Curing of plasmid contents of *Proteus* spp. isolated from urinary tract infections in AL- Diwaniyah city

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الخلاصة :

شملت الدراسة عزل وتشخيص بكتريا *Proteus* spp. من اخماج المسالك البولية من الأطفال المراجعين لمستشفى النسائية والأطفال في مدينة الديوانية، والتحري عن عوامل الضراوة المرتبطة بامراضية هذه البكتريا، والعمل على تحييد المحتوى البلازميدي باستخدام بعض المواد الكيميائية. شكلت عزلات بكتريا *Proteus* Spp. 16% والعائدة للنوعين *P. mirabilis* و *P. vulgaris* من المسببات الجرثومية لخمج المسالك البولية في الأطفال . تميزت عزلات هذه البكتريا بمجموعة من الخصائص الفسلجية التي تمثل عوامل ضراوة ترتبط بقدرة البكتريا على أحداث الخمج حيث أظهرت 80% من عزلاتها قدرة على تلازن كريات الدم الحمر، كما كانت هذه العزلات منتجة لأنزيم الهيمولايسين الذي يحلل الدم وبنسبة 60%، أما أنتاج أنزيمات البيتالاكتاميز فقد أظهرت 40% من عزلات هذه البكتريا قدرتها على إنتاج هذه الأنزيمات . ولم تظهر ألا 20% من عزلات بكتريا *Proteus* spp قدرتها على أنتاج البكتريوسين، كانت جميع هذه العزلات غير قادرة على تصنيع المحفظة.

عند اختبار مقاومة عزلات البكتريا *Proteus* spp تجاه ثمانية مضادات شائعة الاستخدام في علاج اخماج المسالك البولية، أظهرت عزلاتها مقاومات متفاوتة حسب طبيعة المضاد ونوع العزلة، فقد كانت جميع العزلات مقاومة للمضاد Nitrofurantion وبنسبة 100% بينما أظهرت أدنى مقاومة تجاه المضادين Nalidixic acid و Gentamicin بلغت 20% لكليهما. ظهر وجود الدنا البلازميدي في ثلاثة عزلات بكتيرية، لكن لوحظ عدم وجود علاقة بين عدد الحزم البلازميدية التي تمتلكها العزلة وعدد المضادات التي قاومتها . عوملت العزلة *P. mirabilis* التي تحتوي على أربع حزم بلازميدية بمادتي SDS وبروميد الاثيديوم في محاولة لتحييد محتواها البلازميدي، وقد تبين أن هذه العزلة فقدت قدرتها لإنتاج معظم عوامل الضراوة المسببة للمرض، كما أنها فقدت مقاومتها لبعض مضادات الحياة خاصة Nalidixic acid و Ampicillin و Chloralmphnicol وكانت مادة بروميد الاثيديوم هي الأكثر فعالية في عملية التحييد، إذ فقدت السلالة الناتجة جميع حزمها البلازميدية.

Abstract

This study included isolation and identification of *Proteus* spp . from urinary tract infections of Gynecology and padiatrics Hospital teaching in Diwaniyah cityand investigated the virulence factors connected with pathogenicity of this bacteria. It aims also at curing of plasmid contact by means of using some chemical materials. The bacteria *Proteus* spp. Isolates formed 16% which belong

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P. mirabilis and *P. vulgaris* from bacterial causes of urinary tract infection in children. The isolates of this bacteria has some physiological characteristics which represent virulence factors related to the ability of bacteria to make infection .

It seems that 80% from these isolates were capable to Haemagglutination of RBCs . These isolates produce also Haemolysine enzyme. It was found that percentage of production was 60% . As for B-lactamase enzyme production 40% of these bacteria isolates showed ability to produce his enzyme. Moreover, only 20% from *Proteus* isolates showed ability to produce bacteriocin production. All these isolates were not able to produce capsule production.

When testing the resistance of *Proteus* isolates toward 8 different commonly used antibiotics in treatment of urinary tract infection, results showed that the isolates have different resistance depending on the type of antibiotic and the strain of the isolates. All these isolates were completely resistance Nitrofurantion in 100% while the least resistance toward Nalidixic acid and Gentamicin reached 20% for both.

The results showed that there were DNA plasmid in 3 bacteria isolates but there was no relationship between the plasmid bands and resistance toward antibiotics. The P. mirabilis isolate which contained 4 plasmid bands was treated with SDS and Ethidiun bromide in an attempt to curing plasmid bands. The result showed that this isolate lost the ability to produce the majority of virulence factors that caused this disease and lost its resistance for som antibiotics especially Nalidix acid , Ampicillin and Choramphenicol. The ethidium bromide was the most effective in curing process since the resultant strain aest all plasmid bands.

Introduction:

Proteus spp. Represents the most common pathogens of urinary tract infections (UTIs) , especially P. mirabilis which come in second order after the Escherichia coli in (Collee et al., 1996). Proteus spp. Characterized by high ability to secrete urease and causes the stones formation in kidney and urinary bladder (Coker et al., 2000), in addition to that , it has a various virulence factor such an hemolysin, ß- lactamase and colonizing factors which aid it in the pathologenicity and imparement the defense lines of host (Wilson et al., 2002).

Also, this bacteria had awide range to resist antibiotics due to it has some of genetic factors that able it to resust these antibiotics, such as plasmids (Brook et al., 2001), which can transfer between genera and species of bacteria lead to prevalence the resistance by conjugation and transformation (Dionisio et al., 2002).

The plasmid curing may occur under some condition spontanously in nature such as long storage of isolates or lacking the factor that induce pits production plasmid and this process called spontaneous segregation (Barrow et al., 1987). Sometimes this lacking may takeplace by using some chemicals such as sulfate sodium Dodecyl (SDS). Acridine orange stain and ethidium bromide stain, where these chemicals act to induce a disturbance or damage of plasmid DNA which lead to prevent the auto-duplication process (Kulkarni & Kanekar, 1998). The aim of this study was to evaluate the efficiency of some chemicals in ability to plasmid curring of isolates Proteus spp. That isolated from UTIs.

Material and Methods :

- Sample collection: A total of (100) urine samples were collected from patients aged 3-10 years the teaching hospital of Gynecology and pediatrics of AL-Diwania city at the period 2005 to 2006. A mid-stream urine was collected in sterile test tubes after well washing the genital organs with sterile water for 3 times to avoid the contamination of samples (Lipsky, 1999).
- urine culture: A urine sample was cultured after centrifugation and by using a standard loop, 0.001 ml of urine was transferred to plates containing blood agar and macConkey's agar, then incubated aerobically at 37c°for 24-48 hrs.(Atlas, 1995).
- Isolates identification: A bacterial isolates were diagnosed using Api-20E system to identifies the G-ve bacteria and Api-Staph to identify the staphylococci according to instructions of supplied company
- Investigation of some virulence factors:

a- Bacterial capsule: The staining method with Indian ink was used according to Atlas (1995) to investigate the bacterial capsule.

b- Adherence factors: The method of Iwahi et al., (1983) was used to detect the adherence factors by using the RBC agglutination method.

c- Hemolysin production: The bacterial isolates were cultured on blood agar (5%), incubated at for 24 hrs to test there is ability to produce of hemolysin (Senior & Hughes, 1987).

d- B- lactamase production: The quick iodine method described by WHO (1978) to investigate the B-lactamase was used depending on the quick change in color from dark blue to white after less than one min. from reagent addition.

e- Bacteriocin production: The method described in AL-Kasab & AL-Klafaji (1992) was followed to detect the strains that capable to bacteriocin production where the positive result was recorded when

the inhibition zone was formed a round the agar disc that containing the producer stain in brain –heart infusion media.

f- Resistance to antibiotics: the method of Bauer and Kirby that described by Hindeler (1998) was followed using the antibiotics: Amoxicillin, Nitrofurantoin, Nalidixic acid, Chloramphenicol, Gentamicin, Rifapicin, Ampicillin and Lincomycin to detect the ability of bacterial isolates to resist the used antibiotic an Muller-Hinton media. Results were recorded according to measure the diameter of zone inhibition of growth according to NCCLs (2002). A standard strain of E. coli µµ 294 was used for comparison.

❖ Detection of plasmid content:
The boiling method described in Sambrook et al., (1989) was used to segregate the plasmid in bacterial isolates then the Detection of isolated plasmids was done on Agarose gel using Electrophoresis.

❖ Plasmid curring: The Ethidium bromide stain and sodium dodecyle sulfate were used for plasmid curring of proteus spp. according to Sonstein & Baldwin, (1972) where the presence of growth on antibiotics plates after this method indicate that bacteria was loosen the trait of resistance to its antibiotic. Then the plasmid DNA of producer stains resulted from curring process was extracted and electrophoresis on agarose gel to detect the lacking plasmid bands.

Result and Discussion

Table (1) shows the types of bacterial pathogens isolated in this study from patients of UTIs. Results showed that the E. coli was the most common causes in children, where isolated in ratio(56%) and this agreed with study of AL-Abbas (2002), and this may due to highly occurrence of this bacteria in stool which made it a source of Endogenic infection (Chakraborty, 1996). While the Protus spp. came in second order among bacterial pathogens in a ratio 16% for both species (P. mirabilis and P. vugaris). This result was agreed with study of AL-Dabagh (1998). The percent of other bacteria isolated in this study were 11%, 7%, 7%, and 3% for Staphylococcus aureus, Enterobacter spp., Psendomanas aeraginosa and Citrobacter freurdii , respectively. This is in agreement with other studies hat stated that these causative agent play a role in the UTI pathogenicity (Montgomery et al., 1998).

Table (1): Nambers and percent of bacterial causes that isolated from UTLs patients

Bacterial species	No. isolates	percentage
Escherichia coli	17	%56
Proteus mirabilis	4	%13
Proteus vulgaris	1	%3
Enterobacter spp	2	%7
Pseudomonas aeruginosa	2	%7
Citrobacter freundii	1	%3
Staphylococcus aureus	3	%11

In a view of virulence factors (Table 2), results showed that 80% of *Proteus* isolates had an ability to agglutinate the RBC. This is may due to it has the adhesion pili that aid this bacteria to adhesion on the cell surfaces (Mcnamara & Donnerberg-Mis, 2000). Also, 60% of isolates revealed capability to produce the hemolycin which support its pathogenicity of UTIs through its toxic activity on WBC and fibroblast cells in human (Abedel-Wahed *et al*., 2001), whereas the 60% 0f isolates showed an activity of ß-lactamase production, and this was no in agreement with AL-Neimi study which revealed that the ratio was 29.5%.

The bacteriocin production results revealed that only one isolate (20%) produced bacterocin, and this result ageed with study of AL-Mousoy (2006).

Table (2): Certain of virulance factors associated with *Proteus spp.* Pathogenicity isolated from UTLs patients.

Bacteriocin production	B-lactomase productio	Hamolysin production	Haemooagglatination	Capsule prodution	Virulence factors / Bacterial Isolates	No. of isolates
-	+	+	+**	-*	*P. mirabilis*	1
-	-	+	+	-	*P. mirabilis*	2
+	-	+	+	-	*P. mirabilis*	3
-	+	-	+	-	*P. mirabilis*	4
-	-	-	-	-	*P. vulgaris*	5
%20	%40	%60	%80	%0	النسب المئوية	

***- negative result (no producer)**

***+ positive result (producer)**

The antibiotics susceptibity of *Proteus* spp. Against 8 antibiotics that resistance of this bacteria to tested antibiotics (Table 3), where this resistance ranged from 20% -100% and this results agree with AL-Dadagh (1998) and AL-Abasi (2000).

The Electrophoresis results showed the presence of plasmid DNA bands in three isolates, except 2 isolates had no any plasmid in the DAN plasmid samples (Table 3. Fig. 1). There is no correlation was conducted between the no of plasmid bands of isolates and the no. of antibiotics that its resistance and this may due to that this trait of resistance present on chromosom (Nasuda *et al*., 2000).

Table (3): Resistance of bacterial isolates proteus spp to antibiotics.

No. of plasmids	Antibiotics / Bacterial isolates	Gentamicin	Chloramphenicol	Rifampicin	Lincomycin	Amoxicillin	Ampicillin	Nitrofurantoin	Nalidixic acid	No. of plasmids
1	*P. mirabilis*	S	S	S	S	R	R	R**	S*	none
2	*P. mirabilis*	R	S	S	R	R	R	R	S	1
3	*P. mirabilis*	S	R	R	R	S	R	R	R	4
4	*P. mirabilis*	S	R	R	R	R	R	R	S	2
5	*P. vulgaris*	S	R	S	S	R	S	R	S	none
Percentage		%20	%60	%40	%60	%80	%80	%100	%20	

S* Sensitive
R** Resistance

Table (4) shows that the high concentration of SDS allowed the bacterial growth was 7% and 100% using Ethidium bromide.

Table (5) indicates that colonies of *Proteus* spp. Treated with SDS are loosed the resistance to Ampicillin and the ability to hemolysin production, and when detection the plasmid content of resulted stain it noticed had one plasmid band, this is may indicate that the traits which is not lack in the resulted strain may be coded from inhibitor plasmid bands or by genes present on chromosome. While, the colonies treated with ethidium bromide showed a lack of antibiotics resistance to Nalidixic acid, Ampicillin and Chloramphenicol, in addition to lack of bacteriocin and hemolysin production. This is indicate that the lack of resistance and virulence factors production encoded from genes present on lacked plasmids. These results emphasized that both SDS and ethidium bromide had an activity in the plasmid curring (Carlton & Brown, 1981).

Table(4): Effect of SDS and ethidium bromide in growth of *P. mirabilis* isolate (3) on lauria broth.

Growth appearance	Ethidium bromide Mg/ ml	Growth appearance	SDS (٪)
+++	0	+++	%0
+	50	+++	%0.5
±	100	++	%1
_	200	++	%2
_	400	++	%3
_	600	+	%4
_	800	+	%5
_	1000	+	%6
		±	%7
		_	%8
		_	%9
		_	%10

No growth ++ Good growth _ Weak growth ±

Moderate growth + V. good gowth+++

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