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Genetic Relationship Between Heavy Metals Resistance and β- Lactamase Production In *E. Coli* and *Staphylococcus Aureus*

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Abstract

This study is a trail to know if the genes controlling some of heavy metals resistance (lead, zinc, cadmium, cromium) in two types of pathogenic bacteria *E. coli* as gram negative bacteria and *S. aureus* as gram positive bacteria, present on the β-lactamase plasmid. Ten isolates of each bacterial types which produced β-lactamase enzyme, were cultivated in the presence of acridine orange. The growing in the presence of acridine orange resulted in loss of the β-lactamase genes in *S. aureus* and *E. coli*, and loss of the heavy metals resistance in *S. aureus*, while the resistance of *E. coli* against heavy metals still without any change. The results indicate that the genes for heavy metals resistance exist on the β-lactamase plasmid in *S. aureus* only, while in *E. coli* the genes that controlling heavy metals resistance are not on β-lactamase plasmid.

Key words: heavy metals resistance, betalactamase and resistance, heavy metals and betalactamase.

Introduction

Most of pathogenic bacteria produce enzymes that inhibit the antibiotics as a resistance mechanism, one of these enzymes is the β-lactamase. β-lactamase enzymes of gram-positive bacteria such as *staphylococcus aureus* differ from that in gram-negative such as *E. coli* in an important point, they are an extra cellular enzymes in the first group, in the other words bacteria secrete the enzyme to the culture media where that the antibiotic lyse take, while in the gram-negative they are cell-bound enzymes, i.e. the β-lactamase antibiotics lyses inside of bacteria such as β-lactamase of *E. coli* [1].

Bacteria resistant to antibiotics and other antibacterial agents is an increasing problem in today's society. Products such as disinfectants, sterilants and heavy metals used in industry and in household products are, along with antibiotics, creating a selective pressure in the environment that lead to the mutations in microorganisms that will allow them better to survive and multiply [2].

In this study we will try to know if the gene controlling heavy metals resistance in two types of pathogenic bacteria presents on the β-lactamase plasmid.

Materials and Methods

1-Culture media: blood agar, MacConky agar, manitol salt agar, eosin methyline blue(EMB), nutrient agar, brain heart infusion broth, muller hinton agar

2- Solution of standard rapid iodometric method for β-lactamase detecting

a- 1% starch solution
b- Iodin solution
c- Penicillin G solution

The solution were prepared according to (Perret,1994) [3].

3- Heavy metals solutions

Salts of heavy metals were used to prepare certain heavy metals concentrations.

a- Lead nitrate $Pb(No_3)_2$.
b- Cadmium chloride CdCl.
c- Zinc chloride $ZnCl_2$.
d- Chromium Oxide CrO_3.

These salts were used to prepare lead, cadmium, zinc, chromium solutions respectively.

4- Acridin orange

1-Sample collection and identification

25 nasal swabbed samples were obtained from young adults to isolates *S. aureus* bacteria.

30 samples were obtained from the mid stream urine from patients with urinary tract infections to isolates *E. coli* bacteria.

The samples were cultured on the blood agar and macconky agar as a differential medium then incubated at 37ċ for 24 hr. Bacterial isolates identification included: microscopic exam, cultural, morphological, and biochemical characteristics of each isolate [4,5].

2- β-lactamase detection (rapid iodometric method)

Twenty-four hours bacterial growth on nutrient agar was prepared for each isolates then 4-5 colonies were transported to an appendrof tubes containing 100 µl of penicillin G solution and incubated at 37ċ for 30 minutes. Then 50 µl starch solution was added to each tube and mixed with the other content. After that 20 µl of iodin solution was added, a dark blue color will appear immediately due to starch-iodin interaction.

The positive result was recorded if the blue color change to white within one minute [6].

3- Detection of bacterial isolates resistance against some heavy metals

The resistance of bacterial isolates against heavy metals was detected by adding 5mM of each heavy metals ($Pb(No_3)_2$, Cdcl, $Zncl_2$, Cro_3) separately to muller hinton medium after cooling to 45-50 ċ. The mixture was mixed immediately after heavy metals adding and then seeded on plates and kept at 4ċ for 24 hr. after incubation, 5µl of all bacterial isolates were spread on plates which contain 5mM of each heavy metals. The plates were left in room temperature, to dry, then incubated at 37ċ for 18-24 hr [7]. The bacteria that showing good growth on the medium with 5mM of the used heavy metals was considered as a heavy metal resistant isolates.

4- Curing of the β-lactamase plasmid state

Small inocula of β-lactamase positive strains for each bacterial isolates were inculate in nutrient broth containing 12.5 µg/ml of acridin orange for 24 hr at 37 ċ [8].

Mutants that lost the β-lactamase genes were detected by streaking appropriate dilutions of the nutrient broth culture on to nutrient agar medium. After incubation for 24 hr the colonies were sufficiently large to be tested by the rapid iodometric method. The isolates that had lost the capacity to produce β-lactamase were tested for resistance to ($Pb(No_3)_2$,Cdcl, $Zncl_2$ and Cro_3).

Results and Discussion

1-β-lactamase production (rapid iodometric method)

Table (1) shows the results of β-lactamase production for *staphylococcus aureus* and *E. coli* isolates. The results illustrate that all isolates of two bacterial types were producing β-lactamase enzyme except two isolates of *E. coli* did not show production of β-lactamase, because in some gram-negative bacteria the quantity of β-lactamase enzyme is too little to be detected by using rapid iodometric method [6].

2- Bacterial resistance against heavy metals

Table (2) shows the results of bacterial resistance to some heavy metals. The results illustrate that most of isolates were resistant to all heavy metals, because the bacteria have evolved several types of resistance mechanisms. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell and reduction of the heavy metal ions to a less toxic state [8,9].

3- β-lactamase production and heavy metals resistance of staphylococcus aureus and E. coli in the presence of acridin orange

The results shows that all the isolates of *staphylococcus aureus* and *E. coli* lost the ability of β-lactamase production while the resistance of *S. aureus* to the heavy metals changed and the bacteria became sensitive to the used heavy metals. In *E. coli* isolates the resistance to the heavy metals stilled without any change.

The growth in the presence of acridin orange resulted in lossing of β-lactamase production and heavy metals resistance in *S. aureus* because the β-lactamase plasmid in *S. aureus* also carries genes determining resistance to several metal ions. For example, in cadmium resistant S. aureus, two resistant determinants were found on penicillinase-plasmid pI258, called cadA (drives cadmium ion across the membrane by using energy from ATP hydrolysis to confer cadmium resistance) and cadB [10].

In the case of *E. coli* isolates the growth of bacteria in the presence of acridin orange resulted in losing of the β-lactamase production while the resistance of *E. coli* isolates to the heavy metals still without any change, i.e the genes controlling resistance to these heavy metals are not on the β-lactamase plasmid but existing on the other determinants like zntA chromosome which responsible for transporting Zn+2 and Cd+2 in the presence of ATP in E. coli . So, the resistance to these heavy metals was not always associated with β-lactamase production in *E. coli* [11] [12].

Conclusion

Resistant determinants to the heavy metals are carrying on the β-lactemase plasmid in S. aureus only. While in E. coli the resistant determinants are carrying on chromosomal genes.

References

1. Mediaros, A.A. (1997). Evolution and dissemination of β-lactamase accelerated by generations of β-lactamase. Clini. Infec. Dis. 24: 519-525.
2. Baquero, F., Negri, MC., Morosini, MI., and Blazquez, J. (1998). Antibiotics selective environments. Clinical Infection Diseases. 27: S5-S11.
3. Perret, C.J. (1999). Nature. Iodometric assay of penicillinase. 174:1012.
4. Holt, J.J, Krieg, N.R., Seneath, B.H., Staley, J.T., and Williams, S.T. (1994). Bergys manual determinative bacteriology 9th edition. William & Wilken. Baltimore. P: 175-248.
5. Jawetz, E.M., Adelberg, E.A.(1998). Medical Microbiology, 25th edition. Appleton and L ange. USA.
6. WHO. (2002). Techniques for the detection of β-lactamase producing strains of *Neisseria gonorrhoeae*.616: 137-143.

7. Nies, D.H. (1999). Microbial heavy metal resistance. Appl. Microbiol Biotechnol. 51: 730-750.
8. Jack N. Baldwin, Robert H. Strickland, and Marilyn F. Cox. (2000). β-lactamase genes in Staphylococcus epidermidis. Applied microbiology. 18: 628-630.
9. Bouanchaud, D.H., M.R. Scavizzi, and Y.A. Chabbert. (1999). Elimination by ethidium bromide of antibiotic resistance in enterobacter and staphylococci. J. Gen. Microbiol. 54: 417-425.
10. Butaye, P., Cloeckaert, A., and Schwarz, S. (2003). Mobile genes coding for efflux mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. Int J AntimicrobAgents. 22: 205-210.
11. Otitoloju, A. A.; Rogers, G. B.; Bury, N. R. and Bruce, K. D. (2009). Chromosomal genes conferring tolerance to heavy metal (Ag) toxicity. The Enviromeny. Vol 29: 85-92.
12. Piddock, L. V. (2006). Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin. Microbiol. Rev. 19: 382-402

Table (1): β-lactamase production of *staphylococcus aureus* and *E. coli*

s. aureus	β-lactamase production	*E. coli*	β-lactamase production
S1	+	E1	+
S2	+	E2	*
S3	+	E3	+
S4	+	E4	+
S5	+	E5	+
S6	+	E6	+
S7	+	E7	*
S8	+	E8	+
S9	+	E9	+
S10	+	E10	+

Key:(+): positive results, (*) : unknown results

Table (2): the results of S. *aureus* resistance against four types of heavy metals

S. aureus	$Pb(No_3)_2$	Cdcl	$Zncl_2$	Cro_3
S1	R	*	R	R
S2	*	R	R	R
S3	R	R	*	R
S4	R	R	R	*
S5	R	R	R	*
S6	R	R	R	R
S7	R	R	R	R
S8	*	R	R	R
S9	R	R	R	R
S10	R	R	R	R

Key :R= resistant (good growth), (*) =few growth

Table (3):the results of *E. coli* resistance against four types of heavy metals

E. coli	$Pb(No_3)_2$	Cdcl	$Zncl_2$	Cro_3
E1	R	*	R	R
E2	*	R	R	R
E3	*	R	R	R
E4	R	R	R	R
E5	*	R	R	R
E6	R	R	R	R
E7	R	R	R	R
E8	*	R	R	R
E9	R	R	R	R
E10	R	R	R	R

Key :R= resistant (good growth), (*) =few growth

Table (4): the results of β-lactamase production and heavy metal resistance of *S. aureus* in the presenceof acridin orange

S. aureus	β-lactamase production	$Pb(No_3)_2$	Cdcl	$Zncl_2$	Cro_3
S1	-	*	*	*	*
S2	-	*	*	*	*
S3	-	*	*	*	*
S4	-	*	*	*	*
S5	-	*	*	*	*
S6	-	*	*	*	*
S7	-	*	*	*	*
S8	-	*	*	*	*
S9	-	*	*	*	*
S10	-	*	*	*	*

Key :(-)= no β-lactamase production , (*) = no growth of bacterial colonies

Table (5): the results of β-lactamase production and heavy metal resistance of *E. coli* in the presence of acridin orange

E. coli	β-lactamase production	$Pb(No_3)_2$	Cdcl	$Zncl_2$	Cro_3
E1	-	R	**	R	R
E2	*	**	R	R	R
E3	-	**	R	R	R
E4	-	R	R	R	R
E5	-	**	R	R	R
E6	-	R	R	R	R
E7	*	R	R	R	R
E8	-	R	R	R	R
E9	-	R	R	R	R
E10	-	R	R	R	R

Key :(*) = unknown results , (**) = few growth of bacterial colonies

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العلاقة الوراثية بين مقاومة المعادن الثقيلة وإنتاج إنزيم البيتالاكتاميز في بكتريا *E. coli* و *Staphylococcus aureus*

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استلم البحث في :26 نيسان 2011 قبل البحث في :11 تشرين الاول 2011

الخلاصة

هذه الدراسة هي محاولة لمعرفة فيما اذا كان الجين المسيطر على المقاومة لبعض المعادن الثقيلة الرصاص، الزنك، الكادميوم و الكروم في نوعين من البكتريا المرضية (*E. coli*) بكتريا سالبة لصبغة كرام و (*S. aureus*) بكتريا موجبة لصبغة كرام، موجود على البيتالاكتاميز بلازميد. عشر عزلات من كل نوع بكتيري والمنتجة للبيتالاكتاميز قد تم تنميتها بوجود ال acridine orange. التنمية بوجود ال acridine orange نتج عنها فقدان للجينات المسؤولة عن انتاج البيتالاكتاميز انزيم في كلا" من ال *S.aureus* وال *E. coli* وفقدان المقاومة للمعادن الثقيلة في ال *S. aureus* فقط ، بينما مقاومة بكتريا ال *E. coli* للمعادن الثقيلة بقيت نفسها دون أي تغيير.

هذه النتائج تدل على أن الجينات المسؤولة عن المقاومة للمعادن الثقيلة موجودة على البيتالاكتاميز بلازميد في بكتريا ال *S. aureus* فقط ، بينما في بكتريا ال *E. coli* فالجينات المسيطرة على مقاومة البكتريا للمعادن الثقيلة فهي غير موجودة على نفس البلازميد المسؤول عن انتاج البيتلاكتاميز انزيم.

الكلمات المفتاحية: مقاومة المعادن الثقيلة ، إنزيم البيتالاكتاميز، المعادن الثقيلة وإنزيم البيتالاكتاميز