

## Reciprocal Intermolecular antigenic competition between *E. coli* and *P. aeruginosa*

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### الخلاصة

كان قد لوحظ في الخمجات المخاطية الثنائية في السبيل البولي إن عيار الضد المتخصص بإشركيا القولون أعلى من العيار المتخصص بالزوائف الزنجارية . وكان تفسير هذه الملاحظة على أساس التنافس المستضدي . وبناءً على ذلك إختيرت حالة خمج سبيل بولي مختلط من إشركيا القولون والزوائف الزنجارية وتم التعرف على المسببين من خلال الوصف التقليدي ونظام API20E .

جرى تحضير مستضدات مقتولة بالحرارة لكل من إشركيا القولون والزوائف الزنجارية وحضرت خلطات بنسب متوازنة ونسب غير متوازنة بغية التمييز المتخصص في الأرنب . وحضر كذلك مستضد مقتول بالحرارة منفرد لكل من إشركيا القولون والزوائف الزنجارية بهدف تحضير أمصال متخصص بكل منهما على أفراد . أستخدم كل من التلازن في الأنبوبة القياسي و IL4 بطريقة ELIZA للتحري عن المناعة الخلطية والمناعة الخلوية ولمرحلة الإستجابة المناعية الثانوية .

لوحظ حصول إختزال في معدل عيارات الأضداد في المخلوط المستضدي مقارنة بعيارات الأضداد والمحفزة للمستضدات المنفردة وهذا يعني حصول تنافس مستضدي بين الجزينات معتمد على الخلايا اللمفية التائية . كانت التقديرات الكمية لـ IL4 في المصول المحضرة خاصة بالمخاليط المستضدية أعلى من التقديرات الكمية لـ IL4 في المصول المحضرة للمستضدات المنفردة . وهذه الزيادة تؤكد وجود دور لهذا السايبتوكاين في التنافس المستضدي وبناءً على ماتقدم كان التنافس المستضدي بين إشركيا القولون و الزوائف الزنجارية متبادل ، خلطي و خلوي ومن النوع بين الجزينات وأن دور IL4 في هذا التنافس يسجل لأول مرة .

### Abstract

It has been observed that in a dimicrobial mucosal urinary tract infection . The specific antibody titre of *E. coli* was higher than that of *P. aeruginosa*. The interpretation was based on antigenic competition . Hence a case of UTI with combined gram negative infection were chosen and diagnosed as *E. coli* and *P. aeruginosa* (Identified by classical tests and API 20E system) .

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Balanced and unbalanced heat killed *E. coli* and *P. aeruginosa* antigens in different combination proportions were made and used for specific immune priming of rabbits. Monotypic heat killed antigens were made and used for priming rabbits separately standard tube agglutination and ELISA IL-4 were used to match humoral and cellular arms respectively in the state of secondary immune responses. The specific antibody mean titres in combinations was reduced as compared to mean titres of monotypic sera which is an indication for antigenic competition . It was reciprocal intermolecular type (T cell dependent) . IL-4 determinations were increased as compared monotypic and control in the sera react against unbalanced combinations. Such increase indicate its role in antigenic competition .

Thus , the *E. coli-P. aeruginosa* antigenic competition is a reciprocal , humoral and cellular and intermolecular type. The role of IL-4 in antigenic competition is being reported for the first time .

## Introduction

Antigenic competition is a phenomenon of reduction of the specific antibody titres raised against certain antigen in the presence of the other in the combined antigen preparation or in the case of one following the other. It occurs between erythrocytes of different species origin , bacteria , viruses <sup>(1,2)</sup> It is intermolecular and intramolecular type <sup>(3,4)</sup> and can be of reciprocal and nonreciprocal types <sup>(5)</sup> .

Antigenic competition have several practical applications like : immuno diagnosis of dimicrobial infection <sup>(6)</sup> ; combined vaccine preparation <sup>(2,7)</sup> , AIDS vaccine preparations <sup>(8)</sup> and control of diabetes type I <sup>(9)</sup> .

Thus, the objective of the present work was to put forward an experimental lapin model for the antigenic competition between *E. coli* and *P. aeruginosa* that has been observed in this laboratory <sup>(6)</sup> . This objective was fulfilled through going through the following steps:

- 1- Selection of two particulate bacterins of *E. coli* and *P. aeruginosa* from a case of dimicrobial mucosal urinary tract infections.
- 2- Make balanced and non balanced mixtures of these bacteria.
- 3- Use the fixed multisite immunization protocols.
- 4- Match the phenomenon in the state of secondary immune response.
- 5- Assess the competition if any by standard tube agglutination and IL-4 determination .
- 6- Check them by humoral versus cellular antigenic competition .
- 7- Calculate the inhibition percent by the following equation :  
Inhibition percent = (Specific titre in combination / Specific titre in monotype ) X 100 % .
- 8- Check the effect of growth media on antigenic competition .
- 9- Observe the study menu Fig.1.

## Materials and Methods

### 1- Organisms:

Clinical isolates of *E. coli* and *P. aeruginosa* obtained from dimicrobial infection with these isolates. They were characterized by classical tests<sup>(10)</sup> and API 20E Kit (Biomérieux Co. France).

### 2- Monotypic (Single) and combination Antigens:

Monotypic heat killed whole cells antigens (HKWCA) were prepared using minimal media and complete media , from each of *E. coli* and *P. aeruginosa* (Item one) as in<sup>(11)</sup> and standardized to be matching to that of 10 IU WHO opacity tube . From these 10 IU HKWCA Five IU bacterin density were made for each *E. coli* and *P. aeruginosa* antigens. The five and 10 IU bacterin density preparations were used to prepare combinations of EC5 : PA5 IU , EC10 : PA5 IU , EC5 : PA10 IU in equal volume sense . These combinations were mixed prior to specific immune priming of rabbits.

### 3- Rabbits:

From a group of rabbits brought from local breed and proven to be free from ecto , haemo and endo parasites as well as their sera free from *E. coli* and *P. aeruginosa* antibodies . Ten groups each

of three, five groups for antigens prepared on complete media and five for those antigens prepared on minimal media . One group act as saline control. They were kept throughout immunization and adaptation ad libitum conditions .

**4- Immunization Protocols:**

Multisite injection protocol were applied. In which first , second and third weeks rabbits received 1 mL intramuscular and 1 mL subcutaneous at pelvic and subclavian areas. The fifth week animal left then bleed by cardiac puncture. ( Modified from AL-Shahery and Shnawa <sup>(12)</sup> ).

**5- Specific Immune sera:**

The immune sera rabbit blood from test and control rabbits were clotted and sera saved at – 18 C° in aliquots of 0.5 ml for each. They were designated as:

**Table A : Antigens and Antibodies Designations :**

Antigen	Designation	Immune sera	Notes
<i>E. coli</i> antigens in complete media	ECC	Anti ECC	Monotypic
<i>E. coli</i> antigens in minimal media	ECM	Anti ECM	Monotypic
<i>P. aeruginosa</i> in complete media	PAC	Anti PAC	Monotypic
<i>P. aeruginosa</i> in minimal media	PAM	Anti PAM	Monotypic
<i>E. coli</i> CM 10 IU + <i>P. aeruginosa</i> M 5	ECM 10 + PAM 5 (C1)	Anti C1	Unbalanced
<i>E. coli</i> 10 IU + <i>P. aeruginosa</i> C 5 IU	ECC 10 + PAC 5 (C2)	Anti C2	Unbalanced
<i>E. coli</i> M 5 IU + <i>P. aeruginosa</i> M 10 IU	ECM 5 + PAM 10 (C3)	Anti C3	Unbalanced
<i>E. coli</i> C 5 IU + <i>P. aeruginosa</i> C 10 IU	ECC 5 + PAC 10 (C4)	Anti C4	Unbalanced
<i>E. coli</i> M 5 IU+ <i>P. aeruginosa</i> M 5IU	ECM 5 + PAC 5 (C5)	Anti C5	Balanced
<i>E. coli</i> C 5IU + <i>P. aeruginosa</i> C 5IU	ECC 5 + PAC 5 (C6)	Anti C6	Balanced

**6- Immune Function Tests:**

Standard tube agglutination tests were made to monotypic antisera and those sera raised against bacterin combination against theirs on specific antisera <sup>(11)</sup>. IL-4 determinations were done to the same antisera using ELISA assay (Endogen Co. Canada).

## 7- Biometry:

Means and Pie. diagram calculations were made as in <sup>(13)</sup>. Titre inhibition percentage were estimated as in the following formula:

$$\text{Inhibition percentage} = (\text{Specific titre in combination} / \text{Specific titre in monotype}) \times 100 \%$$

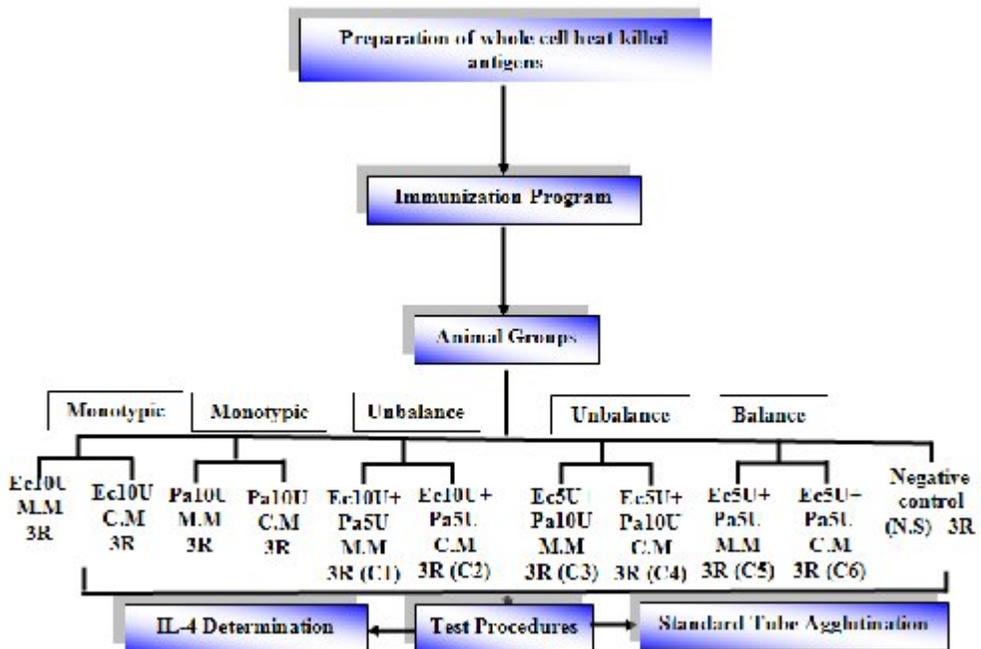


Fig.1: Study menu

Ec : E. coli antigens , Pa : P. aeruginosa antigens , C.M : Complete media , M.M : Minimal media , U : International unit , R : Rabbits , N.S :Normal saline , BHIB : Brain heart infusion broth , C1-C6 : To different antigen combinations .

## Results

### 1- Specific antibody titres as a marker for antigenic competition :

On titration of anti ECC immune sera with ECC antigen , the titre mean was 1280 , while when anti ECM with ECM antigen , the titre mean was 40. Likewise , anti PAC with PAC antigen the

titre mean was 480 and anti PAM with PAM antigen , the titre mean was 30 .

Titration of ECC and PAC as well as C2 , C4 and C6 with their specific immune sera anti C2 , anti C4 and anti C6 , titre means were showing an evident variations As ECC and PAC with anti C1 titration were showing titre means of 213 and 60 respectively . Meantime, reactions of ECC and PAC with anti C2 , the titre means were 266.6 and 80 accordingly .

In comparison , on titration of C1 , C3, C5 with anti C1 , anti C2 and anti C3 , the titre means were nil (Table1 and 2) .

The increase of ECM and PAM from five to ten international units of antigen density as titrated against anti C2 , anti C4 and anti C6 lead to increase in titre means to 133.3 for anti C2 and 426.6 with anti C4 . While it was decreasing with PAM antigen (Table 2).

When ECC and PAC antigens are increased from five and ten units densities as titrated anti C2 , anti C4 and anti C6 , the titre means were increased for ECC but not for PAC (Table 3) .

The titre inhibition percentages of *P. aeruginosa* in combinations , the balanced and unbalanced were ranging between 15.04 to 16.60% . While in the balanced and unbalanced for *E. coli* , they were ranging from 13.3 to 20.78% as showed in Pie diagrams (Fig2 and Fig3) .

#### **- IL4 as a marker for antigenic competition:**

Single *E. coli* as well as single *P. aeruginosa* antigen induce higher IL4 concentrations of immune sera than control sera . Combinations of C2, C4 and C6 were showing higher concentration than those of single specific immune sera for *E. coli* or *P. aeruginosa* and being highest than those of control sera (Table-4) .

IL4 concentrations induced by antigen originated from minimal media were lower than those originated from complete media .

**Table .1 : The effect of antigen quantity per unit volume grown on minimal media and complete media on titres means of monotypic antigen induced by antigens grown on minimal media .**

Test antigens	Titre means of antisera	
	Anti EC 10 From M.M	Anti PA 10 from M.M
EC10 from M.M**	33.3*	0
EC 10 from C.M***	80	0
PA 10 from M.M	0	33.3
PA 10 from C.M	0	80

\*Means of three readings .

\*\* Minimal media , \*\*\* Complete media .

**Table .2 : The effect of grown media and antigen density per unit volume on antigenic competition using sera raised against antigen combination prepared on complete media using titre reduction as a markers .**

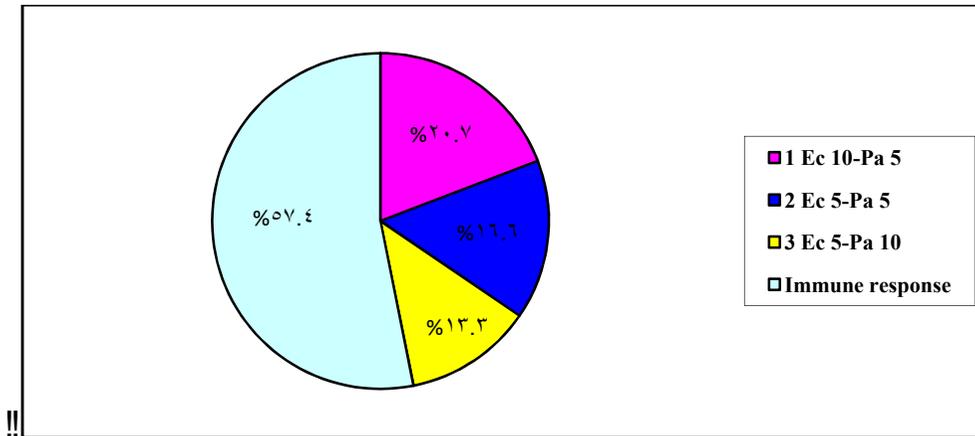
Test antigens prepared from minimal media	Titre means of antisera		
	Anti EC 5-PA 5 From C.M . C6	Anti EC 10-PA 5 from C.M. C2	Anti EC 5-PA 10 from M.M. C4
EC10	240	133.3	426.6
EC 5	33.3	66.6	173.3
PA 10	20	20	20
PA 5	46.6	15	80

**Table.3 : The specific antibody titres as a marker for the antigenic competition in balanced and unbalanced antigen combination of *E. coli* and *P. aeruginosa* grown on complete media .**

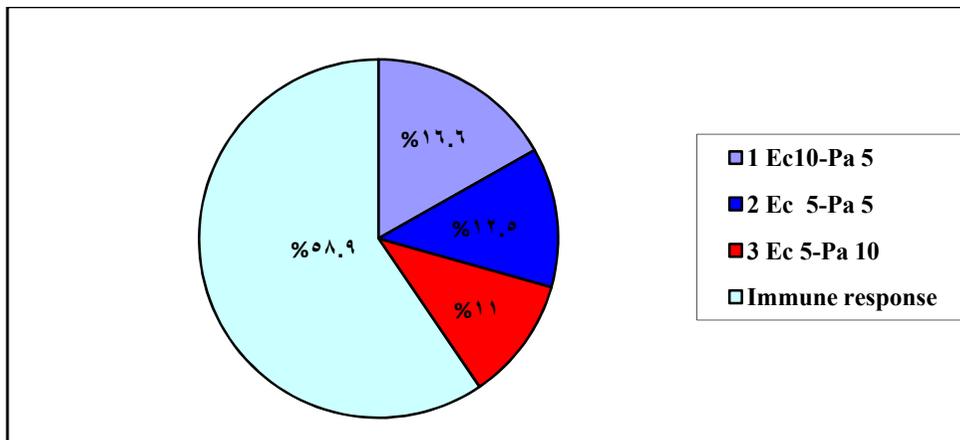
A- Balanced	Anti EC 5-PA 5 antibody titres (C6)			
	R1	R2	R3	Mean
EC5	320	160	160	213.3
PA 5	80	80	20	60
B1- Unbalanced	Anti EC 10-PA 5 antibody titres (C2)			
	R1	R2	R3	Mean
EC10	160	320	320	266.6
PA 5	80	80	80	80
B2- Unbalanced	Anti EC 5-PA 10 antibody titres (C4)			
	R1	R2	R3	Mean
EC5	320	160	40	170.3
PA 10	40	40	80	53.6

**Table .4 : IL-4 concentrations in pg / mL in sera raised against the monotypic and combinations of antigens grown on minimal media and complete media .**

Type of immunizing antigen(s)	IL-4 concentration in pg / mL	
	Complete media	Minimal media
EC 10	156.16	8.993
PA 10	102.108	6.722
EC 5-PA 5	23.074	0.363
EC 10-PA 5	203.853	82.81
EC 5-PA 10	174.324	14.667
Saline	59	59



**Fig.2 : Titre inhibition percentages for *E. coli*.**



**Fig.3 : Titre inhibition percentages for *P. aeruginosa*.**

## Discussion

Neither unilateral nor bilateral shared antigenicity between *E. coli* and *P. aeruginosa* (Table1) antigens were noted. Minimal media yielded antigens of low immunogenic potency, this can be due to limited numbers of the immunodominant epitopes of these antigens to which low immune recognition by Th<sub>2</sub> is ascribed and in turn, mediate low titre antibody response<sup>(1,14,15)</sup>.

In antigenic suspensions, as the antigenic density increased per unit volume their specific antibody titre is increased within the limits of immunotolerance<sup>(16)</sup>. Enriched media like Brain heart infusion agar (BHIA) supports growth of *E. coli* and *P. aeruginosa* as high density antigens raising up high titre antibody responses (Tables 1,2,3) in contrast to those grown on minimal media. Thus, it is a matter of quantity<sup>(15,16)</sup>. Thus, minimal media is not valid for studying antigenic competition. While enriched media is valuable for this purpose.

*E. coli*, *P. aeruginosa* heat killed whole cell antigen as single suspension induce higher specific antibody titres than antigen in antigenic combinations (C1-C6). This indicated reciprocal antigenic competition. However, *E. coli* specific ab titre mean is higher comparison to that of *P. aeruginosa*. This means that *E. coli* is main competitor in antigen combinations<sup>(2,3,5,6)</sup>.

IL4 concentration in sera raised against antigen combinations originated from complete media were higher than those of monotypic *E. coli* and *P. aeruginosa* immune sera. This may indicate the role of IL4 in antigenic competition a finding which is being reported for the first time<sup>(17,18)</sup>.

In an attempt to classify EC-PA antigenic competition one may state the criteria valid for such classification as:

- 1- Both of the antigens are not sharing particulate antigens for whole cells originated from different bacterial genera.
- 2- The prepared antigenic combinations which were balanced and unbalanced in sense of quantity per unit volume.
- 3- All rabbit group immunized with several runs of multisite injection protocols, in fixed manner. So it is of secondary immune response type.

- 4- Reduction in antibody level and increase of IL-4 concentration were the score points.
  - 5- Reduction of both anti EC, anti PA titres in combinations as compared to monotypic mean it is a reciprocal type.
  - 6- Although the anti *E. coli* titres reduced but still higher than anti *P. aeruginosa* in sera specific for combinations which that *E. coli* is main competitor .
  - 7- The antigenic competition is intermolecular type and T cell dependent.
- So what had been observed in this laboratory (Shnawa and Mehdi, 2004) , is being proved as a real case of EC-PA antigen competition and of intermolecular type .

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