The relationship between heavy metals resistance and β- lactamase production in some types of pathogenic bacteria

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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 (***P .aeruginosa***) 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 ***P. aeruginosa***, and loss of the heavy metals resistance in ***S. aureus***, while the resistance of ***P. aeruginosa*** against heavy metals still without any change.

The results indicated that the genes for heavy metals resistance existed on the β-lactamase plasmid in ***S. aureus*** only, while in ***P. aeruginosa*** the genes that controlling heavy metals resistance were not found on β-lactamase plasmid.

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 are extracellular enzymes in gram-positive bacteria such as *staphylococcus aureus* , in 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 *P. aeruginosa* (1).

Bacteria resistance 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).

The gene controlling heavy metals resistance in two types of pathogenic bacteria is present on the β-lactamase plasmid or not is our aim of this research.

Materials and methods

Culture media:

Blood agar, MacConky agar, manitol salt agar, eosin methyline blue(EMB), nutrient agar, brain heart infusion broth, Muller Hinton agar.

Solution of standard rapid iodometric method for β-lactamase detecting

- 1% starch solution.
- Iodine solution.
- Penicillin G solution.

The solutions were prepared according to Perret,1994 (3).

Heavy metals solutions

Salts of heavy metals Lead nitrate ,Cadmium chloride , Zinc chloride , Chromium oxide were used to prepare certain heavy metals concentrations.

Acridin orange

Sample collection and identification

25 nasal swabed 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 *P. aeruginosa* bacteria.

The samples were cultured on the blood agar and MacConky agar as a differential medium and then incubated at 37◦C for 24 hr. Bacterial isolates identification included: microscopic exam, cultural, morphological, and biochemical characteristics of each isolates(4,5).

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 Eppendorf tubes containing 100 µl of penicillin G solution and incubated at 37◦C for 30 min. and then 50 µl starch solution was added to each tube and mixed with the other content. After that 20 µl of iodine solution was added, a dark blue color will appear immediately due to starch-iodine interaction.

The positive result was recorded if the blue color change to white within 1 minute (6).

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◦C. The mixture was mixed immediately after heavy metals were added and then were seeded on plates and kept at 4◦C 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,and then incubated at 37◦C for 18-24 hr (7). The bacteria that showed good growth on the medium with 5mM of the used heavy metals

was considered as a heavy metal resistant isolates.

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

β-lactamase production (rapid iodometric method)

Table (1) showed the results of β-lactamase production for *staphylococcus aureus* and *P.aeruginos* isolates. The results illustrated that all isolates of two bacterial types were producing β-lactamase enzyme except two isolates of *P.aeruginosa* 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).

Bacterial resistance against heavy metals

Table (2) showed the results of bacterial resistance to some heavy metals. The results illustrated that most of isolates were resistant to all heavy metals, because the bacteria have evolved several types of resistance mechanisms. These mechanisms were included 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).

β-lactamase production and heavy metals resistance of *Staphylococcus aureus* and *P. aeruginosa* in the presence of acridin orange

The results showed that all the isolates of *staphylococcus aureus* and *P. aeruginosa* 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 *P. aeruginosa* isolates the resistance to the heavy metals stilled without any change.

The growth in the presence of acridin orange was 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 determing resistance to several metal ions(10,11).

In the case of *P. aeruginosa* isolates the growth of bacteria in the presence of acridin orange resulted in losing of the β-lactamase production while the resistance of *P.aeruginosa* isolates to the heavy metals were still without any change.

The results indicated that the genes controlling resistance to these heavy metals were not on the β-lactamase plasmid (12,13). So, the resistance to these heavy metals was not always associated with β-lactamase production in *P.aeruginosa*.

Tables

Table (1) β-lactamase production of *staphylococcus aureus* and *P. aeruginosa*

s. aureus	β-lactamase production	*P. aeruginosa*	β-lactamase production
S1	+	P1	+
S2	+	P2	*
S3	+	P3	+
S4	+	P4	+
S5	+	P5	+
S6	+	P6	+
S7	+	P7	*
S8	+	p8	+
S9	+	P9	+
S10	+	P10	+

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 *P. aeruginosa* resistance against four types of heavy metals

P.aeruginosa	$Pb(NO_3)_2$	CdCl	$ZnCl_2$	CrO_3
P1	R	*	R	R
P2	*	R	R	R
P3	*	R	R	R
P4	R	R	R	R
P5	*	R	R	R
P6	R	R	R	R
P7	R	R	R	R
P8	*	R	R	R
P9	R	R	R	R
P10	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 *P. aeruginosa* in the presence of acridin orange

P. aeruginosa	β-lactamase production	$Pb(NO_3)_2$	CdCl	$ZnCl_2$	CrO_3
P1	-	R	**	R	R
P2	*	**	R	R	R
P3	-	**	R	R	R
P4	-	R	R	R	R
P5	-	**	R	R	R
P6	-	R	R	R	R
P7	*	R	R	R	R
P8	-	R	R	R	R
P9	-	R	R	R	R
P10	-	R	R	R	R

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

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الخلاصة

تهدف الدراسة لمعرفة فيما اذا كان الجين المسيطر على المقاومة لبعض المعادن الثقيلة (الرصاص، الزنك، الكادميوم، الكروم) في نوعين من البكتريا المرضية (***P.aeruginosa***) كبكتريا سالبة لصبغة كرام و (***S. aureus***) كبكتريا موجبة لصبغة كرام، موجود على البيتالاكتاميز بلازميد.

تمت تنمية عشرعزلات من كل نوع بكتيري والمنتجة للبيتالاكتاميز 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 ***P.aerugi nosa*** وفقدان المقاومة للمعادن الثقيلة في ال ***S. aureus*** فقط ، بينما مقاومة بكتريا ال ***P.aeruginosa*** للمعادن الثقيلة بقيت نفسها دون أي تغيير.

تدل هذه النتائج على أن الجينات المسؤولة عن المقاومة للمعادن الثقيلة موجودة على البيتالاكتاميز بلازميد في بكتريا ال ***S. aureus***فقط، بينما تكون الجينات المسيطرة على مقاومة البكتريا للمعادن الثقيلة في بكتريا ال ***P.aeruginosa***

غير موجودة على البلازميد ذاته المسؤول عن انتاج البيتلاكتاميز انزيم .