



The relationship of plasmid content to the resistance of antibiotics and heavy metals in bacteria isolated from different sources

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Abstract

The present study aimed to finding the relationship between plasmid content with resistance of antibiotic and heavy metal salts in *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated of pathogenic and environmental sources.

The results of antibiotics sensitivity tests showed that all studied isolates had multidrug resistance to these antibiotics and all isolates showed absolute resistance to Amoxicillin, Ampicillin and Streptomycin. The isolates varied in their tolerance to different concentrations of studied heavy metals salts, some isolates showed absolute resistance (concentration 1.6 mg / ml) towards most of these studied minerals (5 out of 6 minerals) and all isolates had absolute resistance to Lead acetate (CH₃CO₂)₂ Pb. The isolates were also varied in their plasmid content (1-2 bundles) depending on their source of isolation.

It found there was no clear relationship between the plasmid content of these isolates and their resistance to some antibiotics and heavy metal salts, that reflects integrative role of plasmids with other factors such as resistance genes carried on chromosome in the emergence of such resistance.

Keywords: Plasmid content, antibiotics, heavy metals

Introduction

Bacteria resistance to Antibiotic poses a serious threat to public health, bacterial resistance to antibiotics is the ability of bacteria to resist the lethal or inhibitory effect of antibiotics, various factors contribute to the increase and spread of this resistance, some of which are the indiscriminate use of antibiotics and the application of antibiotics in agriculture, animal husbandry, and others [2, 17]. Antibiotic resistance can be natural or acquired, and natural resistance is found in all strains of certain species and is naturally determined by genes generally found on the chromosome that are genetically transmitted to subsequent generations by vertical transmission, while acquired resistance depends on changing (modulating) the genetic material through mutations or possessing resistance genes carried on genetic elements (Like plasmids) that are transmitted between bacteria by horizontal transmission [14].

In recent years, the spread and pollution of the environment with heavy metals has become one of the most serious environmental problems, throughout the world by industrial progress by human activities [13]. High concentrations of mineral ions can either inhibit the bacterial community by inhibition of various metabolic activities or that microorganisms can develop resistance or tolerance to elevated levels of minerals, microorganisms may have resistance mechanisms to deal with high concentrations of heavy metals by specialized systems or genes resistant are located on chromosomes, plasmids, and other genetic elements [19].

Heavy metals resistance is often associated with antibiotic resistance. Several studies have shown that antibiotic resistant strains may exhibit common resistance to heavy metals in the environment [3, 8] Plasmids are one of the most important genetic factors that carry resistance genes towards antibiotics or heavy metals, and their presence and spread in

the bacterial community play an important role in the transmission and development of resistance character in this community [17].

The current study aims to find the relationship between the plasmid content to antibiotic and heavy metal resistance in two types of bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from various sources (pathogenic and environmental).

Materials and Methods

Bacterial isolates

The bacterial isolates were obtained from Biology Dept. / College of Science / University of Mosul, three of which (Pathogenic isolate, soil isolate and water isolate) belonging to *Pseudomonas aeruginosa* and three of them (Pathogenic isolate, soil isolate and water isolate) belonging to *Staphylococcus aureus*. The diagnosis of these isolates has been confirmed by appropriate agricultural, biochemical tests based on [4, 16]

Antibiotics

Eight antibiotics tablet were used (Bioanalyse/Turkish): Amoxicillin (AMC) 20µg, Ampicillin (Am) 25 µg, Cephalothin (KS) 30 µg, Ciprofloxacin (CIP) 10 µg, Erythromycin (E) 10µg, Rifampin (RA) 5µg, Streptomycin (S)25 µg, Trimethoprim (TMP) 30 µg.

Heavy metals

Six types of heavy metals were used as a salts obtained from Chemistry Dept. / College of Science / University of Mosul: Aqueous tin chloride SnCl₂.2H₂O, Aqueous zinc sulfate ZnSO₄.7 H₂O, Silver sulfate Ag₂SO₄, Aqueous copper chloride CuCl₂.6 H₂O, Aqueous cobalt chloride CoCl₂.6 H₂O, Lead acetate (CH₃CO₂)₂ Pb.

Antibiotic sensitivity test

The test was conducted using the [5] method, as the bacterial suspensions were prepared in the Tryptic soy broth medium to give turbidity equivalent to the first tube of McFarland tubes, Equivalent to 10^8 cells/ ml. Muller-Hinton agar medium were inoculated with bacterial suspensions using sterile cotton swabs and left for 15 minutes for impregnation, then the antibiotic tablets were placed on the surface of medium. The plates were incubated at $(35)^\circ\text{C}$ for [16-18] hours, and after the incubation the diameter of inhibition zone was measured [20, 23].

Heavy Metal Sensitivity Test

The sensitivity of bacterial isolates to studied heavy metals was tested according to (6) with some modification. Various concentrations of heavy metals were prepared, ranging from (0.02, 0.1, 0.2, 0.4, 0.8, 1.0, 1.6 mg / ml) for each metal [11] sterilized using membrane filters with a diameter of 0.45 micron. Bacterial suspension was prepared and Muller-Hinton agar inoculated with bacterial suspensions as mentioned above (in Antibiotic sensitivity test), then tablets of (What Man No.1) filter paper with 6 mm diameter saturated with different concentrations of minerals by submerging them in this solutions. The tablets were fixed with sterile forceps on the surface of inoculated plates, the plates were incubated at a temperature of 37°C for 24 hours. Inhibition zones around the saturated tablets were observed.

Plasmid extraction

The lysozyme-based plasmid extraction method described by (1) was used. Electrophoresis of the plasmids was carried out in the agarose gel. The gel was examined by a UV

source, then the results was photographed using a digital camera.

Results and discussion

The sensitivity test for antibiotics was conducted for all studied isolates, the results in Table [1] showed in general the prevalence of antibiotic resistance between these isolates depending on the source of their isolation, and all isolates had multidrug resistance to studied antibiotics which confirms that the two types of bacteria have different resistance mechanisms that was indicated by the various studies [9, 14, 17]. Concerning *P. aeruginosa* isolates, it is noted that both pathogenic and water isolates were the most resistant as they resisted six antibiotics and were sensitive to both CIP and E compared to the soil isolate that resisted four antibiotics and was sensitive to four others. As for *S. aureus* isolates, it is noted that pathogenic isolate was the most resistant and showed sensitivity only to RA followed by water isolate, which has resisted six antibiotics were sensitive to both CIP and E, while the soil isolate were sensitive for RA and CIP and E and resistance to other antibiotics, Table 1.

The antibiotic resistance differences in isolates of the same type of bacteria from different isolation sources, confirms that resistance mechanisms influenced by the environment in which they are present, as the transmission of the resistance characteristic depends on the different horizontal transfer methods of resistance genes between bacteria present in the same environment. Resistance genes are usually carried on the chromosome of bacteria or plasmids that contribute to the transmission of genetic material between the same bacterium or different species [18, 21].

Table 1: Sensitivity of studied bacterial isolates to antibiotics.

Bacteria	Source	Antibiotics(μg)							
		AMC (20)	Am (25)	KS (30)	CIP (10)	E (10)	RA (5)	S (25)	TMP (30)
<i>Pseudomonas aeruginosa</i>	Pathogenic	R	R	R	S	S	R	R	R
	Soil	R	R	S	S	S	R	R	S
	Water	R	R	R	S	S	R	R	R
<i>Staphylococcus aureus</i>	Pathogenic	R	R	R	R	R	S	R	R
	Soil	R	R	R	S	S	S	R	R
	Water	R	R	R	S	S	R	R	R

Regarding the effect of heavy metals on the studied isolates, it is noted through the results shown in Table (2) that there is a variation in the effect of heavy metals on these isolates through the different concentrations that resisted them, the concentration 1.6 (mg / ml) was considered (absolute resistance) being the highest concentration of heavy metals

Used in this study. Pathogenic *P. aeruginosa* isolate and pathogenic and water *S. aureus* isolates showed absolute resistance towards five of studied minerals and all isolates had absolute resistance to Lead acetate $(\text{CH}_3\text{CO}_2)_2\text{Pb}$., Table (2).

Table 2: Sensitivity of studied bacterial isolates to heavy metal salts.

Bacteria	Source	Heavy metals (mg/ml)						Absolute Resistance No.*
		Sn	Zn	Ag	Pb	Cu	Co	
<i>Pseudomonas aeruginosa</i>	Pathogenic	1.6	1.6	1.6	1.6	0.02	1.6	5
	Soil	0.1	1.6	0.4	1.6	0.02	0.8	2
	Water	0.8	0.4	0.8	1.6	1.6	1.6	3
<i>Staphylococcus aureus</i>	Pathogenic	1.6	0.8	1.6	1.6	1.6	1.6	5
	Soil	1.0	1.6	1.6	1.6	1.6	0.4	4
	Water	1.6	1.6	0.8	1.6	1.6	1.6	5

* = Resistance to the highest concentration of 1.6 (mg / ml)

Bacteria use different mechanisms to resist different concentrations of heavy metals. These mechanisms adopt several methods, including accumulation and complexation

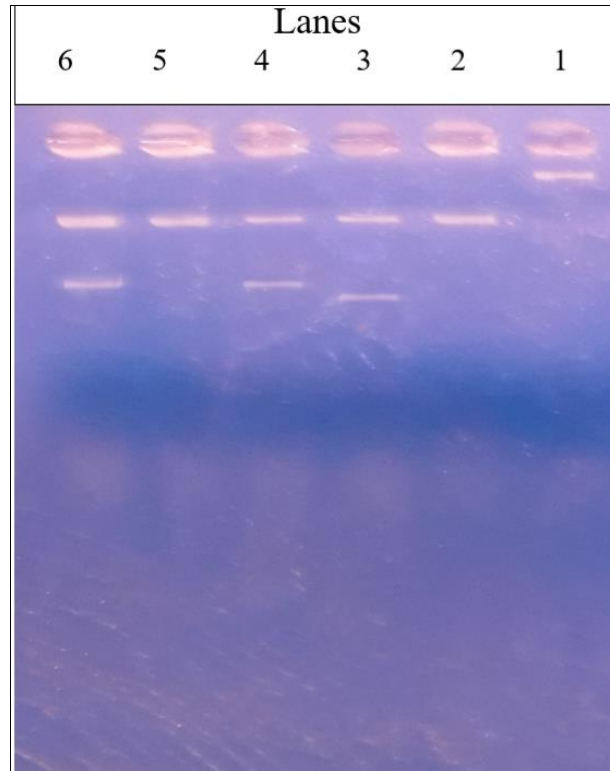
of the metal ions inside the cell, and the efflux of metal ions outside the cell, and the reduction of the heavy metal ions to a less toxic state [7, 10].

Studies indicate that many genes responsible for resistance to heavy metals are portable by plasmids, and there is a relationship between antibiotic resistance and heavy metals resistance [12, 15, 19].

The emergence and spread of multidrug resistance in bacteria is observed due to co-contaminations of heavy metal and antibiotics, and relation is existed between heavy metals tolerant genes and increased concentrations of both heavy metals and antibiotics in environments [3, 22].

Figure (1) shows the plasmid content of the studied isolates, which notes that there is a variation in the number of

plasmids possessed by these isolates (1-2 bundles), both pathogenic and soil isolates of *P. aeruginosa* showed the presence of one plasmid bundle while water isolate showed the presence of two plasmid bundles, on the other hand pathogenic and water *S. aureus* isolates showed the presence of two plasmid bundles and soil isolates showed the presence of one plasmid bundle, and these results confirm other research results which indicates variation in the plasmid content of bacteria isolates depending on their source of isolation [9, 12].



Lane (1) Plasmid content of pathogenic *P. aeruginosa*. Lane (2) Plasmid content of soil isolate *P. aeruginosa*. Lane (3) Plasmid content water isolate *P. aeruginosa* Lane (4) Plasmid content of pathogenic *S. aureus*. Lane (5) Plasmid content of soil isolate *S. aureus*. Lane (6) Plasmid content of water isolate *S. aureus*.

Fig 1: The plasmid content of studied isolates.

Table (3) shows the relationship of antibiotic resistance and heavy metals with the plasmid content of the studied isolates the results indicates that there is no consistent pattern that combines isolates of single bacterial species, except in the case of pathogenic and water isolates of *S. aures*, which showed similarities in resistance pattern and plasmid content.

The resistance characteristic of antibiotics and heavy metals clearly observed in the studied isolates reflects that these

Isolates have different resistance genes coded for these resistance characteristics, and these resistance genes may be carried on plasmids, but some of them may be carried on the chromosome or transposons [14, 17]. Hence, an additional study that includes plasmids curing may explains the resistance genes carried on these plasmids, then the relationship between plasmid content with antibiotic resistance, and heavy metals became more clear.

Table 3: The relationship between antibiotic resistance and heavy metals and the plasmid content of studied isolates.

Bacteria	Source	Resistance of Antibiotics & Heavy metals		Plasmids No.
		Antibiotics	Heavy metals	
<i>Pseudomonas aeruginosa</i>	Pathogenic	AMC, Am, KS, S, TMP, RA	Sn, Zn, Ag, Co, Pb	1
	Soil	AMC, Am, S, RA	Zn, Pb	1
	Water	Am, KS, S, TMP, RA, AMC	Cu, Co, Pb	2
<i>Staphylococcus aureus</i>	Pathogenic	AMC, Am, TMP, KS, CIP, E, S	Cu, Sn, Ag, Co, Pb	2
	Soil	AMC, Am, KS, S, TMP	Zn, Ag, Pb, Cu	1
	Water	AMC, Am, KS, S, TMP, RA	Cu, Sn, Zn, Co, Pb	2

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