Detection of the Transposition Property of Genes that Confer Resistance to Antibiotics, Heavy Metals and Minimal Media (M9)

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Abstract

This study involved fifty four bacterial isolates of different species collected from patients with urinary tract infection in TIkrit city during February-June 2008. Bacterial isolates were identified by using morphological and cultural characteristics and biochemical tests.Results showed that17(31.48%) bacterial isolates were gram negative and the rest were gram positive. The resistant bacterial isolates were examined for 17 antibiotics belonging to various group of antibiotics and also to 5 salts of heavy metals, in addition to the ability of utilize of different sugar as carbon source. These isolates revealed different patterns of resistance and utilizing ability of sugar. Another character of plasmid DNA in the bacterial isolates was investigated. Character of plasmid DNA in bacterial isolates was also included in this study where the transposition property for genes coding for antibiotics and heavy metals resistance and utilizing ability of sugar was detected using the incubation temperature 40 °C to 90 minutes as inducer factor for transposing genes jumping. in this concern results showed that in all isolates genes coding for all antibiotics except (Ciprofloxacin and Nitrofurantion), heavy metal ($HgCl_2$) and sugar (Lactose).

Key words: Transposon, UTI, Heavy Metal,Minimal Media

الكشف عن صفة القفز للجينات مانحة المقاومة للمضادات الحيوية ، المعادن الثقيلة والوسط الغذائي الادنى (M9)

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الملخص

تضمنت هذه الدراسة جمع (54) عينة من الإدرار من المصابين بالتهاب المجاري البولية في مدينة تكريت للفترة من شهر شباط - حزيران 2008، شخصت العزلات البكتيرية باستخدام الاختبارات المظهرية والفسلجية والكيموحيوية. أظهرت النتائج 17 (31.48%) عزلة بكتيرية سالبة لصبغة كرام ، و المتبقي هي عزلات بكتيرية موجبة لصبغة كرام. اختبرت قابلية العزلات البكتيرية على مقاومة 17 مضادا حيويا تعود الى مجاميع مختلفة ، كذلك فقد اختبرت مقاومتها لـ 5 املاح من المعادن الثقيلة، فضلا عن قابليتها على استخدام سكريات مختلفة مصدرا للكربون، وأظهرت الأنواع السائدة اختلافا في مقاومتها للمضادات الحيوية والمعادن الثقيلة، وكذلك في قابليتها على استخدام السكريات مصدرا للكربون . من بين الصفات الوراثية التي تمت دراستها لتوصيف محتوى الـ DNA البلازميدي للعزلات البكتيرية فقد تمت دراسة صفة قفز الجينات مانحة المقاومة للمضادات الحيوية والمعادن الثقيلة وذات القابلية على استخدام السكريات مصدرا للكربون . حفزت الجينات باستخدام درجة تحضين (40°C) مدة (90) دقيقة. اظهرت النتائج ان جميع العزلات البكتيرية تمتلك قابلية القفز في جيناتها مانحة المقاومة للمضادات الحيوية والمعادن الثقيلة وفي

استخدام السكريات كمصدر للكربون ماعدا المضادين Ciprofloxacin, Nitrofurantion والمعدن الثقيل كلوريد الزئبق وسكر اللاكتوز والذي لم يكن للعزلات البكتيرية اي قابلية قفز تجاهها.

Introduction

Urinary tract infection (UTI) is a heterogeneous disease, which can be divided into several types of infection, such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria. The urinary tract is generally a hostile environment for bacteria and except for the distal urethra it is usually sterile. Infection results when the bacteria virulence factor overcomes the numerous host defence mechanism [(1)]. UTI are one of the most common infectious diseases diagnosed in outpatients as well as in hospitalized patients, and can lead to significant mortality [(2)]. UTI account for a large proportion of antibacterial drug consumption and have large socio-economic impacts [(3)]. UTI is a common condition in children. Approximately 1 in 10 girls and 1 in 30 boys will have a UTI by the age of 16 years [(4)] It occurs in 3-5% of girls and 1% of boys up to 5 years, and peaks during infancy and toilet training period [(5,6)]. in males most often occur in neonates and in older men [(7)].

Urinary tract infection remains a major medical problem in terms of number of women afflicted each year. Although anti-microbial agents are generally effective at eradicating these infections, there is a high incidence of recurrence [(7)]. The patient's quality of life is affected and many women become frustrated by cycle of repeated antimicrobial agents whose effectiveness is diminishing due to increasing development of microbial resistance. In addition, the use of antimicrobial agents is not only selects resistance bacteria but it can also disturb the balance of body by killing useful bacteria, when this happens bacteria and yeast can move in and flourish leading to uro-genital tract infection [(8)].

Transposons are DNA elements with the ability to move, or transpose, to new locations within a genome. Several modified transposon systems have proven useful as genetic tools for insertional inactivation of genes, identifying conditionally regulated genes, and genome sequencing [(9)]. These techniques have identified microbial genes essential for growth and virulence, as well as loci affecting physiology and morphology [(10,11,12,13)]. Mobile DNA elements, originally discovered in maize more than fifty years ago, have become the indispensable tools for bacterial genetics: so many different types of specialised transposon derivatives were constructed so far [(14)].

The transposable elements divided into three distinct classes, based on the structural properties, mechanism of transposition and DNA sequence homology[(15)]: *Class I* - the insertion sequence (IS) modules and composite elements formed from them. IS modules are short elements, less than 2 kb in size, encoding only determinants relevant to their own transposition (IS1-IS5, IS102, ISR1). Two copies of certain ISs flanking a DNA segment were termed the composite transposons. *Class II* - the transposon (Tn) family, sized more than 5 kb, containing 38-40 bp inverted repeats at their ends, which generate 5 bp repeats of target DNA during insertion. These usually encode, in addition to transposition functions, the accessory determinants, such as antibiotic and heavy metal resistance. *Class III* - presents the transposing bacteriophages, such as Mu or its derivatives. These possess the genes and sites for transposition as well as the genes for DNA replication, phage development and cell lysis [(14,15)].

An IS is a discrete segment of DNA, commonly between 1 and 1.5 kb, that can transpose directly to new sites on the

genome. An IS element usually has a short inverted repeat (IR) sequence at both ends. ISs are found on the chromosomes of many different sequenced bacterial and archaeal genomes [16]. In some bacterial lineages, many IS elements are found in high copy numbers These elements play an important role in chromosomal rearrangements and generation of pseudo genes in bacteria [17].A transposon, unlike an IS element, is a more complex type of mobile genetic element. Transposons contain genes that encode proteins required for transposition and proteins that have other functions including, for example, resistance to antibiotics or heavy metals. An important group of transposons is the conjugative transposon (CTn), which is able to mobilize from one bacterial cell to another of the same, or different, species by a conjugation-like process that requires cell-to-cell contact[18].

Material and Methods

Specimens Collection and Identification

A total of 54 samples of urine collected from patients of both sexes of the age group 4-60 years admitted and hospitalized patients in Tikrit Teaching Hospital in Tikrit city from 1st February 2008 to 30th June 2008. Collected urine from patients were transported to the laboratory within two hours, enriched in nutrient broth and incubated at 37 ºC aerobically for 18-24 hours. Each sample is sub-cultured on blood agar and MacConkey agar and EMB (Difco, USA) [19]. The identification of tests is including cultural, morphological and biochemical characteristics for each isolate. Colonies of the bacterial isolates were described according to their shapes, color, diameter, odor, and other characteristics [20].

Preparation Antibiotic and Heavy metals stock solutions

Stock solutions in mg/ml of seventeen different antibiotics (table 1), were prepared by using ethanol and distaled water as solvents. They were added in the final concentration µg/ml. in addition, stock solutions of the five heavy metals in mg/ml were prepared (table 1), and they were used in the final concentration µg/ml [21].

Table (1) Stock solutions and Final concentration and Solvent to antibiotics and heavy metals

Antibiotics	Symbol	Stock solutions mg/ml	Final con. µg/ml	Solvent
Amoxicillin	Ax	25	50	Ethanol 70%
Ampicillin	Am	25	50	Ethanol 70%
Cefotaxime	CTX	25	50	Distal water
Cephalexin	CL	20	30	Distal water
Chloramphenicol	Cm	20	10	Absolute Ethanol
Ciprofloxacin	Cf	30	30	Ethanol 70%
Clarithromicine	Cw	20	10	Ethanol 70%
Erythromycin	E	20	15	Distal water
Gentamicin	GN	80	30	Distal water
Metronidazole	Mt	25	50	Ethanol 70%
Nitrofurantoin	Nf	30	30	Ethanol 70%
Penicillin G	P	25	50	Distal water
Refampicin	RA	5	5	Distal water
Streptomycin	S	25	25	Distal water
Tetracycline	TE	12.5	15	Ethanol 70%
Trimethoprim	Tr	20	10	Distal water
Vancomycin	Va	25	50	Ethanol 70%
mercury chloride	$HgCl_2$	50	25	Distal water

Cadmium Chloride	$CdCl_2$	50	25	Distal water
Cobalt Chloride	$CoCl_2$	50	25	Distal water
Zink Chloride	$ZnCl_2$	50	25	Distal water
Nickel Chloride	$NiCl_2$	50	25	Distal water

To determine antibiotics and heavy metals resistance patterns, the nutrient agar plates with final concentrations of antibiotics and heavy metals listed above were prepared. The bacterial species were streaked onto these plates, incubated at 37 ℃ overnight and the results of the bacterial group were recorded [22].

Preparation Minimal Medium (M9)

6 g Na_2HPO_4 , 3 gKH_2PO_4 , 0.5 g NaCl, 1 g NH_4Cl) solubilize in 1 L distal water adjust pH to 7.4 . Add 2 ml $MgSO_4$ 1 *M*, 10 ml sugar 20% (w/v), 0.1 ml $CaCl_2$ 1*M*) all autoclaved without the sugar sterilize by filtration [23].

Inducing the Transposition of Jumping Genes that Confer Resistance

Inducing the transposition was carried out according to the method mentioned by Dulaimy and Ghelawy (2007)[24]. The bacterial isolates 0.1 ml (approximately 5-9×10^7 cells/ml as determined by dark field microscopy using a Petroff-Hausser chamber) were grown in 10 ml Nutrient broth with antibiotics and heavy metals in shaking incubator for 90 minute at 40 ℃ until the optical density at 600 nm of the culture reached more than 0.5.

Master Plate Preparation for Bacterial Isolates Growing in the Presence of Antibiotics and Heavy Metals

Prepared ten serial culture dilutions that grow of induction process and transferring 0.1 ml from the last three dilutions to Nutrient agar plate which contain the final concentration of antibiotics and heavy metals. The plates were incubated for 24 hours at 37 ℃ . The master plate prepared that transfer 100 random bacterial colonies .

Detection of Mutations that Generated from Transposing Genes as Result of their Induction.

Transfer the bacterial colonies after 24 hours to Nutrient agar plates which contain the final concentration of antibiotics and heavy metals. Also, colonies tested on minimal media which are additive the sugar. The plates were incubated for 24 hours at 37 ℃ .Compute the percentage value of mutant colonies through limiting the percentage value of non growth of colonies isolates on the plates.

Results and Discussion

The genetic transposones play an important role due to their mutations and taking part in rearrangement the genome of organism, these element lead therefore to gain new genes and help in their dissemination in the bacteria. The insertion of these transposons into the sequence of the target genes results in its inactivation, as well as their participation in deletion or their multiplying the genes and inverting the DNA segments and helping in integration of the multipliers[24]. The molecular mutation is conducted by induction the transposition of the responsible genes on the resistance by thermal induction at 40C for 90 minutes to induce the mutant genes to transpose and insert at random into devours sites of the bacterial chromosome to plasmid as well as making the genetic mutation by inactivation through the insertion of the transposed genes. Thus it is the generated mutation by the transposition of the genes that give the resistance of the antibiotics to the plasmid through transposing the growing colonies on the master plate to plates contained the nutrient agar in which added the separated studied antibiotics, and the plates contained the separately heavy metals. In

addition, they are transported to the plates contained the minimal media (M9)in which added the suitable sugar and after their incubation in 37C for 24 hour, the results are shown in table (2).

The resulted illustrated in the table revealed the difference into the resistance loss ratios to the antibiotics and the higher resistance loss ratio is registered by the *S.aureus* which reached to 44% for the Tr antibiotic. The genetic transposition is indicated that the genes of resistance to this antibiotic is located on the chromosome of the bacterial isolate and due to the thermal induction, the responsible genes on resistance to this antibiotic may enable to transpose and insert into the genome of the bacterial cell. As the results explained. There is a resistance loss to some of the antibiotics, so the percentage of the resistance loss for the isolates 1 and 51 in *C. diversus* ranged 2-37% to the listed antibiotics in the table, while the *P. valgaris* isolate 5 is detected the resistance loss to the couple antibiotics Va and E with the ratios 8% and 21% respectively. Besides, the percentages of the resistance loss for the isolates 7 in *K. oxytoca* were 2%, 6% and 11% to the antibiotics Va, E and Am, respectively. Whereas the percentage of the resistance loss for the couple isolates 30 and 43 in *E. coli* represented 5-40%. The *Se. marcescens* isolate 18 had but one chance represented by the resistance loss to the antibiotic Cm with ratio 15%. While the *Y. enterocolitica* isolate no.19 had the ratios of the resistance loss to antibiotics ranged between 6-43%. Through the table, the isolates are discovered the multiplier in the most of the bacterial species in ratios of the resistance loss to the antibiotics.

Table (2) the percentage of colonies lose its resistance to antibiotics

No. of Isolates	Isolates Name	Number of colonies tested	The percentage of colonies devoid of antibiotic resistance using a final concentration of µg / ml															
			Vancomycin (50)	Penicillin G (50)	Gentamicin(80)	Erythromycin (15)	Amoxicillin (50)	Ampicillin (50)	Tetracycline (15)	Ciprofloxacin(30)	Refampicin(5)	Cephalexin(20)	Chloramphenicol (10)	Trimethoprime (10)	Clarithromicine (10)	Cefotaxime (50)	Nitrofurantoin (30)	Metronidazole (50)	Streptomycin (25)
1	*C. diversus*	100 Colony each one	S	S	S	32	25	6	S	S	S	S	S	22	S	S	S	S	S
51	*C. diversus*		0	0	0	0	0	0	37	0	0	2	0	8	12	6	0	16	0
5	*Pr. Vulgaris*		8	S	S	21	S	S	0	S	S	S	S	S	S	S	S	0	S
7	*K. oxytoca*		2	S	S	6	0	11	S	S	S	0	S	0	S	S	S	S	S
30	*E. coli*		0	6	0	23	0	0	0	S	S	0	S	0	0	0	S	0	S
43	*E. coli*		0	0	5	S	40	0	S	S	S	0	6	0	S	0	S	0	0
18	*Se. marcescens*		0	0	0	S	S	0	0	S	S	0	15	0	S	0	S	0	0
19	*Y. enterocolitica*		S	S	S	S	34	43	19	S	S	6	8	29	36	21	S	0	S
8	*S. sciuri*		0	0	S	4	0	0	12	S	19	0	0	0	0	0	S	0	S
22	*S. xylosus*		0	0	0	0	0	22	5	S	S	0	S	0	0	0	S	0	0
34	*S. heamolyticus*		0	0	S	S	0	0	0	S	S	0	0	0	0	0	S	0	0
28	*S. lentus*		0	0	0	0	8	3	0	S	S	0	S	0	0	0	S	0	S
33	*S. lentus*		5	S	S	S	15	12	S	S	S	0	S	22	0	18	S	0	24

21	*S.aureus*	S	S	S	S	S	0	7	S	S	S	S	44	S	S	S	0	S
46	*S. aureus*	0	3	32	S	0	7	0	0	0	0	32	0	0	0	0	0	0
9	*S. saprophyticus*	4	7	S	0	0	0	0	S	S	0	S	0	0	5	S	6	22
13	*S. saprophyticus*	0	0	6	33	0	0	9	S	S	0	4	0	0	0	S	0	0
44	*S. saprophyticus*	0	0	5	S	0	0	S	S	S	0	0	0	S	0	S	0	S
54	*S. saprophyticus*	0	0	1	S	0	2	S	S	S	0	4	0	S	0	S	0	S

S:senestive

The staphylococcus isolates are recognized the difference in ratios of the resistance loss to antibiotics, while *S. sciari* isolate 8 is showed ratios of the resistance loss to antibiotics E, TE and RA that represented 4%, 12% and 19% respectively, but the *S. xylosus* isolate 22 appeared the ratios of the resistance loss up to 22% and 5% to antibiotics Am and TE respectively. Although the *S. heamolyticus* isolate no 34 had no ratios of the resistance loss to any antibiotics, but the ratios of the resistance loss ranged between 3% to 22% to antibiotics for the two isolates 28 and 33 in *S. lentus* that showed a difference in the transposition where as the isolate no. 28 is less in the resistance loss ratios that of the isolate 33 that appeared more numerous in the resistance loss ratios. We observed also the cut-clear variation between the both isolate 21 and 46 in *S.aureus* in ratios of the resistance loss to antibiotics which are ranged between 3-44%. Finally, the three isolates 13, 44 and 54 belonged to the species *S. saprophyticus* are shared the resistance loss ratios to the antibiotics GN but differed in the rest ratios for the isolate 9, and the resistance loss ratios for the four isolates are ranged between 1-33%.

The two antibiotics CF and NF had no ratios of the resistance loss for any studied bacterial isolates, but the antibiotic Am had the higher ratios of the resistance loss compared with the other antibiotics and there are difference in ratios of the resistance loss to other antibiotics, where as the antibiotic RA had but 19% in *S. sciuri*. The higher ratios of the resistance loss to the antibiotic Am may be relative to the transposon *Tn3* in the bacteria that showed the mutant nature and had the capacity of coding to resisting this antibiotic. The bacterial species which appeared the higher loss ratios to Am may contain a group of the variable transposons that represented by *Tn* family. It is characterized by the longer Inverted Repeats (IR), non containing the Insertion Sequence (IS), as well as the middle repeats coding the transposing enzymes and resolves[25]. When observed the resistance loss ratios to the antibiotics in gram positive bacterial isolates, we found a cut-clear variation in these ratios among the species, it is likely belonged to being have these species the conjugative transposon which is aimed to obtain a doublement into the DNA segment in which is targed and inserted the transposone as it is done in the resistance bacterium TE that indicated being have the *Tn 917* conjugated transposon which is coding the resistance to this antibiotic by the gram positive bacteria and it is showed that the conjugated transposon is the cause of the spread resistance of these species.

The difference in the ratios of the resistance loss to antibiotics that appeared by the bacterial isolates which are induced by the higher heat, this difference may indicated that these isolates had the

mutant by the genes bearing on their chromosomes or the plasmids of these bacteria, but the temperature used in this operation played an important role. the transporation of the transposons from one site to another into the genetic content is caused the mutation into the new site in which it is transferred [(26)]. In addition, the bacterial species had the (IS) and their composite transposons that are coding the existing DNA among the insertion repeats for certain function, on of which the resistance to one or most of the antibiotics as it is happened with the transposon *Tn10* that is coding the resistance to the TE antibiotic and the bacterial isolates had it with the resistance loss ratio to this antibiotic. It is believed that the transposons arecaused the resistance to the antibiotics in view of the isolated plasmids from the present infections are bearing a number of the transposon that or coding the resistance to the existing antibiotics located on their various sites. The integration resultant of the genetic transposons from their hybridization led to the various resistance to antibiotics. Having the transposon the limitations of the resistance may result in, some cases, creation of the complex transposon coding the resistance to some of the antibiotics, and what increased the existence and influence of this transposon is the provision of the selective pressure that is representing by using the antibiotics in treatment, as well as the horizontal genetic transpositions among the cells as the transformation and conjugation , helped in spreading the plasmids that are bearing the transposons as R-factor, and after entering these plasmids into the cells. The transposon is enable to transpose to the plasmids of the inter cell, and then transpose to the cell chromosome, so the stability of the mobile plasmids into the cell did not debar against the resistance spread existed on the transposons[(25)].

The resistance loss ratios that are considered lower as compared with it appeared in the antibiotic Am case, perhaps resulted in absence of the mutation because these antibiotics whose the giving resistance genes are invaded by the transposing genes, as well as they are resulted in the condition of the culture as the repeated culture or by the spontaneous mutation in these genes. This study is agreed with the study conducted by Ahmed and Sphora (2007)[(27)], and study conducted by al-Rifaay (2008)[(28)] but it is in difference with the resistance loss ratios to some antibiotics in their studies and it is not agreed with the study of Takamatsu *et al*., (2003)[(29)] who mentioned that the insertion of certain transposon into the plasmid RP4 that giving the resistance to both antibiotics TE and Am. This is led to resistance loss to antibiotic TE with ratio 1-2% while the ratio in loss to this antibiotic, ranged among 5-37%.

When tested the colony growing in the used heavy metal salts,table (3) we observed the lower resistance loss ratios in all of the used heavy metals, so we detected the resistance loss ratios in $NiCl_2$ only in the *K. oxytoca* and *S. saprophyticus* reached 8% and 3% respectively. But the $ZnCl_2$ had but the resistance loss ratios in isolates 34, 33 and 46% with the percentage 4%, 22% and 6% respectively, it is noting that the *S. heamolyticus* isolate 34 did not show any loss ratios to the antibiotics and appeared

the ratio 4% as resistance to the $ZnCl_2$, this indicated that it is having the mutant

The percentage of colonies loss of resistance to heavy metals by final concentration (25µg/ml)					Number of colonies tested	Isolates Name	No. of Isolates
$CdCl_2$	$HgCl_2$	$CoCl_2$	$ZnCl_2$	$NiCl_2$			
S	S	S	0	0	100 Colony each one	*C. diversus*	1
0	0	5	0	0		*C. diversus*	51
S	S	S	0	0		*P. vulgaris*	5
0	S	S	0	8		*K. oxytoca*	7
7	0	S	0	S		*E. coli*	30
S	S	S	0	S		*E. coli*	43
6	S	S	0	S		*S. marcescens*	18
S	S	S	S	S		*Y. enterocolitica*	19
6	0	S	0	0		*S. sciuri*	8
4	S	S	0	S		*S. xylosus*	22
0	0	S	4	0		*S. heamolyticus*	34
S	S	S	0	0		*S. lentus*	28
S	S	0	22	0		*S. lentus*	33
0	0	S	S	S		*S.aureus*	21
0	S	0	6	0		*S. aureus*	46
0	0	S	0	3		*S. saprophyticus*	9
0	0	S	0	S		*S. saprophyticus*	13
S	S	S	0	S		*S. saprophyticus*	44
22	0	0	0	0		*S. saprophyticus*	54

Table (3) the percentage of colonies lost its resistance to heavy metals

S:senestive

genes responsible to resist this metal without the antibiotics. Besides, the metal $CoCl_2$ had but the ratio of the resistance loss amounted 5%for the isolate 51 in *C. diversus* only.While the resistance loss ration for $CdCl_2$ ranged between 4-22%, it is the highest ratios in the metals. $HgCl_2$ has no resistance loss ratios in all isolates. These results are not correspondent in ratios with[27]that results ranged between 55-100% and 5-10% for the two metals $HgCl_2$ and $CdCl_2$ respectively.

When investigated the colonies of the growing loss in the minimal media (M9)table (4) that contained the various sugars separately, we found that these ratios are variable among them. We found the higher loss ratios in Arabinose sugar metabolism, which ranged between 2-53% while the higher ratio of the growing loss in the M9 that contained glucose is ranged between 3-62%, as well as the maltose is registered the growing loss ratios that are 3-44%. These ratios of the three sugars are somewhat equivalent in regard of their represented isolates as

compared with the absence of any growing ratios into the M9 contained Lactose sugar.

Table (4) the percentage of colonies has lost the ability to use sugars

The percentage of colonies has lost the ability to grow on various sugars				Number of colonies tested	Isolates Name	No. of Isolates
Maltose	Glucose	Lactose	Arabinose			
ng	Ng	0	Ng	100 Colony each one	*C. diversus*	1
0	0	0	44		*C. diversus*	51
ng	Ng	Ng	Ng		*P. vulgaris*	5
ng	Ng	0	Ng		*K. oxytoca*	7
ng	0	0	26		*E. coli*	30
0	0	0	0		*E. coli*	43
0	0	0	2		*S. marcescens*	18
ng	Ng	Ng	Ng		*Y. enterocolitica*	19
0	0	Ng	0		*S. sciuri*	8
3	22	0	5		*S. xylosus*	22
0	0	0	53		*S. heamolyticus*	34
0	0	0	0		*S. lentus*	28
4	0	0	33		*S. lentus*	33
ng	4	Ng	0		*S.aureus*	21
0	Ng	0	Ng		*S. aureus*	46
44	3	0	0		*S. saprophyticus*	9
0	0	Ng	0		*S. saprophyticus*	13
0	0	0	0		*S. saprophyticus*	44
9	62	0	0		*S. saprophyticus*	54

ng: no growth

These results are not correspondent with these obtained by [27] and with these of Kroose *et al* (1986)[30], who reported that the insertion of the transposon Tn5 into the genes coding the responsible genes on the Lactose metabolism led to reduce the genetic expression of the B-galactosidease enzyme, thus resulted in a mutation into the gene coding this enzyme.

The induction by heat that is used in the transposition and lead to the resistance loss to the heavy metals and loss of the capacity of using the sugars may be due to the mutation and may be due to being the giving genes of the resistance to the antibiotics and the responsible genes on the sugar metabolism located on the plasmids that are giving the resistance to the antibiotics, so when these elements are transposing, this may affect into the closed genes of the region in which the genes located on these plasmids are invalid.

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