قسم : الميكروبيولوجيا.
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دراسات مبدئية عن نواتج نمو عصويات الصديد الأخضر

اسماعيل صديق ، شعبان أحمد ، أماني ثابت

لقد أجريت هذه الدراسة على نواتج النمو الخارجية لخمسة عشرة عترة من ميكروب عصويات الصديد الأخضر التي تم عزلها من الجروح المتقيحة والتهابات الأذن الوسطى والتهاب القناة البولية لبعض المرضى.

وقد لوحظ من هذه الدراسة أن لهذه النواتج التأثيرات التالية :

١- تأثير قاتل على فئران التجارب المعملية التي تم حقنها بواسطتها.
٢- نشاط تنكرزى عند حقنها بجلد الأرانب .
٣- لها القدرة على تكسير كرات الدم الحمراء .
٤- تأثير قاتل على كثير من الميكروبات الايجابية لصبغة الجرام وبعض الميكروبات السلبية لصبغة الجرام.

وقد توصلت هذه الدراسة الى اثبات أن هذه الصبغات جميعها تختفي مع التسخين عند ٦٠ °م لمدة عشرة دقائق .

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PRELIMINARY STUDIES ON THE EXOPRODUCTS OF PSEUDOMONAS AERUGINOSA

(With Two Tables)

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SUMMARY

Study was carried out on the expoproducts of 15 strains of Ps.aeruginosa isolated from wound infection, otitis media and urinary tract infection. It was noticed that the exoproducts exhibited lethal effect when injected intraperitonealy into white mice as well as dermonecrotic activity when injected into skin of rabbits. As regards the haemolysin activity, it was detected for all the products by agar well technique and the tube method. Also, the exoproducts had antimicrobial activity on Gram-positive and some Gram-negative organisms. All these activities were thermolabile at 60°C for 10 minutes.

INTRODUCTION

The emergence of ps.aeruginosa as an important pathogen in the last 20 years has been due primarily to its resistance to antibiotics. Also, the increasing number of patients receiving broad spectrum antibiotics, immunosuppressive agents, and other metabolic inhibitors have placed the opportunistic and antibiotic resistant ps.aeruginosa in the forefront as a primary cause of clinical infections. This led to attempts to use immunological methods in control of this infection, and require to study the mode of pathogenesis of this organism.

It was generally held that the pathogenesis of Gram-negative bacteria was due to their endotoxins. The failure of large number of dead ps.aeruginosa cell to reproduce the pathological picture as that had been seen in infections with live cells should have indicated that endotoxin alone was not sufficient to explain the pathogenesis of the organism (LIU, 1957; LIU, et al. 1961). This evidence confirms that the pathogenesis of the organism is mostly due to its extracellular products (LIU, 1974; BLACKWOOD, et al. 1983).

Our attention is now being focused on the prevention and control of these problems. In our work trials were made to evaluate the roles played by extracellular products of ps.aeruginosa in its pathogenesis. The pathological picture produced by exotoxins as well as the antibacterial effect of the extracellular products against a variety of Gram-positive and Gram-negative bacteria was studied.

MATERIAL and METHODS

A total number of 15 strains of Ps.aeruginosa was isolated from cases of urinary tract infection, otitis media and wound infection atending Assiut University Hospital.

They were identified according to CRUICKSHANK, et al. (1975) as well as WILSON and MILES (1983) by their motility, reduction of nitrate, production of oxidase and pigments, ability to liquefy gelatin and urease production. The motility of Ps.aeruginosa strains was tested by measuring migration in soft agar tubes, all strains were motile. The oxidase test was performed as described by STANIER, et al. (1966), cultures grown for 24 hours on cetrimide agar medium.

Gelatin was rapidly liquefied by all strains, in litmus milk an alkaline reaction and clotting were noticed.

All the strains produced diffusible pigments. The identified strains were evaluated for the production of the extracellular products which are important virulent factors in Ps.aeruginosa infections.

Two drops of 16 hours nutrient broth culture of each strain were inoculated into each of 5 flasks each containing 200 ml. nutrient broth and incubated at 37°C for either 16, 48, 72, 96 or 120 hours. Each medium was centrifugated at 4000 r.p.m. for 20 minutes and the supernatent was further filtered using sterile Seitz filter. The filtrates then preserved in refrigerator at 4°C for further use in the following tests.

1- Toxigenicity:

The filtrates of different strains were tested for their toxigenicity by intraperitoneal injection into white mice. The inoculated doses were 0.2, 0.5 and 1 ml. of each strain into 3 groups each contained 4 mice.

The inoculated animals were kept under observation and the number of dead animals were recorded. A control was included and injected with sterile broth.

2- Haemolytic activity:

It was tested by two methods. The first was the agar well technique using plates containing 5% human blood group "O" and sheep blood and the second was the tube method using 2.5% of the same RBCs of human blood group "O" and sheep blood.

3- Dermonecrotic activity:

This was done by the I.D. injection of 0.2 ml. of each filtrate into rabbits after shaving the site of inoculation; sterile broth was used as control.

4- Detection of alkaline phosphatase:

Release of alkaline phosphatase was also detected in the extracellular product, this was done by using photometer apparatus and sterile medium was used as control (CHENG, et al. 1970).

5- Antibacterial effect:

The antibacterial effect of the products was determined by its serial dilutions in physiological saline being prepared first then using cup-diffusion method against a variety of Gram-positive and Gram-negative microorganisms.

These organisms were Staph. aureus, Staph. albus, Staph. citreus, Strept. pyogenes, Pneumococci, Corynebacterium mitis. Bacillus cereus, E.coli, Enteropathogenic E.coli, Klebs. pneumonia, Proteus vulgaris and Ps.aeruginosa.

6- Effect of heat on filtrates:

The filtrates were heated at 60°C for either 10, 20 or 30 minutes and retested for toxigenicity and inhibitory effects.

RESULTS

From the table No. 1 it was noticed that untreated crude products killed all the injected mice at doses of 0.5 and 1 ml. while the 0.2 ml. dose did not kill the injected mice in case of culture incubated for 16 and 48 hrs. The same dose of cultures incubated for longer periods had a mortality range between 25-50%.

From the same table it was observed that all the crude products heated at 60°C for 10, 20 or 30 minutes were non toxic to mice.

As regards the haemolytic activity, it was observed that all the strains produced haemolytic extracellular product. It was also noticed that the haemolysin produced by filtrate of strains isolated from wound infection was somewhat, stronger than the other strains as its activity was noted with dilution up to 1/16 while that of the other strains did not exceed 1/4 dilution. The dermonecrotic activity was demonstrated by the filtrate of all strains. It started with ulceration and papule formation and ended with necrosis.

As regards the alkaline phosphatase, it was released by all the isolated strains in a considerable quantity.

From table No. 2 it was observed that the exoproducts of Ps.aeruginosa had antimicrobial activity against many microorganisms such as Staph. aureus, Staph. albus, Staph. citreus, pneumococci, Strept.pyogenes, Prot. vulgaris, E.coli, Ps.aeruginosa and Bacillus cereus. The diameter of zone of inbibition was measured. On the other hand entero-pathogenic E.coli, Coryne. diphtheriae (mitis) and Kleb.pneumonia were not affected at all by the filtrates.

The inhibitory effect of the exoproduct as well as its haemolytic activity, toxigenicity or dermonecrotic activity was abolished by heating at 60°C for 10 minutes.

DISCUSSION

The pathogenesis of Ps.aeruginosa is mostly due to its extracellular products which produced most of the histopathological effects of the infection (LIU, 1957; LIU, et al. 1961 and BLACKWOOD, et al. 1983). In our work, the lethality of the exoproduct on I.p injection to white mice mainly was due to the effect of the exotoxin. It was noticed that the filtrate of broth culture incubated for 16 hours was sufficient to kill the white mice after 12 hours and maximum mortality rate was observed after 24 hours. This result is similar to that obtained by LIU (1973) and PAVLOVASKIS and SHACKEFLORD (1974). The virulence of Ps.aeruginosa appears to be correlated with its ability to produce an exotoxin (IGLWSKI and KABAT, 1975 and BERDAL, et al. 1982).

All Ps.aeruginosa strains used in our work were able to produce proteolytic activity and lead to complete liquefaction of gelatin within 48 hours. The strains were also evaluated according to its protease activity as it plays a role in its effect; HOLDER and HAIDERIS (1974) and SNELL, et al. (1978) had mentioned that protease would play a role in virulence of ps.aeruginosa infection.

The exoproducts produced characteristic necrotic lesion in the skin of rabbits. Such lesion is most probably due to protease, phospholipase and phosphatase produced by different strains. This type of skin lesion was found to be associated with the protease of Ps.aeruginosa recorded by LIU (1966).

In our work it was noticed that the crude products of Ps.aeruginosa exhibited a high inhibitory effect on most Gram-positive organisms (Staph.aureus, albus, citrus, Strept. pyogenes, pneumococci and Bacillus cereus as well as some Gram-negative bacilli (E.coli, Prot.vulgaris, and 2 strains of Ps.aeruginosa isolated from urinary tract infection).

The significance of the pigments of Ps.aeruginosa particularly pyocyanine in the pathogenesis of this organism probably lies in its inhibitory effect on other bacteria or even other strains of the same species. Therefore, the pigment may play role in the suppression of other bacterial flora and their replacement by pseudomonas; a common event in long lasting infections.

In our work it was observed that the inhibitory effect, the haemolytic activity, dermonecrotic activity as well as the lethal effect were heat labile. The aforementioned observations are sufficient to indicate that the pathogenesis of Ps.aeruginosa is in large part due to the heat labile extracellular toxins. It is believed that these correlated characteristics presented here will necessitate that immunological studies should be attempted in order to prevent Ps.aeruginosa infection.

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Table (1)

Toxigenicity of the exo-products of Ps.aeruginosa on intraperitoneal injection of white mice

Origin of strains	Incubation period of culture	Untreated toxin Dose			Treated toxin by heating at 60°C for a period of					
					10 minutes		20 minutes		30 minutes	
		0.2 ml	0.5 ml	1 ml	0.5 ml	1 ml	0.5 ml	1 ml	0.5 ml	1 ml
Otitis media	16 hrs.	*0/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	48 hrs.	0/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	72 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	96 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	120 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
Urinary tract infection.	16 hrs.	0/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	48 hrs.	0/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	72 hrs.	1/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	96 hrs.	1/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	120 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
Wounds.	16 hrs.	0/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	48 hrs.	0/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	72 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	96 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	120 hrs.	2/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
Sterile broth (control)		0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

* N.B.: The numerator gives the number of dead mice and the denominator represents the number of inoculated animals.

Table (2)
The effect of Pseud.aeruginosa filtrate on the growth of gram positive and gram negative organisms using agar diffusion method

	Serial dilution of the filtrate								
	Unit	1/2	1/4	1/8	1/16	1/32	1/64	1/128	cont.
Staph.aureus.	+++	++	++	+	+	--	--	--	--
Staph.albus.	+++	+++	++	+	+	--	--	--	--
Staph.citreus.	+++	+++	++	++	++	--	--	--	--
Pneumococci	++	++	+	+	--	--	--	--	--
Strept.pyogenes	++	++	+	+	+	--	--	--	--
Bacillus.cereus	+++	+++	+++	++	++	++	+	--	--
Coryn.diphtheriae (mitis)	--	--	--	--	--	--	--	--	--
E.coli	++	+	+	--	--	--	--	--	--
Enteropathogenic E.coli	--	--	--	--	--	--	--	--	--
Prot.vulgaris	++	++	++	+	+	+	+	--	--
Klebs.pneumonia	--	--	--	--	--	--	--	--	--
Pseud.aeruginosa	++	+	+	--	--	--	--	--	--

Abbreviations:

+++, ++, + the extent of the inhibition zone.
-- = No effect.