fast traveling deeply stained bands are also found to be exceptionally toxic. However, the lowest level of toxicity is shown by LPS of fast traveling lightly stained molecules, which are hardly considered as toxic LPS (see relative LD_{50} values Fig. 1).

Discussion

Although, the LPS of *Pseudomonas aerüginosa* elucidate the classic biological effects on animals, its variable values of LD_{50s} raised some questions to the importance of this part of the cell in the pathogenesis of the organisms. Jarrell and Kropinski (1977) deprived LPS of *P. aeruginosa* of being even toxic. In the light of the analytical electrophoretic partition of LPS performed during this investigation, qualitative and quantitative differences were observed. Quantitatively, the electrophoretic partitions of LPS from the studied isolates projected various patterns; those include LPS molecules with different number of repeating oligosaccharide units distributed through the lanes showing variable response to the silver stain which resulted in light and deep stained patterns. Molecules of homogenous (or closely related) repeating oligosaccharide units forming patterns of deeply stained bands aggregates and molecules classified as oligosaccharide deficient or peering limited chains were characterized by their fast traveling to the far end of the lanes. The migration distance of LPS molecules has been attributed to the number of oligosaccharide repeating units (Palva and Makela 1980, Tsai and Frasch 1982).

Qualitatively, the migrating distance of independent staining (gray, yellow and red) of the bands (forming certain patterns) depend upon the fatty acid constituent of the LPS molecules (Hitchcock and Brown 1983, Formsgaard *et al.* 1990). However, analytical differences in LPS appear to correlate with its toxicity; thus staining intensity, migration distance and number of bands are the limiting factors for LD_{50s} values. Therefore, LPS showing profiles of deeply stained molecules with various oligosaccharide repeating units are more toxic than the same profiles of lightly stained molecules.

The reason underlying the great difference in toxicity of LPS could be associated with lipid A content of the two profiles. Such a conclusion is clearly supported by the evidence reported in the study of Formsgaard *et al.* (1990) in which they indicated that lightly stained profiles of LPS consist of lipid *A* deficient molecules. Consequently and owing to the fact that lipid *A* is the toxic part of LPS; toxicity should be a reflection of its relative existence. In addition, profiles showing deeply stained aggregate of molecules

(having homogenous oligosaccharide repeating units) are found to be as toxic as those of deeply stained having various oligosaccharide repeating units, which also support our above mentioned interpretation.

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References

Barasanin, I., Alonso, M.L., Rojo, J.M., Ronda, E. and **Portoles, A.** (1978) Comparative study of immunobiological activities of *Pseudomonas aeruginosa* and *Brucella melitensis* lipopolysaccharides. *Curr. Microbiol.* **1:** 263-267.

Cho, Y., Tanamoto, K., Oh, Y. and **Homma, J.Y.** (1979) Differences of chemical structures of *Pseudomonas aeruginosa* lipopolysaccharide essential for adjunanticity and antitumor and interferoninducing activities. *FEBS* Letters, **105:** 120-122.

Cryz, S.J., Jr., Pitt, T.L., Germanier, R. and **Furer, E.** (1984) Role of lipopolysaccharide in virulence of *Pseudomonas aeruginosa. Infect. Immun.* **44:** 508-513.

Formsgaard, A., Freadenburg, M.A. and **Galanos, C.** (1990) Modification of the silver staining technique to detect lipopolysaccharide in polyacrylamide gels. *Microbiol.* **28:** 2627-2631.

Fyre, J.A., Harris, G. and **Govan, J.W.R.** (1984) Revised pyocin typing method for *Pseudomonas aerüginosa. J. Clin. Microb.* **20:** 47-50.

Gillies, R.R. and **Govan, J.R.W.** (1966) Typing of *Pseudomonas pyocganea* by pyocine production. *J. Path. Bact.* **91:** 339-345.

Hitchcock, P.J. and **Brown, T.M.** (1983) Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver -stained polyacrylamide gels. *J. Bact.* **154:** 267-277.

Homma, J.Y. (1971) Recent investigation on *Pseudomonas aeruginosa. Jap. J. Exp. Med.* **41**: 387-400.

Jarrell, K.F. and **Kropinski, A.M.** (1977) The chemical composition of the lipopolysaccharide from *Pseudomonas aeruginosa* PAO and sponstaneously derived rough mutant. *Microbios* **19:** 103-116.

King, A., Warren, C., Shenon, K. and **Philips, I.** (1980) The *in vitro* antibacterial activity of cefotaxin compared with that of cefuroxime and cefoxitim. *J. Antimicrob. Chemother.* **6:** 479-494.

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

Mlynarcik, D. and **Muszynski, Z.** (1974) Immunochemical and toxic properties of the lipopolysaccharide from highly and low virulent *Pseudomonas aeruginosa* strains. *J. Hyg. Epidemiol. Microbiol. Immunol.* **18:** 425-428.

Palva, E.T. and **Makela, P.M.** (1980) Lipopolysaccharide heterogeneity in *Salmonella typhimarium* analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Europ. J. Biochem.* **107:** 137-143.

Reed, L.I. and **Muench, H.A.** (1935) A simple method of estimating fifty percent endpoint. *Ame. J.* Hyg. **279:** 493-497.

Tsai, C.M. and **Frasch, C.E.** (1982) A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Analyt. Biochem.* **119:** 115-119.

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نمط الفصل الكهربائي على هلام لعديدات السكاكر البكتيرية يتوافق مع سميتها

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أظهر التحليل الكهربائي لعديدات السكاكر البكتيرية المحضرة *Pseudomonas aerüginosa* من أنماط فصل مختلفة توحي بتباين كمي وكيفي لهذه السكاكر ، ومن هذه الأنماط :

١ـ التركيب السلمي لجزيئات تتميز بوجود وحدات متتابعة من السكاكر مختلفة الاستجابة لصبغة الفضة فهي ذات أصطباغ عميق أو باهت .

٢ـ نمط يميزة تراص جزيئات ذات وحدات سكرية متتابعة ومتجانسة وذات أصطباغ عميق .

٣ـ نمط يميزة قلة السكاكر المفصولة وهجرتها إلى طرف الهلام واصطباغها متباين .

أما سمية عديد السكاكر البكتيرية فتحددها عدد الجزيئات ذات الأصطباغ العميق .